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 ammonia. 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.

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

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

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

3.2.1 Inhalation Exposure

3.2.1.1 Death

There are many reports in the literature of human deaths resulting from inhalation of ammonia (Arwood et al. 1985; Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; George et al. 2000; Heifer 1971; Price et al. 1983; Sobonya 1977; Walton 1973; Weiser and Mackenroth 1989; Yang et al. 1987). Most of these reports are of acute accidental exposure to ammonia gas. A review of the early literature on ammonia toxicity cites acute exposure to 5,000–10,000 ppm as being rapidly fatal in humans (Henderson and Haggard 1927; Mulder and Van der Zalm 1967) and exposure to 2,500–4,500 ppm as being fatal in about 30 minutes (Helmers et al. 1971; Millea et al. 1989). Immediate deaths resulting from acute exposure to ammonia appear to be caused by airway obstruction while infections and other secondary complications are lethal factors among those who survive for several days or weeks. Chemical burns and edema of exposed tissues, including the respiratory tract, eyes, and exposed skin, are often observed after exposure to lethal levels. Post-mortem findings in the fatal case described by Walton (1973) included extensive edema and burns affecting the mouth, faces, trunk, arms, and upper part of the trunk. The airway at the larynx was almost blocked and the lungs were greatly distended and congested. Histological examination of the lungs showed acute congestion and edema. The bronchial walls were stripped of their epithelial lining, and some smaller bronchi contained plugs of debris, which included epithelial cells, red blood cells, and dust cells. No reports of human death due to intermediate or chronic exposure to ammonia were located.

Studies in animals indicate that the acutely lethal exposure concentration depends on the exposure duration. The lethal concentration in rats and mice increases 5–10 times as the exposure duration decreases from 16 hours to several minutes (Hilado et al. 1977, 1978; Kapeghian et al. 1982; Morgan 1997; Prokop'eva et al. 1973; Weedon et al. 1940). Exposure frequency also appears to be an important factor in determining lethality. Continuous exposure to 653 ppm for 25 days resulted in nearly 64% lethality in rats, whereas intermittent exposure (5 days/week, 8 hours/day) to nearly twice this concentration was tolerated for 42 days (Coon et al. 1970). It appears that male rats are more sensitive than female rats to the lethal effects of ammonia (Appelman et al. 1982; Stupfel et al. 1971). Animals exposed to acutely lethal concentrations show severe lesions in the respiratory tract that are similar to those observed in humans. Less severe lesions of the liver, heart, and kidney have been observed following continuous long-term exposure to lethal concentrations in rats, guinea pigs, rabbits, and dogs (Coon et al. 1970). However, these may represent secondary complications from chronic respiratory tract injury.

3.2.1.2 Systemic Effects

Respiratory Effects. Ammonia is an upper respiratory irritant in humans. Exposures to levels exceeding 30 ppm result in immediate irritation to the nose and throat (Industrial Bio-Test Laboratories 1973; MacEwen et al. 1970; Sekizawa and Tsubone 1994; Verberk 1977). Four out of six human subjects described moderate irritation of the nose and eyes when exposed to 50, but not 30, ppm ammonia gas for 10 minutes (MacEwen et al. 1970). Twenty to 30% of subjects exposed to 72, but not 50, ppm ammonia gas for 5 minutes experienced eye, nasal, and throat irritation (Industrial Bio-Test Laboratories 1973). However, tolerance appears to develop with repeated exposure (Sekizawa and Tsubone 1994; Verberk 1977). Thus, subjects exposed to 50 ppm ammonia 6 hours/day, 5 days/week for 6 weeks experienced nose and throat irritation only during the first week (Ferguson et al. 1977). Acute exposure to higher levels (500 ppm) has been shown to alter respiratory minute volume (Cole et al. 1977; Silverman et al. 1949). Buff and Koller (1974) suggest that this is due to an effect on "irritant receptors" in the lungs resulting in increased activity of reflex respiratory muscles. This mechanism is also suggested by Cole et al. (1977), who exposed men to 100–331 ppm ammonia gas for 8–11 minutes while they were exercising on a stationary bicycle. Respiratory minute volume was decreased at concentrations of 150–331 ppm (but not at 100 ppm), and tidal volume was increased at 100 ppm ammonia, but decreased at higher concentrations (Cole et al. 1977). Accidental exposures to concentrated aerosols of ammonium solutions, high concentrations of ammonia gas, or anhydrous ammonia fumes have resulted in nasopharyngeal and tracheal burns, airway obstruction and respiratory distress, and bronchiolar and alveolar edema (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kass et al. 1972; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; O'Kane 1983; Price et al. 1983; Sobonya 1977; Taplin et al. 1976; Walton 1973; Weiser and Mackenroth 1989). Chronic occupational exposure (about 14 years) to low levels of airborne ammonia (12.5 ppm) had no significant effect on pulmonary function or odor sensitivity in a group of workers at a soda ash factory compared to a control group from the same factory that was not exposed to ammonia (Holness et al. 1989). An acute-duration inhalation MRL of 1.7 ppm was derived from the Verberk (1977) study, and a chronic inhalation MRL of 0.1 ppm was derived from the Holness et al. (1989) study; MRLs are presented in Table 3-1 and Figure 3-1 and are discussed in Section 2.3.

One human study with somewhat controlled exposure to ammonia showed that pulmonary function was not affected by low levels (25–100 ppm) of ammonia (Ferguson et al. 1977). Transient nose and throat

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Human	1 d 0.5 hr/d				5000 (rapidly fatal)	Henderson & Haggard 1927
2	Rat	1 d 15 min/d				17401 (LC50)	Prokop'eva et al. 1973
3	Rat	1 d 16 hr/d				1000 (LC50)	Weedon et al. 1940
4	Mouse	1 d 30 min/d				21430 (LC50)	Hilado et al. 1977
5	Mouse	1 d 1 hr/d				4230 (LC50)	Kapeghian et al. 1982
6	Mouse	1 d 60 min/d				11299 (LC50)	Prokop'eva et al. 1973
7	Mouse	1 d 16 hr/d				1000 (LC50)	Weedon et al. 1940

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
8	Rabbit	1 d 1 hr/d				5025 (LC50)	Boyd et al. 1944
9	Cat	1 d 1 hr/d				5025 (LC50)	Boyd et al. 1944
	Systemic						
10	Human	8-11 min	Resp	100 M	150 M (decreased minute volume; increased tidal volume)		Cole et al. 1977
11	Human	5 min	Resp	50	72 (nasal and throat irritation)		Industrial Bio-Test Laboratories, Inc. 1973
12	Human	10 min	Resp	30	50 (moderate nasal irritation)		MacEwen et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
13	Human	1 d 30 min/d	Resp		500 M (nasal and throat irritation; increased minute volume and respiratory rate)		Silverman et al. 1949
			Cardio	500 M			
			Hemato	500 M			
			Ocular		500 M (lacrimation)		
14	Human	1 d 2 hr/d	Resp		50[b] (urge to cough; irritation to nose and throat)		Verberk 1977
			Ocular		50 (irritation to eyes)		
15	Rat (Sherman)	4 wk 24 hr/d	Ocular		100 (eye irritation)		Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)	Reference Chemical Form
16	Rat (OFA)	1 wk 24 hr/d	Resp		500	(irritation)		Richard et al. 1978a
			Renal		500	(increased kidney weight)		
			Bd Wt		500	(body weight and food intake decreased 21%)		
17	Rat	7 d	Resp	714				Schaerdel et al. 1983
			Gastro	714				
			Hemato		15	(slight increase blood pO2)		
			Renal	714				
			Dermal	714				
			Other	714				

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
18	Mouse (ICR)	1 d 1 hr/d	Resp		3440 M (dyspnea; nasal irritation)	4220 M (congestive intraalveloar hemorrhage, 24% increased relative lung weight)	Kapeghian et al. 1982
			Hepatic			3440 M (degenerative changes; increased relative liver weight)	
			Bd Wt	3440 M	4220 M (12% reduction in body weight)		
19	Dog (Beagle)	1 wk 5 d/wk 8 hr/d	Resp	218.6 M	1085.7 M (temporary dyspnea during first week of exposure)		Coon et al. 1970
20	Rabbit (New Zealand)	1 wk 5 d/wk 8 hr/d	Resp	218.6 M	1085.7 M (temporary dyspnea during first week of exposure)		Coon et al. 1970
			Ocular	218.6 M	1085.7 M (temporary lacrimation)		
21	Rabbit	1 d 1 hr/d	Resp			5000 (acute pulmonary edema)	Richard et al. 1978b
			Cardio		2500 (bradycardia)	5000 (hypertension, acidosis, EKG change)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
22	Cat (Mongrel)	1 x 10 min (IT)	Resp		1000 (dyspnea; rhonchi; rales)		Dodd and Gross 1980
23	Pig (Belgian Landrace)	6 d	Resp	50 M	100 M (decreased pulmonary vascular response to endotoxin challenge)		Gustin et al. 1994
			Cardio	50 M	100 M (decreased pulmonary vascular response to endotoxin challenge)		
			Hemato	100 M			
			Endocr	100 M			
			Bd Wt	25 M	50 M (3% weight loss)		
24	Pig (Duroc)	1-2 wk	Resp	10	50 (frequent coughing)		Stombaugh et al. 1969
			Dermal	10	50 (oral and nasal irritation)		
			Bd Wt	10	50 (reduced weight gain and food intake)		

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	Immuno/ Lymphoret						
25	Mouse	7 d 24 hr/d			500 M (decreased resistance to infection)		Richard et al. 1978a
26	Pig (Belgian Landrace)	6 d		50 M	100 M (decreased pulmonary response to endotoxin challenge)		Gustin et al. 1994
	Neurological						
27	Rat	1 d 6 hr/d			100 (sensory irritation)		Tepper et al. 1985
28	Mouse	1 d 6 hr/d			100 (sensory irritation)		Tepper et al. 1985
	INTERMEDIATE EXPOSURE						
	Death						
29	Rat	90 d				641.6 (98% lethality)	Coon et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	Systemic						
30	Human	6 wk 5 d/wk 6 hr/d	Resp	25	50 (transient irritation of nose and throat)		Ferguson et al. 1977
			Cardio	100			
			Ocular	25	50 (transient eye irritation)		
31	Monkey (Squirrel monkey)	6 wk 5 hr/wk 8 hr/d	Resp		218.6 M (focal pneumonitis)		Coon et al. 1970
			Cardio	1085.7 M			
			Hemato	1085.7 M			
			Hepatic	1085.7 M			
			Renal	1085.7 M			
			Dermal	1085.7 M			
32	Rat (Sherman)	5-10 wk 24 hr/d	Resp		250 (nasal lesions, epithelial hyperplasia)		Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
33	Rat	6 wk 5 d/wk 8 hr/d	Resp	218.6	1085.7	(nonspecific inflammation)			Coon et al. 1970
			Cardio	1085.7					
			Hemato	1085.7					
			Hepatic	1085.7					
			Renal	1085.7					
			Ocular	1085.7					
34	Rat (Sprague-Dawley)	90 or 114 d	Resp	179.1	369.4	(mild nasal discharge in 25% of animals)	641.6	(interstitial pneumonitis)	Coon et al. 1970
			Cardio	369.4			641.6	(myocardial fibrosis)	
			Hepatic	369.4	641.6	(fatty changes of liver plate cells)			
			Renal	369.4			641.6	(renal tubular calcification)	

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
35	Gn Pig	6 wk 5 d/wk 8 hr/d	Resp	218.6	1085.7 (non specific inflammation)		Coon et al. 1970
			Cardio	1085.7			
			Hemato	1085.7			
			Hepatic	1085.7			
			Renal	1085.7			
			Dermal	1085.7			
36	Gn Pig (Hartley)	3 wk 24 hr/d	Resp	90			Targowski et al. 1984
			Hemato	90			
			Ocular	90			
			Bd Wt	90			

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
37	Gn Pig	18 wk 5 d/wk 6 hr/d	Resp	170			Weatherby 1952
			Cardio	170			
			Gastro	170			
			Hemato		170 (increased hemosiderin)		
			Hepatic		170 (congestion)		
			Renal		170 (congestion)		

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
38	Rabbit	114 d	Resp	57			Coon et al. 1970
			Cardio	57			
			Hemato	57			
			Hepatic	57			
			Renal	57			
			Dermal	57			
39	Pig (NS)	4 wks	Resp		100 (excessive nasal secretion, coughing, tracheal inflammation)		Drummond et al. 1980
			Ocular		50 (excessive lacrimation)		
			Bd Wt	50	100 (18.6% reduction in final body weight)		
	Immuno/ Lymphoret						
40	Rat (Sherman and Fischer)	4 wk 24 hr/d			25 (increased severity of infection by mycoplasma)		Broderson et al. 1976

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
41	Rat	3 wk 24 hr/d			500 M (reduced resistance to infection)		Richard et al. 1978a
42	Gn Pig (Hartley)	3 wk 24 hr/d			90 (significantly reduced delayed-type response to tuberculin)		Targowski et al. 1984
				50			
43	Pig (NS)	31-45 d 24 hr/d			100 (decreased serum concentration of gamma globulin)		Neumann et al. 1987
	Neurological						
44	Monkey (Squirrel monkey)	6 wk 5 hr/wk 8 hr/d		1085.7 M			Coon et al. 1970
45	Gn Pig	6 wk 5 d/wk 8 hr/d		1085.7			Coon et al. 1970

Table 3-1 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
46	Pig	4 wks		50	100 (lethargy)		Drummond et al. 1980
	CHRONIC EXPOSURE						
	Systemic						
47	Human	12.2 yr 5 d/wk 8 hr/d	Resp	9.2[c]			Holness et al. 1989
			Ocular	9.2			

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

b Used to derived an acute-duration inhalation MRL of 1.7 ppm; the MRL was derived by dividing the LOAEL of 50 ppm by an uncertainty factor of 30 (10 for variation in sensitivity among humans and 3 for use of a minimal LOAEL).

c Used to derive a chronic-duration inhalation MRL of 0.1 ppm; the MRL was derived by adjusting the NOAEL of 9.2 ppm for continuous exposure (9.2 x 8/24 hours x 5/7 days) and dividing by an uncertainty factor of 30 (10 for the protection of sensitive individuals, and 3 for the lack of reproductive and developmental studies).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; gastro = gastrointestinal; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; metab = metabolic; min = minute; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation

Acute (≤14 days)

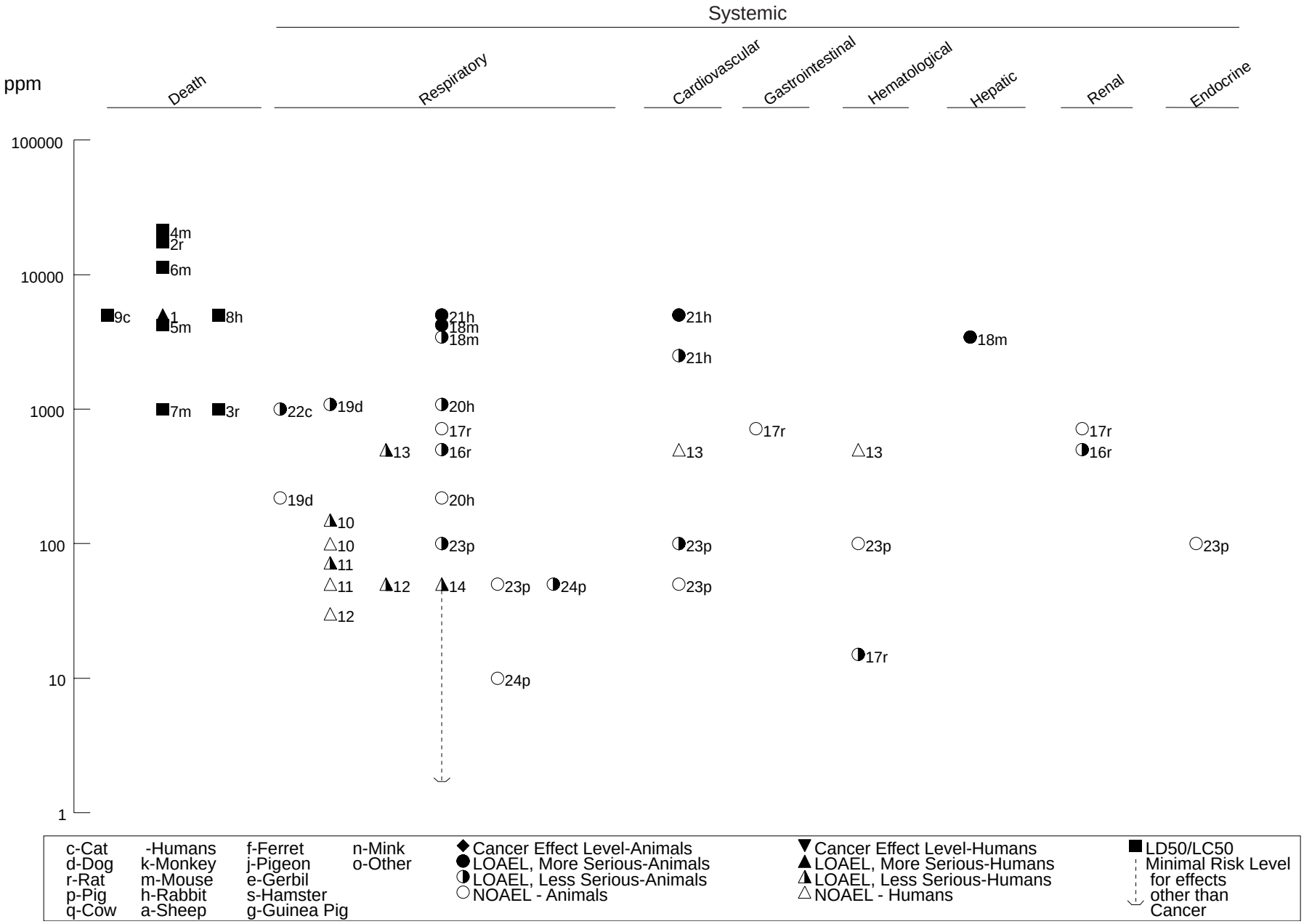


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (*Continued*)

Acute (≤14 days)

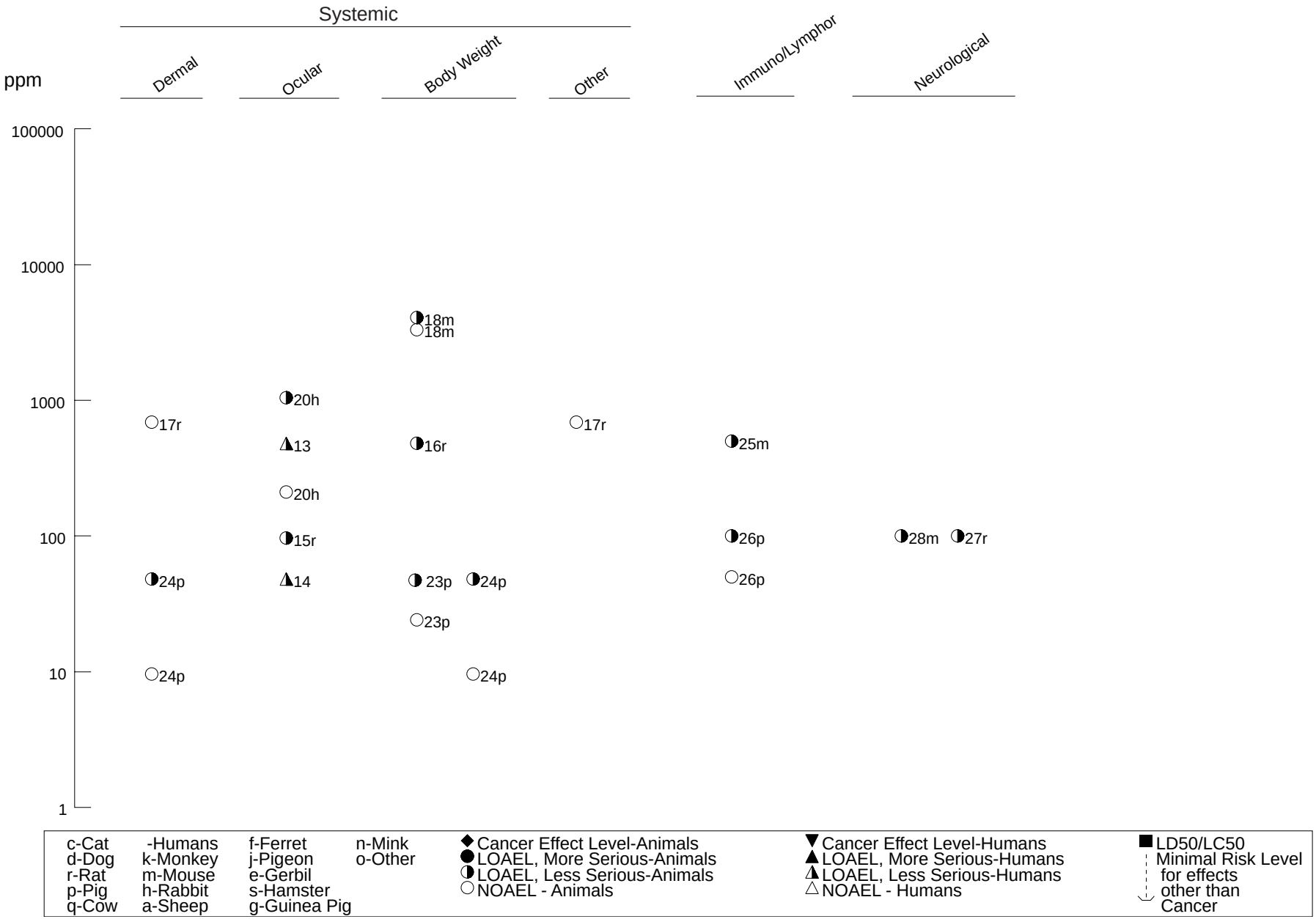


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (*Continued*)

Intermediate (15-364 days)

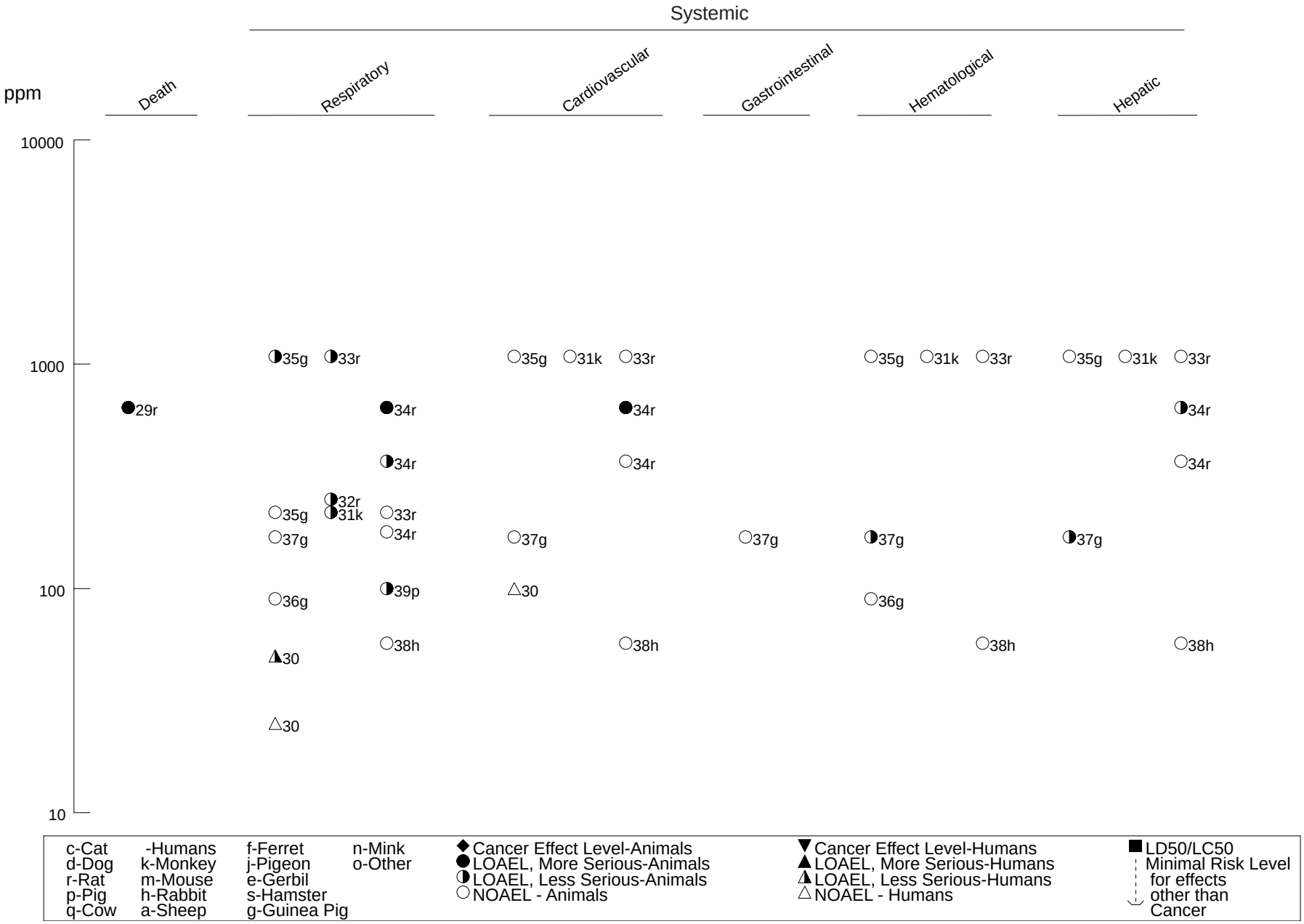


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Inhalation (*Continued*)

Intermediate (15-364 days)

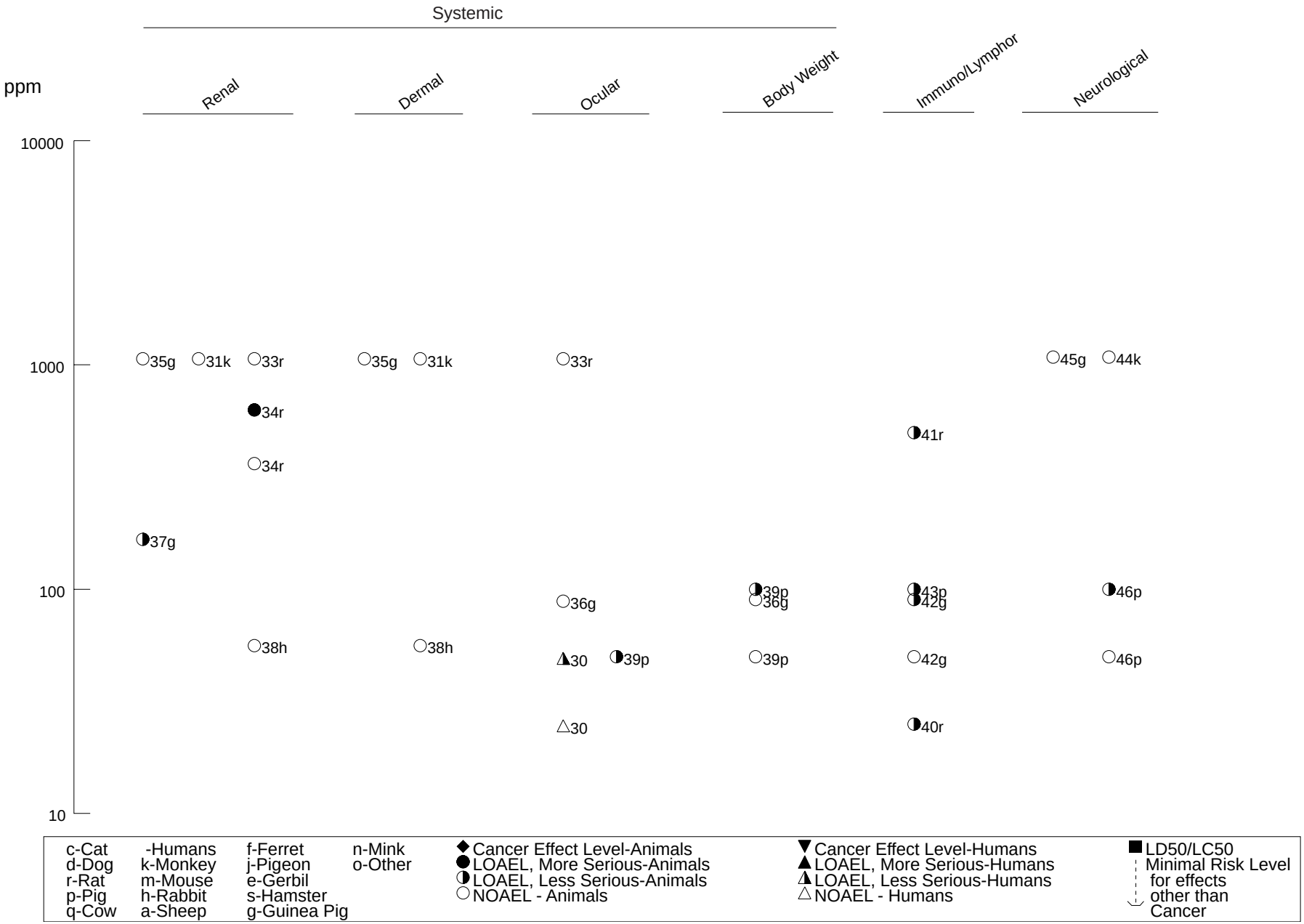
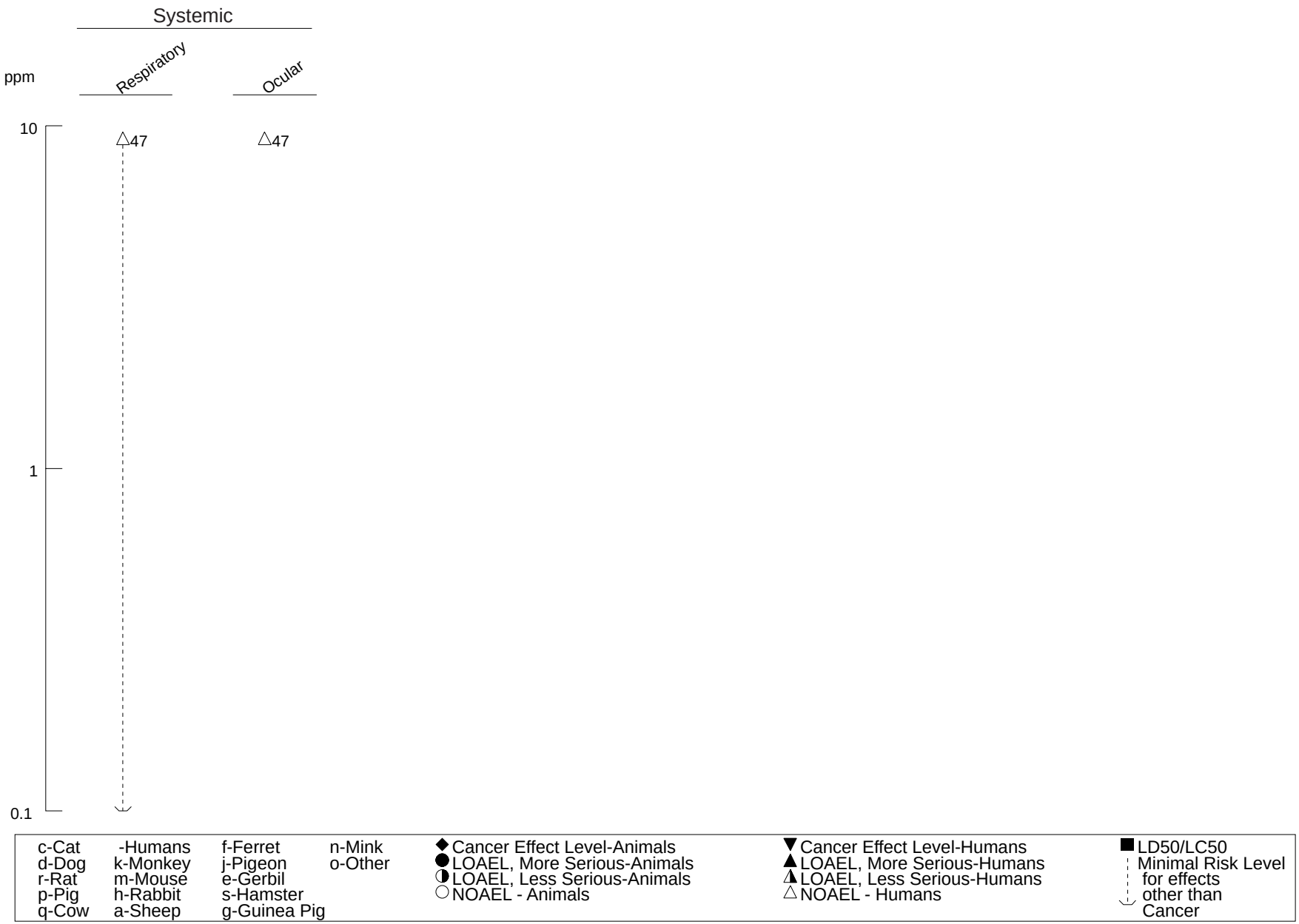


Figure 3-1. Levels of Significant Exposure to Ammonia And Ammonium Compounds- Inhalation (*Continued*)

Chronic (≥365 days)



irritation was noted the first week of exposure in male and female volunteers exposed to 50 ppm, but not 25 ppm ammonia 5 days/week, for 6 weeks. No significant differences were noted between exposed and control groups for pulmonary function tests, physical examinations, or performance of normal job duties (Ferguson et al. 1977).

A number of occupational cohort studies that examined farmers who worked in enclosed livestock buildings have been conducted. These studies all included measurements of ammonia in the livestock confinement buildings, as well as measurements of one or more of the following: total dust, respirable dust, carbon dioxide, total endotoxins, respirable endotoxins, fungi, bacteria, and molds (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Melbostad and Eduard 2001; Reynolds et al. 1996; Vogelzang et al. 1997, 2000). Of the pollutants measured, ammonia and dust were most frequently associated with respiratory effects, many of which were temporary and disappeared with cessation of exposure. Ammonia levels ranged from 2.3 to 20.7 ppm and total dust levels from 0.04 to 5.64 mg/m^3. Most of these studies reported an association between exposure to pollutants, including ammonia, in livestock confinement buildings and an increase in respiratory symptoms (such as bronchial reactivity/hyperresponsiveness, inflammation, cough, wheezing, or shortness of breath) and/or a decrease in pulmonary function (such as forced expiratory volume in the first second [$FEV_{1.0}$], maximum expiratory flow rates [MEF_{50} and MEF_{75}], and maximal mid-expiratory flow rate [MMEF]) (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990; Reynolds et al. 1996; Vogelzang et al. 1997, 2000). One study, however, reported correlations only between total dust, fungal spore, and endotoxin mean exposure levels and task-specific prevalences (Melbostad and Eduard 2001). Another study reported no significant correlations between lung function or chronic respiratory symptoms and dust or ammonia levels, but suggested that endotoxins and bacteria levels may play a role (Heederik et al. 1991). Most studies adjusted for confounding factors, such as smoking and number of years worked on a farm, in their statistical analyses. All of the studies concluded that prevalence of respiratory symptoms of some type was higher in the farmer cohort than in the respective control group. It is not clear from these studies what the contribution of ammonia is to the respiratory changes, but the cumulative data indicate that ammonia may contribute to transient respiratory distress in farmers working in enclosed livestock facilities.

A cross-sectional study of male workers at two fertilizer factories in Saudi Arabia showed a significant association between exposure to ammonia gas and respiratory symptoms including bronchial asthma (Ballal et al. 1998). Workers in factory one were exposed to air ammonia levels of 2.82–183.86 ppm (2.0–130.4 mg/m^3), and workers in factory two were exposed to 0.03–9.87 ppm (0.02–7.0 mg/m^3).

However, continuous exposure levels for workers could not be calculated because the number of days worked per week was not provided by the study authors. Logistic regression analysis showed that ammonia concentration was significantly related to cough, phlegm, wheezing (with and without shortness of breath), and asthma, whereas smoking was only a factor for wheezing and phlegm. Additionally, those workers exposed to ammonia levels above the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 25.4 ppm (18 mg/m^3) had significantly higher relative risks for cough, phlegm, wheezing, dyspnea, and asthma than workers exposed to levels below the TLV. Incidence of wheezing was also elevated in workers exposed to ammonia levels below the TLV. Cumulative ammonia concentration (CAC) of >50 mg/m^3-years also showed a significantly increased relative risk for all of the above symptoms compared to workers with a CAC of ≤50 mg/m^3-years. None of the relative risks for workers in the second factory (ammonia levels <25.4 ppm) were significant.

Other occupational studies also evaluated the effects of ammonia exposure and pulmonary function. Firefighters who reported exposure to ammonia while working had a rate of decline of FEV_1 of 1.7 times that of nonexposed firefighters over a period of 6–10 years (Tepper et al. 1991).

Children (8–9 years old) who attended two schools in the vicinity of a fertilizer plant had higher incidences of acute respiratory diseases than children of the same age who attended a school 20 kilometers away (Gomzi and Šarić 1997). Incidence was related to levels of measured pollutants (ammonia, hydrogen fluoride, nitrogen dioxide, total suspended particulate matter, and smoke) in the inside and outside air. Forced expiratory volumes were not statistically different between the three schools. These results indicate that exposure to low levels of ammonia (0.04–0.23 ppm) and other air-borne pollutants may not cause functional respiratory deficits, but may lower the resistance to respiratory pathogens in children. These effects may be due in part or in whole to toxicants other than ammonia, such as nitrogen dioxide.

Case reports of individuals acutely exposed to anhydrous or aqueous ammonia reported respiratory effects including nasal irritation; epiglottic, laryngeal, pharyngeal, tracheal, and pulmonary edema; dyspnea; wheezing; coughing; rhonchi; pneumonia; and cardio-respiratory arrest (de la Hoz et al. 1996; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Lee et al. 1993; Millea et al. 1989; Morgan 1997; Prudhomme et al. 1998; Weiser and Mackenroth 1989). de la Hoz et al. (1996) described the initial and residual effects of three adult males who had been acutely exposed to ammonia

gas in separate incidents. All three men complained of burning eyes, throat, and skin, cough, and wheezing, and all had been treated at hospitals shortly after exposure. Follow-up examinations 2–2½ years later showed persistent dyspnea, cough, and wheezing at rest and/or on exertion, which is consistent with restrictive lung disease secondary to acute ammonia inhalation injury. Another man exposed to anhydrous ammonia gas experienced pharyngeal and laryngeal edema, dyspnea, chest tightness, copious bronchial secretions, and wheezing (Leduc et al. 1992); 12 years postexposure, he continued to have cough, exertional dyspnea, and recurrent bronchial infections. Similar cases were reported by Kerstein et al. (2001) and Latenser and Lucktong (2000); no follow-up reports were available.

A more severe exposure was reported by George et al. (2000). An adult male was found unconscious next to a burst pipe carrying liquefied ammonia. He had ocular and cutaneous burns and severe difficulty breathing. Over the next 27 days, he suffered many medical setbacks, including attacks of bradycardia, a complete circulatory collapse from which he was resuscitated, and finally, a fatal cardiac arrest from severe bleeding. Another similar severe exposure resulted in the death of the patient 13 days postexposure due to treatment-resistant bronchopneumonia; histological examination showed massive, hemorrhagic pulmonary edema, regions of emphysema, and edema of the epiglottis and glottis (Weiser and Mackenroth 1989).

Reports of apparently rare effects have been found. A man exposed occupationally for 5 months to low levels of ammonia gas (8–15 ppm) from ammonia-containing silver polish developed asthma-like symptoms (Lee et al. 1993). Separate specific bronchial provocation tests to the silver polish and to 12 ppm ammonia produced asthmatic reactions, implicating the ammonia in the silver polish as the cause. Another study reported hyposmia (loss of the sense of smell) in a man following acute inhalation exposure (for several hours) to an unknown concentration of ammonia gas; the hyposmia had not resolved 30 months after exposure (Prudhomme et al. 1998).

Studies in animals have demonstrated similar dose-effect and duration-effect patterns for the respiratory tract. Acute exposures (1 hour to 1 week) to low concentrations of ammonia (≤1,000 ppm) irritate the upper respiratory tract whereas exposures (3 hours to 2 weeks) to high concentrations (≥4,000 ppm) result in severe damage to the upper and lower respiratory tract and alveolar capillaries (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969). Prolonged or repeated exposures to lower levels (≥150 ppm) produce inflammation and lesions of the upper respiratory tract (Broderson et al. 1976; Coon et al. 1970).

Clinical and histological effects have been seen in the lungs of animals following exposure to ammonia gas (Dodd and Gross 1980; Gaafar et al. 1992; Sjöblom et al. 1999). Cats exposed to 1,000 ppm ammonia gas for 10 minutes and observed for up to 35 days showed a biphasic course of respiratory pathology (Dodd and Gross 1980). Effects seen at 24 hours post-exposure included severe dyspnea, anorexia, and dehydration, with rhonchi and coarse rales evident upon auscultation. Microscopy of lung samples on day 1 showed necrotizing bronchitis in the large conducting airways, and necrosis and sloughing of the epithelium and acute inflammatory reaction in the bronchi. On day 7, the mucosal lesions had resolved, but on day 35, varying degrees of bronchitis and early bronchopneumonia with areas of bulbous emphysema were seen. Gross pathology revealed varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance was increased throughout the study (Dodd and Gross 1980). Swiss mice exposed to 909 ppm, but not 303 ppm, ammonia gas 6 hours/day, 5 days/week for 4–14 days had histological lesions in the respiratory epithelium in the nasal cavity (Zissu 1995); no lesions were observed in the trachea or lungs. Nasal mucosa was adversely affected in adult male mice exposed to vapor of 12% ammonia solution for 15 minutes/day, 6 days/week for 4, 5, 6, 7, or 8 weeks (Gaafar et al. 1992). Histological changes progressed from weeks 4–8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One animal that had loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane also had a carcinoma *in situ* in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histochemical results were also abnormal. The levels and cell locations of succinic dehydrogenase, acid phosphatase, alkaline phosphatase, and nonspecific esterase activities were altered, indicating altered cell metabolism and energy production, cell injury, proliferation, and possibly chronic inflammation and neoplastic transformation (Gaafar et al. 1992).

Anesthetized, mechanically ventilated rabbits exposed to high levels of nebulized ammonia (2 mL of 23–27% ammonia solution; estimated by the study authors as peak ammonia concentrations of 35,000–39,000 ppm) for 4 minutes had a decrease in blood oxygen saturation and an increase in airway pressure (a measure of changes in airway resistance) (Sjöblom et al. 1999). Arterial oxygen tension decreased from 23.3 (±3.6) to 11.0 (±3.6) kPa and peak airway pressure increased from 13 (±2) to 17 (±2) cm H_2O. At baseline and 5 and 15 minutes after ammonia administration, measurements were taken via a catheter in the left auricular artery, which monitored pressure and sampled for arterial blood gases, and via transducers in the ventilator. Thirty and 150 minutes after ammonia exposure, rabbits received inhalation therapy of either 0.5 mg budesonide (a steroid) or a placebo, and airway pressure, hemodynamics, and gas exchange were measured every 30 minutes for 6 hours. Slight, gradual

improvement of blood gas parameters was noted over the 6-hour observation period in all rabbits, with or without steroid treatment; however, no parameters approached normal during that time period.

Other studies examined pigs in normal swine production facilities (Donham 1991) or in environmentally regulated enclosures (Diekman et al. 1993; Gustin et al. 1994; Urbain et al. 1994). Donham (1991) investigated the correlation of housing air environment (in the finishing barn) to swine diseases and productivity over 12 months on 28 swine farms. Total dust, respirable dust, endotoxin activity of the dust, and hydrogen sulfide levels were determined, and area dust and microbial counts were monitored at 1.2 meters above the floor (the human breathing zone). Ammonia and carbon dioxide levels were determined 1.2 meters and 20 cm (swine breathing zone) above the floor. The average ammonia concentration in the human breathing zone for all farms was 9.1 ppm; the mean concentration in the swine breathing zone was 14.5 ppm. The mean concentrations of environmental contaminants were calculated for the most productive farrowing operations and the least productive ones and compared with lower production variables (Donham 1991). Ammonia concentration was related to number of pigs weaned per litter, and total and respirable dust concentrations were related to prolonged age to reach a weight of 25 kg. Another comparison involved the stratification of the finishing farms into quartiles according to percentage of pigs with specified disease conditions and comparison of mean concentrations of various environmental contaminants for each farm in each strata (Donham 1991). Levels of ammonia (in the animal breathing zone) greater than 25, 29, and 23 ppm were associated with buildings in which pneumonia, pleuritis, and arthritis, respectively, were greater than the mean value for the group. Overall, respirable dust (>0.8 mg/m^3), ammonia (>23 ppm in the animal breathing zone), and carbon dioxide (>2,000 ppm) levels were most often associated with increased disease. Possible study shortcomings noted by the study author were that pre-existing conditions could have been present in the pigs (before they entered the finishing barns) and that nasal turbinates were not routinely examined for abnormalities. These data suggest that ammonia may contribute to respiratory and other pathological conditions in pigs raised in crowded, enclosed conditions, but the exact contribution of ammonia is difficult to assess.

The lungs of young pigs exposed continuously to 0, 25, 50, or 100 ppm ammonia gas for 6 days in air-pollutant exposure chambers were removed, ventilated, and perfused, and the pulmonary vascular hemodynamics and permeability and the endotoxin-induced vascular response were assessed (Gustin et al. 1994). In lungs from pigs exposed to 100 ppm, but not 25 or 50 ppm ammonia, the endotoxin-induced vascular response seen in lungs from control pigs was abolished. The study authors suggested that this is due to a modification of the balance between vasodilators (such as cyclooxygenase products and platelet activating factor) and vasoconstrictors (such as prostacyclin). Since vasoconstriction, as induced by

endotoxin, may serve as a protective mechanism in the lungs and attenuate edema formation, the effective abolishment of this effect by ammonia may be detrimental.

Young pigs exposed continuously to ammonia vapors (0, 25, 50, or 100 ppm) for 6 days in air-pollutant exposure chambers had increased numbers of neutrophils in nasal lavage fluid in all exposure groups (Urbain et al. 1994) and increased porcine serum albumin at 100 ppm.

Not all studies have shown adverse respiratory effects from intermediate exposure to ammonia vapors. Groups of gilts (virgin female pigs) were raised from the age of 2–4.5 months in a conventional grower unit where they were naturally exposed to mycoplasmal and bacterial pathogens that cause enzootic pneumonia and atrophic rhinitis (Diekman et al. 1993). The pigs were then transferred to environmentally regulated rooms, where they were exposed continuously to low (mean 7 ppm) or moderate (mean 35 ppm) levels of ammonia for 6 weeks. No statistically significant differences were seen in the percent of lung tissue containing lesions or in snout grade (Diekman et al. 1993). Ninety-five percent of all gilts had lung lesions, with a wide range of degree of severity. Snouts were graded at the level of the second deciduous premolar as having normal turbinates (grade of 0), slight to moderate degeneration (grade of 1–3), or severe degeneration to complete loss of turbinates (grade of 4 or 5). Some of the gilts were continuously exposed through puberty and breeding (around 205 days of age) and the lungs and turbinates were examined at 30 days of gestation. No statistically significant differences were observed in percent of lung tissue containing lesions or in snout grade (Diekman et al. 1993).

A number of cattle were acutely exposed to anhydrous ammonia when a pipeline running through their pasture ruptured and leaked 1,800 barrels of ammonia in a short period of time (Morgan 1997). The ammonia combined with moisture and formed a white cloud (ammonia aerosol), which drifted south across two additional fields containing cattle. In the field where the rupture occurred, four head of cattle were found dead and two others were euthanized because of blindness and respiratory distress. Cattle in the adjacent pasture had runny eyes and noses and were coughing and wheezing. Eight days after the pipeline rupture, the cloud of ammona aerosol, which had apparently settled in a low-lying protected area, blew back up the valley and exposed the remaining cattle again and also exposed a horse in the same pasture. All animals had respiratory distress, elevated body temperatures, and one cow and the horse had swollen tongues and enlarged lymph glands. All cattle were given antibiotics and some were treated specifically for respiratory problems. No measurements or estimations of ammonia concentrations were provided and no follow-up examinations were available to assess long-term effects from the exposures.

All reliable LOAELs and highest NOAELs are presented in Table 3-1 and Figure 3-1.

Cardiovascular Effects. Acute exposure to highly concentrated aerosols of ammonium compounds may cause elevated pulse and blood pressure, bradycardia, and cardiac arrest in humans (George et al. 2000; Hatton et al. 1979; Montague and Macneil 1980; White 1971). These effects did not occur after acute exposure to 500 ppm ammonia or repeated exposure to 100 ppm ammonia (Ferguson et al. 1977; Silverman et al. 1949).

Cardiovascular changes that may be analogous to those observed in humans have been observed in rabbits exposed to high concentrations of ammonia (Richard et al. 1978b). Bradycardia was seen at 2,500 ppm, and hypertension and cardiac arrhythmias leading to cardiovascular collapse followed acute exposures to concentrations exceeding 5,000 ppm. Pathological correlates for these effects have not been demonstrated. Atrophy of pericardial fat has been observed in mice exposed to 4,000 ppm ammonia (Kapeghian et al. 1982). Myocardial fibrosis has been observed in rats, guinea pigs, rabbits, dogs, and monkeys after prolonged (90 days) continuous exposure to 653 ppm (Coon et al. 1970). The contribution of these lesions to the morbidity and mortality of affected animals has not been determined.

Exposure of pigs *in vivo* to up to 100 ppm ammonia for 6 days did not alter the baseline values of any hemodynamic or permeability parameters (arterial, pre- or postcapillary, or venous blood flow resistance, or total pulmonary blood flow resistance), but did eliminate the hemodynamic response to *Escherichia coli* endotoxins in the lungs (Gustin et al. 1994). This may affect the ability of the lungs to resist bacterial infection. The pulmonary blood flow resistance measurements were taken *in vitro* in ventilated and perfused lungs from pigs exposed to ammonia *in vivo* (Gustin et al. 1994). Reliable LOAELs and highest NOAELs for cardiovascular effects are presented in Table 3-1 and Figure 3-1.

Gastrointestinal Effects. Exposure to highly concentrated aerosols of ammonium compounds can produce burns of the lips, oral cavity, and pharynx, along with edema of these areas (Hatton et al. 1979; Kass et al. 1972; Leduc et al. 1992; Levy et al. 1964; Price et al. 1983; Stroud 1981; Ward et al. 1983; Yang et al. 1987). Gastrointestinal effects of ammonia in animals have not been reported. As shown in Table 3-1, pathological changes in the gastrointestinal tract were not observed in guinea pigs exposed repeatedly to 170 ppm ammonia (Weatherby 1952).

Hematological Effects. Cyanosis, elevated white blood cell count, and pulmonary artery thrombosis have been observed in humans exposed to highly concentrated aerosols of ammonium compounds (Sobonya 1977; Taplin et al. 1976; Voisin et al. 1970; Ward et al. 1983; White 1971).

Standard hematological measurements, including blood hemoglobin and differential cell counts, have been reported for a few animal species. As shown in Table 3-1 and Figure 3-1, acute hematological effects of ammonia have not been demonstrated (Doig and Willoughby 1971; Gustin et al. 1994). Pigs exposed to up to 100 ppm ammonia for 6 days had no statistically significant differences from controls in total leukocytes or percent lymphocytes, neutrophils, or eosinophils (Gustin et al. 1994). Repeated exposure to 1,100 ppm had no effect on hematological parameters in guinea pigs, rats, and rabbits (Coon et al. 1970). Weatherby (1952) reported increased concentrations of hemosiderin in the spleen of guinea pigs exposed to 170 ppm ammonia for 18 weeks. This suggests the possibility of increased turnover of red blood cells; however, this has not been corroborated.

Musculoskeletal Effects. Spasms of muscles of the extremities have resulted from an acute exposure of a man to anhydrous ammonia gas (White 1971), but this was probably caused by an effect of ammonia on the nervous system.

Hepatic Effects. Hemorrhagic necrosis of the liver was observed in an individual exposed to a lethal concentration of ammonia gas and liquid for a short period of time (<45 minutes) (Heifer 1971). No other cases of hepatic effects have been reported in humans. Hepatic effects are usually not seen in animals exposed to ammonia gas. As shown in Table 3-1, liver necrosis has been observed following acute lethal exposure of mice to 3,440 ppm ammonia for 1 hour (Kapeghian et al. 1982). Fatty changes of liver plate cells were seen in rats following continuous long-term exposure to 642 ppm ammonia for 90 days, but no such changes were seen in rats, squirrel monkeys, and guinea pigs exposed to 1,086 ppm ammonia 8 hours/day, 5 days/week for 6 weeks (Coon et al. 1970).

Renal Effects. No studies were located regarding renal effects in humans after inhalation exposure to ammonia. In animals, renal effects do not appear to be an important feature of the toxicity of inhaled ammonia. Effects reported have not been corroborated or cannot be interpreted. Mild abnormalities in the renal tubules have been described in guinea pigs exposed to 170 ppm for 12 weeks, 5 days/week, 6 hours/day; however, renal effects at this relatively low level have not been corroborated (Weatherby 1952). Exposure to more than 6 times this concentration for 6 weeks, 5 days/week, 8 hours/day did not

result in pathological changes to the kidney (Coon et al. 1970). Renal tubular calcification (severity not reported) has been reported in rats continuously exposed to near lethal levels (Coon et al. 1970).

Endocrine Effects. Adrenaline levels in urine, 17-oxycorticosteroids in the urine, and 11-oxycorticosteroid levels in blood were increased in humans exposed to 3.0 ppm ammonia for 37 days (Kalandarov et al. 1984). Exposure to 7.2 ppm for 17 days also increased adrenaline levels in urine and 17-oxycorticosteroids in the urine, and increased free, but not total, 11-oxycorticosteroid levels in blood (Kalandarov et al. 1984). Experimental details were lacking in this study; additionally, no clinical or histological data were provided for this or other end points in this study and no supporting data are available in the literature. Therefore, the significance of these effects is unclear. Exposure of pigs to up to 100 ppm ammonia for 6 days did not significantly alter the plasma cortisol concentration (Gustin et al. 1994). No statistically significant difference was seen in adrenal gland weight of female pigs exposed to about 35 ppm ammonia for 6 weeks or for 6 weeks plus through day 30 of gestation compared to pigs exposed for similar time frames to about 7 ppm ammonia (Diekman et al. 1993). No unexposed controls were included in that study. The endocrine system does not appear to be a primary target of inhaled ammonia.

Dermal Effects. Ammonia gas and aerosols of ammonium compounds derived from anhydrous ammonia are dermal irritants in humans and animals. These effects are described in the discussion of dermal effects associated with dermal exposure (Section 3.2.3.2).

Ocular Effects. Ammonia gas and aerosols of ammonium compounds derived from anhydrous ammonia are ocular irritants in humans and animals. These effects are described in the discussion of ocular effects associated with dermal exposure (Section 3.2.3.2).

Body Weight Effects. Reduced body weight has been observed in rats exposed via inhalation to 500 ppm (Richard et al. 1978a) and in pigs exposed to 50 ppm or more ammonia for 6 days (Gustin et al. 1994; Urbain et al. 1994). Pigs gained less weight and showed decreased food consumption when exposed to 100 ppm ammonia for 4 or 5 weeks (Drummond et al. 1980; Stombaugh et al. 1969). Female pigs exposed to about 35 ppm for 6 weeks gained less weight than those exposed to only about 7 ppm (Diekman et al. 1993). However, females that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation had no statistically significant difference in body weight (Diekman et al. 1993); however, no controls were included in this study.

3.2.1.3 Immunological and Lymphoreticular Effects

Several case reports describe occupational asthma that developed due to exposure to aerosols that contained ammonium compounds (Ballal et al. 1998; Lee et al. 1993; Weir et al. 1989).

Secondary infections often complicate the clinical outcome of burns and respiratory lesions related to exposure to highly concentrated aerosols derived from anhydrous ammonia (Sobonya 1977; Taplin et al. 1976). However, there is no evidence that the decreased immunological resistance represents a primary impairment of the immune system in humans following exposure to ammonia. Nevertheless, as shown in Table 3-1 and Figure 3-1, studies in animals have shown that acute and long-term exposure to ammonia can decrease the resistance to bacterial infection and decrease immune response to infection. A significant increase in mortality was observed in mice exposed to ammonia for 168 hours followed by exposure to the LD_{50} of *Pasteurella multocida* (Richard et al. 1978a). Exposure of rats to ammonia at ≥25 ppm for 4–6 weeks following inoculation with *Mycoplasma pulmonis* intranasally significantly increased the severity of respiratory signs characteristic of murine respiratory mycoplasmosis (Broderson et al. 1976). Guinea pigs exposed to 90 ppm ammonia for 3 weeks developed a significant decrease in the cell-mediated immune response to challenge with a derivative of tuberculin (Targowski et al. 1984). Furthermore, the response of blood and bronchial lymphocytes to mitogens (phytohemagglutinin, concanavalin A, purified protein derivative of tuberculin) was markedly reduced. The hemodynamic response (increased total pulmonary blood flow resistance) to *E. coli* endotoxins in the lungs of pigs was eliminated by exposure to up to 100 ppm ammonia for 6 days, which may affect the ability of the lungs to resist bacterial infection (Gustin et al. 1994). Also, a reduction in gamma globulin concentration was reported in pigs exposed to 100 ppm ammonia for 31–45 days (Neumann et al. 1987).

3.2.1.4 Neurological Effects

Case reports of accident victims exposed to highly concentrated aerosols derived from anhydrous ammonia describe blurred vision, diffuse nonspecific encephalopathy, loss of consciousness, muscle weakness, and decreased deep tendon reflexes (George et al. 2000; Hatton et al. 1979; Latenser and Lucktong 2000; White 1971). Acute exposure to low levels of ammonia (100 ppm) has been shown to depress free-access wheel running behavior in rodents (Tepper et al. 1985). No overt symptoms of neurological disorders were reported in guinea pigs or monkeys that were exposed to up to 1,105 ppm ammonia for 6 weeks (Coon et al. 1970). Exposure of the nasal mucosa to ammonia-saturated air elicited vasodilatation and corresponding increased blood flow and reflex hypertension in the lower lip of cats

(Izumi and Karita 1993). Stimulation of the nasal mucosa by chemical irritants has been shown to elicit changes in the respiratory system, such as apnea, laryngeal spasm, and bronchoconstriction, and in the cardiovascular system, such as bradycardia and variable blood pressure changes (Izumi and Karita 1993). Brain glutamine levels have also been shown to increase in rats that inhaled 25 or 300 ppm ammonia vapor for 6 hours/day for 5 days, which is likely a result of ammonia metabolism by the astrocytic glutamate-glutamine cycle (Manninen and Savolainen 1989; Manninen et al. 1988).

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to ammonia. No statistically significant differences were noted in ovarian or uterine weights of pigs exposed to about 7 or 35 ppm ammonia for 6 weeks (Diekman et al. 1993). Female pigs that were continuously exposed to about 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation had no statistically significant differences in age at puberty, number of live fetuses, or fetus-to-corpus luteum ratio compared to pigs exposed to only about 7 ppm (Diekman et al. 1993). No unexposed controls were included in that study.

3.2.1.6 Developmental Effects

No information was located regarding developmental effects of ammonia in humans following inhalation exposure. No statistically significant difference in fetal length was evident at 30 days of gestation in offspring of pig dams that were continuously exposed to about 7 or 35 ppm ammonia from 6 weeks before breeding until day 30 of gestation (Diekman et al. 1993).

3.2.1.7 Cancer

Carcinogenic potential of ammonia by the inhalation route has not been assessed in humans or animals. One case report was found of an individual who developed epidermal carcinoma of the nasal septum 6 months after being badly burned by accidental contact with a refrigeration ammonia-oil mixture (Shimkin et al. 1954). However, the role of ammonia is impossible to ascertain and no conclusion can be drawn from this study. Shimkin et al. (1954) indicated that “no single case can prove a general principle, and it is only by the publication of additional reports of similar cases that enough data can become available for critical analysis.” No other such reports were located, although other cases of inhalation

exposure to ammonia from spills have been followed for more than 6 months after exposure. One of 10 adult male mice exposed to ammonia gas for 15 minutes/day 6 days/week for 8 weeks had mitotic figures with an intact basement membrane and a carcinoma *in situ* in one nostril and one mouse had an invasive adenocarcinoma of the nasal mucosa (Gaafar et al. 1992). Again, there is no conclusive evidence that ammonia played a role in the induction of the carcinoma. Gaafar et al. (1992) provided an alternate explanation by stating that "prolonged exposure to ammonia may interfere with the normal protective reflexes of the respiratory nasal mucosa resulting in the accumulation of particulate matter initiating or promoting a neoplastic process." However, the plausibility of tumor formation in only 8 weeks by a weak carcinogen such as particulate matter is debatable.

3.2.2 Oral Exposure

As discussed in Chapter 4, ammonia in aqueous solution exists in equilibrium with ammonium hydroxide, a weak base, which is partially ionized in water. Degree of ionization is dependent on pH; at physiological pH, ammonium hydroxide is 99% ionized, but at pH 9.25, is only 50% ionized. Information available for humans exposed to ammonia by the oral route usually involved case reports of people who swallowed household ammonia (ammonium hydroxide). Studies by the oral route in animals generally have used ammonium salts or ammonium hydroxide. For these reasons, oral doses are expressed as mg NH_4^+/kg/day, given as the particular ammonium compound.

In many animal studies, the animals were administered ammonium chloride. Ammonium chloride is commonly used to induce metabolic acidosis in experimental animals. The acidosis is due to the formation of hydrogen ions from the metabolism of ammonium ions to urea. WHO (1986) notes that the ingestion of ammonium chloride in doses around 500–1,000 mg/kg/day for 1–8 days (longer treatment would worsen the condition) has induced metabolic acidosis in mice, guinea pigs, rats, rabbits, and dogs. Metabolic acidosis can result in a variety of nonspecific changes in neurological, cardiovascular, pulmonary, gastrointestinal, and musculoskeletal function, as well as in changes in hematological and clinical chemistry parameters.

3.2.2.1 Death

Human deaths due to ingestion of household ammonium salts have been reported (Klein et al. 1985; Klendshoj and Rejent 1966), but no quantitative data for oral exposure in humans were located. A

69-year-old woman who ingested an unknown quantity of lemon ammonia (3% ammonium ion) was found semi-conscious and making gurgling respiratory sounds (Klein et al. 1985). Radiographic results were consistent with aspiration pneumonia, and endoscopy showed laryngeal and epiglottic edema and a friable, erythematous esophagus with severe corrosive injury. The woman died several days later after developing acute respiratory distress syndrome and renal failure (Klein et al. 1985). A 57-year-old man was found dead with a glass containing dilute ammonium hydroxide (2.4% ammonium ion) nearby (Klendshoj and Rejent 1966); autopsy showed hemorrhagic esophagus, stomach, and duodenum. As shown in Table 3-2 and Figure 3-2, 303 mg ammonium/kg as ammonium chloride is a lethal dose in guinea pigs when given as single gavage dose (30/40 died) (Koenig and Koenig 1949). Death, in this study, resulted from pulmonary edema. No deaths were seen in cats, rabbits, guinea pigs, or rats after a similar dose of ammonium (337 mg ammonium/kg given as ammonium chloride) (Boyd and Seymour 1946).

3.2.2.2 Systemic Effects

No information was located regarding dermal or ocular effects of ammonia or ammonium compounds in humans or animals following oral exposure.

Respiratory Effects. No information was located regarding respiratory effects of ammonia or ammonium compounds in humans following oral exposure. Guinea pigs that received a single gavage dose of ammonium chloride developed serious respiratory effects including increased rate and depth of respiration, pulmonary edema, and death by respiratory failure (Koenig and Koenig 1949). Because the blood pH of the guinea pigs decreased after administration of ammonium chloride, adjustments in respiratory rate and depth may have been a compensatory mechanism for acidosis. Similarly, administration of ammonium chloride in doses of approximately 100 mg of NH_4^+/kg/day (as ammonium chloride) or higher for up to a year to rabbits resulted in metabolic acidosis and compensatory changes in respiratory rate and tidal volume (Seegal 1927). The low blood pH results in increased lung ventilation, which increases the elimination of carbon dioxide from the blood, and therefore, can be considered a compensatory response to acidosis rather than a direct effect of ammonium ion on the lungs or respiratory system.

Cardiovascular Effects. No information was located regarding cardiovascular effects of ammonia or ammonium compounds in humans following oral exposure. No pathological abnormalities were noted in the hearts of adult and weanling rats fed doses of up to 79 mg ammonium/kg/day as ammonium

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
	ACUTE EXPOSURE								
	Death								
1	Gn Pig	1 d					286	(death due to pulmonary edema)	Koenig & Koenig 1949 NH4CL
	Systemic								
2	Rat (Sprague-Dawley)	6 d (W)	Hemato		2325	(elevated serum calcium)			Barzel 1975 NH4Cl
3	Rat (Wistar)	3 or 7 d (F)	Bd Wt	22 F	3150.4 F	(10% reduction in final body weight)			Bodega et al. 1993 ammonium acetate
4	Rat (Wistar)	3 or 7 d (F)	Bd Wt	22	3102.2	(final body weight decreased by 15%)			Boyano-Adanez et al. 1996 ammonium acetate
5	Rat (Sprague-Dawley)	7 d Gavage - NS	Renal		433	(renal enlargement due to cell hypertrophy)			Janicki 1970 NH4Cl
6	Gn Pig	1 d Gavage - NS	Resp				303	(pulmonary edema)	Koenig & Koenig 1949 NH4CL

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Neurological						
7	Rat (Wistar)	3 or 7 d (F)		22	3102.2 (decreased binding of somatostatin to receptors in frontoparietal cortex and hippocampus)		Boyano-Adanez et al. 1996 ammonium acetate
	INTERMEDIATE EXPOSURE						
	Systemic						
8	Rat	330 d (W)	Musc/skel		991 (reduced calcium less fat-free solid)		Barzel & Jowsey 1969 NH4Cl
			Bd Wt		991 (reduced body weight)		
9	Rat (Wistar)	90 d (F)	Bd Wt	22 F	3150 F (15% reduction in final body weight after 90 days)		Bodega et al. 1993 ammonium acetate
10	Rat (Wistar)	15 d (F)	Bd Wt	22	3102.2 (final body weight gain decreased by 10%)		Boyano-Adanez et al. 1996 ammonium acetate
11	Rat	3 wk (W)	Renal	412			Freedman & Beeson 1961 NH4CL

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
12	Rat (albino)	90 d 6 d/wk (W)	Cardio	79 F					Gupta et al. 1979 NH4NH2SO3
			Gastro	79 F					
			Hemato	79 F					
			Hepatic	79 F					
			Renal	79 F					
			Bd Wt	39.5 F	79 F	(body weight decreased by 16%)			
13	Dog	11 wk Gavage - NS	Musc/skel				337	(bone deformity and softening)	Bodansky et al. 1932 NH4Cl
	Neurological								
14	Rat (Wistar)	15 d (F)		22	3102.2	(decreased binding of somatostatin to receptors in frontoparietal cortex and hippocampus)			Boyano-Adanez et al. 1996 ammonium acetate

Table 3-2 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
	Developmental							
15	Rat (Wistar)	Gd 1-ppd 21 (F)				4293	(BW decreased by 16-27%; decreased NMDA receptor function in neurons)	Minana et al. 1995 NH3CH3CO2

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); DW = drinking water; (F)= feed; F = female; Gd = gestation day; G = gavage; gastro = gastrointestinal; hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level;min = minute(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; NS = not specified; wk = week(s)

Figure 3-2. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral

Acute (≤14 days)

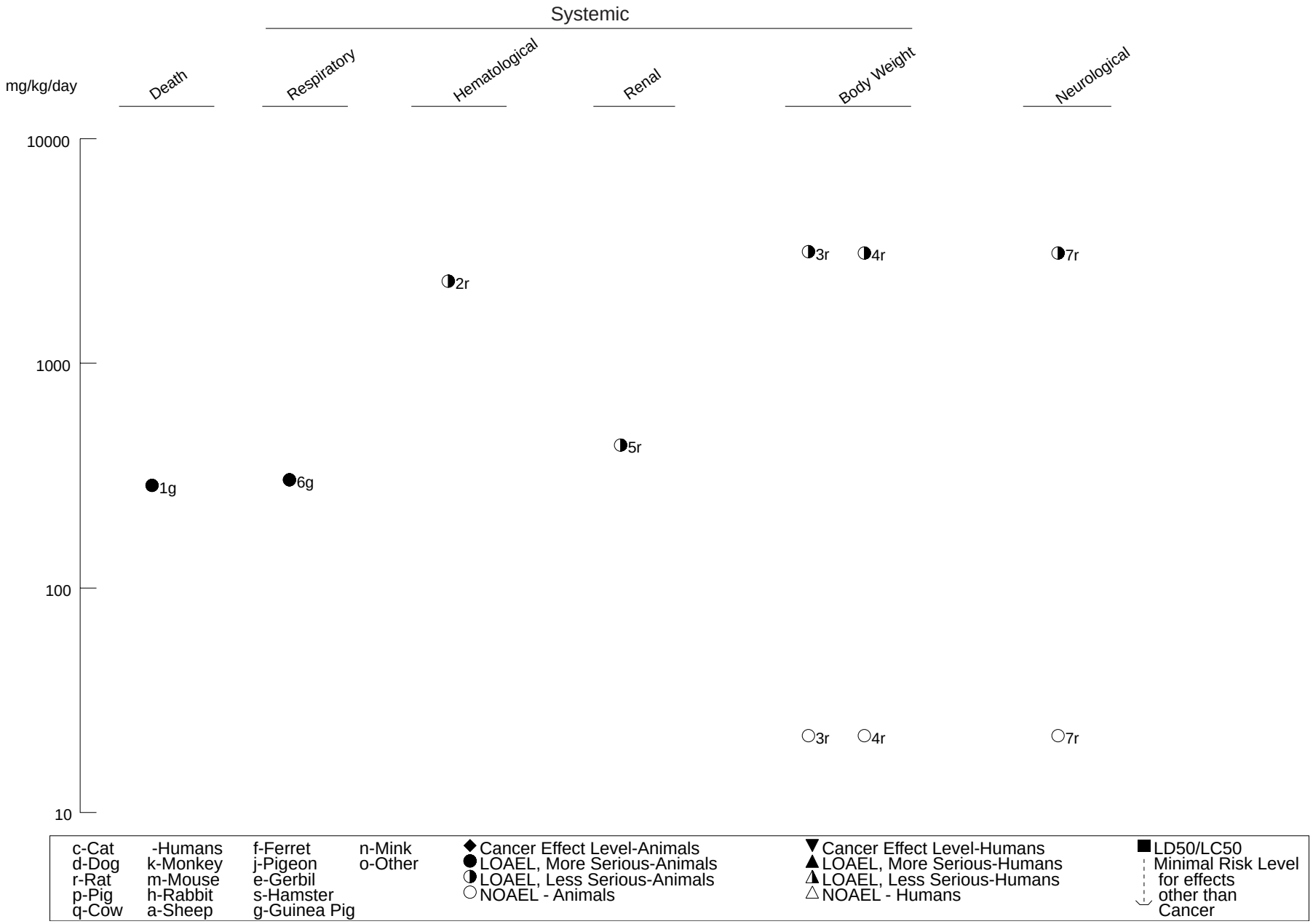
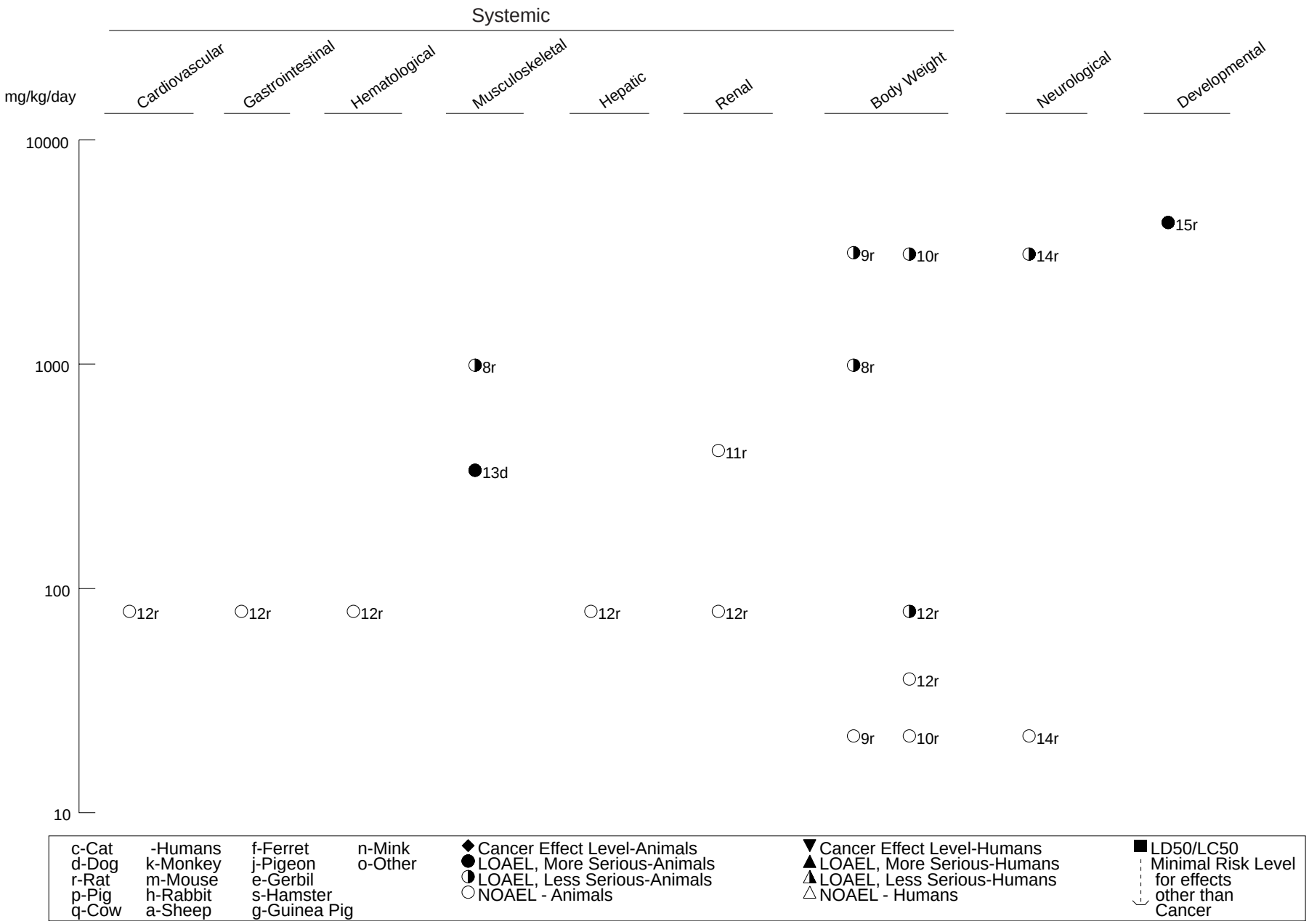


Figure 3-2. Levels of Significant Exposure to Ammonia And Ammonium Compounds - Oral (*Continued*)

Intermediate (15-364 days)



sulfamate for 90 days in drinking water (Gupta et al. 1979). These data are presented in Table 3-2 and Figure 3-2.

Gastrointestinal Effects. Several cases have been described of young children (2–3 years old) who bit into ammonia pellets/capsules (Lopez et al. 1988; Rosenbaum et al. 1998). Two of the children drooled and had ulcerative lesions on the tongue and/or on the buccal mucosa; one child had superficial ulcerations on the posterior esophageal wall and the other child had edematous, erythematous upper and lower lips with areas of desquamation, eschar of the hard palate, and edema and erythema of the supraglottic structures and upper trachea (Rosenbaum et al. 1998). All of the children experienced one or more of the following symptoms: vomiting, drooling, dysphagia, cough, or oral or pharyngeal burns (Lopez et al. 1988; Rosenbaum et al. 1998). None of the children had esophageal or respiratory burns and all healed within a few days. Esophageal lesions and edema were reported in five persons who ingested household ammonia (ammonium hydroxide), one of whom experienced acute respiratory obstruction (Christesen 1995; Klein et al. 1985). These observations were not quantified. The effects are probably due to the alkaline nature of ammonium hydroxide. A single case report described a self-administered ammonia solution enema that resulted clinically in anal pain, diffuse abdominal colic, and tenesmus (da Fonseca et al. 1998). Sigmoidoscopy showed diffuse erythematous friable mucosa with large ulcerations covered by yellowish exudate that receded in a few days, but chronic inflammation and fibrosis of the rectum and sigmoid colon was noted 3 months postexposure (da Fonseca et al. 1998).

No histopathological abnormalities of the gastrointestinal tract were observed in adult or weanling rats administered doses of up to 79 mg ammonium/kg/day as ammonium sulfamate for 90 days via drinking water (Gupta et al. 1979). Likewise, a 3% solution of ammonium chloride administered to rats via gastric tube produced no gastric mucosal damage in 1 hour and a 10% solution produced only a minimum of hemorrhagic lesions (about 9 mm^2) (Takeuchi et al. 1995). However, similar administration of 1 or 3% ammonium hydroxide in rats produced severe hemorrhagic lesions (about 26.6 or 97.7 mm^2, respectively) (Takeuchi et al. 1995). Gavage administration in rats of 0.3% ammonia (33.3 mg/kg) produced gastric mucosal lesions within 5 minutes with corresponding decreases in gastric wall immunoreactive endothelin-1 (ET-1) and immunoreactive thyrotropin-releasing hormone (TRH) concentrations and increases in gastric juice ET-1 and TRH concentrations (Mori et al. 1998). *In situ* gastric exposure has also shown ammonia-induced gastric mucosal damage (Murakami et al. 1995; Nagy et al. 1996). These lesions are exacerbated by neutrophil products, especially hypochlorous acid (Murakami et al. 1995) and cysteine proteases, such as some of the cathepsins (Nagy et al. 1996). Administration of 0.01% ammonia in drinking water to rats (approximately 42 mg/kg/day) for 8 weeks resulted in acceleration of cell

migration leading to mucosal atrophy in the stomach antrum, increased labeling indices, and enlargement of the proliferative zone in the antral and body mucosa (Tsujii et al. 1993).

Hematological Effects. No information was located regarding the hematological effects of ammonia or ammonium compounds in humans following oral exposure. Repeated exposure to ammonium chloride in animals resulted in metabolic acidosis with related changes in bone metabolism and serum calcium. For example, rats fed diets containing high levels of ammonium chloride had increased serum calcium (Barzel 1975). The increased serum calcium resulted from enhanced demineralization of bone in response to chronic acidosis. This effect was not found to be a specific effect of ammonium and was reported to occur in states of chronic metabolic acidosis produced from repeated doses of acidifying agents (e.g., hydrochloric acid, sulfuric acid). Decreased blood pH was seen in cats fed an acidifying diet containing ammonium chloride for several weeks (Kienzle and Wilms-Eilers 1994). As shown in Table 3-2 and Figure 3-2, no effects on blood hemoglobin or blood cell counts were observed in adult or weanling rats that received doses of up to 79 mg ammonium/kg/day administered as ammonium sulfamate in drinking water (Gupta et al. 1979).

Musculoskeletal Effects. No information was located regarding musculoskeletal effects of ammonia or ammonium compounds in humans following oral exposure. Guinea pigs and rats that received lethal gavage doses of ammonium chloride developed muscle weakness, fasciculation, and incoordination (Koenig and Koenig 1949). In other animal studies, repeated ingestion of ammonium salts resulted in metabolic acidosis, which stimulated bone demineralization. As is shown in Table 3-2 and Figure 3-2, repeated ingestion of ammonium chloride in drinking water resulted in net bone resorption in rats and bone deformities in dogs (Barzel and Jowsey 1969; Bodansky et al. 1932). This effect can be anticipated with repeated exposure to any acidifying agent.

Hepatic Effects. No information was located regarding hepatic effects of ammonia or ammonium compounds in humans following oral exposure. As shown in Table 3-2 and Figure 3-2, no toxic effects were noted in livers of adult or weanling rats fed doses of up to 79 mg ammonium/kg/day as ammonium sulfamate for 90 days in drinking water (Gupta et al. 1979).

Renal Effects. Renal failure was identified as the cause of death in humans after ingestion of an unknown amount of household ammonia (ammonium hydroxide) (Klein et al. 1985). It is not certain if this represents a primary effect of ammonium or is secondary to massive burns to the gastrointestinal tract.

Renal effects have been observed in animals following repeated oral doses of ammonium chloride. These effects may be secondary to chronic acidosis produced from the interaction of ammonium chloride with water (which results in an increased H^+ concentration) rather than from a direct effect of ammonium ion on the kidney. Renal enlargement, increased blood ammonia content, and increased urinary ammonia have been reported in rats exposed to 180–433 mg/kg/day for 3–7 days (Benyajati and Goldstein 1975; Janicki 1970; Lotspeich 1965;), but are unlikely to be indicative of renal pathology. The highest NOAELs and LOAELs are presented in Table 3-2 and Figure 3-2.

Endocrine Effects. No information was located regarding endocrine effects of ammonia or ammonium compounds in humans following oral exposure. Enlarged adrenal glands were observed in rabbits that received 124 mg ammonium/kg/day as ammonium hydroxide by gavage in water for 17 months (Fazikas 1939). These limited data suggest that the endocrine system is not a primary target for ammonia or ammonium compounds.

Body Weight Effects. Decreased body weight or weight gain has been observed in animals following oral exposure to ammonium ion (Barzel and Jowsey 1969; Bodega et al. 1993; Boyano-Adánez et al. 1996; Gupta et al. 1979; Noda and Chikamori 1976). Rats exposed to ammonium ion *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 21) and then received a normal diet had showed reduced body weight gain (Miñana et al. 1995); body weight gain was reduced by 25 and 16% in male and female offspring, respectively, at 120 days of age. Rats that were continued on the same ammonia diet as their dams had an even greater reduction in body weight gain (27 and 26% for males and females, respectively) (Miñana et al. 1995). Birth weights were not reported in the Miñana et al. (1995) study. Gupta et al. (1979) noted increased water intake and reduced food intake in weanling rats, and decreased body weight in adults but not weanlings fed 79 mg ammonium/kg/day in drinking water for 90 days as ammonium sulfamate. This represents the LOAEL for this effect. A NOAEL of 39.5 mg ammonium/kg/day was also identified in this study (see Table 3-2 and Figure 3-2).

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological effects of ammonia or ammonium compounds in humans or animals after oral exposure.

3.2.2.4 Neurological Effects

No information was located regarding neurological effects of ammonia or ammonium compounds in humans after oral exposure.

Guinea pigs that received lethal gavage doses of ammonium chloride (303.5–404.7 mg NH_4^+/kg) developed neuromuscular effects including fasciculation; incoordination; hyperexcitability to tactile, auditory, and painful stimuli; and tonic convulsions (Koenig and Koenig 1949).

A number of studies have indicated that increased ammonium ion levels in the brain may disrupt energy production and modify the availability of some receptors that are involved in neurotransmission. Administration of 20% ammonium acetate in the diet of rats for 20 days resulted in statistically significant increases in brain ammonium ion (12.8-fold), glutamine (37%), and alanine (93%) and in some TCA cycle-associated components in the brain including glucose, lactate, and pyruvate, and decreases in brain cytosolic NAD^+/NADH ratio, β-hydroxybutarate, and ATP content (Kosenko et al. 1993). Rats with high NH_4^+ intake from administration of 20% ammonium acetate in the diet and 5 mM ammonium acetate in the water for up to 15 days had a decreased number of available somatostatin receptors in the frontoparietal cortex and hippocampus (Boyano-Adánez et al. 1996). Since somatostatin hyperpolarizes neurons in the cerebral cortex, the study authors speculated that this reduction in available receptors may contribute to the alteration of electrophysiological properties of neural tissue caused by excess NH_4^+ (Boyano-Adánez et al. 1996). Binding of [H^3]MK-801 (an NMDA receptor antagonist) to NMDA receptors was reduced by approximately 60% in cerebellar cell cultures from 8-day-old rats exposed to NH_4^+ *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 8) (Miñana et al. 1995). Additionally, aspartate aminotransferase (AST) induction was absent in treated neurons (occurred in neurons from control rats), which also indicates impairment of NMDA receptors. Treated neurons were much more resistant to the toxic effects of glutamate than control neurons; since glutamate toxicity is mediated by NMDA receptors, attenuation of glutamate toxicity is indicative of impaired NMDA receptor function (Miñana et al. 1995). Loss of glial fibrillary acidic protein (GFAP) has been shown to occur in human spontaneous hyperammonemia (Kimura and Budka 1986; Kretzschmar et al. 1985; Sobel et al. 1981) and in other hyperammonemia models, such as portacaval shunt rats (Bodega et al. 1991; Suárez et al. 1992), but was not evident after exposure of rats to ammonium acetate (20% ammonium acetate in the diet and 5 mM ammonium acetate in the water for up to 90 days) (Bodega et al. 1993). Rats fed 19.5% ammonium acetate in the diet had increased NH_4^+ levels in the brain and altered assembly and disassembly of tubulin, an essential

component of the axonal transport system in the brain (Miñana et al. 1989a). The amount of polymerized tubulin increased but the amount of free tubulin was not affected. *In vitro* experiments using brains of rats fed a diet high in NH_4^+ indicated that the cause of the alteration might be a modification of the tubulin (and not the microtubule-associated proteins [MAPs], which modulate polymerization of the tubulin), which may result in a disruption in neurotransmission (Miñana et al. 1989a). Additional studies in rats brain showed that tubulin was significantly increased specifically in the septum, ventral hippocampus, dorsal hippocampus, hypothalamus, reticular formation, and frontal cortex, but not in the temporal amigdala, mammillary nucleus, locus coeruleus, caudate nucleus, or cingulate cortex after 2 months on the high ammonia diet (Miñana et al. 1989b).

3.2.2.5 Reproductive Effects

No information was located regarding reproductive effects of ammonia or ammonium compounds in humans or animals following oral exposure.

3.2.2.6 Developmental Effects

No information was located regarding the developmental effects of ammonia or ammonium compounds in humans. Rats exposed to NH_4^+ *in utero* and during lactation (dams received 4,293 mg ammonium/kg/day in the diet from gestational day 1 through lactation day 21), which then received a normal diet, had a statistically significant reduction in body weight gain (Miñana et al. 1995); body weight was reduced by 25 and 16% in male and female offspring, respectively, at 120 days of age. Rats that were continued on the same ammonia diet as their dams had an even greater decrease in body weight gain (27 and 26% for males and females, respectively) at 120 days of age (Miñana et al. 1995). No information was provided in the study regarding the health of the dams, but it is likely that the high ammonium dietary concentration made them hyperammonemic. Body weights and food consumption by the dams throughout the study were not reported.

3.2.2.7 Cancer

No information was located regarding carcinogenic effects of ammonia or ammonium compounds in humans following oral exposure. Exposure of mice to 193 mg ammonium/kg/day as ammonium hydroxide in drinking water for 2 years did not produce carcinogenic effects, nor did it affect spontaneous

development of breast cancer, which is common to C3H female mice (Toth 1972). No evidence of a carcinogenic effect was found in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg NH_4^+/kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors (Uzvolgyi and Bojan 1980). The ammonia and pyrocarbonate may have reacted *in vivo* to form the carcinogen, urethane. In a group of mice treated with urethane, the incidence of lung tumors was 9 of 9. Data from studies *in vitro* cited by Uzvolgyi and Bojan (1980) demonstrated the formation of urethane from diethyl pyrocarbonate added to beverages containing ammonia. No lung tumors were observed in the offspring of mice exposed similarly to NH_4^+ and diethyl pyrocarbonate during pregnancy or during lactation (Uzvolgyi and Bojan 1985). In another study, Tsujii et al. (1992a, 1995) tested the hypothesis that ammonia produced in the stomach in humans infected with *Helicobacter pylori* may play a role in the development of gastric cancer. Rats pretreated with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for 24 weeks before receiving 0.01% ammonia solution in the drinking water for 24 weeks had a statistically significantly greater incidence of gastric cancer (70% of rats) and number of tumors per tumor-bearing rat (2.1) than rats receiving only MNNG and tap water (31% and 1.3 tumors/rat) (Tsujii et al. 1992a). Additionally, the size, depth, and metastasis of the MNNG-initiated tumors were enhanced in the rats treated with ammonia (Tjusii et al. 1995). It can be estimated that the dose of ammonia received by the rats was approximately 42 mg/kg/day (the daily intake from food and water for the general population is approximately 0.3 mg/kg/day [WHO 1986]). The relevance of these studies to assess the cancer risk of oral exposure to ammonia is uncertain.

3.2.3 Dermal Exposure

Dermal exposure to ammonia may also result in some inhalation exposure. Therefore, based on the available data, it is not always clear to what extent each route of exposure contributes to the toxicity observed in dermal exposure studies.

3.2.3.1 Death

Human and animal deaths involving dermal exposure to ammonia and ammonium have been reported (Prokop'eva et al. 1973; Slot 1938; Sobonya 1977), but the extent of exposure is not known, and effects were probably due to inhalation exposure as well. A 25-year-old woman exposed to ammonia gas from a broken pipe had burns on her face, arms, and torso, and had difficulty breathing and swallowing (Slot

1938). She was treated symptomatically and with supportive treatment, but died about a month after exposure (Slot 1938). Autopsy showed edematous, inflamed, hemorrhagic epiglottis, trachea, and lungs. Petechial hemorrhages were found on the heart and the kidneys were congested with hemorrhagic nephritis. A 25-year-old man died after a tank of anhydrous ammonia exploded near him while he was farming (Sobonya 1977). Immediately after exposure he had mild bilateral conjunctival edema, burns over about 30% of his body surface, bilateral pulmonary edema, and severe respiratory distress. He developed pneumonia and died on the sixtieth day post-exposure. In rats, LC_{50} values of 112, 71.9, and 48.4 mg ammonia/L were determined for exposures of 15, 30, and 60 minutes, respectively (Prokop'eva et al. 1973). These data are presented in Table 3-3.

3.2.3.2 Systemic Effects

No information was located on hematological, musculoskeletal, hepatic, endocrine, or body weight effects in humans or animals after dermal exposure to ammonia or ammonium.

Dermal or multiple route exposure to ammonia or ammonium has produced respiratory, cardiovascular, gastrointestinal, renal, dermal, and ocular effects.

Respiratory Effects. Respiratory effects have been reported in humans from exposure to massive amounts of ammonia gas, but no quantitative data were located. It is also unclear as to what extent the effects were a result of inhalation and dermal exposure. Tracheitis, bronchitis, edema, and bronchopneumonia were reported by Slot (1938). Lung infection and respiratory distress were reported in one case (Sobonya 1977). Dyspnea, rales, rhonchi, and blocked airways were found by Levy et al. (1964). The effects probably resulted from concurrent inhalation and dermal exposure. No information was located regarding respiratory effects of ammonia or ammonium in animals following dermal or ocular exposure.

Cardiovascular Effects. Elevated pulse, shock, and cardiac failure were reported in humans from accidental exposures to massive amounts of ammonia gas, but the extent of exposure was not quantified (Slot 1938). No information was located regarding cardiovascular effects of ammonia or ammonium in animals following dermal or ocular exposure.

Gastrointestinal Effects. Persistent vomiting was noted by Slot (1938) in human accidental massive exposure cases, but the extent of exposure was not quantified. Oral and pharyngeal burns and edema

Table 3-3 Levels of Significant Exposure to Ammonia And Ammonium Compounds - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious		Reference Chemical Form
ACUTE EXPOSURE								
Death								
Rat	1 d 30 min/d					71.9 mg/L	(LC50)	Prokop'eva et al. 1973
Rat	1 d 60 min/d					48.4 mg/L	(LC50)	Prokop'eva et al. 1973
Systemic								
Human	5 min	Ocular	50 ppm	72 ppm	(eye irritation)			Industrial Bio-Test Laboratories, Inc. 1973
Human	10 min	Ocular	30 ppm	50 ppm	(moderate ocular irritation)			MacEwen et al. 1970
Pig (Duroc)	5 wk min/d	Ocular	10 ppm	50 ppm	(ocular irritation)			Stombaugh et al. 1969
INTERMEDIATE EXPOSURE								
Systemic								
Human	6 wk 5 d/wk 6 hr/d	Ocular	25 ppm	50 ppm	(transitory eye irritation)			Ferguson et al. 1977

d = day(s); hr = hour; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; wk = week(s)

were reported by Levy et al. (1964) in four human males accidentally exposed to an unknown quantity of anhydrous ammonia. Inhalation exposure may have contributed to these effects also.

Renal Effects. Renal congestion and hemorrhagic nephritis were reported by Slot (1938) in six cases of accidental human exposures to highly concentrated aerosols of ammonium derived from anhydrous ammonia. The exposure level cannot be determined from the available data.

Dermal Effects. Skin and eyes are extremely sensitive to airborne ammonia or ammonium in water. The topical damage caused by ammonia is probably due mainly to its alkaline properties. Its high water solubility allows it to dissolve in moisture on these surfaces, react with fatty substances in the corneal layer, be absorbed into deeper layers, and inflict extensive damage (Jarudi and Golden 1973). Reports of skin damage in humans are numerous, but good quantitative data are lacking. The severity of the damage is proportional to concentration and duration of exposure; flushing with water immediately after contact alleviates or prevents effects. Burns, blisters, and lesions of the skin have been reported (Close et al. 1980; Flury et al. 1983; Shimkin et al. 1954; Slot 1938; Taplin et al. 1976; Walton 1973). Exposure levels associated with dermal effects are presented in Table 3-3.

Several case reports described exposure of individuals to ammonia liquid and/or gas that resulted in cutaneous burns (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). All exposures were occupationally related. Total body surface area burned ranged from 14 to 45% and most had at least small areas of full-thickness burns that required skin grafting. A summary of 12 case reports of liquid anhydrous ammonia injuries reported a range of percent body surface area burned of 3–22%, with 25% of the patients having full-thickness burn injuries (Millea et al. 1989). One case report included a skin litmus paper test that showed the pH of the skin to be 10 at the time of hospital admission (Amshel et al. 2000). Infection of the burn wounds was not uncommon, with most of the patients responding to antibiotic treatment. One person had facial and neck hyperemia, erythematous petechiae on one ear, and edematous and peeling lips (Latenser and Lucktong 2000). The individual with 45% total body surface area burned had additional severe injuries, including respiratory and ocular, and developed circulatory and hematological problems, which led to his death (George et al. 2000).

Rosenbaum et al. (1998) described two cases of young children (2–3 years old) who bit into ammonia pellets/capsules. Both children drooled and had ulcerative lesions on the tongue and/or on the buccal mucosa. One child had superficial ulcerations on the posterior esophageal wall and the other child had

edematous, erythematous upper and lower lips with areas of desquamation, eschar of the hard palate, and edema and erythema of the supraglottic structures and upper trachea. Both children recovered without incident.

A single case report described a self-administered ammonium solution enema that resulted in anal pain, diffuse abdominal colic, and tenesmus (da Fonseca et al. 1998). Sigmoidoscopy showed diffuse erythematous friable mucosa with large ulcerations covered by yellowish exudate. Six days later, the ulcers had receded, but the colon was still erythematous. Three months postexposure, biopsies showed chronic inflammation and fibrosis of the rectum and sigmoid colon, but no stenosis.

Animal data regarding dermal and ocular effects of exposure to ammonia support the findings in humans. A number of cattle were acutely exposed to anhydrous ammonia fumes when a pipeline running through their pasture ruptured and leaked 1,800 barrels of ammonia in a short period of time (Morgan 1997). The ammonia combined with moisture in the air and formed a white cloud, which drifted south across two additional fields containing cattle. The noses of the cattle in the field with the pipeline turned black and peeled and the horns of cattle in an adjacent field turned black and peeled. Hair coats on all livestock within a 2-mile radius of the rupture were singed.

Ocular Effects. Reported ocular effects in humans following ammonia or ammonium exposure increase in severity with dose and duration. Good quantitative data are lacking, but symptoms progress as follows: inflamed eyes, lacrimation, swelling of the eyelids (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Jarudi and Golden 1973; Legters et al. 1981; Montague and Macneil 1980; O'Kane 1983; Price et al. 1983; Silverman et al. 1949; Stombaugh 1969; Verberk 1977; Ward et al. 1983), hyperemic conjunctiva (Caplin 1941; Hatton et al. 1979; Levy et al. 1964; Slot 1938; Sobonya 1977), transient blindness, blurred vision, and corneal abrasions (Latenser and Lucktong 2000), and sustained corneal damage (Caplin 1941; Grant 1974: Kass et al. 1972; McGuinness 1969; Stroud 1981; Yang et al. 1987). Ammonia is slightly irritating to human eyes at concentrations of 100 ppm (Ferguson et al. 1977), and immediately irritating to the eyes and throat at 698 ppm (Henderson and Haggard 1927). Exposure to an air concentration of 250 ppm is bearable for most persons for 30–60 minutes (Withers et al. 1986). Exposure levels associated with ocular effects are presented in Table 3-3.

Animal data regarding ocular effects of exposure to ammonium support the findings in humans. Corneal opacity has been observed in rabbits following brief exposures (2 seconds) to a solution of 28.5% ammonium hydroxide (Grant 1974). Volume administered was not reported. Cattle in an adjacent field

to a pipeline that ruptured and released 1,800 barrels of ammonia had runny eyes; two cows in the same field with the ruptured pipeline were euthanized because of blindness and respiratory distress (Morgan 1997).

3.2.3.3 Immunological and Lymphoreticular Effects

Secondary infections often complicate the clinical outcome of burns and respiratory lesions related to exposures to highly concentrated aerosols derived from anhydrous ammonia in which dermal and ocular exposure accompanies inhalation exposure (Sobonya 1977; Taplin et al. 1976). However, there is no evidence that the decreased immunological resistance represents a primary impairment of the immune system in humans. No information was located regarding the immunological effects of ammonia or ammonium in animals following dermal or ocular exposure.

No information was located regarding the following effects of ammonia or ammonium compounds in humans or animals following dermal or ocular exposure:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

Carcinogenic potential of ammonia has not been established in humans or animals by the dermal route of exposure. One case report was found of a person who developed epidermal carcinoma of the nasal septum 6 months after being badly burned by accidental contact with a refrigeration ammonia-oil mixture (Shimkin et al. 1954). It is unclear whether ammonia played a role in this tumor development. No other reports were located, although many cases of contact with ammonia from spills have been followed for more than 6 months after exposure.

3.2.4 Other Routes of Exposure

There are limited *in vitro* data suggesting that ammonium ion may affect fetal development (Lane and Gardner 1994). Mouse embryos (conceived *in vivo*) were cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 µmol/L ammonium ion for 48, 69, or 93 hours before being transferred to pseudopregnant mouse dams (Lane and Gardner 1994). Examination on gestational day 15 showed an apparent relationship between the duration of exposure and the incidence of exencephaly. Embryos that were cultured with various concentrations of ammonium ion before being transferred to recipient dams showed increased incidence of exencephaly with increased ammonium concentration (38–300 µmol/L) and decreased percentage of implantation sites with increased ammonium concentration. It is unclear how embryos might be exposed to ammonia or ammonium *in vivo* or if *in vivo* exposure would affect fetal development and implantation in a way similar to that described in the Lane and Gardner (1994) study.

3.3 GENOTOXICITY

A single study examined the genotoxic effect of ammonia in humans (Yadav and Kaushik 1997). Analysis of blood samples from 22 workers exposed to ammonia in a fertilizer factory and 42 control workers not exposed to ammonia showed increased frequency of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs), increased mitotic index (MI), and increased frequency of CAs and SCEs with increasing length of exposure.

Swiss albino mice administered a single dose of 12, 25, or 50 mg/kg ammonium intraperitoneally had an increased frequency of micronuclei compared to controls (Yadav and Kaushik 1997).

All remaining tests of ammonia's mutagenicity consist of studies in *E. coli*, chick fibroblast cells, and *Drosophila melanogaster* (Table 3-4). Demerec et al. (1951) noted positive effects in a reverse mutation test in *E. coli*, but only in treatments using toxic levels of NH_4^+ (98% lethality). Lobasov and Smirnov (1934) found slight mutagenic activity in *Drosophila* following exposure to ammonia gas, but once again, survival after treatment was <2%. Auerbach and Robson (1947) tested Lobasov and Smirnov's results and noted 0.5% sex-linked lethals. The authors concluded that although their data did not support the earlier study's findings, it is possible that ammonia has a very slight mutagenic action. In their data presentation, however, they report their findings as negative, qualifying it as doubtful and probably negative.

Table 3-4. Genotoxicity of Ammonia *In Vitro* and *In Vivo*

Species (test system)	End point	Form	Activation system	Results		Reference
				With activation	Without activation	
In vitro:						
Escherichia coli	Reverse mutation	NH_3		NT	+ (at toxic levels)	Demerec et al. 1951
Chick fibroblasts	Chromosomal aberrations	NH_4Cl^+ NH_4OH buffer		NT	+	Rosenfeld 1932
Mouse fibroblasts	Reduced cell division	$NH_3^+NH_4Cl$		NT	+	Visek et al. 1972
Mouse fibroblasts (3T3)	Reduced cell division			NT	+	Capuco 1977
Mouse fibroblasts	DNA repair inhibition	NH_4Cl		NT	+	Capuco 1977
In vivo:						
Drosophila melanogaster	Mutagenic lethality	NH_3		+	NT	Lobasov and Smirnov 1934
D. melanogaster	Sex-linked recessive lethal mutations	NH_3		– (doubtful, probably negative)	NT	Auerbach and Robson 1947
D. melanogaster	Dominant lethality	NH_3		–	NT	Auerbach and Robson 1947
Mouse ileal and colonic mucosa cells	Decreased rate of DNA synthesis	NH_4Cl		NT	+	Zimber and Visek 1972a

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NH_3 = ammonia; NH_4Cl = ammonia chloride; NH_4OH = ammonium hydroxide; NT = not tested

In vitro tests of chick fibroblast cells showed that buffered ammonia-ammonium chloride solutions can induce clumping of chromosomes, inhibit spindle formation, and result in polyploidy (Rosenfeld 1932). Visek et al. (1972) noted reduced cell division in mouse fibroblasts cultured in media to which ammonia and ammonium chloride were added. The effect was noted in cultures irrespective of pH. Decreased rate of DNA synthesis was noted in mouse mucosal cells in the ileum and colon when serum NH_4^+ levels were significantly elevated over normal levels; these elevated levels were induced by intraperitoneal injection of urease or infusion of ammonium chloride (Zimber and Visek 1972a).

Iwaoka et al. (1981), responding to controversy regarding mutagenicity in fried hamburgers, found that extraction of organic ingredients from fried hamburger and refrigerated biscuit products with ammonium hydroxide or ammonium sulfate increased mutagenic activities in *Salmonella typhimurium* T98 and TA1538 Ames' microsomal systems, while negative results were obtained from extraction with sodium sulfate. The mode of action is unclear; ammonium salts may in some way affect the mutagenic activities of some agents, or they may simply be more efficient extractors of mutagenic components from these foods.

Taken together, the data indicate that ammonia and ammonium ion may have clastogenic and mutagenic properties.

3.4 TOXICOKINETICS

Studies suggest that ammonia can be absorbed by the inhalation and oral routes of exposure, but there is less certainty regarding absorption through the skin. Absorption through the eye has been documented. Most of the inhaled ammonia is retained in the upper respiratory tract and is subsequently eliminated in expired air. Almost all of the ammonia produced endogenously in the intestinal tract is absorbed. Exogenous ammonia is also readily absorbed in the intestinal tract. Ammonia that reaches the circulation is widely distributed to all body compartments although substantial first pass metabolism occurs in the liver where it is transformed into urea and glutamine. Ammonia or ammonium ion reaching the tissues is taken up by glutamic acid, which participates in transamination and other reactions. The principal means of excretion of ammonia that reaches the circulation in mammals is as urinary urea; minimal amounts are excreted in the feces and in expired air.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Experiments with volunteers show that ammonia, regardless of its tested concentration in air (range, 57–500 ppm), is almost completely retained in the nasal mucosa (83–92%) during short-term exposure, i.e., up to 120 seconds (Landahl and Herrmann 1950). However, longer-term exposure (10–27 minutes) to a concentration of 500 ppm resulted in lower retention (4–30%), with 350–400 ppm eliminated in expired air by the end of the exposure period (Silverman et al. 1949), suggesting an adaptive capability or saturation of the absorptive process. Nasal and pharyngeal irritation, but not tracheal irritation, suggests that ammonia is retained in the upper respiratory tract. Unchanged levels of blood-urea-nitrogen (BUN), non-protein nitrogen, urinary-urea, and urinary-ammonia are evidence of low absorption into the blood. Exposure to common occupational limits of ammonia in air (25 ppm) with 30% retention (and assuming this quantity is absorbed into the blood stream) would yield an increase in blood ammonium concentration of 0.09 mg/L (calculated by WHO 1986). This calculated rise is only 10% above fasting levels, as reported by Conn (1972).

Animal data provide supporting evidence for high-percentage nasal retention, thus protecting the lower respiratory tract from exposure (Dalhamn [1963] and Boyd et al. [1944], rabbit; Egle [1973], dog). Continuous exposure of rats for 24 hours to concentrations up to 32 ppm resulted in significant increase in blood ammonia levels (Schaerdel et al. 1983). Exposures to 310–1,157 ppm led to significantly increased blood concentrations of ammonia within 8 hours of exposure initiation, but blood ammonia returned to pre-exposure values within 12 hours of continuous exposure and remained so over the remaining of the 24-hour exposure period. This suggests an adaptive response mechanism may be activated with longer-term exposure (Schaerdel et al. 1983).

3.4.1.2 Oral Exposure

Case reports of human ingestion of household ammonia (ammonium hydroxide) provide evidence of its absorption by this route, but few provide quantitative data. For example, in a fatal case of a man who drank an unknown amount of a 2.4% solution of ammonium hydroxide, analysis of the contents of the stomach and blood showed ammonium ion concentrations of 153 and 33 ppm, respectively (Klendshoj and Rejent 1966). In a study of volunteers, ingestion of a single ammonium chloride tablet (approximately 15 mg NH_4^+/kg/day) led to a small transient increase (33% above fasting levels) in arterial

blood concentrations of ammonium ion in 11 out of 20 subjects (Conn 1972); no change was noted in the remaining nine subjects in this group. Among 50 cirrhotic patients, increases of about 150% were noted in arterial blood concentrations of ammonium ion and return to normal levels was slow (Conn 1972). These data indicate that ingested ammonia is readily absorbed from the digestive tract and that the liver plays a large role in removing it from the blood (Conn 1972). Analysis of urine samples from subjects on high and low protein diets and given ^{15}N-ammonium chloride, showed that 30–65% of labeled nitrogen from ^{15}N-ammonium chloride is absorbed and metabolized (Richards et al. 1975). Oral administration of $^{15}NH_4Cl$ to a group of six subjects for six days resulted in absorption of at least 38.7% of the administered radioactivity as determined by the amount of ^{15}N that appeared in urinary urea within 24 hours of the last $^{15}NH_4Cl$ ingestion (Metges et al. 1999).

Ammonium ion is endogenously produced in the human digestive tract, much of it arising from the bacterial degradation of nitrogenous compounds from ingested food. About 4,200 mg/day are produced, greater than 70% of which is synthesized or liberated within the colon and its fecal contents. The total amount absorbed is about 4,150 mg/day, or 99% of the amount produced (Summerskill and Wolpert 1970); absorption after oral loading of NH_4^+ is similarly complete (Fürst et al. 1969). Evidence from Castell and Moore (1971) and Mossberg and Ross (1967) suggests that absorption of NH_4^+ increases as the pH of the contents of the lumen increases, and that the ammonium ion is actively transported at the lower pH levels (pH 5 was lowest detected absorption). Ammonium ion absorbed from the gastrointestinal tract travels via the hepatic portal vein directly to the liver, where in healthy individuals, most of it is converted to urea and glutamine. Human and animal data show that little of it reaches the systemic circulation as ammonia or ammonium compounds, but that it is a normal constituent of plasma at low levels (Brown et al. 1957; Pitts 1971; Salvatore et al. 1963; Summerskill and Wolpert 1970). Analysis of plasma drawn from 10 healthy young male subjects yielded endogenously derived NH_4^+ concentrations ranging from 30 to 55 μg NH_3/100 mL, with a mean of 39 μg/100 mL (Brown et al. 1957).

3.4.1.3 Dermal Exposure

Quantitative data on absorption from exposure by the dermal route were not located in the available literature. Human case reports of dermal exposure describe local damage (burns, irritations). One report of case histories of five persons exposed to an exploding, bursting anhydrous ammonia gas pipe indicated there was systemic toxicity (vomiting, renal congestion, delirium), but exposure was by inhalation as well as dermal route, and it is impossible to delineate a systemic dermal exposure contribution (Slot 1938).

WHO (1986) concluded that systemic effects from skin and eye exposure are not quantitatively important. Ammonia is readily absorbed into the eye; it was found to diffuse within seconds into cornea, lens, drainage system, and retina (Beare et al. 1988; Jarudi and Golden 1973). However, amounts absorbed were not quantified, and absorption into systemic circulation was not investigated.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No quantitative reports of distribution of ammonia from inhalation exposure were found in the available literature. Absorption data from human inhalation exposure suggest that only small amounts of ammonia are absorbed into the systemic circulation (Silverman et al. 1949; WHO 1986). Initial retention of inhaled ammonia in the mucus of the upper respiratory tract may be 80% or more, but after equilibrium is established (within 30 minutes) 70–80% of inspired ammonia is expired in exhaled air (Silverman et al. 1949). The lack of change in blood nitrogen compounds and urinary-ammonia compounds lends further support to a limited absorption into the systemic circulation (Silverman et al. 1949). Toxic effects reported from inhalation exposure suggest local damage, or changes resulting from necrotic tissue degradation, rather than the presence of elevated levels of NH_4^+, *per se*, in tissues other than the respiratory/pharyngeal tissues. Information on the distribution of endogenously-produced ammonia suggests that any NH_4^+ absorbed through inhalation would be distributed to all body compartments via the blood, where it would be used in protein synthesis or as a buffer, and that excess levels would be reduced to normal by urinary excretion, or converted by the liver to glutamine and urea. If present in quantities that overtax these organs, NH_4^+ is distributed to other tissues and is known to be detoxified in the brain (Takagaki et al. 1961; Warren and Schenker 1964).

3.4.2.2 Oral Exposure

Human oral exposure data for NH_4^+ clearly indicate that it readily enters the portal circulation and is delivered to the liver (Conn 1972; Fürst et al. 1969), as has been shown to be the case for endogenously produced NH_4^+ (Pitts 1971; Summerskill and Wolpert 1970). In nitrogen-deficient persons, NH_4^+ (as ammonium acetate) administered orally was absorbed and carried directly to the liver where most of it was converted to urea and excreted in the urine; little change in the negative nitrogen balance was observed (Fürst et al. 1969). Output of urea from the liver corresponded to the amount of NH_4^+ ingested (Fürst et al. 1969).

Un-ionized ammonia is freely diffusible, whereas the ammonium ion is less so and is relatively confined to the extracellular compartment (Stabenau et al. 1958). However, ammonium ion is in dynamic equilibrium with dissolved ammonia. Therefore, ammonium compounds that enter the circulatory system or other body fluids can thus freely penetrate tissue cells as ammonia. In hypophysectomized rats that were administered ^{15}N-ammonium citrate orally by gavage, labeled protein was found in liver, kidney, spleen, heart, and skeletal muscle 6–72 hours after ^{15}N-ammonium citrate administration (Vitti et al. 1964). The percentages of ingested label absorbed and then excreted as urea in the urine were not provided (Vitti et al. 1964).

3.4.2.3 Dermal Exposure

No quantitative data on distribution of ammonia from dermal exposure were located in the available literature. Toxic effects from dermal exposure suggest that little or no ammonia gains entry into the systemic circulation by this route.

3.4.2.4 Other Routes of Exposure

Intravenous administration of NH_4^+ (as ammonium salts) to people with a nitrogen deficiency (in negative nitrogen balance) resulted in an increase in the peripheral blood NH_4^+ level and a shift in the nitrogen balance from negative to positive; no increase in urinary urea was seen (Fürst et al. 1969). The nitrogen from NH_4^+, which gains entry into the general circulation, is distributed to cells throughout the body and incorporated into tissues (Fürst et al. 1969; Vitti et al. 1964). After intraperitoneal injection of ammonium chloride in mice, ammonia distributes to brain tissues within 20 seconds (Warren and Schenker 1964), and in rats, brain concentrations increase dramatically within 5 minutes (Salvatore et al. 1963). Tissues other than blood and brain were not analyzed by these researchers. Comparative patterns of distribution of ^{15}N-labeled ammonium citrate indicate that the amount of NH_4^+ taken up by tissues other than the liver is greatest by subcutaneous injection, less by intraperitoneal injection, and least following intragastric administration. Intravenous administration of ^{15}N-labeled ammonium salts leads to rapid distribution of ^{15}N-labeled metabolites throughout the body, with the highest levels of labeled urea appearing in the kidney and liver, and lesser amounts in heart, spleen, brain, testes, and carcass. Highest levels of labeled glutamine were found in heart and liver, with lesser amounts in brain, spleen, carcass, kidney, and testes (Duda and Handler 1958).

3.4.3 Metabolism

Quantitative data on human metabolism of exogenously introduced ammonia were not located in the available literature. Ammonia and ammonium ion are metabolized to urea and glutamine mainly in the liver by the process diagrammed in Figures 3-3 and 3-4 and described by Fürst et al. (1969) and Pitts (1971). However, it can be rapidly converted to glutamine in the brain and other tissues as well (Takagaki et al. 1961; Warren and Schenker 1964). The nitrogen is released from glutamine within tissue cells and used for protein synthesis as needed (Duda and Handler 1958; Fürst et al. 1969; Richards et al. 1975; Vitti et al. 1964). Ingestion of ammonium salts leads to almost complete conversion of ammonium ion into urea in the liver, whereas exposure by other routes may lead to its metabolism in body tissues to glutamine or tissue protein (Fürst et al. 1969; Vitti et al. 1964).

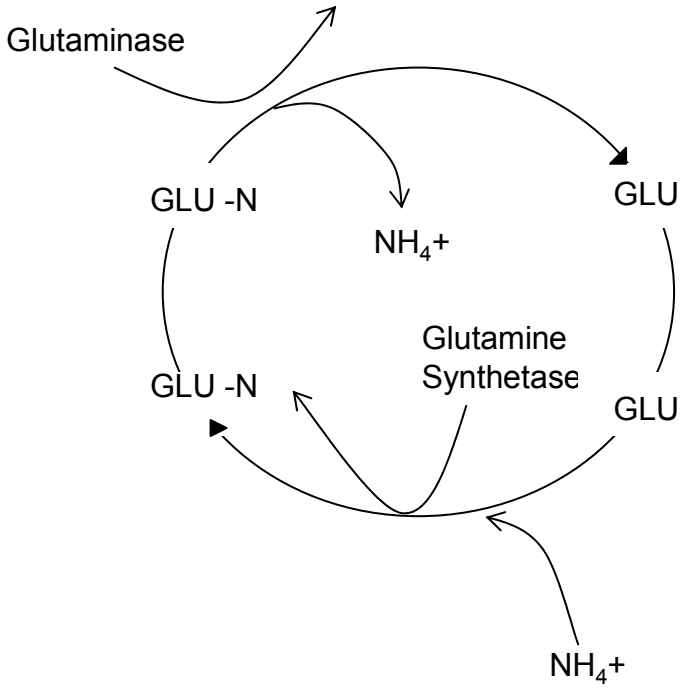
Duda and Handler (1958) administered 0.03 mg/kg body weight of ^{15}N-ammonium acetate intravenously to rats and noted that 90% was converted to glutamine and urea within 30 minutes, with glutamine being the major early product. Labeled nitrogen was also found in amino acids, purines, pyrimidines, and other nitrogenous compounds. Morimoto et al. (1988) found that the amount of ^{15}N from an intravenous injection of ammonium chloride to rats that was taken up into glutamine-amide-N and urea-N reached a peak at 5 minutes and decreased gradually from 15 to 60 minutes after the injection. This finding suggests that urea synthesis and glutamine synthesis occurred simultaneously within minutes after the injection, and glutamine-amide-N is gradually transferred to the urea cycle from 15 to 60 minutes following dosing. Low amounts (0.008% of a 17 mg oral dose) of ^{15}N-ammonium chloride administered repeatedly to rats were converted to ^{15}N-nitrate in the urine (Saul and Archer 1984).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Studies using low levels of ammonia show that inhaled ammonia is temporarily dissolved in the mucus of the upper respiratory tract, and then a high percentage of it is released back into the expired air. Following exposure to 500 ppm ammonia for 10–27 minutes, healthy male subjects eliminated 70–80% of the inspired ammonia by this route (Silverman et al. 1949). Analysis of endogenous ammonia levels in the expired air of rats showed concentrations ranging from 10–353 ppb (mean=78 ppb) in nose-breathing animals (Barrow and Steinhagen 1980).

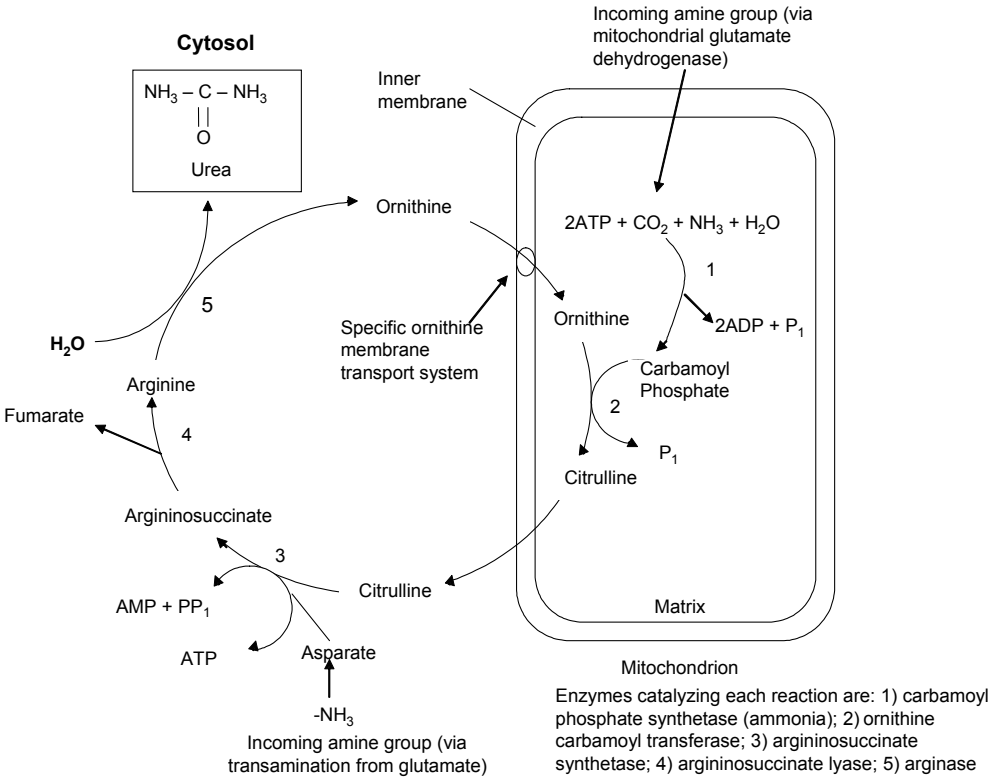
Figure 3-3. Glutamine Cycle



GLU = Glutaminate; GLU-N = Glutamine

Source: Brunner and Thaler 1981

Figure 3-4. The Urea Cycle Showing the Compartmentalization of its Steps Within Liver Cells



Source: Lehninger 1975

The quantitative difference between inspired and expired ammonia suggests that small amounts are absorbed across the nasopharyngeal membranes into the systemic circulation. Absorbed ammonia is excreted by the kidneys as urea and urinary ammonium compounds (Gay et al. 1969; Pitts 1971; Richards et al. 1975; Summerskill and Wolpert 1970), as urea in feces (Richards et al. 1975), and as components of sweat (Guyton 1981; Wands 1981), but quantitative data are lacking. Toxic levels do not develop as a result of chronic inhalation exposure because the body has multiple effective mechanisms for detoxifying and excreting it.

3.4.4.2 Oral Exposure

Excretion data for humans orally exposed to ammonia have been quantified with respect to excretion of isotope from ^{15}N-labeled ammonium salts, thus providing an indication of the turnover rate of the compound within the body and excretion route of its metabolites. Approximately 72% of a dose of ^{15}N was excreted in the urine of three subjects within 3 days of ingestion of ammonium salts in drinking water; 25% (24% urinary urea and 1% urinary NH_4^+) was eliminated within the first 6 hours after exposure. Ammonium salt administered by gavage to humans led to a corresponding increase in blood urea concentration transported out of the liver, leading the authors (Fürst et al. 1969) to conclude that orally ingested ammonium salt is quickly and almost completely converted in the liver and eliminated from the body as urinary urea. Analysis of urine samples from subjects on high and low protein diets showed higher cumulative excretion of ^{15}N (percent of dose) in the urine of the high protein group (approximately 70%) than that of the low protein group (35%). Small amounts of labeled nitrogen were also excreted as urea in feces (Richards et al. 1975).

These data correspond to that for excretion of endogenously produced ammonia (Davies and Yudkin 1952; Muntwyler et al. 1956; Summerskill and Wolpert 1970; Van Slyke et al. 1943). Ammonia is also known to be excreted via sweat (Guyton 1981; Wands 1981) and expired air (Barrow and Steinhagen 1980; Larson et al. 1980; Robin et al. 1959; Utell et al. 1989); quantitative data are unavailable for excretion via sweat.

3.4.4.3 Dermal Exposure

Data regarding excretion of ammonia absorbed following dermal exposure were not located in the available literature.

3.4.4.4 Other Routes of Exposure

Data are available on exposure of humans and dogs to ammonium salts by intravenous injection. Excretion of isotope after ^{15}N-ammonium lactate injection in three human subjects yielded 5–7% of isotope excreted as urinary NH_4^+ in the first 6 hours postexposure, and another 2% within 3 days. Approximately 6% of the isotope was excreted as urea in urine in the first 6 hours. An average of approximately 60% of the dose of label was excreted in urine within 3 days. These data are considerably different from that resulting from oral loading (as described in Section 3.4.4.2). Intravenous loading led to decreased labeling of urinary urea and grossly increased labeling of urinary ammonia; the differences are attributed by the authors to a "first pass" effect from oral loading (Gay et al. 1969). The hepatic transformation of ammonium ion to urea is so efficient that relatively little unconverted ammonium salt is released to the general circulation.

Intravenous exposure of seven dogs to 107 mg/kg ammonium acetate led to amounts ranging from 0.044 to 0.073 mg ammonia excreted in expired air. No measurable amount of ammonia was present in expired air during the pre-exposure control period (Robin et al. 1959).

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 et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can

be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

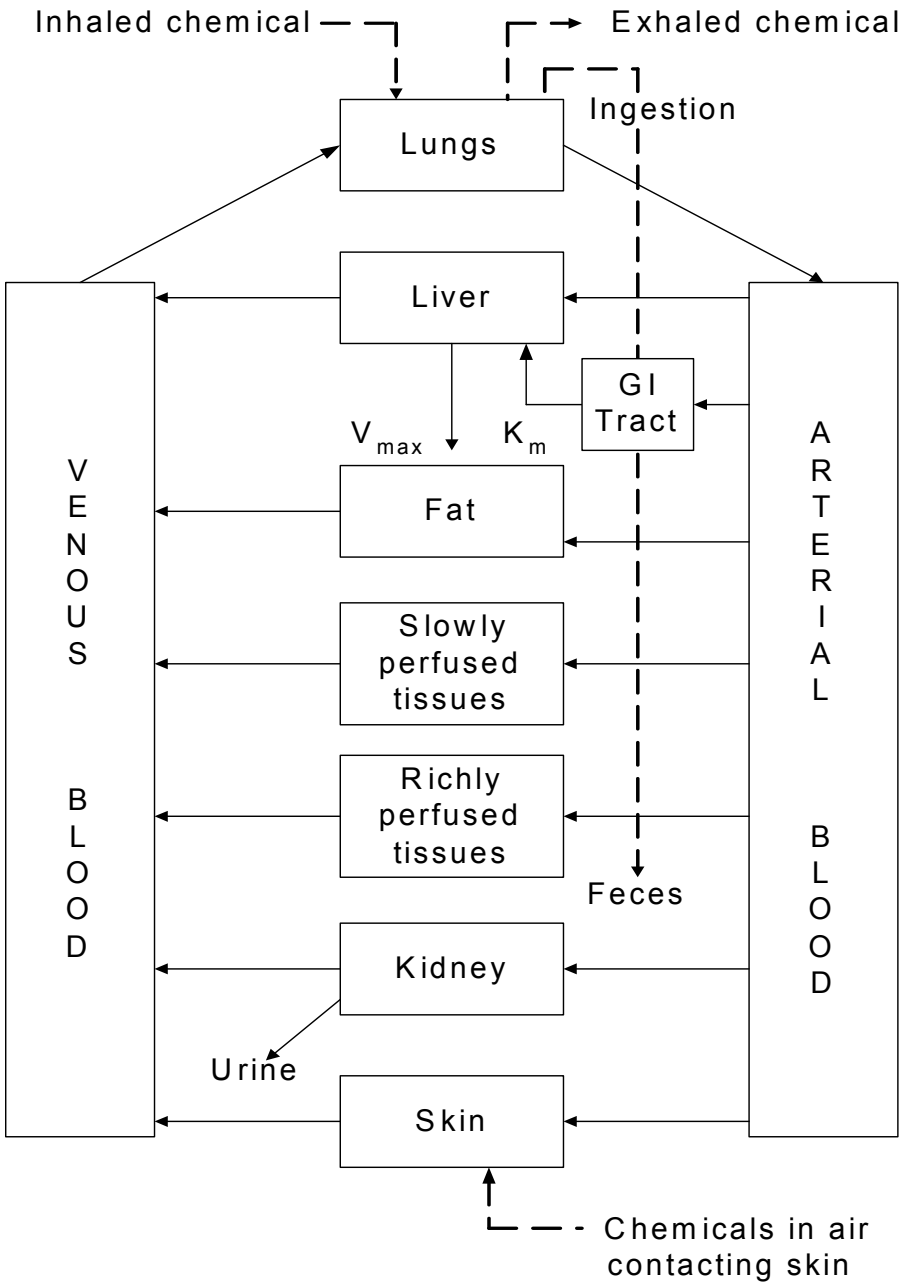
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically 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) is 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 3-5 shows a conceptualized representation of a PBPK model.

No data regarding PBPK models for ammonia were located.

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Data regarding the pharmacokinetic mechanisms of ammonia were not located in the available literature.

3.5.2 Mechanisms of Toxicity

Ammonia is an irritant and the primary and most immediate effect of ammonia exposure is burns to the skin, eyes, and respiratory tract. The topical damage caused by ammonia is probably due mainly to its alkaline properties. Its high water solubility allows it to dissolve in moisture on the mucous membranes, skin, and eyes, forming ammonium hydroxide, which causes liquefaction necrosis of the tissues (Jarudi and Golden 1973). Specifically, ammonium hydroxide causes saponification of cell membrane lipids, resulting in cell disruption and death. Additionally, it extracts water from the cells and initiates an inflammatory response, which further damages the surrounding tissues (Amshel et al. 2000). Contact with liquid ammonia results in cryogenic injury in addition to the alkali burns (Amshel et al. 2000; Wibbenmeyer et al. 1999).

Excess circulating levels of ammonia (hyperammonemia) can cause serious neurological effects. Hyperammonemias can be inherited (i.e., inborn errors of urea cycle enzymes) or acquired (i.e., liver toxicity caused by ingested toxins, viral infections, autoimmune disease) (Felipo and Butterworth 2002). An extensively studied neuropsychiatric disorder known as hepatic encephalopathy develops when liver function is impaired and the organ cannot metabolize ammonia. This results in an increased concentration of ammonia in the blood and brain. The mechanism of ammonia-induced encephalopathies has not been definitively elucidated, but is thought to involve the alteration of glutamate metabolism in the brain and resultant increased activation of NMDA receptors (Felipo et al. 1993; Marcaida et al. 1992), which causes decreased protein kinase C-mediated phosphorylation of Na^+/K^+ ATPase, increased activity of Na^+/K^+ ATPase, and depletion of ATP (Kosenko et al. 1994). Antagonists of NMDA receptors, agonists of metabotropic glutamate receptors, agonists of muscarinic receptors, and inhibitors of protein kinase C, calcineurin, or nitric oxide synthase prevent glutamate toxicity, indicating that all of these play a role in acute ammonia neurotoxicity (Felipo et al. 1998). Additional evidence of altered energy levels include changes in some TCA cycle-associated components including acetoacetate, and NAD^+/NADH ratio, 2-oxoglutarate, and 3-hydroxybutarate (Kosenko et al. 1993). A disruption in neurotransmission

has also been suggested by alteration of brain tubulin, which is an essential component of the axonal transport system (Miñana et al. 1989a, 1989b).

During certain disease states that result in renal tubular injury, NH_4^+ production by renal proximal tubules may increase in order to maintain net acid excretion. However, this may also contribute to further renal damage by modifying the third component of complement and initiating the alternative complement pathway (Clark et al. 1990). Ammonia can chemically interact with an internal thiolester bond of complement 3 (C3), resulting in an amide linkage and a subsequent conformational change of the C3. The altered C3 then activates the alternative complement pathway, which causes the release of chemoattractants and the assembly of the membrane attack complex of complement (Clark et al. 1990). Amidated C3 can also bind directly to phagocyte complement receptors, which causes the release of toxic oxygen species (Clark et al. 1990). It has also been suggested that NH_4^+ depresses protein degradation in renal cells and inhibits renal cell replication, which supports the findings of renal hypertrophy in renal injury and indicates that NH_4^+ may inhibit recovery from injury (Rabkin et al. 1993).

Ammonium ion may also contribute to adverse effects of *Helicobacter pylori* on the stomach. *H. pylori* produces urease, which breaks down urea that is normally present in the stomach into ammonia (Mégraud et al. 1992; Tsujii et al. 1992a). An *in vitro* study that examined the effects of ammonia produced by *H. pylori* on HEp2 cells showed increased cell vacuolation and viability of the cells compared to a urease-negative variant of the same cells (Mégraud et al. 1992). An *in vivo* study suggested that NH_4^+ also causes macroscopic gastric lesions and increases the release of endothelin-1 (ET-1) and thyrotropin releasing hormone (TRH) from the gastric mucosa, probably via an endothelin A (ET_A) receptor, which exerts ulcerogenic action on the gastric mucosa (Mori et al. 1998). Ammonia may also trigger the release of cysteine proteases in the stomach that contribute to the development of gastric hemorrhagic mucosal lesions (Nagy et al. 1996). Neutrophils that migrate to the gastric mucosa in response to the presence of *H. pylori* may release hypochlorous acid, which can interact with NH_4^+ to produce the powerful cytotoxic oxidizing agent monochloramine (Murakami et al. 1995).

3.5.3 Animal-to-Human Extrapolations

The primary effects of ammonia in humans are due to its corrosive and irritative properties. Exposure to ammonia gas causes damage to the respiratory tract, eyes, and skin when the ammonia combines with water to become ammonium hydroxide, which results in liquefaction necrosis of the tissues, cell structural breakdown, and inflammatory damage (Amshel et al. 2000; Wibbenmeyer et al. 1999). Animal studies

have indicated similar types of injuries of the respiratory tract (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969), eyes, and skin (Morgan 1997).

Oral exposure of humans to high concentrations of ammonia and ammonium hydroxide has been shown to result in buccal, esophageal, and upper tracheal burns and edema (Christesen 1995; Klein et al. 1985; Rosenbaum et al. 1998), but no reports of the effects of ammonia on the stomach or upper gastrointestinal tract in humans have been found. One report of an ammonia enema in a human showed diffuse erythematous, friable mucosa, and large exudative ulcerations in the sigmoid colon and rectum (da Fonseca et al. 1998). Gavage studies in rats have shown similar lesions of the gastric mucosa with notable histopathological effects (Mori et al. 1998; Takeuchi et al. 1995; Tsujii et al. 1993). Rats, therefore, appear to be an adequate model for the primary effects of ammonia in humans. However, humans are unlikely to be orally exposed to amounts of ammonia that would result in the gastric lesions seen in rats. Elevated levels of endogenously produced ammonia resulting from disease states apparently may cause or contribute to gastric pathology (Mégraud et al. 1992; Mori et al. 1998; Tsujii et al. 1992a).

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 Colborn and Clement (1992), was also used in 1996 when Congress mandated the Environmental Protection Agency (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), which in 1998 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).

No studies were located regarding toxicity mediated through the endocrine axis in humans after exposure to ammonia. Two studies examined the effects of induced hyperammonemia (with infusion of ammonium chloride) in steers on circulating and portal-drained visceral flux of metabolites and on pancreatic hormones (Fernandez et al. 1988, 1990). Plasma glucose increased 12% during infusion of ammonium chloride (Fernandez et al. 1988, 1990). Plasma insulin decreased up to 46% during ammonium chloride infusion, and then increased up to 122% after infusion was halted (Fernandez et al. 1988); portal-drained visceral release of insulin did not increase during ammonium chloride infusion even with the rise in plasma glucose levels, but increased 109% after cessation of infusion (Fernandez et al. 1990). These data indicate that hyperammonemia in steers may cause reduced hepatic glucose output and glucose-mediated pancreatic insulin release.

No *in vitro* studies were located regarding toxicity mediated through the endocrine axis by ammonia.

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).

Human and animal data indicate that the primary effects of ammonia are irritation and burns and that the primary targets of ammonia are the respiratory tract, eyes, and skin (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Flury et al. 1983; George et al. 2000; Hatton et al. 1979; Heifer 1971; Holness et al. 1989; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; Price et al. 1983; Shimkin et al. 1954; Slot 1938; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989). There are limited data on the toxicity of ammonia in children and no information on effects in adults who were exposed as children. Children (8–9 years old) who attended two schools in the vicinity of a fertilizer plant had higher incidences of acute respiratory diseases than children who attended a school 20 kilometers away (Gomzi 1999; Gomzi and Šarić 1997). Incidence was related to levels of measured pollutants (ammonia, hydrogen fluoride, nitrogen dioxide, total suspended particulate matter, and smoke) in the inside and outside air (Gomzi and Šarić 1997). Forced expiratory volumes were not statistically different between the three schools (Gomzi and Šarić 1997). These results indicate that exposure to low levels of ammonia (0.04–0.23 ppm) or other airborne pollutants may not cause functional respiratory deficits, but may lower the resistance to respiratory pathogens in children. There is no indication that children are more susceptible to the effects of ammonia than adults. However, children have greater surface area to body weight and lung surface area to body weight ratios, and increased minute volume to weight ratio, so they may receive a higher dose than adults in the same situation. Children may also tend to be exposed longer than adults because they may not be as quick as adults to evacuate a contaminated area.

There are no studies that indicate that metabolism of ammonia differs between children and adults. Ammonia is eliminated from the body mainly by processing through the urea cycle in the liver, and urea is then eliminated in the urine and feces. The urea cycle is fully functional in infants at birth; therefore, it is not expected that infants or children are at greater risk of hyperammonemia. Neurotoxicity resulting from hyperammonemia involves alteration of levels of some components of the citric acid cycle, which leads to depletion of ATP, and starvation of brain cells, and depletion of glutamate, a precursor to the neurotransmitter γ-aminobutyrate (GABA). It is not expected that children are more susceptible than adults to ATP depletion via this mechanism.

Infants under 6 months of age may be more sensitive than adults to the effects of high levels of nitrates (from nitrification of ammonia in fertilizers) that may be present in groundwater and well water (Payne

1981). Infants who consume formula and food made with contaminated water from these sources may develop methemoglobinemia, which results in decreased delivery of oxygen to the tissues.

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).

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

There are no known specific biomarkers of exposure for ammonia. Identification of biomarkers of exposure to ammonia is confounded because large amounts of ammonia are produced endogenously. Pharmacokinetic studies reveal that after inhalation exposure to low levels of ammonia, BUN, nonprotein nitrogen, urinary-urea, and urinary-ammonia levels do not change (Silverman et al. 1949). Exposure to common occupational limits of ammonia in air (25 ppm) yield increased blood-ammonia levels only 10% above fasting levels (WHO 1986). In one human study, oral ingestion of ammonium chloride tablets (approximately 15 mg NH_4^+/kg) yielded only a transient increase in blood-ammonia above fasting levels in 11 out of 20 subjects tested; no increase was observed in the remaining 9 subjects (Conn 1972).

3.8.2 Biomarkers Used to Characterize Effects Caused by Ammonia

Effect biomarkers of ammonia exposure are limited to site-of-contact tissue injuries. Upon inhalation exposure, distribution of ammonia is usually limited to the respiratory tract and involves irritation and, at higher concentrations, pulmonary edema and necrosis (Kapeghian et al. 1982; Richard et al. 1978b; Silverman et al. 1949). Oral exposure to high doses of ammonium chloride has produced pulmonary edema in animals (Koenig and Koenig 1949). Dermal exposure to ammonia causes skin and eye irritation and, at higher concentrations, necrosis (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). The severity of injuries by all routes of exposure are dose-related. Unfortunately, these effect biomarkers are not specific for ammonia and can be caused by a variety of caustic substances.

The tissues and organs most sensitive to ammonia exposure are mainly dependent on route of exposure. After inhalation exposure, which can involve a significant dermal exposure, the skin and eyes and the respiratory tract, including the lungs, are most sensitive. Direct dermal exposure produces dose-related effects from irritation to necrosis. Ingestion of ammonium hydroxide has resulted in oral, pharyngeal, and

esophageal lesions (Christesen 1995; Klein et al. 1985). The tissue and organ injuries produced by ammonia, however, are of limited value as biomarkers to characterize the effects caused by ammonia because many other caustic chemicals can produce similar injuries.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Exposure to substances that would increase the pH of exposed tissues could be expected to enhance the alkalotic effects of ammonia, and vice versa. Agents acting to elevate the intestinal-tract pH would increase its local irritant effect, and would promote its absorption as well (Castell and Moore 1971).

Co-administration of ammonia and diethyl pyrocarbonate induced lung tumors in Kid:CFLP mice, while neither agent administered intragastrically and separately was carcinogenic; this effect is believed to be a result of a compound, urethane (a known carcinogen), produced by their interaction (Uzvolgyi and Bojan 1980, 1985). Sprague-Dawley rats given intrarectal doses of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and ammonium acetate had a higher incidence of tumors than did controls that were administered distilled water in place of ammonium acetate (Clinton et al. 1988). The role of acetate was not ruled out. Ammonia acted synergistically with potassium ions on pyruvate kinase, a known Ehrlich ascites tumor enzyme (Olavarria et al. 1986).

Some compounds play a synergistic role with ammonia in producing hepatic coma. Simultaneous injection of an ammonium salt and a fatty acid in Holtman or Sprague-Dawley rats produced coma at lower plasma levels than did injection of either compound separately. Inhalation of methanethiol or injection with sodium octanoate blocked metabolism of an injected dose of ammonium acetate and led to elevated blood ammonia levels (Zieve et al. 1974).

Data regarding exposure to mixtures of atmospheric contaminants indicate that, contrary to what might be expected, increased carbon dioxide concentration (up to 5% in air) does not alter the hyperventilatory rate induced by hyperammonemia in dogs (Herrera and Kazemi 1980). Ammonia in expired air may neutralize inhaled acid aerosols (EPA 1979; Larson et al. 1980; Utell et al. 1989).

Other substances to which people have been exposed have been shown to alter the toxic effects of ammonia. Methionine sulfoximine, administered by intraperitoneal injection, suppressed the tonic convulsions produced by intravenous injection of ammonium chloride in mice (Hindfelt and Plum 1975; Warren and Schenker 1964). Intraperitoneal injection of alpha-methylglutamic acid also exerts a

protective effect against hyperammonemia in rats (Lamar 1970). Nicotinohydroxamic acid and neomycin administered orally reduce blood ammonia levels and increase excretion of urea in treated rats (Harada et al. 1985). Ethanol exerted a protective effect on acute ammonia intoxication in mice (O'Connor et al. 1982), although ethanol was reported to increase ammonia concentrations in body tissues of treated rats (Mohanachari et al. 1984).

Sodium benzoate decreased urea production in ammonia challenged rats (Maswoswe et al. 1986) and hyperammonemic mice (O'Connor et al. 1987). Valproate, a widely used antiepileptic drug, has a hyperammonemic effect in Wistar rats (Ferrier et al. 1988) and may therefore predispose to ammonia intoxication. Ammonia interferes with the metabolism of pent-4-enoic acid in cultured rat hepatocytes and may dramatically potentiate its toxicity (Coude and Grimber 1984).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to ammonia than will most persons exposed to the same level of ammonia 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 ammonia, or compromised function of organs affected by ammonia. Populations who are at greater risk due to their unusually high exposure to ammonia are discussed in Section 6.7, Populations With Potentially High Exposures.

Persons who suffer from severe liver or kidney disease may be susceptible to ammonia intoxication, as it is chiefly by the actions of these organs that NH_4^+ is biotransformed and excreted (Córdoba et al. 1998; Gilbert 1988; Jeffers et al. 1988); individuals with hereditary urea cycle disorders are also at risk (Schubiger et al. 1991). In these individuals, the levels produced endogenously are sufficient to produce toxicity. Levels that are likely to be encountered in the environment, with the exception of those resulting from high-level accidental exposures, are insignificant, due to the low absorption rate, in comparison with levels produced within the body (WHO 1986).

Since ammonia is a respiratory tract irritant, persons who are hyperreactive to other respiratory irritants, or who are asthmatic, would be expected to be more susceptible to ammonia inhalation effects. The results of an epidemiological study of a group of workers chronically exposed to airborne ammonia indicate that ammonia inhalation can exacerbate existing symptoms including cough, wheeze, nasal complaints, eye irritation, throat discomfort, and skin irritation (Ballal et al. 1998).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to ammonia. 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 ammonia. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide general information about treatment following exposures alkalies:

Ellenhorn MJ, Barceloux DG. 1997. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier.

Haddad LM, Shannon MW, Winchester JF, eds. 1998. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W.B. Sanders Company.

3.11.1 Reducing Peak Absorption Following Exposure

Absorption of ammonia via dermal exposure is not sufficient to be of concern, but immediate flushing of exposed skin with water or saline will limit dermal damage and reduce dermal absorption of ammonia. It is highly unlikely that enough ammonia could be ingested to be of danger via absorption from the intestines; however, in individuals with liver disease, endogenous production of ammonia may cause toxicity. Emesis should not be induced in case of ingestion of ammonia, but administration of activated charcoal, gastric lavage, or neutralization with weak acids is recommended HSDB (2003). Elimination of urease-producing enteric bacteria with oral antibiotics decreases the amount of ammonia absorbed from the gut (Gilbert 1988). Because ammonia is readily soluble in water at low concentrations, very little may reach and be absorbed in the lungs. Instead, ammonia at low concentrations may be absorbed in the mucosa of the upper respiratory tract and swallowed. Movement to an area of fresh air as quickly as possible would limit respiratory damage and absorption via the lungs.

3.11.2 Reducing Body Burden

No experimental data regarding methods for reducing the ammonia body burden were located. In healthy people, ammonia is efficiently metabolized via the urea cycle, primarily in the liver, and eliminated in the urine and feces (Fürst et al. 1969; Richards et al. 1975).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The primary effects of ammonia are related to its alkalinity and its solubility in water, which results in rapid and severe tissue damage. It is extremely important to get to an area free of ammonia gas and to remove all clothing contaminated with ammonia as quickly as possible. Skin and eyes should be irrigated with water for at least 15–20 minutes at the time of exposure and periodically for 24 hours after exposure (Millea et al. 1989). Irrigation of the eye should continue until the pH of the conjunctival sac is less than 8.5 (Grant 1974). This should be followed with proper medical treatment for respiratory symptoms and dermal and ocular burns.

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 ammonia 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 ammonia.

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 Ammonia

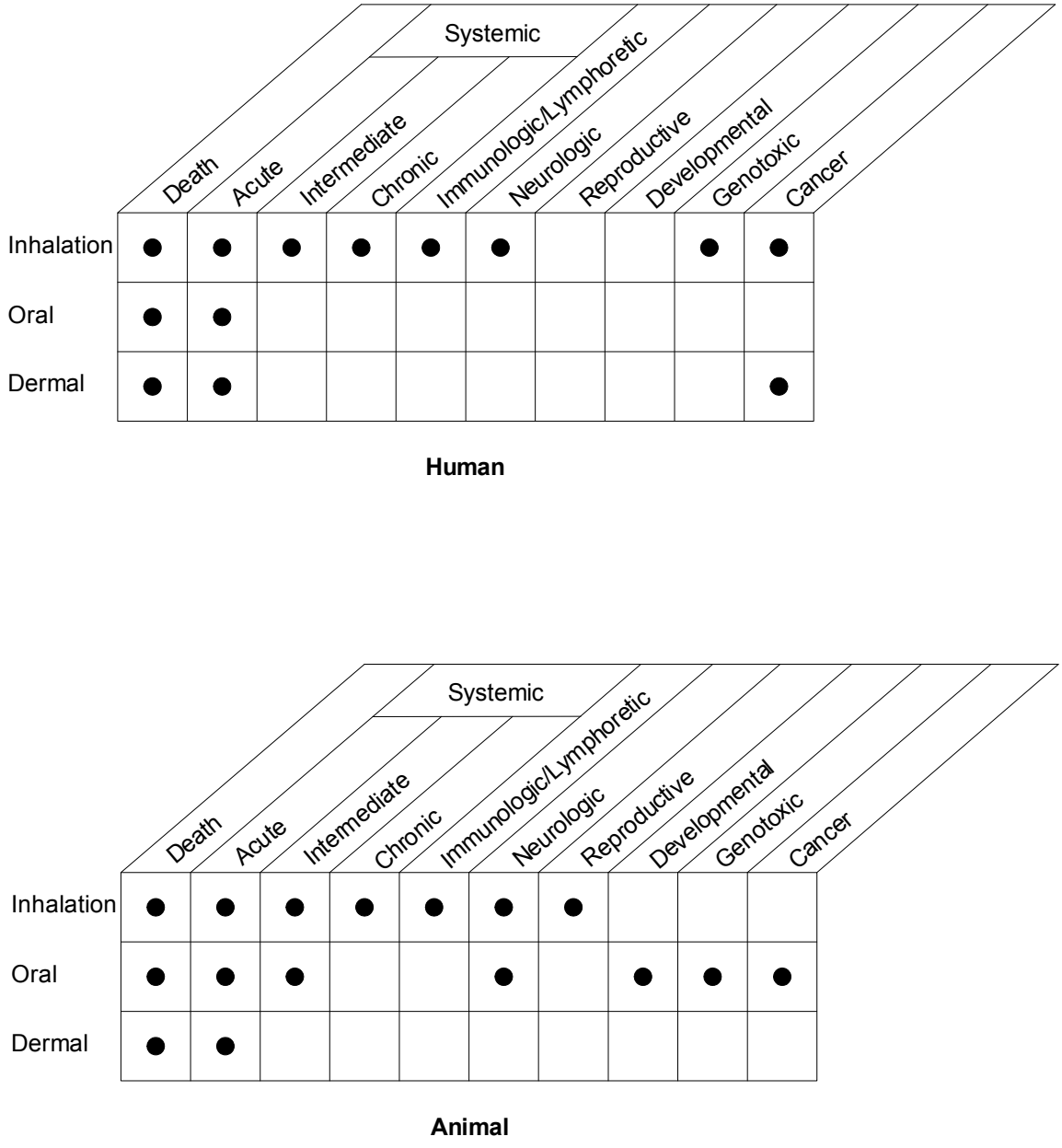
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to ammonia are summarized in Figure 3-6. The purpose of this figure is to illustrate the existing information concerning the health effects of ammonia. Each dot in the figure indicates that one or more 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 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.

Information regarding health effects of ammonia in humans consists largely of case reports of fatalities or illnesses following massive inhalation and/or dermal exposures resulting from accidental explosions or leakages. A few controlled studies have been conducted on inhalation and oral exposure effects. Health effects of ammonia in animals have been investigated in numerous inhalation studies, and a few oral and dermal exposure studies. Clearly, ammonia is an acutely toxic chemical in high concentrations. As indicated in Figure 3-6, available data address these concerns, both in humans and animals. The data indicate that airway blockage, edema, burns, and lesions of tissues directly exposed to ammonia or NH_4^+ are the most prominent ammonia-related effects. Secondary effects include liver and kidney damage, along with decreased resistance to infection.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The available human and animal data provide strong evidence that acute-duration exposure to ammonia can result in site-of-contact lesions primarily of the skin, eyes, and respiratory tract. In some cases, exposure to very high ammonia concentrations has resulted in death (Arwood et al. 1985; Walton 1973). Respiratory tract irritation (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Ferguson et al. 1977; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; Price et al. 1983; Sekizawa and Tsubone 1994; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989) and impaired pulmonary function (Kass et al. 1972; Silverman et al. 1949) have been observed in humans acutely exposed to ammonia gas. Animal studies support the

Figure 3-6. Existing Information on Health Effects of Ammonia



identification of the respiratory tract as a sensitive target of toxicity (Coon et al. 1970; Kapeghian et al. 1982; Mayan and Merilan 1972; Richard et al. 1978a, 1978b; Schaerdel et al. 1983; Stombaugh et al. 1969). Nonrespiratory tract effects (e.g., cardiovascular effects, renal effects) have also been observed following inhalation exposure. However, these effects were not consistently observed or may be secondary to the respiratory tract damage. Additional studies would be useful to assess the potential toxicity of NH_4^+ to remote tissues. The available acute data were considered adequate to derive an acute-duration inhalation MRL (Verbeck et al. 1977). No acute-duration oral MRL was derived for NH_4^+. The acute-duration oral database consists of case reports with no dose information (Klein et al. 1985; Klendshoj and Rejent 1966; Lopez et al. 1988) and several animal studies that examined a limited number of end points (Noda and Chikamori 1976), involved a single exposure resulting in no effect, serious effects, or unsupported effects (Benyajati and Goldstein 1975; Koenig and Koenig 1949), and a repeated exposure study that found effects at high dosages (Barzel 1975). In addition, many animal studies have been conducted with ammonium chloride, a substance widely used experimentally to induce metabolic acidosis in animals. Metabolic acidosis is not a consequence of the ammonium ion, but is due to the formation of hydrogen chloride. Metabolic acidosis can affect the lungs, kidney, nervous system, liver, and bone. Ingestion of concentrated ammonia will cause irritation and damage to the mouth, throat, and gastrointestinal tract. Given the levels of ammonia in the environment, such exposure scenario is unlikely. Additional oral exposure studies do not seem warranted at this time. The available data on the dermal toxicity of ammonia suggest that the skin is a sensitive target of toxicity. Cutaneous burns have been reported in humans exposed to ammonia liquid and/or airborne ammonia (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989). Ocular effects (inflamed eyes, lacrimation, swelling of the eyelids, transient blindness, blurred vision, and corneal abrasions) have been reported in humans exposed to ammonia (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Grant 1974; Hatton et al. 1979; Jarudi and Golden 1973; Kass et al. 1972; Latenser and Lucktong 2000; Legters et al. 1981; Levy et al. 1964; McGuinness 1969; Montague and Macneil 1980; Price et al. 1983; Silverman et al. 1949; Slot 1938; Sobonya 1977; Stombaugh 1969; Stroud 1981; Verberk 1977; Ward et al. 1983; Yang et al. 1987). Additional studies to examine the dermal toxicity of ammonia are unlikely to provide new key information.

Intermediate-Duration Exposure. Information on the toxicity of inhaled ammonia in humans exposed for an intermediate duration is limited to a case report of an individual who developed asthma-like symptoms following exposure to ammonia gas for 5 months (Lee et al. 1993) and a study with volunteers exposed intermittently for 5–6 weeks (Ferguson et al. 1977). The latter study found no

significant changes in pulmonary function in subjects exposed to up to 100 ppm ammonia, but eye and throat irritation occurred at ≥50 ppm. Because some uncertainties regarding the study design and reporting, the Fergusson et al. (1977) study could not be used as basis for an intermediate-duration inhalation MRL. Several animal studies examined the toxicity of ammonia following intermittent or continuous exposure to ammonia. As with acute-duration exposure, these studies suggest that the respiratory tract is the most sensitive target of toxicity. Symptoms of irritation, nasal lesions, dyspnea, and pulmonary inflammation have been observed in several animal species (Broderson et al. 1976; Coon et al. 1970; Drummond et al. 1980; Gaafar et al. 1992; Sjöblom et al. 1999; Stombaugh et al. 1969). In general, the concentrations used in these studies were higher than the lowest adverse effect levels identified for acute-duration exposure. No intermediate-duration oral MRL was derived for some of the same reasons mentioned under acute-duration exposure. No human studies or reports of intermediate-duration oral exposure to NH_4^+ were located. Animal studies have reported decreases in body weight gain in rats exposed via drinking water (Gupta et al. 1979) or diet (Boyano-Adanez et al. 1996). It should be mentioned that Gupta et al. (1979) administered ammonium sulfamate to the rats. Ammonium sulfamate is an herbicide for which there is little toxicity information in the open literature. The EPA (IRIS 2004) has derived an oral RfD for the sulfamate moiety based on the results of Gupta et al. (1979). Following gavage administration of ammonium salts, bone, blood pressure, adrenal gland, and renal effects have been observed in early studies of generally poor quality (Bodansky et al. 1932; Fazekas 1939; Seegal 1927). Additional intermediate-duration oral low to moderate dose studies are unlikely to provide new valuable information. No intermediate-duration dermal exposure studies were identified. Based on the irritant properties of ammonia, it is reasonable to assume that direct contact of the skin with ammonia for a prolonged time will produce irritation.

Chronic-Duration Exposure and Cancer. Several studies have examined the relationship between chronic exposure to ammonia in the air and respiratory effects. Studies of farmers working in enclosed livestock facilities provide evidence that ammonia may contribute to transient respiratory distress (Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Melbostad and Eduard 2001; Reynolds et al. 1996; Vogelzang et al. 1997, 2000); however, co-exposure to total dust, respirable dust, carbon dioxide, total endotoxins, respirable endotoxins, fungi, bacteria, and/or molds complicates the interpretation of these studies. A study of workers at a fertilizer production facility found an association between respiratory effects and ammonia exposure at levels of ammonia higher than 25 ppm (Ballal et al. 1998). Another long-term study of workers exposed to an average of 9.2 ppm ammonia did not find respiratory effects (Holness et al. 1989). A chronic-duration inhalation MRL was derived based on the findings of Holness et al. (1989). Animal studies examining the chronic

toxicity of inhaled ammonia were not identified. No chronic-duration oral or dermal data were located. A need for such studies is not apparent at this time.

There are limited data to assess the carcinogenic potential of ammonia. Nasal cancer was reported in an individual accidentally exposed to a refrigeration-oil mixture, but the role of ammonia, if any, is unknown (Shimkin et al. 1954). Animal carcinogenicity data consist of several oral exposure studies. Ammonia was not found to increase the occurrence of tumors following oral exposure to relatively low doses (Toth 1972; Uzvolgyi and Bojan 1980). Another study found evidence that ammonia administered as drinking fluid may act as a cancer promoter (Tsujii et al. 1992a, 1995). The dose of ammonia administered can be estimated at 200 mg/day, compared to the estimated 0.36 mg/day from water ingestion for the general population (WHO 1986). The relevance of these studies to exposures in humans is unknown. The available information does not suggest that ammonia is carcinogenic, but well-designed studies in animals have not been conducted and may be warranted.

Genotoxicity. Data on the genotoxicity of ammonia in humans are limited to a study of workers at a fertilizer factory that found an increase in clastogenic effects (Yadav and Kaushik 1997). *In vivo* animal data consist of a study in mice that found alterations in the occurrence of micronuclei (Yadav and Kaushik 1997) and several studies in *D. melanogaster* that resulted in a positive response for mutagenic lethality (Lobasov and Smirnov 1934), but negative responses for sex-linked recessive lethal mutations and dominant lethality (Auerbach and Robson 1947). *In vitro* studies revealed positive responses for genotoxicity in *E. coli* (Demerec et al. 1951), and chick (Rosenfeld 1932) and mouse (Capuco 1977; Visek et al. 1972) fibroblasts. It would be valuable to further assess the genotoxicity of ammonia with mutagenicity assays in *S. typhimurium* and *in vitro* and/or *in vivo* tests for chromosomal aberrations in mammalian systems.

Reproductive Toxicity. No information was located regarding reproductive effects of ammonia in humans. Reproductive toxicity data in animals are limited to a study in pigs exposed prior to mating and until gestation day 30 (Diekman et al. 1993). This study did not find alterations in fetus-to-corpus luteum ratio, number of live fetuses, or ovarian or uterine weights (6 weeks of exposure only). This study is not adequate for assessing reproductive toxicity because very low concentrations were used, there were no unexposed controls, and only females were exposed to ammonia. Additional studies are needed to assess ammonia's potential to induce reproductive effects.

Developmental Toxicity. No information was located regarding developmental effects of ammonia in humans and very limited data were located for animals. No alterations in number of live fetuses or fetal length were observed in a study of pigs exposed to a relatively low concentration of ammonia for 6 weeks prior to mating and until gestation day 30 (Diekman et al. 1993). A reduction in body weight gain was observed in the offspring of rats orally exposed to high doses of ammonia, but no information was provided regarding the health of the dams (Miñana et al. 1995). Developmental toxicity studies are needed to assess the potential of ammonia to damage the developing organism.

Immunotoxicity. Secondary infection has been observed in humans who have received severe burns from exposure to highly concentrated aerosols derived from ammonia (Sobonya 1977; Taplin et al. 1976). It is not known if this represents a primary effect on the immune system in humans since necrosis of exposed tissues facilitates infection by pathogenic organisms. Animal studies have shown that exposure to airborne ammonia may impair immune function (Broderson et al. 1976; Gustin et al. 1994; Richard et al. 1978a; Targowski et al. 1984). No oral immunotoxicity data were located. The available data provide suggestive evidence that ammonia may be an immunotoxicant. It would be valuable to assess the potential for immunotoxicity of ammonia with a battery of immune function tests.

Neurotoxicity. Neurological effects have been observed in humans who received extensive and serious burns from exposure to anhydrous ammonia (George et al. 2000; Hatton et al. 1979; Latenser and Lucktong 2000; White 1971). These effects may be secondary to trauma, rather than direct effects of ammonia on the central nervous system. There are limited data on the potential of NH_4^+ to induce overt neurological effects in animals. A decrease in motor activity has been observed in rodents following an acute exposure to low levels of airborne ammonia (Tepper et al. 1985); no overt signs of neurological impairment were observed following sublethal inhalation exposure (Coon et al. 1970). However, numerous animal studies have found evidence that orally administered ammonia may disrupt normal energy production in the brain and impair neurotransmitter receptors (Bodega et al. 1991; Boyano-Adánez et al. 1996; Kimura and Budka 1986; Kosenko et al. 1993; Kretzschmar et al. 1985; Miñana et al. 1989b; Sobel et al. 1981; Suárez et al. 1992). Additional studies following inhalation and oral exposure would be useful to determine if the neurochemical alterations would result in clinical impairment. No dermal studies examining neurological end points were identified.

Epidemiological and Human Dosimetry Studies. Several studies have examined the toxicity of airborne ammonia in workers (Ballal et al. 1998; Choudat et al. 1994; Cormier et al. 2000; Donham et al. 1995, 2000; Heederik et al. 1990, 1991; Holness et al. 1989; Melbostad and Eduard 2001; Reynolds et al.

1996; Vogelzang et al. 1997, 2000). These studies have primarily focused on the respiratory tract, which is the most sensitive target of toxicity. Interpretation of many of these studies is complicated by co-exposure to other chemicals and microorganisms. In addition to these studies, there are reports of acute-duration exposure to ammonia via inhalation (Burns et al. 1985; Close et al. 1980; Couturier et al. 1971; de la Hoz et al. 1996; Ferguson et al. 1977; George et al. 2000; Hatton et al. 1979; Heifer 1971; Kass et al. 1972; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Millea et al. 1989; Morgan 1997; O'Kane 1983; Price et al. 1983; Sekizawa and Tsubone 1994; Sobonya 1977; Taplin et al. 1976; Verberk 1977; Weiser and Mackenroth 1989), ingestion (Klein et al. 1985; Klendshoj and Rejent 1966; Lopez et al. 1988), dermal contact (Amshel et al. 2000; da Fonseca et al. 1998; George et al. 2000; Kerstein et al. 2001; Latenser and Lucktong 2000; Leduc et al. 1992; Rosenbaum et al. 1998; Weiser and Mackenroth 1989), or ocular contact (Beare et al. 1988; Caplin 1941; Close et al. 1980; Ferguson et al. 1977; Grant 1974; Hatton et al. 1979; Jarudi and Golden 1973; Latenser and Lucktong 2000; Legters et al. 1981; Levy et al. 1964; McGuinness 1969; Montague and Macneil 1980; Price et al. 1983; Silverman et al. 1949; Slot 1938; Sobonya 1977; Stombaugh 1969; Stroud 1981; Verberk 1977; Ward et al. 1983; Yang et al. 1987). These studies suggest that the most sensitive target is the site of contact. The carcinogenic potential of ammonia has not been assessed in humans. There are several subpopulations of individuals exposed to higher than normal levels of ammonia; these groups include farmers and communities living near fertilizer plants. Studies of these groups that involved examination for a variety of potential effects could provide useful information on the toxicity of ammonia in humans. In addition, if the study group included both children and adults, these data would address the issue of age-related differences in toxicity.

Biomarkers of Exposure and Effect.

Exposure. There are no known specific biomarkers of exposure for ammonia in humans or animals. Furthermore, no evidence for alterations in clinical indices of body ammonia or nitrogen levels after exposure to exogenous ammonia has been reported. It does not seem useful at this time to develop biomarkers of exposure for ammonia because after exposure to low levels, ammonia is either rapidly cleared from the body or metabolized to compounds found endogenously at appreciable levels. Exposure to high concentrations is immediately and overtly toxic, which eliminates the need for a more subtle biomarker.

Effect. There are no known specific biomarkers of effect for ammonia in humans or animals. Lesions produced by exposure to high concentrations of ammonia are similar to those produced by other caustic substances.

Absorption, Distribution, Metabolism, and Excretion. Measurement of ammonia absorption is complicated by the appreciable levels of endogenously produced ammonia. Although, most of the inhaled ammonia is retained in the tissues of the upper respiratory tract, inhalation exposure to low levels of ammonia can result in a small amount of absorption (Silverman et al. 1949). As the ammonia concentration increases, the ability of the upper respiratory tract to retain ammonia is saturated, and a larger percentage is absorbed into the blood stream (Silverman et al. 1949). Absorption into the systemic circulation after oral exposure is limited (Metges et al. 1999). Ammonium ions absorbed from the gastrointestinal tract travels via the hepatic portal vein directly to the liver where, in healthy individuals, most of it is converted to urea and glutamine. Although it has not been extensively studied, dermal absorption of ammonia does not occur to a great extent; WHO (1986) concluded that systemic effects from skin and eye exposure to ammonia are not quantitatively important. Data are not available to assess the distribution of ammonia in humans or animals. Studies examining the distribution of ammonia would be useful for identifying potential targets of toxicity. Studies on endogenously produced ammonia, however, indicate that it is distributed to most of the organs and tissues of the body. Extensive work has been completed on the metabolism of ammonia and its participation in the glutamine cycle and the urea cycle (Duda and Handler 1958; Fürst et al. 1969; Richards et al. 1975; Vitti et al. 1964). Data regarding excretion are limited but it is known that ammonia inhaled at low levels is excreted primarily unchanged in the expired breath (Silverman et al. 1949); NH_4^+ absorbed from the gastrointestinal tract is excreted primarily in the urine as urea and other urinary nitrogen compounds (Gay et al. 1969; Pitts 1971; Richards et al. 1975; Summerskill and Wolpert 1970). No information regarding excretion after dermal exposure was located.

Comparative Toxicokinetics. Available data indicate that ammonia has similar targets of toxicity in humans and animals. Ammonia is most hazardous as a site-of-contact toxicant; therefore, the respiratory system is most vulnerable after inhalation exposure, the gastrointestinal tract is most vulnerable after oral exposure, and the skin and eyes are most vulnerable after dermal/ocular exposure. Limited human and animal data are available for toxicokinetics; however, these data indicate that humans and animals are probably very similar regarding the toxicokinetic disposition of ammonia. Furthermore, it is reasonable to expect, especially given the biochemical importance of ammonia, that humans and animals would handle this compound similarly.

Methods for Reducing Toxic Effects. There are limited specific data on reducing the toxic effects of ammonia. Many of the methods are generic approaches, such as getting to an area with fresh air and removal of contaminated clothing. No methods were identified for reducing the body burden or interfering with the mechanisms of toxicity. Studies designed to identify methods for interfering with the damage associated with direct contact with ammonia would be useful.

Children's Susceptibility. There are limited data on the toxicity of ammonia in children and no information on effects in adults who were exposed as children. A higher incidence of respiratory diseases was found in school children exposed to airborne ammonia and other chemicals (Gomzi 1999; Gomzi and Šarić 1997). There is no indication that children are more susceptible to the effects of ammonia than adults; studies of children and adults exposed to ammonia would be useful for assessing potential age-related differences in ammonia toxicity.

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

Ongoing studies pertaining to ammonia have been identified and are shown in Table 3-5.

Table 3-5. Ongoing Studies on the Health Effects of Ammonia

Investigator	Affiliation	Research description	Sponsor
Seashore MR	Yale University	Sodium phenylbutyrate treatment of inborn errors of ammonia metabolism	National Center for Research Resources
Wall SM	University of Texas Health Science Center	$NH4^+$ transport in renal inner medullary collecting duct	National Institute of Diabetes and Digestive and Kidney Diseases
Matthews JB	University of Cincinnati	Intestinal secretion and inflammation; impact of ammonia	National Institute of Diabetes and Digestive and Kidney Diseases
Wall SM	University of Texas Health Science Center	Renal net acid secretion and $Na^+/K^+/2Cl$- cotransporter	National Institute of Diabetes and Digestive and Kidney Diseases
Weiner ID	University of Florida	Localization of ammonia transporters in human liver	National Institute of Diabetes and Digestive and Kidney Diseases
Raushel FM	Texas A&M University System	Mechanism and control of urea biosynthesis	National Institute of Diabetes and Digestive and Kidney Diseases
Hammon DS	Utah State University, Animal Dairy and Vet Science	Gamete and embryo toxic effects of ammonium in cattle	Animal Health Award
Nagami GT	Department of Veterans Affairs, Medical Center West Los Angeles, California	Effect of angiotensin II on ammonia production by tubule cells. Effect of acidosis on renin-angiotensin system	Department of Veterans Affairs, Research and Development, Washington, DC
Nagami GT	Department of Veterans Affairs, Medical Center West Los Angeles, California	Ammonia production and transport by the proximal tubule	Department of Veterans Affairs, Research and Development, Washington, DC
Feldman GM	Department of Veterans Affairs, Medical Center Richmond, Virginia	Colonic transport of bicarbonate, ammonium and small organic anions	Department of Veterans Affairs, Research and Development, Washington, DC
Walsch PJ	University of Miami	Mechanism of tolerance of extreme ammonia	National Institute of Environmental Health Sciences
Weiner ID	Department of Veterans Affairs, Medical Center Gainesville, Florida	Effect of ammonia on IMCD H-K-ATPase. Expression of an ammonia-sensitive protein in brain that mediates ammonia's neurological effects	Department of Veterans Affairs, Research and Development, Washington, DC
Sastrasinh S	Department of Veterans Affairs, Medical Center East Orange, New Jersey	Na^+/H^+ antiport in renal mitochondria	Department of Veterans Affairs, Research and Development, Washington, DC

Source: FEDRIP (2003)