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TOXICOLOGICAL REVIEW

OF

AMMONIA

(CAS No. 7664-41-7)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

October 2011

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LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMP	adenosine monophosphate
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
ATSG	acid-treated silica gel
BMD	benchmark dose
BrDU	5-bromo-2-deoxyuridine
BUN	blood urea nitrogen
C3	complement 3
CAC	cumulative ammonia concentration
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
FEF	forced expiratory flow
FEV	forced expiratory volume
FVC	forced vital capacity
GABA	γ-amino butyric acid
IgE	immunoglobin E
IgG	immunoglobin G
IRIS	Integrated Risk Information System
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MMEF	mean midexpiratory flow
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRM	murine respiratory mycoplasmosis
NAD^+	nicotineamide adenine dinucleotide, oxidized
NADH	nicotineamide adenine dinucleotide, reduced
NH_3	ammonia
NH_4^+	ammonium ion
NIOSH	National Institute for Occupational Safety and Health
NMDA	N-methyl D-aspartate
NOAEL	no-observed-adverse-effect level
NOx	nitrogen oxides
NRC	National Research Council
OR	odds ratio
PARP	poly(ADP-ribose) polymerase
PEF	peak expiratory flow
PEFR	peak expiratory flow rate
PHA	phytohemagglutin
POD	point of departure
PPD	purified protein derivative
PRRSV	porcine reproductive and respiratory syndrome virus
RfC	reference concentration
RfD	reference dose
SIFT-MS	selected ion flow tube mass spectrometry

TLV	threshold limit value
TWA	time-weighted average
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to ammonia. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of ammonia.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration, and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of ammonia. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for ammonia has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA,

1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b), and *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011a).

The literature search strategy employed for ammonia was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. Primary, peer-reviewed literature identified through June 2011 was included where that literature was determined to be critical to the assessment. The relevant literature included publications on ammonia which were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate.

2. CHEMICAL AND PHYSICAL INFORMATION

Ammonia is a corrosive gas with a very pungent odor (O'Neil et al., 2006). It is highly soluble in water (4.82×10^5 mg/L) and is a weak base (Lide, 2008; Eggeman, 2001; Dean, 1985). When ammonia (NH_3^+) is present in water at environmental pH, a pKa of 9.25 indicates that the equilibrium will favor the formation of the conjugate acid, the ammonium ion (NH_4^+) (Lide, 2008). A solution of ammonia in water is sometimes referred to as ammonium hydroxide because the ammonia and water both ionize to form ammonium cations and hydroxide anions (Eggeman, 2001). Ammonium salts are easily dissolved in water and disassociate into the ammonium ion and the anion.

Many physical and chemical properties of ammonia are related to the pH of ammonia in solution (ammonium hydroxide). Ammonium hydroxide is a weak base that is partially ionized in water with a dissociation constant of 1.77×10^{-5} at 25 °C that increases slightly with increasing temperature (Reed, 1982). At a pH of 8.25, 90% of ammonia will be protonated. At a pH of 7.25, 99% of ammonia will be protonated. Thus, a decrease in pH would result in an increase in the ammonium ion concentration and an increase in solubility of ammonia in water. At physiological pH (7.4), the equilibrium between NH_3 and NH_4^+ favors the formation of NH_4^+.

Chemical and physical properties of ammonia are listed in Table 2-1.

Table 2-1. Chemical and physical properties of ammonia

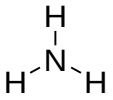
Chemical name	Ammonia[a]	
Synonym(s)	AM-Fol; anhydrous ammonia; ammonia gas; Nitro-sil; R 717; Spirit of hartshorn	ChemID Plus, 2009
Structure		ChemID Plus, 2009
Chemical formula	NH_3	ChemID Plus, 2009
CASRN	7664-41-7[a]	ChemID Plus, 2009
Molecular weight	17.031	Lide, 2008
Form	Colorless gas; corrosive	O'Neil et al., 2006
Melting point	-77.73°C	Lide, 2008
Boiling point	-33.33°C	Lide, 2008
Odor threshold	53 ppm	O'Neil et al., 2006
Density	0.7714 g/L at 25°C	O'Neil et al., 2006
Vapor density	0.5967 (air = 1)	O'Neil et al., 2006
pK_a (ammonium ion)	9.25	Lide, 2008
Solubility: Water Organic solvents	 4.82×10^5 mg/L at 24°C Soluble in ethanol, chloroform, and ether	 Dean, 1985 Lide, 2008; O'Neil et al., 2006

Table 2-1. Chemical and physical properties of ammonia

Vapor pressure	7.51×10^3 mm Hg at 25°C	AIChE, 1999
Henry's law constant	1.61×10^{-5} atm-m^3/mol at 25°C	Betterton, 1992
Conversion factors ppm to mg/m^3 mg/m^3 to ppm	 1 ppm = 0.707 mg/m^3 1 mg/m^3 = 1.414 ppm	 Verschueren, 2001 Verschueren, 2001

[a]Ammonia dissolved in water is sometimes referred to as ammonium hydroxide (CASRN 1336-21-6). Ammonium hydroxide does not exist outside of solution.

Ammonia is a major component of the geochemical nitrogen cycle and is essential for many biological processes (Rosswall, 1981). Nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia available for plant uptake (Socolow, 1999; Rosswall, 1981). Organic nitrogen released from biota is converted into ammonia through nitrogen mineralization (Rosswall, 1981). Ammonia in water and soil is naturally converted into nitrite and nitrate through the process of nitrification (Rosswall, 1981).

Commercially produced ammonia is obtained through the Haber-Bosch process, which involves mixing nitrogen from the atmosphere with hydrogen obtained from natural gas in a 1 to 3 ratio and passing the mixture over a catalyst at high temperature and pressure (Eggeman, 2001). Over the past century, world-wide anthropogenic nitrogen fixation (conversion to ammonia) has risen to approximately 140 million metric tons, exceeding the amount of nitrogen fixed through natural processes (NSF, 1999; Socolow, 1999).

Large amounts (thousands of tons) of ammonia are transported and stored using large pipelines and refrigerated, low pressure tanks (Eggeman, 2001). Approximately 80–85% of commercially produced ammonia is used in the production of agricultural fertilizers in the form of urea, ammonium nitrate, ammonium sulfate, ammonium phosphate, and other nitrogen compounds (Eggeman, 2001). The remaining uses of ammonia mostly involve formation of chemical intermediates (Eggeman, 2001). Ammonia is used in metal treating operations, in water treatment operations, in catalytic reactors, as a convenient source of hydrogen for the hydrogenation of fats and oils, as a neutralizer in the petroleum industry, and as a stabilizer in the rubber industry (HSDB, 2009). Ammonia may be emitted during these processes and may also be emitted from light duty vehicles, heavy-duty diesel trucks and some non-road engines. Ammonia has been used to reduce nitrogen oxides (NOx) emissions from the exhaust of stationary combustion sources such as industrial and municipal boilers and power generators since the 1980's (Johnson et al., 2009), but more recently, ammonia (generated from urea injected into the exhaust stream) is being used in a selective catalytic reduction-based diesel engine aftertreatment technology to reduce NOx emissions.

3. TOXICOKINETICS[1]

Ammonia can be absorbed by the inhalation and oral routes of exposure. There is less certainty regarding absorption through the skin, although 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. Ammonia that reaches systemic circulation is widely distributed to all body compartments, although substantial first-pass metabolism occurs in the liver, where biotransformation into urea and glutamine occur. Ammonia exists in the blood as ammonium ion (NH_4^+). Ammonia is transported in the circulatory system primarily via glutamine and alanine, amino acids that are used to transport ammonia to and from tissues. When transported to the liver and kidney, the amide moiety is hydrolyzed via glutaminase forming glutamatic acid (glutamate) and ammonium ion, which is synthesized into urea and excreted in the urine. Ammonia or ammonium ion reaching the tissues is utilized for glutamate production, which participates in transamination and other reactions. The principal means of excretion of absorbed ammonia in mammals is as urinary urea; minimal amounts are excreted in the feces and in expired air.

Ammonia is endogenously produced in humans and animals. It is an essential mammalian metabolite used in nucleic acid and protein synthesis, is necessary for maintaining acid-base balance, and an integral part of nitrogen homeostasis. Given its important metabolic role, ammonia exists in a homeostatically regulated equilibrium in the body.

3.1. ABSORPTION

3.1.1. Inhalation Exposure

Experiments with volunteers[2] show that ammonia, regardless of its tested concentration in air (range, 57–500 ppm or 40–354 mg/m^3), is almost completely retained in the nasal mucosa (83–92%) during short-term acute exposure, i.e., up to 120 seconds (Landahl and Hermann, 1950). However, longer-term acute exposure (10–27 minutes) to a concentration of 500 ppm (354 mg/m^3) resulted in lower retention (4–30%), with expired breath concentrations of 350–400 ppm (247–283 mg/m^3) observed by the end of the exposure period (Silverman et al., 1949), suggesting saturation of absorption into the nasal mucosa. Nasal and pharyngeal irritation, but not tracheal irritation, suggests that ammonia is retained in the upper respiratory tract.

[1]Portions of this section were adapted from the Toxicokinetics Section (Section 3.4) of the *Toxicological Profile for Ammonia* (ATSDR, 2004) under a Memorandum of Understanding (MOU) with ATSDR.

[2]The human toxicokinetic studies cited in this section did not provide information on the human subjects research ethics procedures undertaken in the studies; however, there is no evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

Unchanged levels of blood urea nitrogen (BUN), nonprotein nitrogen, urinary urea, and urinary ammonia following these acute exposures are evidence of low absorption into the blood. Exposure to a common occupational limit of ammonia in air (25 ppm or 18 mg/m^3), assuming 30% uptake into blood, would yield an increase in blood ammonia concentration of 0.09 µg/mL (calculated by WHO, 1986). This calculated rise would likely be indistinguishable from the observed baseline levels of 0.1–1.0 µg/mL (Monsen, 1987; Conn, 1972; Brown et al., 1957) for healthy controls.

Data in rabbits and dogs provide supporting evidence for high-percentage nasal retention, resulting in a lower fraction of the inhaled dose reaching the lower respiratory tract (Egle, 1973; Dalhamn, 1963; Boyd et al., 1944). Continuous exposure of rats to up to 32 ppm (23 mg/m^3) for 24 hours did not result in a statistically significant increase in blood ammonia levels (0.1 µg/mL above preexposure levels), whereas exposures to 310–1,157 ppm (219–818 mg/m^3) led to significantly increased blood concentrations of ammonia within 8 hours of exposure initiation; blood ammonia returned to preexposure values within 12 hours of continuous exposure (Schaerdel et al., 1983).

3.1.2. Oral Exposure

Case reports of human ingestion of household ammonia (ammonium hydroxide) provide evidence of oral absorption, but few quantitative data are available. 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 levels of 15.3 mg in the stomach and 33 µg/mL in the blood (Klendshoj and Rejent, 1966). This blood concentration is about 30-fold higher than the concentration of 1 µg/mL in fasting volunteers, as reported by Conn (1972).

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, >70% of which is synthesized or liberated within the colon and its fecal contents (Summerskill and Wolpert, 1970). About 99% of the total amount produced (4,150 mg) is systemically absorbed. Evidence suggests that fractional absorption of ammonia increases as the lumen pH increases, and that active transport occurs at the lower pH levels (absorption has been detected at a pH as low as 5) (Castell and Moore, 1971; Mossberg and Ross, 1967). 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.

3.1.3. Dermal Exposure

Quantitative data on absorption from exposure by the dermal route are not available. One report of five case histories of workers exposed to anhydrous ammonia via a burst gas pipe indicated that there was systemic toxicity (vomiting, renal congestion, and delirium), suggesting

dermal absorption; however, the fractional dose from dermal exposure could not be determined (Slot, 1938). WHO (1986) concluded that systemic effects from skin and eye exposure are not quantitatively important. Ammonia is readily absorbed into the eye; and it was found to diffuse within seconds into the 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.

3.2. DISTRIBUTION

The range of mean ammonia concentrations in humans as a result of endogenous production was reported as 0.1 to 0.6 µg/mL in arterial blood and 0.2 to 1.7 µg/mL in venous blood (Huizenga et al., 1994). Other baseline levels observed in experimental volunteers range from 1 to 5.5 µg/mL (Conn, 1972; Brown et al., 1957). Ammonia is homeostatically regulated to remain at low concentrations, with 95–98% existing in the blood (at physiological pH) as NH_4^+ ion (da Fonseca-Wolhheim, 1995; Souba, 1987).

Ammonia is present in fetal circulation. In vivo studies in several animal species and in vitro studies of human placenta suggest that ammonia is produced within the uteroplacenta and released into the fetal and maternal circulations (Bell et al., 1989; Johnson et al., 1986; Haugel et al., 1983; Meschia et al., 1980; Remesar et al., 1980; Holzman et al., 1979, 1977; Rubaltelli et al., 1968; Luschinsky, 1951). Jóźwik et al. (2005) reported that ammonia levels in human fetal blood (specifically, umbilical arterial and venous blood) at birth were 1.0–1.4 µg/mL, compared to 0.5 µg/mL in the mothers' venous blood. DeSanto et al. (1993) similarly collected human umbilical arterial and venous blood at delivery, and found umbilical arterial ammonia concentrations were significantly higher than venous concentrations; there was no correlation between umbilical ammonia levels and gestational age (range of 25–43 weeks of gestation). In sheep, uteroplacental tissues are the main site of ammonia production, with outputs of ammonia into both the uterine and umbilical circulations (Jóźwik et al., 1999). In late-gestation pregnant sheep that were catheterized to allow measurement of ammonia exposure to the fetus, concentrations of ammonia in umbilical arterial and venous blood and uterine arterial and venous blood ranged from about 0.39–0.60 µg/mL (Jóźwik et al., 2005, 1999).

Ammonia is present in human breast milk as one of the sources of nonprotein nitrogen (Atkinson et al., 1980).

3.2.1. Inhalation Exposure

Little information was found in the available literature for distribution of inhaled ammonia. Information on the distribution of endogenously-produced ammonia suggests that any ammonia absorbed through inhalation would be distributed to all body compartments via the blood, where it would be used in protein synthesis, as a buffer, reduced to normal concentrations by urinary excretion, or converted by the liver to glutamine and urea (Takagaki et al., 1961).

Rats inhaling 300 ppm (212 mg/m^3) ammonia for 6 hours/day for 15 days exhibited increased blood ammonia (200%) and brain (28%) glutamine levels at 5 days of exposure, but not at 10 or 15 days (Manninen et al., 1988), demonstrating transient distribution of ammonia to the brain (metabolic adaptation).

3.2.2. Oral Exposure

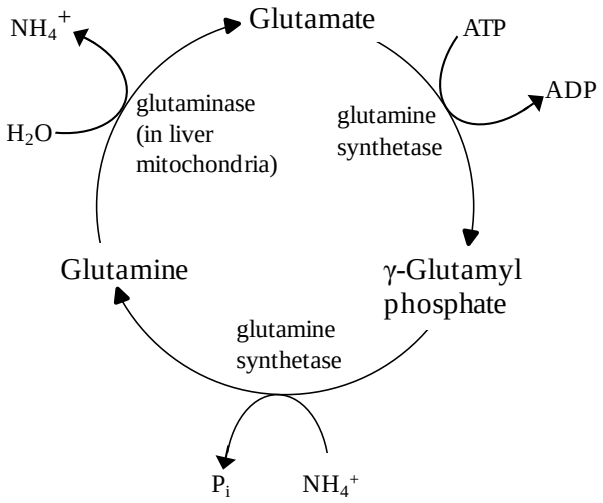
Human oral exposure data indicate that ammonia readily enters the portal circulation and is delivered to the liver, as has been shown to be the case for endogenously produced ammonia (Pitts, 1971; Summerskill and Wolpert, 1970). Unionized ammonia is freely diffusible, whereas the ammonium ion is less so, and is relatively confined to the extracellular compartment (Stabenau et al., 1958).

3.2.3. Dermal Exposure

No quantitative data on distribution of ammonia from dermal exposure were located in the available literature.

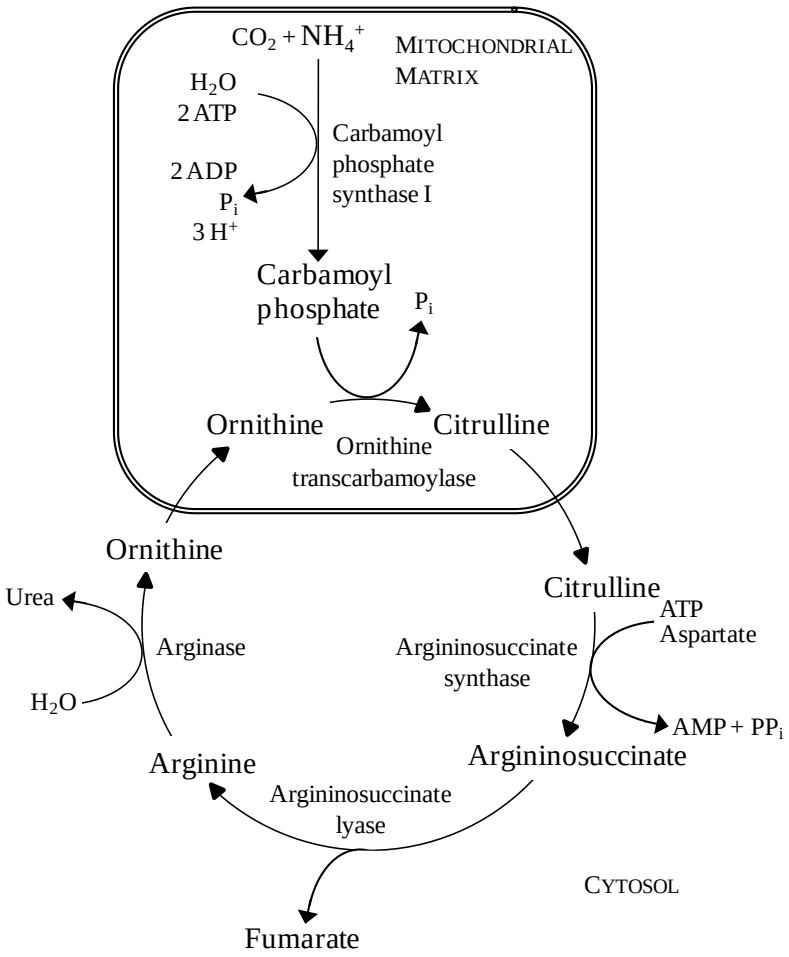
3.3. METABOLISM

Endogenously, ammonia is produced by catabolism of amino acids by glutamate dehydrogenase primarily in the liver and renal cortex, but also in the brain and heart (Souba, 1987). In skeletal muscle, ammonia may be produced by metabolism of adenosine monophosphate (AMP) via adenylate deaminase. Information on the metabolism of exogenously-introduced ammonia was not found in the available literature. Ammonia and ammonium ion are metabolized to glutamine mainly in the liver via glutamine synthetase in the glutamine cycle (Figure 3-1), or incorporated into urea as part of the urea cycle as observed in the hepatic mitochondria and cytosol (Figure 3-2) (Souba, 1987). Ammonia can be rapidly converted to glutamine in the brain as well (Takagaki et al., 1961). Van de Poll (2008) reported that the liver removes an amount of ammonia from circulation equal to the amount added by the intestines at metabolic steady state, indicating that the gut does not contribute significantly to systemic ammonia release.



Adapted from: Nelson and Cox (2008).

Figure 3-1. Glutamine cycle.



Adapted from: Nelson and Cox (2008).

Figure 3-2. The urea cycle showing the compartmentalization of its steps within liver cells.

Given its important metabolic role, ammonia exists in a homeostatically regulated equilibrium in the body. In particular, free ammonia has been shown to be homeostatically regulated to remain at low concentrations, with 95–98% of body burden existing in the blood (at physiological pH) as NH_4^+ ion (da Fonseca-Wolhheim, 1995; Souba, 1987). Two studies in rats (Manninen et al., 1988; Schaerdel et al., 1983) provided evidence that ammonia concentrations in air below 25 ppm (18 mg/m^3) do not alter blood ammonia concentrations. Schaerdel et al. (1983) exposed rats to ammonia for 24 hours at concentrations ranging from 15–1,157 ppm (11-818 mg/m^3). Exposure to 15 ppm (11 mg/m^3) ammonia did not increase blood ammonia concentrations after 24 hours; concentrations of ≥32 ppm caused an exposure-released increase in blood ammonia, but concentrations at 12- and 24-hour sampling periods were lower than at 8 hours, suggesting compensation by increasing ammonia metabolism through conversion to urea, pyrimidine and polyamine synthesis, incorporation into amino acid substrates, and metabolism in nervous system tissue. Rats inhaling 25 ppm (18 mg/m^3) ammonia for 6 hours/day for 15 days

did not exhibit blood or brain ammonia or glutamine levels that were different from controls; however, rats inhaling 300 ppm (212 mg/m^3) for the same duration exhibited statistically significantly increased levels of blood ammonia (threefold) and brain glutamine (approximately 40%) at 5 days of exposure, but not at 10 or 15 days (Manninen et al., 1988). The return of blood and brain ammonia and glutamine levels to control levels with time is consistent with metabolic adaptation, and these data suggest that animals have a large capacity to handle high concentrations of inhaled ammonia.

Various disease states can affect the rate of glutamine uptake and catabolism and, thereby, affect the blood and tissue levels of ammonia. Abnormally elevated levels of ammonia are indicative of end-stage renal failure (Davies et al., 1997). Acute renal failure can result in increased renal glutamine consumption and ammonia production with a decreased capability of eliminating urea in the urine (Souba, 1987). End-stage liver failure due to fulminant hepatitis or hepatic cirrhosis may result in decreased ureagenesis and increased levels of ammonia in blood (hyperammonemia), leading to increased uptake into the brain and the onset of hepatic encephalopathy. The increased metabolic alkalosis associated with hepatic encephalopathy may result in a shift in the NH_4^+/NH_3 ratio in the direction of ammonia, which could pass through the blood-brain barrier (Katayama, 2004). In patients with liver cirrhosis and acute clinical hepatic encephalopathy, the observed trapping of [^{13}N]-ammonia in the brain appeared to be related to a fivefold increase, relative to healthy controls, of ammonia permeability across the blood-brain barrier (Keiding et al., 2010, 2006). Furthermore, Sorensen et al. (2009) demonstrated greater unidirectional clearance of ammonia from the blood to brain cells than metabolic clearance of ammonia from the blood in both healthy controls and in cirrhotic patients with and without hepatic encephalopathy.

3.4. ELIMINATION

Absorbed ammonia, as well as endogenously produced ammonia, is excreted by the kidneys as urea (Summerskill and Wolpert, 1970; Gay et al., 1969; Muntwyler et al., 1956; Davies and Yudkin, 1952; Van Slyke et al., 1943) and is a component of sweat (Guyton, 1981; Wands, 1981). Lee and colleagues observed that acidosis-stimulated renal excretion of ammonia is mediated by intercalated cell-specific Rh B glycoprotein expression in mice (Bishop et al., 2010; Lee et al., 2010, 2009). In rat kidney, ammonium ion is secreted into the lumen of the outer medullary collecting duct via H^+ secretion and parallels ammonia diffusion (Flessner et al., 1992). The inner medullary collecting duct exhibits a Na^+- and K^+-independent NH_4^+/H^+ exchange activity that may be mediated by an Rh C glycoprotein (Handlogten et al., 2005), which is also expressed in human kidneys (Han et al., 2006).

Additionally, ammonia is known to be excreted through expired air and is present in the expired air of all humans (Manolis, 1983). Two investigators specifically measured ammonia in breath exhaled from the nose (Smith et al., 2008; Larson et al., 1977). Smith et al. (2008)

reported median ammonia concentrations in exhaled breath from the nose of three healthy volunteers (with samples collected daily over a 4-week period) of 0.059–0.078 mg/m^3; these concentrations were similar to or slightly higher than the mean laboratory air level of ammonia of 0.056 mg/m^3 reported in this study. Larson et al. (1977) reported that the median concentration of ammonia collected from air samples exhaled from the nose ranged from 0.013 to 0.046 mg/m^3. One sample collected from the trachea (via a tube inserted through the nose of one subject) was 0.029 mg/mg^3—a concentration within the range of concentrations in breath exhaled through the nose (Larson et al., 1977).

Higher and more variable ammonia concentrations are reported in breath exhaled from the mouth or oral cavity than from air exhaled from the nose. In studies that reported ammonia in breath samples from the mouth or oral cavity, the majority of ammonia concentrations ranged from 0.085 to 2.1 mg/m^3 (Smith et al., 2008; Spanel et al., 2007a; Spanel et al., 2007b; Turner et al., 2006; Diskin et al., 2003; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977). These higher concentrations are largely attributed to the production of ammonia by bacterial degradation of food protein in the oral cavity or GI tract (Turner et al., 2006; Smith et al., 1999; Vollmuth and Schlesinger, 1984). This source of ammonia in breath was demonstrated by Smith et al. (1999), who observed elevated ammonia concentrations in the expired air of 6 healthy volunteers following the ingestion of a protein-rich meal.

Other factors that can affect ammonia levels in breath exhaled from the mouth or oral cavity include diet, oral hygiene, age, living conditions, and disease state. Norwood et al. (1992) reported decreases in baseline ammonia levels (0.085–0.905 mg/m^3) in exhaled breath following tooth brushing (<50% depletion), a distilled water oral rinse (<50% depletion), and an acid oral rinse (80–90% depletion). These findings are consistent with ammonia generation in the oral cavity by bacterial and/or enzymatic activity. Several investigators have reported that ammonia in breath from the mouth and oral cavity increases with age (Spanel et al., 2007a; Spanel et al., 2007b; Turner et al., 2006), with ammonia concentrations increasing, on average, by about 0.1 mg/m^3 for each 10 years of life (Spanel et al., 2007b). Turner et al. (2006) reported that the age of the individual accounts for about 25% of the variation in observed mean breath ammonia levels and the remaining 75% is due to factors other than age. Certain disease states can also influence ammonia levels in exhaled breath. Ammonia is greatly elevated in the breath of patients in renal failure (Spanel et al., 2007b; Davies, 1997). Increases in breath ammonia are associated with decreasing kidney function with increasing age (Diskin et al., 2003; Epstein, 1996). These studies are further described in Appendix C.1 in Table C-1.

Because ammonia measured in samples of breath exhaled from the mouth or oral cavity can be generated in the oral cavity and thus may be substantially influenced by diet and other factors, ammonia levels measured in mouth or oral cavity breath samples do not likely reflect systemic (blood) levels of ammonia. Ammonia concentrations in breath exhaled from the nose appear to better represent systemic or background levels (Smith et al., 2008).

Ammonia has also been detected in the expired air of animals. Whittaker et al. (2009) observed a significant association between ambient ammonia concentrations and increases in exhaled ammonia in stabled horses. Analysis of endogenous ammonia levels in the expired air of rats showed concentrations ranging from 0.01 to 0.353 ppm (0.007–0.250 mg/m^3) (mean = 0.08 ppm or 0.06 mg/m^3) in nose-breathing animals (Barrow and Steinhagen, 1980). Larson et al. (1980) reported ammonia concentrations measured in the larynx of dogs exposed to sulfuric acid ranging between 0.03 and 0.225 ppm (0.02 and 0.16 mg/m^3) following mouth breathing, and between 0.05 and 0.220 ppm (0.04 and 0.16 mg/m^3) following nose breathing.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

No physiologically based pharmacokinetic (PBPK) models have been developed for ammonia. An expanded one-compartment toxicokinetic model in rats was developed by Diack and Bois (2005), which used physiological values to represent first-order uptake and elimination of inhaled ammonia (and other chemicals). The model is not useful for dose-response assessment of ammonia because: (1) it cannot specify time-dependent amounts or concentrations of ammonia in specific target tissues, (2) it has not been verified against experimental data for ammonia, glutamate, or urea levels in tissues, and (3) it cannot extrapolate internal doses of ammonia between animals and humans.

4. HAZARD IDENTIFICATION

As noted in Section 2, ammonium salts (e.g., ammonium acetate, chloride, and sulfate) readily dissolve in water through disassociation into the ammonium ion (NH_4^+) and the anion. At physiological pH (7.4), the equilibrium favors the formation of NH_4^+. Because of this equilibrium at physiological pH, the literature on the toxicity of ammonium salts was reviewed to determine whether this literature could inform the toxicity of ammonia. In rats following exposure to ammonium chloride in the diet for subchronic and chronic exposure durations at doses ranging from approximately 500 to 1,800 mg/kg-day, the primary effect of this salt was related to the acid-base balance in the body (Lina and Kuijpers, 2004; Barzel et al., 1969). Ammonium chloride treatment induced a dose-related hyperchloremic metabolic acidosis in rats as evidenced by decreases in blood pH, base excess, and bicarbonate concentration, and increased plasma chloride levels. Lina and Kuijpers (2004) also observed a significant dose-related depression in body weight gain at exposure to ammonium chloride doses ≥1,590 mg/kg-day for 13 weeks. The administration of ammonium chloride was also associated with zona glomerulosa hypertrophy of the adrenal gland in both the 13-week and 18- and 30-month studies by Lina and Kuijpers (2004).

In contrast, metabolic acidosis was not induced in rats exposed to ammonium sulfate in the diet at doses up to 1,527 mg/kg-day for 52 weeks or histopathologic changes in the adrenal gland at doses up to 1,371 mg/kg-day for 104 weeks (Ota et al., 2006). The only dose-related effects associated with the 52-week exposure to ammonium sulfate were increased liver and kidney weights (~7 and 10%, respectively) at a dose of 1,490 mg/kg-day (female rat) or 1,527 mg/kg-day (male rat) (Ota et al., 2006). In the 104-week study, the incidence of chronic nephropathy was statistically significantly increased in low-dose (564 mg/kg-day), but not in high-dose (1,288 mg/kg-day) male rats (Ota et al., 2006).

Appendix B, Table B-1, presents a summary of repeat-dose oral toxicity studies for selected ammonium salts. Studies of ammonium sulfamate, a broad spectrum herbicide, were not included in this summary table because the pesticidal properties of this ammonium salt were not considered relevant to ammonia toxicity. No studies of the toxicity of ammonium salts by the inhalation pathway are available.

Because the toxicity information for the ammonium salts that have been adequately studied (i.e., the chloride and sulfate) indicates that their toxicity profiles differ, it appears that the anion can influence the toxicity of the ammonium salt. Therefore, it is uncertain whether toxicity data for ammonium salts can be used to inform the toxicity of ammonia. Accordingly, the toxicity of ammonium salts as the basis for characterizing the toxicity of ammonia was not further evaluated in this assessment.

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Case Reports – Oral and Inhalation Exposures

Ammonia exposure has occurred frequently in occupational settings (both industrial and agricultural) when equipment failure or operator error resulted in the sudden release of pressurized ammonia. Transportation accidents and catastrophic releases have also resulted in exposure to high concentrations of ammonia. Oral exposure to ammonia has resulted from intentional or accidental ingestion of household products containing ammonia. Numerous case reports of injury in adults and children due to exposure to ammonia via inhalation of vapors, dermal contact, or ingestion of household cleaning solutions or ammonia inhalant capsules exist.

These case reports indicate that the clinical signs of acute oral exposure to ammonia were headache, stomachache, nausea, dizziness, diarrhea, drooling, erythematous and edematous lips, reddened and blistered tongues, dysphagia, vomiting, oropharyngeal burns, laryngeal and epiglottal edema, erythmatous esophagus with severe corrosive injury, and hemorrhagic esophago-gastro-duodeno-enteritis. Acute inhalation or dermal exposure to ammonia resulted in first-, second-, and third-degree burns to body surfaces, mild to severe erythema and edema throughout the mouth, nasal passages, pharynx, larynx, trachea and esophagus, mild to severe respiratory distress with diffuse or scattered rales and rhonchi, hypoxemia, wheezing, and upper airway obstruction, eye irritation, blepharospasm, lacrimation, ocular erythema, edema, and death. In addition, delayed effects and complications involving the eyes and respiratory system were often encountered even after initial improvement in acute conditions. Delayed effects included complete or partial loss of vision, persistent and/or progressive shortness of breath (especially on exertion), persistent, productive cough, and progressive obstructive and restrictive pulmonary disease with development of cylindrical and/or saccular bronchiectasis with or without obliterative fibrous adhesions in the pleural space, and death. The details of these case reports are given in Appendix C.2.

4.1.2. Controlled Human Inhalation Exposure Studies

Several controlled exposure studies were conducted in volunteers to evaluate irritation effects and changes in pulmonary function following acute inhalation exposure to ammonia; some of these studies describe the occurrence of eye, nose, and throat irritation. These studies are presented in more detail in Appendix C.3. Some of the studies included in Appendix C.3 did not provide information on the human subjects research ethics procedures undertaken in the study (Altmann et al., 2006 (abstract only); Douglas and Coe, 1987; Kalandarov et al., 1984; Ferguson et al., 1977; Verberk, 1977; Silverman et al., 1949), but there is no evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted. Other studies reported that informed consent by volunteers and/or study approval by local boards/officials regarding ethical

conduct was obtained (Petrova et al., 2008; Smeets et al., 2007; Ihrig et al., 2006; Sigurdarson et al., 2004; Sundblad et al., 2004; Cole et al., 1977; MacEwan et al., 1970).

Altmann et al. (2006) showed a dose-dependent increase in the intensity of odor annoyance and irritation in healthy male and female volunteers during inhalation exposure to ammonia; strong olfactory and moderate to strong irritation sensations occurred at concentrations >15 ppm (11 mg/m^3), with odor detection thresholds at <20 ppm (14 mg/m^3). In another study, 12 healthy volunteers exposed to 5 and 25 ppm (4 and 18 mg/m^3) ammonia on three different occasions for 1.5 hours in an exposure chamber while exercising on a stationary bike reported discomfort in the eyes and odor detection at 5 ppm (4 mg/m^3) (Sundblad et al., 2004). Eye irritation was also shown to increase in a concentration-dependent manner in 15 volunteers exposed to ammonia for 2 hours in an exposure chamber at concentrations of 50, 80, 110, and 140 ppm (35, 57, 78, and 99 mg/m^3); ammonia concentrations of 140 ppm (99 mg/m^3) caused severe and intolerable irritation (Verberk, 1977). The lachrymatory threshold was determined to be 55 ppm (39 mg/m^3) in volunteers exposed to ammonia gas inside tight-fitting goggles for an acute duration of up to 15 seconds (Douglas and Coe, 1987). In contrast, exposures to up to 90 ppm (64 mg/m^3) ammonia gas did not produce severe lacrimation in seven volunteers after 10 minutes in an exposure chamber, although increased eye erythema was reported (MacEwen et al., 1970). Exposure to 500 ppm (354 mg/m^3) of ammonia gas for 30 minutes through a masked nose and throat inhalation apparatus resulted in 2/7 volunteers reporting lacrimation, and 2/7 reporting nose and throat irritation that lasted up to 24 hours after exposure (Silverman et al., 1949).

Petrova et al. (2008) investigated irritation threshold differences between 25 healthy volunteers and 15 mild-to-moderate persistent asthmatic volunteers exposed to ammonia via the eyes and nose at concentrations ranging from 2 to 500 ppm (1–354 mg/m^3) for durations lasting up to 2.5 hours. Irritation threshold, odor intensity, and annoyance were not significantly different between the two groups. The nasal and eye irritation thresholds were reported to be 129 ppm (91 mg/m^3) and 175 ppm (124 mg/m^3), respectively. Smeets et al. (2007) investigated odor and irritation thresholds for ammonia vapor in 24 healthy female volunteers at concentrations ranging from 0.03 to 615 ppm (0.02 to 435 mg/m^3). This study observed a mean odor detection threshold of 2.6 ppm (2 mg/m^3) and a mean irritation threshold of 31.7 or 60.9 ppm (22 or 43 mg/m^3), depending on the olfactometry methodology followed (static versus dynamic, respectively). Irritation thresholds may be higher in people who have had prior experience with ammonia exposure (Ihrig et al., 2006). Thirty male volunteers who had not experienced the smell of ammonia and 10 male volunteers who had regular workplace exposure to ammonia were exposed to ammonia vapors at concentrations of 0, 10, 20, and 50 ppm (0, 7, 14, and 35 mg/m^3) on 5 consecutive days (4 hours/day) in an exposure chamber; volunteers in the group familiar to the smell of ammonia reported fewer symptoms than the nonhabituated group, but at a concentration of 20 ppm (14 mg/m^3), there were no differences in perceived

symptoms between the groups. However, the perceived intensity of symptoms was concentration-dependent in both groups, but was only significant in the group of volunteers not familiar with ammonia exposure (Ihrig et al., 2006). Ferguson et al. (1977) reported habituation to eye, nose, and throat irritation in six male and female volunteers after 2–3 weeks of exposure to ammonia concentrations of 25, 50, and 100 ppm (18, 35, and 71 mg/m^3) during a 6-week study (6 hours/day, 1 time/week). Continuous exposure to even the highest concentration tested became easily tolerated with no general health effects occurring after acclimation occurred.

Several studies evaluated pulmonary functions following acute inhalation exposure to ammonia. Volunteers exposed to ammonia (lung only) through a mouthpiece for 10 inhaled breaths of gas experienced bronchioconstriction at a concentration of 85 ppm (60 mg/m^3) (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in seven volunteers exposed to ammonia at concentrations of 30, 50, or 90 ppm (21, 35, and 64 mg/m^3) for 10 minutes in an exposure chamber (MacEwen et al., 1970). Similarly, 12 healthy volunteers exposed to ammonia on three separate occasions to 5 and 25 ppm (4 and 18 mg/m^3) for 1.5 hours in an exposure chamber while exercising on a stationary bike did not have changes in bronchial responsiveness, upper airway inflammation, exhaled nitric oxide levels, or lung function as measured by vital capacity and FEV_1 (Sundblad et al., 2004). In another study, 18 healthy servicemen volunteers were placed in an exposure chamber for 3 consecutive half-day sessions. Exposure to ammonia at concentrations of 50–344 mg/m^3 (70–486 ppm) occurred on the 2nd session, with sessions 1 and 3 acting as controls (Cole et al., 1977). The no-effect concentration was determined to be 71 mg/m^3 (100 ppm). Exercise tidal volume was increased at 106 mg/m^3 (150 ppm), but then decreased at higher concentrations in a concentration-dependent manner (Cole et al., 1977). Decreased FEV_1 and forced vital capacity (FVC) were reported in eight healthy male volunteers exposed to a mean airborne ammonia concentration of 20.7 ppm (15 mg/m^3) in swine confinement buildings for 4 hours at one-week intervals; however, swine confinement buildings also include confounding exposures to dust, bacteria, endotoxin, and molds, thereby making measurement of effects due to ammonia uncertain in this study (Cormier et al., 2000).

Differences in pulmonary function between healthy and asthmatic volunteers exposed to ammonia were evaluated in several studies. There were no changes in lung function as measured by FEV_1 in 25 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers after ocular and nasal exposure to 2–500 ppm (1–354 mg/m^3) ammonia at durations lasting up to 2.5 hours (Petrova et al., 2008). In another study, six healthy volunteers and eight mildly asthmatic volunteers were exposed to 16–25 ppm (11–18 mg/m^3) ammonia, ammonia and dust, and dust alone for 30-minute sessions, with 1 week between sessions (Sigurdarson et al., 2004). There were no significant changes in pulmonary function as measured by FEV_1 in the healthy volunteers for any exposure. A decrease in FEV_1 was reported in asthmatics exposed to dust and ammonia, but not ammonia alone; similarly, increased bronchial hyperreactivity was reported in

asthmatics after exposure to dust and ammonia, but not to ammonia alone. Exposure to dust alone caused similar effects, suggesting that dust was responsible for decreased pulmonary function (Sigurdarson et al., 2004).

Kalandarov et al. (1984) investigated the effect of ammonia exposure on the function of the sympathico-adrenal system and the adrenal cortex in male volunteers and found that exposure to 2 mg/m^3 (3.0 ppm) ammonia in combination with increased temperature and humidity did not result in any significant changes to the function of the sympathico-adrenal system, although the adrenal cortex function was affected exhibiting increased 11-oxycorticosteroids in plasma. Ammonia concentrations of 5 mg/m^3 (7.2 ppm), in combination with increased temperature and humidity, resulted in increased levels of adrenaline, 17-oxycorticosteroids, and free 11-oxycorticosteroids fraction in plasma. These results suggest that both sympathico-adrenal system and adrenal cortex function is altered at an ammonia concentration of 5 mg/m^3 (7.2 ppm) (Kalandarov et al., 1984).

In summary, volunteer studies demonstrate that eye irritation can occur following acute exposure to ammonia at concentrations as low as 5 ppm (4 mg/m^3). Irritation thresholds may be higher in people who have had prior experience with ammonia exposure and habituation to eye, nose, and throat irritation occurs over time. Pulmonary function was not affected in workers acutely exposed to ammonia concentrations as high as 71 mg/m^3 (100 ppm). Studies comparing the pulmonary function of asthmatics and healthy volunteers exposed to ammonia do not suggest that asthmatics are more sensitive to the pulmonary effects of ammonia.

4.1.3. Cross Sectional Studies in Farmers Exposed to Inhaled Ammonia

Several studies have evaluated respiratory symptoms and changes in pulmonary function in livestock farmers and stable workers exposed to ammonia (see Appendix C.4 for detailed descriptions). In addition to ammonia, these studies also documented exposures to airborne dust, bacteria, fungal spores, endotoxin, and mold. The release of other volatiles on livestock farms is likely, but measurements for other volatile chemicals were not conducted. Although studies of farm workers summarized here focused on exposure to ammonia, these and other studies have also demonstrated respiratory effects associated with exposure to other constituents in farm worker air (e.g., respirable dust, endotoxin).

Swine and dairy farmers had a higher prevalence of respiratory symptoms including cough, phlegm, wheezing, chest tightness, and eye, nasal and throat irritation compared to controls (Melbostad and Eduard, 2001; Preller et al., 1995; Choudat et al., 1994; Zejda et al., 1994; Crook et al., 1991; Heederik et al., 1990). Impaired respiratory function in farmers was associated with ammonia exposure in several studies (e.g., decreased FEV_1, FVC) (Cormier et al., 2000; Donham et al., 2000, 1995; Vogelzang et al., 1998; Reynolds et al., 1996; Preller et al., 1995; Crook et al., 1991; Heederik et al., 1990). Bronchial hyperreactivity to methacholine or histamine challenge was increased in farmers exposed to ammonia compared to control workers

(Vogelzang et al., 2000, 1997; Choudat et al., 1994). Stable workers showed signs of bronchial obstruction with increased peak expiratory flow (PEF) variability as well as increased pulmonary inflammation related to allergies (Elfman et al., 2009). Other findings that suggest an allergic or inflammatory response in livestock farmers exposed to ammonia include the presence of immunoglobin E (IgE) and immunoglobin G (IgG) antibodies to pig squames and urine in blood (Crook et al., 1991), increased neutrophils in the nasal wash (Cormier et al., 2000) and increased white blood cell count (Cormier et al., 2000). In summary, several studies have demonstrated an association between ammonia exposure in livestock farmers and respiratory symptoms and impaired respiratory function; however, farmers are additionally exposed to several constituents that likely contribute to these effects, including respirable dust, endotoxin, bacteria, fungi, and mold.

4.1.4. Occupational Studies in Industrial Worker Populations

Holness et al. (1989) conducted a cross-sectional study of workers in a soda ash (sodium carbonate) plant[3] who had chronic low-level exposure to ammonia. The cohort consisted of 58 workers and 31 controls from stores and office areas of the plant. All workers were males (average age 40.5 years) and the average exposure duration for the exposed workers at the plant was 12.2 years. The mean time-weighted average (TWA) ammonia exposure of the exposed group based on personal sampling over one work shift (mean sample collection time, 8.4 hours) was 9.2 ppm (6.5 mg/m^3), compared to 0.3 ppm (0.2 mg/m^3) for the control group. The average concentrations of ammonia to which workers were exposed were determined using the procedure recommended by the National Institute for Occupational Safety and Health (NIOSH) which involves the collection of air samples on sulfuric acid-treated silica gel (ATSG) adsorption tubes (NIOSH, 1979).

No statistically significant differences were observed in age, height, years worked, percentage of smokers, or pack-years smoked for exposed versus control workers. Exposed workers weighed approximately 8% ($p < 0.05$) more than control workers. Information regarding past occupational exposures, working conditions, and medical and smoking history, as well as respiratory symptoms and eye and skin complaints was obtained by means of a questionnaire that was based on an American Thoracic Society questionnaire (Ferris, 1978). Each participant's sense of smell was evaluated at the beginning and end of the work week using several concentrations of pyridine (0.4, 0.66, or 10 ppm). Lung function tests were conducted at the beginning and end of the work shift on the first and last days of their work week (four tests administered). Differences in reported symptoms and lung function between groups were evaluated using the actual exposure values with age, height, and pack-years smoked as covariates

[3]At this plant, ammonia, carbon dioxide, and water were the reactants used to form ammonium bicarbonate, which in turn was reacted with salt to produce sodium bicarbonate and subsequently processed to form sodium carbonate. Ammonia and carbon dioxide were recoved in the process and reused.

in linear regression analysis. Exposed workers were grouped into three exposure categories (high = >12.5 ppm [>8.8 mg/m^3], medium = 6.25–12.5 ppm [4.4–8.8 mg/m^3], and low = <6.25 ppm [<4.4 mg/m^3]) for analysis of symptom reporting and pulmonary function data.

Endpoints evaluated in the study included sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, skin problems and lung function parameters (FVC, FEV_1, FEV_1/FVC, forced expiratory flow [FEF_{50}], and FEF_{75}). No statistical differences in the prevalence of respiratory symptoms or eye irritation were evident between the exposed and control groups (Table 4-1). There was a statistically significant increase ($p < 0.05$) in the prevalence of skin problems in workers in the lowest exposure category (<4.4 mg/m^3) compared to controls; however, the prevalence was not increased among workers in the two higher exposure groups. Workers also reported that exposure at the plant had aggravated specific symptoms including coughing, wheezing, nasal complaints, eye irritation, throat discomfort, and skin problems. Odor detection threshold and baseline lung functions were similar in the exposed and control groups. No changes in lung function were demonstrated over either work shift (days 1 or 2) or over the work week in the exposed group compared with controls. No relationship was demonstrated between chronic ammonia exposure and baseline lung function changes either in terms of the level or duration of exposure. Study investigators noted that this finding was limited by the lack of adequate exposure data collected over time, precluding development of a meaningful index accounting for both level and length of exposure. Based on the lack of exposure-related differences in subjective symptomatology, sense of smell, and measures of lung function, EPA identified 8.8 mg/m^3 (12.5 ppm) as the no-observed-adverse-effect level (NOAEL). A lowest-observed-adverse-effect level (LOAEL) was not identified for this study.

Table 4-1. Symptoms and lung function results of workers exposed to different levels of TWA ammonia concentrations

Parameter	Ammonia concentration			
	Control 0.2 mg/m^3 (0.3 ppm)	Exposed <4.4 mg/m^3 (<6.25 ppm)	Exposed 4.4–8.8 mg/m^3 (6.25–12.5 ppm)	Exposed >8.8 mg/m^3 (>12.5 ppm)
Symptom				
Cough	3/31(10)[a]	6/34 (18)	1/12 (8)	2/12 (17)
Sputum	5/31 (16)	9/34 (26)	3/12 (25)	1/12 (8)
Wheeze	3/31 (10)	5/34 (15)	1/12 (8)	0/12 (0)
Chest tightness	2/31 (6)	2/34 (6)	0/12 (0)	0/12 (0)
Shortness of breath	4/31 (13)	3/34 (9)	1/12 (8)	0/12 (0)
Nasal complaints	6/31 (19)	4/34 (12)	2/12 (17)	0/12 (0)
Eye irritation	6/31 (19)	2/34 (6)	2/12 (17)	1/12 (8)
Throat irritation	1/31 (3)	2/34 (6)	1/12 (8)	1/12 (8)
Skin problems	2/31 (6)	10/34[b] (29)	1/12 (8)	1/12 (8)
Lung function (% predicted)				
FVC	98.6	96.7	96.9	96.8
FEV_1	95.1	93.7	93.9	95.3
FEF_{50}	108.4	106.9	106.2	111.2
FEF_{75}	65.2	71.0	67.8	78.8

FVC = forced vital capacity; FEV_1=forced expiratory volume in 1 second; FEF_{50}=forced expiratory flow rates at 50%; FEF_{75}=forced expiratory flow rates at 75%

[a]Number affected/number examined. The percentage of workers reporting symptoms is indicated in parentheses.
[b]Significantly different from controls, $p < 0.05$, by Fisher's exact test performed for this review.

Source: Holness et al. (1989).

Ballal et al. (1998) conducted a cross-sectional study of male workers at two urea fertilizer factories in Saudi Arabia[4]. The cohort consisted of 161 exposed subjects (84 from factory A and 77 from factory B) and 355 unexposed controls. Workers in factory A were exposed to air ammonia levels of 2–130 mg/m^3 (2.8–184.4 ppm), and workers in factory B were exposed to 0.02–7 mg/m^3 (0.03–9.9 ppm). Mean duration of employment was 51.8 months for exposed workers and 73.1 months for controls. Exposure levels were estimated by analyzing a total of 97 air samples collected over 8-hour shifts close to the employee's work site. The prevalence of respiratory symptoms and diseases was determined by administration of a questionnaire. The authors stated that there were no other chemical pollutants in the workplace that might have affected the respiratory system. Smoking habits were similar for exposed workers and controls. Stratifying the workers by ammonia exposure levels (above or below the American Conference of Governmental Industrial Hygienists [ACGIH] threshold limit value

[4]The process of fertilizer production involved synthesis of ammonia from natural gas, followed by reaction of the ammonia and carbon dioxide to form ammonium carbamide, which was then converted to urea.

[TLV] of 18 mg/m^3 [25 ppm]) showed that those exposed to ammonia concentrations higher than the TLV had significantly higher relative risks for cough, phlegm, wheezing, dyspnea, and asthma than workers exposed to levels below the TLV (Table 4-2). The relative risk for wheezing was also elevated among those exposed to ammonia levels at or below the TLV. Distribution of symptoms by cumulative ammonia concentration (CAC, mg/m^3-years) also showed significantly higher relative risk for all the above symptoms among those with higher CAC (Table 4-2). Results of the logistic regression analysis showed that ammonia concentration was significantly related to cough, phlegm, wheezing with and without shortness of breath, and asthma (Table 4-3).

Table 4-2. The prevalence of respiratory symptoms and disease in urea fertilizer workers exposed to ammonia

Respiratory symptom/disease	Relative risk (95% CI)			
	Exposure category		Cumulative concentration (mg/m^3 of air-yrs)	
	≤ACGIH TLV (18 mg/m^3) (n = 138)	>ACGIH TLV (18 mg/m^3) (n = 17)	≤50 (n = 130)	>50 (n = 30)
Cough	0.86 (0.48–1.52)	3.48 (1.84–6.57)	0.72 (0.38–1.35)	2.82 (1.58–5.03)
Wheezing	2.26 (1.32–3.88)	5.01 (2.38–10.57)	1.86 (1.04–3.32)	5.24 (2.85–9.52)
Phlegm	0.79 (0.43–1.47)	3.75 (1.97–7.11)	0.63 (0.31–1.26)	3.03 (1.69–5.45)
Dyspnea	1.13 (0.62–2.04)	4.57 (2.37–8.81)	1.19 (0.66–2.17)	2.59 (1.25–5.36)
Chronic bronchitis	1.43 (0.49–4.19)	2.32 (0.31–17.28)	0.61 (0.13–2.77)	5.32 (1.72–16.08)
Bronchial asthma	1.15 (0.62–2.15)	4.32 (2.08–8.98)	1.22 (0.66–2.28)	2.44 (1.10–5.43)
Chronic bronchitis and bronchial asthma	2.57 (0.53–12.59)	6.96 (0.76–63.47)	1.82 (0.31–10.77)	8.38 (1.37–45.4)

Source: Ballal et al. (1998).

Table 4-3. Logistic regression analysis of the relationship between ammonia concentration and respiratory symptoms or disease in exposed urea fertilizer workers

Respiratory symptom/disease	OR (95% CI)
Cough	1.32 (1.08–1.62)[a]
Phlegm	1.36 (1.10–1.67)[a]
Shortness of breath with wheezing	1.26 (1.04–1.54)[a]
Wheezing alone	1.55 (1.17–2.06)[a]
Dyspnea on effort	0.83 (0.68–1.02)
Diagnosis of asthma	1.33 (1.07–1.65)[a]

[a]$p \leq 0.05$.

Source: Ballal et al. (1998).

Results from limited spirometry testing of workers from factory A were reported in a followup study (Ali, 2001). The pulmonary function indices measured in 73 ammonia workers and 343 control workers included FEV_1 and FVC. Prediction equations for these indices were developed for several nationalities (Saudis, Arabs, Indians, and other Asians) and corrected values were expressed as the percentage of the predicted value for age and height. The FVC% predicted was higher in exposed workers than in controls (4.6% increase, $p \leq 0.002$); however, workers with cumulative exposure ≥50 mg/m^3-years had significantly lower FEV_1% predicted (7.4% decrease, $p < 0.006$) and FVC% predicted (5.4% decrease, $p \leq 0.030$) than workers with cumulative exposure ≤50 mg/m^3-years. A comparison between symptomatic and asymptomatic exposed workers showed that FEV_1% predicted and FEV_1/FVC% were significantly lower among symptomatic workers (9.2% decrease in FEV_1% predicted, $p < 0.001$ and 4.6% decrease in FEV_1/FVC%, $p < 0.02$). Although Ballal et al. (1998) and Ali (2001) suggest that exposure to ammonia concentrations above 18 mg/m^3 (50 mg/m^3-years) is associated with respiratory symptoms and altered pulmonary function, NOAEL and LOAEL values could not be identified by EPA from these studies due to inadequate reporting of exposure concentrations.

Rahman et al. (2007) conducted a cross-sectional study of workers at a urea fertilizer factory in Bangladesh that consisted of an ammonia plant and a urea plant. The exposed group studied consisted of 63 operators in the ammonia plant and 77 in the urea plant; 25 individuals from the administration building served as a control group. Mean duration of employment exceeded 16 years in all groups. Personal ammonia exposures were measured by two different methods (Dräger PAC III and Dräger tube) in five to nine exposed workers per day for 10 morning shifts in the urea plant (for a total of 64 workers) and in five to nine exposed workers per day for 4 morning shifts from the ammonia plant (for a total of 24 workers). Four to seven volunteer workers per day were selected from the administration building as controls for a total of 25 workers over a five-day period. Questionnaires were administered to inquire about demographics, past chronic respiratory disease, past and present occupational history, smoking status, acute respiratory symptoms (cough, chest tightness, runny nose, stuffy nose, and sneezing), and use of protective devices. Lung function tests (FVC, FEV_1, and peak expiratory flow rate [PEFR]) were administered preshift and postshift (8-hour shifts) to the 88 exposed workers after exclusion of workers who planned to have less than a four-hour working day; lung function was not tested in the control group. Personal ammonia exposure and pulmonary function were measured on the same shift for 28 exposed workers. Linear multiple regression was used to analyze the relationship between workplace and the percentage cross-shift change in FEV_1 (ΔFEV_1%) while adjusting for current smoking.

Mean exposure levels at the ammonia plant determined by the Dräger tube and Dräger PAC III methods were 25.0 and 6.9 ppm (17.7 and 4.9 mg/m^3), respectively; the corresponding means in the urea plant were 124.6 and 26.1 ppm (88.1 and 18.5 mg/m^3) (Rahman et al., 2007). Although the Dräger tube measurements indicated ammonia exposure about 4–5 times higher

than those obtained with the PAC III instrument, there was a significant correlation between the ammonia concentrations measured by the two methods ($p = 0.001$). No ammonia was detected in the control area using the Dräger tube (concentrations less than the measuring range of 2.5 to 200 ppm [1.8 to 141 mg/m^3]). Based on an evaluation of the two monitoring methods and communication with technical support at Dräger[5], EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, the PAC III air measurements were considered the more reliable measurement of exposure to ammonia for the Rahman et al. (2007) study. The study authors, however, observed that their measurements indicated only relative differences in exposures between workers and production areas, and that the validity of the exposure measures could not be evaluated based on their results.

The prevalence of acute respiratory symptoms was higher in the urea plant than in the ammonia plant or in the administration building. Comparison between the urea plant and the administration building showed that cough and chest tightness were statistically higher in the former; a similar comparison of the ammonia plant and the administration building showed no statistical difference in symptom prevalence between the two groups (Table 4-4). Preshift measurement of FVC, FEV_1, and PEFR did not differ between urea plant and ammonia plant workers. Significant cross-shift reductions in FVC and FEV_1 were reported in the urea plant (2 and 3%, respectively, $p \leq 0.05$), but not in the ammonia plant. When controlled for current smoking, a significant decrease in ΔFEV_1% was observed in the urea plant ($p \leq 0.05$). Among 23 workers with concurrent measurements of ammonia and lung function on the same shift, ammonia exposure was correlated with a cross-shift decline in FEV_1 of 3.9% per unit of log-transformed ammonia concentration in ppm. EPA identified a NOAEL of 6.9 ppm (4.9 mg/m^3) and a LOAEL of 26.1 ppm (18.5 mg/m^3) in the Rahman et al. (2007) study based on increased prevalence of respiratory symptoms and an acute decrease in lung function.

[5]Telephone conversations and e-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, Syracuse Research Corporation [contractor to National Center for Environmental Assessment (NCEA), ORD, U.S. EPA].

Table 4-4. Prevalence of acute respiratory symptoms and cross-shift changes in lung function among workers exposed to ammonia in a urea fertilizer factory

Parameter	Ammonia plant (4.9 mg/m^3)[a]	Urea plant (18.5 mg/m^3)[a]	Administration building (concentration not determined)[b]
Respiratory symptoms			
Cough	4/24 (17%)[c]	18/64 (28%)[d]	2/25 (8%)
Chest tightness	4/24 (17%)	21/64 (33%)[d]	2/25 (8%)
Stuffy nose	3/24 (12%)	10/64 (16%)	1/25 (4%)
Runny nose	1/24 (4%)	10/64 (16%)	1/25 (4%)
Sneeze	0/24 (0%)	14/64 (22%)	2/25 (8%)
Lung function parameters (cross-shift percentage change)[e, f]			
FVC	0.2 ± 9.3	-2.3 ± 8.8	ND
FEV_1	3.4 ± 13.3	-1.4 ± 8.9	ND
PEFR	2.9 ± 11.1	-1.0 ± 16.2	ND

ND: no data
[a]Mean ammonia concentrations measured by the Dräger PAC III method.
[b]Concentrations in the administration building were rejected by study authors due to relatively large drift in the zero levels.
[c]Values are presented as incidence (prevalence expressed as a percentage).
[d]$p \leq 0.05$ by Fisher's exact test, comparing exposed workers to administrators.
[e]Calculated as ((Post shift-preshift)/preshift) x 100.
[f]Values are presented as mean ± SD.

Source: Rahman et al. (2007).

Tepper et al. (1991) evaluated pulmonary function (FEV_1) changes among firefighters from the city of Baltimore 6–10 years after a baseline examination. The eligible study population consisted of 963 firefighters of which 695 participated in the follow-up study. Pulmonary function tests were performed for 628 firefighters. Information about exposures was obtained by questionnaire and by combining data from fire department records regarding the number of fires fought by fire fighting units with individual work histories. To determine the effects of occupational exposures while accounting for confounding by or interaction with other risk factors, multiple linear regression techniques with dichotomous indicator variables were used. Reported exposure to specific chemicals was rare, except for ammonia and chlorine; 160 men reported exposure to ammonia and 128 to chlorine. Men with self-reported ammonia exposure experienced a rate of decline in FEV_1 1.7 times greater than men without ammonia exposure, but the difference was not statistically significant. NOAEL and LOAEL values were not identified in this study.

Hamid and El-Gazzar (1996) evaluated changes in serum clinical chemistry as measures of neurochemical alterations and liver function among workers at a urea production plant in Alexandria, Egypt. The study group consisted of 60 male workers from the fertilizer plant, including 30 workers with known exposures to ammonia and 30 workers from the administrative departments with no known history of exposure to ammonia. The authors indicated that the exposed population had worked at the fertilizer plant on average for 12 years. The exposed and reference populations were matched on demographic characteristics including age, educational status, and socioeconomic status. No information is reported on exposure levels. Blood samples were collected from each subject and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, blood urea, and monoamine oxidase (MAO) and catalase activity. Table 4-5 shows statistically significant changes in hemoglobin and serum chemistry. Mean levels of AST, ALT, and blood urea were significantly elevated among exposed workers over controls. Mean levels of hemoglobin were significantly lower, and MAO and catalase enzyme activities were significantly depressed among exposed workers compared to controls. A correlation analysis showed a positive correlation between catalase activity and levels of hemoglobin, AST and ALT, and MAO activities. Hamid and El-Gazaar (1996) noted that inhibition of catalase can affect electrical stability, permeability, and fluidity of membranes, which may lead to hepatotoxic and neurotoxic alterations in occupationally exposed workers. NOAEL and LOAEL values were not identified in this study due to the absence of information on exposures at this fertilizer plant.

Table 4-5. Summary of significant changes in serum from workers occupationally exposed to ammonia at a fertilizer plant

Parameter	Controls[a]	Exposed[a]
ALT (U/mL)	16.0 ± 5.59	19.4 ± 5.69[b]
AST (U/mL)	14.5 ± 4.67	17.9 ± 4.14[b]
Hb (%)	14.8 ± 2.62	12.2 ± 2.29[c]
Blood urea (mg/mL)	0.203 ± 0.0512	0.319 ± 0.0755[c]
MAO (units)	31.9 ± 10.1	20.8 ± 4.30[c]
Catalase (IU/mL)	119.3 ± 4.76	80.9 ± 9.31[c]

[a]Mean ± standard deviation.
[b]Significantly different from controls ($p < 0.05$).
[c]Significantly different from controls ($p < 0.01$).

Source: Hamid and El-Gazzar (1996).

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

Kawano et al. (1991) investigated the hypothesis that the bacterium *Helicobacter pylori* (*H. pylori*), which produces a potent urease that increases ammonia production, plays a significant role in the etiology of chronic atropic gastritis. Male Sprague Dawley rats (6/group) were given tap water, 0.01%, or 0.1% ammonia ad libitum for two or four weeks. The daily dose of 0.01% and 0.1% ammonia in drinking water, based on a weight of 230 g for male rats and a water consumption of 50 mL/day, was estimated to be 22 and 220 mg/kg-day, respectively. The effect of ammonia on the antral mucosa was estimated by three measurements of the thickness of the mucosa about 175 microns from the pyloric ring in the antral mucosa. The parietal cell number per gland was determined at three locations in the oxyntic glandular area. Mucosal lesions were not observed macro- or microscopically. There was a statistically significant decrease in mean antral mucosal thickness with increasing dose and duration of exposure (Table 4-6). Parietal cell number per oxyntic gland decreased in a statistically significant dose- and time-dependent fashion. The index of periodic acid-Schiff Alician blue positive intracellular mucin was significantly lower in the antral and body mucosa with 0.1% ammonia; the index was significantly lower only for the antral mucosa with 0.01% ammonia. The authors suggested that administration of ammonia in drinking water causes gastric mucosal atrophy. Based on the reduction in antral mucosal thickness, EPA identified a LOAEL of 22 mg/kg-day; a NOAEL was not identified.

Table 4-6. Effect of ammonia in drinking water on the thickness of the gastric antral and body mucosa of the rat stomach

	Thickness of mucosa (µM); mean ± sem		
		Percent ammonia in drinking water	
Length of treatment	**Control (tap water)**	0.01%	0.1%
Antral mucosa			
2 weeks	270 ± 18	258 ± 22	217 ± 40[a]
4 weeks	276 ± 39	171 ± 22[a]	109 ± 12[b,c]
Body mucosa			
2 weeks	574 ± 116	568 ± 159	591 ± 183
4 weeks	618 ± 154	484 ± 123	440 ± 80[a,c]

[a] $p < 0.05$ vs. control group
[b] $p < 0.01$ vs. control group
[c] $p < 0.01$ versus 2 week treatment group

Source: Kawano et al. (1991).

In a follow-up study of the effect of ammonia produced from *H. pylori*, Tsujii et al. (1993) studied the subchronic effect of ammonia in drinking water on the cell kinetics of the gastric mucosa of the stomach. Six groups of Sprague Dawley male rats (36 rats/group) were

given 0.01% ammonia in drinking water for 3 days, or 1, 2, 4 or 8 weeks; ammonia solutions were changed daily. Tap water was provided for the balance of the 8-week study. A control group was given tap water for eight weeks. Based on the initial body weight (150 g) and estimated daily water intake (50 mL), the daily dose at a drinking water concentration of 0.01% ammonia was estimated to be 33 mg/kg-day. Cellular migration was measured by labeling cells with 5-bromo-2-deoxyuridine (BrDU) at different time periods and measuring the incorporation of this modified nucleoside with a histochemical technique using anti BrDU monoclonal antibodies. Antral and body mucosa thickness was measured as described in Kawano et al. (1991). The measurement of cell proliferation in the gastric mucosa was estimated using the labeling index in gastric pits (ratio of labeled nuclei to total nuclei in the proliferation zone). The antral mucosal thickness decreased significantly at 4 and 8 weeks of treatment (Table 4-7) but there was no effect on the body mucosa. Cell migration preceded the decrease in thickness of the antral mucosa. The rate of cell migration (cells/day) toward the mucosal surface was significantly greater for 0.01% ammonia-treated rats compared to the control at 4 and 8 weeks of treatment. Cell proliferation, as estimated from the labeling index, was significantly increased after one week for the antral and body mucosa. The authors concluded that 0.01% ammonia increased epithelial cell migration in the antrum leading to mucosal atrophy. The EPA identified a LOAEL of 33 mg/kg-day based on decreased thickness of the gastric antrum; a NOAEL was not identified.

Table 4-7. Effect of ammonia in drinking water on gastric antral and body mucosa in the stomach of Sprague-Dawley rats administered 0.01% ammonia in drinking water

Length of treatment	Thickness of mucosa (µM)	
	Antral mucosa	Body mucosa
Control (tap water only)	283 ± 26	534 ± 27
3 Days	305 ± 45	559 ± 50
1 Week	272 ± 31	542 ± 28
2 Weeks	299 ± 26	555 ± 37
4 Weeks	159 ± 29[b]	531 ± 32
8 Weeks	168 ± 26[b]	508 ± 29

[a]Extracted from Figure 3 of Tsujii et al. (1993); mean ± SD
[b]$p < 0.05$ vs. control (tap water only) group

Source: Tsujii et al. (1993)

Fazekas (1939) administered ammonium hydroxide to 51 rabbits (strain and sex not specified) via gavage every other day initially and later daily in increasing amounts of 50–80 mL as either a 0.5 or 1.0% solution over a long period of time. The daily dose (mg/kg-day) was estimated using the weight of adult rabbits from standard growth curve for rabbits (3.5–4.1 kg) (U.S. EPA, 1988). Based on daily water consumption of 50–80 mL, a daily dose for the rabbits

receiving 0.5 and 1.0% ammonia in drinking water was approximately 61–110 mg/kg-day and 120–230 mg/kg-day, respectively. The exact duration of the study is not reported, but it is clear from the data that by the end of the experiment some rabbits received only three or four doses before dying as a result of intoxication in 5.5 days and other rabbits received over 80 doses and survived for up to 17 months. Toxicological endpoints evaluated included fluctuations in body weights, changes in blood pressure measured at the central artery of the ear in 10 rabbits after lengthy treatment, and changes in the weight, fat and cholesterol content of adrenals. For comparison purposes, the weight of the adrenals from 41 healthy rabbits of similar age and body weight were also determined. The average weight of adrenals from these 41 control rabbits was 400.0 ± 13.4 mg.

Fazekas (1939) reported that differences in mean adrenal weight in ammonium hydroxide-treated animals were significant, although there was no description of the statistical analysis performed in this study. Chemical evaluation of the adrenals from treated rabbits revealed fat content 4.5 times greater and cholesterol content 6.5 times greater than controls. At the beginning of the experiment, a greater weight loss was observed among those rabbits receiving ammonium hydroxide more frequently (daily) at higher doses. Body weights fluctuated among treated rabbits and generally decreased initially and gradually increased in the later months only to drop again a few weeks before death. Body weights for controls were not reported. Thirteen rabbits exhibited weight increases after the initial loss that persisted until the end of the experiment. Dissection of these rabbits revealed enlarged adrenals (800–1,340 mg), and fatty tissue surrounding the kidneys, mesentery, and the pericardium. This fat accumulation was not observed in untreated controls. Histology revealed enlarged cells of the zona fasciculata of the adrenal cortex that were rich in lipid. The blood pressure of rabbits before dosing ranged from 60 to 74 mm Hg and dropped with initial exposure (during the first 5–10 minutes that lasted up to 7 hours) to 20–30 Hg/mm. Following several months of ammonium hydroxide treatment, a moderate elevation in blood pressure of 10–30 mm Hg was found in 8/10 rabbits. In the other two rabbits, the blood pressure increased from the initial values of 62 and 65 to 90 Hg/mm during the first 7 months of treatment and remained almost unchanged at this level until sacrifice.

In summary, Fazekas (1939) concluded that initial decreases in blood pressure and effects of emaciation in rabbits following gavage treatment with ammonium hydroxide is associated with the hypofunction of the cortical or medullary substance of the adrenal gland. The authors also concluded that the subsequent increases in blood pressure and body weight could be attributed to hypertrophy of the adrenal cortex. This study is limited by lack of reporting detail and inadequate study design. The EPA did not identify a NOAEL or LOAEL from this study.

Toth (1972) evaluated whether hydrazine, methylhydrazines, and ammonium hydroxide play a role in tumorigenesis in mice. Solutions of hydrazine (0.001%), methyl hydrazine (0.01%), methyl hydrazine sulfate (0.001%), and ammonium hydroxide (0.1, 0.2, and 0.3%) were administered continuously in the drinking water of 5- and 6-week-old randomly bred Swiss

mice (50/sex) for their entire lifetime. For ammonium hydroxide, the study authors reported the average daily drinking water intakes as 9.2, 8.2, and 6.5 mL/day for males for the 0.1, 0.2, and 0.3% groups, respectively, and as 8.3, 6.5, and 4.8 mL/day for females, respectively. Given these rates and assuming average default body weights of 37.3 and 35.3 g for males and females, respectively (U.S. EPA, 1988), the approximate continuous doses for ammonium hydroxide are 250, 440, and 520 mg/kg-day for males and 240, 370, and 410 mg/kg-day for females. Additionally, groups of C_3H mice (40/sex) were exposed to ammonium hydroxide in the drinking water at a concentration of 0.1% for their lifetime. Average daily water consumption for these mice was reported as 7.9 and 8.4 mL/day for males and females, respectively. The approximate equivalent doses for these mice assuming the same default body weights as above (U.S. EPA, 1988) are 191 and 214 mg/kg-day for males and females, respectively. Data were not reported for a concurrent control group. Mice were monitored weekly for changes in body weights and gross pathological changes were recorded. The animals were either allowed to die or were killed when found in poor condition. Complete necropsies were performed on all mice and the liver, kidney, spleen, lung, and organs with gross lesions were processed for histopathological examination. Data on body weights were not reported.

For Swiss mice, tumor incidence at the 0.3% ammonium hydroxide concentration was as follows: malignant lymphomas: 3/50 (males), 9/50 (females); and lung adenoma or adenocarcinoma: 7/50 (male), 4/50 (female). Tumor incidence at the 0.2% ammonium hydroxide concentration was: malignant lymphomas: 7/50 (males), 10/50 (females); lung adenoma or adenocarcinoma: 5/50 (male), 8/50 (female); and breast tumors: 4/50 (females). Tumor incidence at the 0.1% ammonium hydroxide concentration was: malignant lymphomas: 4/50 (males), 10/50 (females); lung adenoma or adenocarcinoma: 5/50 (male), 12/50 (female); and breast tumors: 1/50 (females). The denominators were not adjusted for survival, and concurrent control data were not provided. For a second strain of mice (C_3H) that received 0.1% ammonium hydroxide in drinking water, the incidence of adenocarcinomas of the mammary gland in female mice was 60%. The incidence of breast tumors in the corresponding untreated control mice was 76%. Other tumors were identified in treated rats, but were of low incidence. Toth (1972) concluded that ammonium hydroxide was not carcinogenic in either strain of mouse. Because concurrent control tumor incidence was not provided other than the incidence of breast tumors in C_3H female mice, the incidence of tumors in treated mice cannot be independently compared to control tumor incidence.

Tsujii et al. (1995, 1992a) evaluated the role of ammonia in *H. pylori*-related gastric carcinogenesis. *H. pylori* is a bacterium that produces a potent urease, which generates ammonia from urea in the stomach, and has been implicated in the development of gastric cancer. Tsujii et al. (1995, 1992a) pretreated groups of 40–44 male Sprague-Dawley rats with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for 24 weeks before administering 0.01% ammonium solution as a drinking fluid for 24 weeks. Based on an average

body weight of 523 g for male Sprague-Dawley rats during chronic exposure (U.S. EPA, 1988) and a reported water consumption rate of 0.05 L/day, the approximate continuous dose administered to these rats is 10 mg/kg-day. In each study, an additional group of 40–43 rats given tap water for 24 weeks following pretreatment with MNNG served as controls. The study protocol did not include a dose group that received ammonia only in drinking water. Stomachs from rats surviving beyond 45 weeks were examined histologically for evidence of ulcers, lesions, and tumors. Tsujii et al. (1995) also evaluated serum gastrin levels from blood collected at 30 and 46 weeks and mucosal cell proliferation in animals surviving to 48 weeks by calculating the labeling index (percentage ratio of labeled nuclei to total number of nuclei in the proliferation zone) and the proliferation zone index (fraction of the gastric pit occupied by the proliferation zone).

Tsujii et al. (1995, 1992a) observed a significantly greater incidence of gastric cancers among rats receiving ammonia after pretreatment with MNNG compared to rats receiving only MNNG and tap water ($p < 0.01$, χ^2 test). Seventy percent of MNNG+ammonia-treated rats versus 31% of control rats developed gastric tumors in the first study (Tsujii et al., 1992a). The number of gastric cancers per tumor-bearing rat in this study was 2.1 ± 1.4 among treated rats and 1.3 ± 0.6 among control rats ($p < 0.01$, χ^2 test).

In the second study, 66% of rats dosed with ammonia and pretreated with MNNG developed gastric cancers compared to 30% of the control rats (Tsujii et al., 1995). The numbers of gastric tumors per rat in this study were also significantly higher among MNNG+ammonia-exposed rats than controls ($p < 0.001$, Mann-Whitney test) suggesting that ammonia was a promoter. In the absence of an ammonia-only treatment group, however, it is not possible to distinguish with certainty between possible promotion and initiator activity. The degree of differentiation of adenocarcinomas in control and ammonia-treated rats was significantly different. Ammonia-treated rats also demonstrated a significantly higher incidence of larger tumors (5.3 mm compared to 4.4 mm for controls) and of gastric cancers penetrating the muscularis propria or deeper ($p < 0.01$, 22% compared to controls 12%). In this study, the labeling index and the proliferation zone index were statistically significantly elevated in ammonia exposed rats compared to controls in the fundic mucosa and antral mucosa.

Tsujii et al. (1995) explored the hypothesis that ammonia might increase intragastric pH, leading to an increase in serum gastrin, a trophic hormone in the gastric fundus mucosa, and a possible proliferating factor in gastric epithelial cells. The investigators found no significant effects on serum gastrin levels and concluded that serum gastrin does not appear to play a significant role in ammonia-induced promotion.

4.2.2. Inhalation Exposure

Anderson et al. (1964) exposed a group of 10 guinea pigs (strain not given) and 10 Swiss albino mice of both sexes continuously to 20 ppm (14 mg/m^3) ammonia vapors for up to 6 weeks

(anhydrous ammonia, purity not reported). Controls (number not specified) were maintained under identical conditions except for the exposure to ammonia. An additional group of six guinea pigs was exposed to 50 ppm (35 mg/m^3) for 6 weeks. The animals were observed daily for abnormal signs or lesions. At termination, the mice and guinea pigs were sacrificed (two per group at 1, 2, 3, 4, and 6 weeks of exposure) and selected tissues (lungs, trachea, turbinates, liver, and spleen) were examined for gross and microscopic pathological changes. No significant effects were observed in animals exposed for up to 4 weeks, but exposure to 20 ppm (14 mg/m^3) for 6 weeks caused darkening, edema, congestion, and hemorrhage in the lung. Exposure of guinea pigs to 50 ppm (35 mg/m^3) ammonia for 6 weeks caused grossly enlarged and congested spleens, congested livers and lungs, and pulmonary edema. NOAEL and LOAEL values were not identified in this study because only two animals per group were examined at a single time period for the 20 ppm (14 mg/m^3) exposure groups (mice and guinea pigs).

Coon et al. (1970) exposed groups of male and female Sprague-Dawley and Long-Evans rats, male and female Princeton-derived guinea pigs, male New Zealand rabbits, male squirrel monkeys, and purebred male beagle dogs to 0, 155, or 770 mg/m^3 ammonia (0, 219, or 1,089 ppm) 8 hours/day, 5 days/week for 6 weeks (anhydrous ammonia, >99% pure). The investigators stated that a typical loaded chamber contained 15 rats, 15 guinea pigs, 3 rabbits, 3 monkeys, and 2 dogs. Blood samples were taken before and after the exposures for determination of hemoglobin concentration, packed erythrocyte volume, and total leukocyte counts. Animals were routinely checked for clinical signs of toxicity. At termination, sections of the heart, lung, liver, kidney, and spleen were processed for microscopic examination in approximately half of the surviving rats and guinea pigs and all of the surviving dogs and monkeys. Sections of the brain, spinal cord, and adrenals from dogs and monkeys were also retained, as were sections of the thyroid from the dogs. The nasal passages were not examined in this study.

Exposure to 155 mg/m^3 ammonia did not result in any deaths or adverse clinical signs of toxicity in any of the animals. Hematological values were within normal limits for the laboratory and there were no significant gross alterations in the organs examined. Microscopic examination showed evidence of focal pneumonitis in the lung of one of three monkeys. Exposure to 770 mg/m^3 caused initial mild to moderate lacrimation and dyspnea in rabbits and dogs. However, these clinical signs disappeared by the second week of exposure. No significant alterations were observed in hematology tests or upon gross or microscopic examinations at the highest dose. However, consistent nonspecific inflammatory changes (not further described) were observed in the lungs from rats and guinea pigs in the high-dose group that were more extensive than in control animals (incidence not reported).

Coon et al. (1970) also exposed rats (15–51/group) continuously to ammonia (anhydrous ammonia, >99% pure) at 0, 40, 127, 262, 455, or 470 mg/m^3 (0, 57, 180, 371, 644, or 665 ppm) for 90–114 days. Fifteen guinea pigs, three rabbits, two dogs, and three monkeys were also

exposed continuously under similar conditions to ammonia at 40 mg/m^3 (57 ppm) or 470 mg/m^3 (665 ppm). No significant effects were reported in any animals exposed to 40 mg/m^3 and nonspecific inflammatory changes in the lungs and kidneys (not further described) were seen in rats exposed to 127 mg/m^3 (180 ppm) ammonia. Exposure to 262 mg/m^3 (371 ppm) caused nasal discharge in 25% of the rats and nonspecific circulatory and degenerative changes in the lungs and kidneys (not further described, incidence not reported). A frank effect level at 455 mg/m^3 (644 ppm) was observed due to high mortality in the rats (50/51). Thirty-two of 51 rats died by day 25 of exposure; no histopathological examinations were conducted in these rats. Exposure to 470 mg/m^3 (665 ppm) caused death in 13/15 rats and 4/15 guinea pigs and marked eye irritation in dogs and rabbits. Dogs experienced heavy lacrimation and nasal discharge, and corneal opacity was noted in rabbits. Hematological values did not differ significantly from controls in animals exposed to 470 mg/m^3 (665 ppm) ammonia. Histopathological evaluation of animals exposed to 470 mg/m^3 (665 ppm) consistently showed focal or diffuse interstitial pneumonitis in all animals and also alterations in the kidneys (calcification and proliferation of tubular epithelium), heart (myocardial fibrosis), and liver (fatty change) in several animals of each species (incidence not reported). The study authors did not determine a NOAEL or LOAEL concentration from this study. EPA identified a NOAEL of 40 mg/m^3 (57 ppm) and a LOAEL of 127 mg/m^3 (180 ppm) based on nonspecific inflammatory changes in the lungs and kidneys in rats exposed to ammonia for 90 days.

Stombaugh et al. (1969) exposed groups of Duroc pigs (9/group) to measured concentrations of 12, 61, 103, or 145 ppm ammonia (8, 43, 73, or 103 mg/m^3) continuously for 5 weeks (anhydrous ammonia, purity not reported). Endpoints evaluated included clinical signs, food consumption (measured 3 times/week), weight gain (measured weekly), and gross and microscopic examination of the respiratory tract at termination. A control group was not included. In general, exposure to ammonia reduced food consumption and body weight gain, but since a control group was not used, it is impossible to determine whether this reduction was statistically significant. Food efficiency (food consumed/kg body weight gain) was not affected. Exposure to ≥103 ppm (>73 mg/m^3) ammonia appeared to cause excessive nasal, lacrimal, and mouth secretions and increased the frequency of cough (incidence data for these effects were not reported). Examination of the respiratory tract did not reveal any significant exposure-related alterations. The study authors did not identify a NOAEL or LOAEL concentration from this study. EPA did not identify a NOAEL or LOAEL value for this study due to the absence of a control group.

Doig and Willoughby (1971) exposed groups of six specific-pathogen-free derived Yorkshire Landrace pigs to 0 or 100 ppm ammonia (0 or 71 mg/m^3) continuously for up to 6 weeks. The mean concentration of ammonia in the control chamber was 8 ppm (6 mg/m^3). Additional groups of pigs were exposed to similar levels of ammonia as well as to 0.3 mg/ft^3 of ground corn dust to simulate conditions on commercial farms. Pigs were monitored daily for

clinical signs and changes in behavior. Initial and terminal body weights were measured to determine body weight gain during the exposure period. Blood samples were collected prior to the start of each experiment and at study termination for hematology (packed cell volume, white blood cell, differential leukocyte percentage, and total serum lactate dehydrogenase). Two pigs (one exposed and one control) were necropsied at weekly intervals and tracheal swabs for bacterial and fungal culture were taken. Histological examination was conducted on tissue samples from the lung, trachea, and bronchial lymph nodes.

During the first week of exposure, exposed pigs exhibited slight signs of conjunctival irritation including photophobia and excessive lacrimation. These irritation effects were not apparent beyond the first week. Measured air concentrations in the exposure chambers increased to more than 150 ppm (106 mg/m^3) on two occasions. Doig and Willoughby (1971) reported that at this concentration, the signs of conjunctival irritation were more pronounced in all pigs. No adverse effects on body weight gain were apparent. Hematological parameters and gross pathology were comparable between exposed and control pigs. Histopathology revealed epithelial thickening in the trachea of exposed pigs and a corresponding decrease in the numbers of goblet cells as shown in Table 4-8. Tracheal thickening was characterized by thinning and irregularity of the ciliated brush border and an increased number of cell layers. Changes in bronchi and bronchioles characterized as lymphocytic cuffing were comparable between exposed and control pigs. Similarly, intraalveolar hemorrhage and lobular atelectasis were common findings in both exposed and control pigs. Pigs exposed to both ammonia and dust exhibited similar reactions as those pigs exposed only to ammonia although initial signs of conjunctival irritation were more severe in these pigs and these pigs demonstrated lesions in the nasal epithelium similar to those observed in the tracheal epithelium of pigs exposed only to ammonia.

Table 4-8. Summary of histological changes observed in rats exposed to ammonia for 6 weeks

Duration of exposure (wks)	Thickness of tracheal epithelium (µm)		Number of tracheal goblet cells (per 500 µm)	
	Control	71 mg/m^3 NH_3	Control	71 mg/m^3 NH_3
1	15.7	21.0	13.6	24.0
2	20.4	29.3	22.7	10.3
3	20.4	36.6	18.9	7.3
4	21.8	36.2	18.3	10.7
5	19.3	33.2	20.2	10.0
6	18.9	41.6	20.0	1.3
Mean ± standard deviation	19.4 ± 2.1	32.9 ± 7.2	18.9 ± 3.0	10.6 ± 7.5

Source: Doig and Willoughby (1971).

Doig and Willoughby (1971) concluded that ammonia exposure at 71 mg/m^3 may be detrimental to young pigs. The authors suggested that although the structural damage to the upper respiratory epithelium was slight, such changes may cause severe functional impairment. The study authors did not identify a NOAEL or LOAEL concentration from this study. EPA identified a LOAEL of 71 mg/m^3 (100 ppm), based on damage to the upper respiratory epithelium. A NOAEL could not be identified from this single-concentration study.

Broderson et al. (1976) exposed groups of Sherman rats (5/sex/dose) continuously to 10 or 150 ppm ammonia (7 or 106 mg/m^3, respectively) for 75 days (anhydrous ammonia, purity not reported). The 10-ppm exposure level represented the background ammonia concentration resulting from cage bedding that was changed 3 times/week. The 150-ppm concentration resulted from cage bedding that was replaced occasionally, but never completely changed. F344 rats (6/sex/group) were exposed to ammonia in an inhalation chamber at concentrations of 0 or 250 ppm (177 mg/m^3) continuously for 35 days. Rats were sacrificed at the end of the exposure period and tissues were prepared for histopathological examination of nasal passages, middle ear, trachea, lungs, liver, kidneys, adrenal, pancreas, testicle, mediastinal lymph nodes, and spleen.

Histopathological changes were observed in the nasal passage of rats exposed to 150 ppm (106 mg/m^3) for 75 days (from bedding) or 250 ppm (177 mg/m^3) for 35 days (inhalation chamber). Nasal lesions were most extensive in the anterior portions of the nose compared with posterior sections of the nasal cavity. The respiratory and olfactory mucosa was similarly affected with a three- to fourfold increase in the thickness of the epithelium. Pyknotic nuclei and eosinophilic cytoplasm were observed in epithelial cells located along the basement membrane. Epithelial cell hyperplasia and formation of glandular crypts were observed and neutrophils were located in the epithelial layer, the lumina of submucosal glands and the nasal passages. Dilation of small blood vessels and edema were observed in the submucosa of affected areas. Collagen replacement of submucosal glands and the presence of lymphocytes and neutrophils were also observed. No histopathological alterations were seen in control rats (10 ppm from bedding or 0 ppm from the inhalation chamber). Broderson et al. (1976) did not identify a NOAEL or LOAEL from this study. EPA identified a NOAEL of 10 ppm (7 mg/m^3) and a LOAEL of 150 ppm (106 mg/m^3) based on nasal lesions in rats exposed to ammonia (from bedding) for 75 days. Broderson et al. (1976) also studied the effect of ammonia inhalation on the incidence and severity of murine respiratory plasmosis in mice inoculated with *Mycoplasma pulmonis*. These results are presented in Section 4.4.3.

Gaafar et al. (1992) exposed 50 adult male white albino mice under unspecified conditions to ammonia vapor derived from a 12% ammonia solution (air concentrations were not reported) 15 minutes/day, 6 days/week for up to 8 weeks. Twenty-five additional mice served as controls. Starting the fourth week, 10 exposed and five control mice were sacrificed weekly. Following sacrifice, the nasal mucosa was removed and examined histologically. Frozen sections of the nasal mucosa were subjected to histochemical analysis (succinic dehydrogenase,

nonspecific estrase, acid phosphatase, and alkaline phosphatase [ALP]). Histological examination revealed a progression of changes in the nasal mucosa from exposed rats from the formation of crypts and irregular cell arrangements at 4 and 5 weeks, epithelial hyperplasia, patches of squamous metaplasia, and loss of cilia at 6 weeks, and dysplasia in the nasal epithelium at 7 weeks. Similar changes were exaggerated in the nasal mucosa from rats sacrificed at 8 weeks. Neoplastic changes included a carcinoma in situ in the nostril of one rat sacrificed at 7 weeks that presented with loss of polarity of the epithelium, hyperchromatism and mitotic figures with an intact basement membrane, and an invasive adenocarcinoma in one rat sacrificed at 8 weeks. Histochemical results revealed changes in succinic dehydrogenase, acid phosphatase, and ALP in exposed mice compared to controls (magnitude of change not reported), especially in areas of the epithelium characterized by dysplasia. Succinic dehydrogenase and acid phosphatase changes were largest in the superficial layer of the epithelium, although the acid phosphatase reaction was stronger in the basal and intermediate layers in areas of squamous metaplasia. ALP was strongest in the goblet cells from the basal part of the epithelium and basement membrane.

In summary, Gaafar et al. (1992) observed that ammonia exposure induces histological changes in the nasal mucosa of male mice that increase in severity over longer exposure periods. Corresponding abnormalities in histochemistry suggest altered cell metabolism and energy production, cell injury, cell proliferation, and possible chronic inflammation and neoplastic transformation. The study authors did not determine a NOAEL or LOAEL concentration from this study. EPA did not identify a NOAEL or LOAEL because air concentrations were not reported in the study.

Done et al. (2005) continuously exposed groups of 24 weaned pigs of several breeds in an experimental facility to atmospheric ammonia at 0, 0.6, 10, 18.8, or 37 ppm (0, 0.4, 7, 13.3, or 26 mg/m^3) and 1.2, 2.7, 5.1, or 9.9 mg/m^3 inhalable dust for 5 weeks (16 treatment combinations). The concentrations of ammonia and dust used were representative of those found commercially. A split-plot design was used in which one dust concentration was allocated to a "batch" (which involved five lots of 24 pigs each) and the four ammonia concentrations were allocated to the four lots within that batch. The fifth lot served as a control. Each batch was replicated. In other words, there were four dust concentrations × four ammonia concentrations plus four controls each replicated once giving 40 lots in total. In total, 960 pigs (460 males and 500 females) were used in the study; 560 pigs were given postmortem examinations. Blood was collected from 15 sows before the start of the experiment and tested for porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza. Five sentinel pigs were sacrificed at the start of each batch and lung, nasal cavity, and trachea, together with material from any lesions, were examined postmortem and subjected to bacteriological examination. Postmortem examination involved examining the pigs' external surfaces for condition and abnormalities, examination of the abdomen for peritonitis and lymph node size, internal gross examination of the stomach for

abnormalities, and gross examination of the nasal turbinates, thorax, larynx, trachea, tracheobronchial lymph nodes, and lung. Pigs were monitored for clinical signs (daily), growth rate, feed consumption, and feed conversion efficiency (frequency of observations not specified). After 37 days of exposure, eight pigs from each lot were sacrificed. Swabs of the nasal cavity and trachea were taken immediately after death for microbiological analysis and the pigs were grossly examined postmortem. On day 42, the remaining pigs were removed from the exposure facility and transferred to a naturally ventilated building for a recovery period of 2 weeks. Six pigs from each lot were assessed for evidence of recovery and the remaining 10 pigs were sacrificed and examined postmortem.

The pigs in this study demonstrated signs of respiratory infection and disease common to young pigs raised on a commercial farm (Done et al., 2005). The different concentrations of ammonia and dust did not have a significant effect on the pathological findings in pigs or on the incidence of pathogens. In summary, exposure to ammonia and inhalable dust at concentrations commonly found at pig farms was not associated with increase in the incidence of respiratory or other disease. The study authors did not identify a NOAEL or LOAEL concentration from this study. EPA identified a NOAEL of 26 mg/m^3 (37 ppm), based on the lack of respiratory or other disease following exposure to ammonia in the presence of respirable dust.

Weatherby (1952) exposed a group of 12 guinea pigs (strain not reported) to a target concentration of 170 ppm (120 mg/m^3) 6 hours/day, 5 days/week for up to 18 weeks (anhydrous ammonia, purity not reported). The actual concentration measured in the exposure chamber varied between 140 (99 mg/m^3) and 200 ppm (141 mg/m^3). A control group of six guinea pigs was exposed to room air. All animals were weighed weekly. Interim sacrifices were conducted at intervals of 6 weeks (four exposed and two control guinea pigs) and the heart, lungs, liver, stomach and small intestine, spleen, kidneys, and adrenal glands were removed for microscopic examination; the upper respiratory tract was not examined. No exposure-related effects were observed in guinea pigs sacrificed after 6 or 12 weeks of exposure. However, guinea pigs exposed to ammonia for 18 weeks showed considerable congestion of the spleen, liver, and kidneys, and early degenerative changes in the adrenal gland. The most severe changes occurred in the spleen and the least severe changes occurred in the liver. The spleen of exposed guinea pigs contained a large amount of hemosiderin and kidney tubules showed cloudy swelling with precipitated albumin in the lumens and some urinary casts (cylindrical structures indicative of disease). The incidence of histopathological lesions was not reported. EPA identified the ammonia concentration of 170 ppm (120 mg/m^3) to be a LOAEL based on congestion of the spleen, liver, and kidneys and early degenerative changes in the adrenal gland. A NOAEL could not be identified in this single-concentration study.

Curtis et al. (1975) exposed groups of crossbred pigs (4–8/group) to 0, 50, or 75 ppm ammonia (0, 35, or 53 mg/m^3) continuously for up to 109 days (anhydrous ammonia, >99.9% pure). Endpoints evaluated included clinical signs and body weight gain. At termination, all

pigs were subjected to a complete gross examination and representative tissues from the respiratory tract, the eye and its associated structures, and the visceral organs (not specified) were taken for subsequent microscopic examination. Weight gain was not significantly affected by exposure to ammonia and the results of the evaluations of tissues and organs were unremarkable. The turbinates, trachea, and lungs of all pigs were classified as normal. The study authors did not identify a NOAEL or LOAEL from this study. EPA identified a NOAEL of 75 ppm (53 mg/m^3) based on the absence of effects occurring in pigs exposed to ammonia; a LOAEL was not identified from this study.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Diekman et al. (1993) reared 80 crossbred gilts (young female pigs) in a conventional grower from 2 to 4.5 months of age; pigs were exposed naturally during that time to *Mycoplasma hypopneumoniae* and *Pasteurella multocida*, which causes pneumonia and atrophic rhinitis, respectively. At 4.5 months of age, the pigs were transferred to environmentally regulated rooms where they were exposed continuously to a mean concentration of ammonia of 7 ppm (range, 4–12 ppm) (5 mg/m^3; range, 3–8.5 mg/m^3) or 35 ppm (range, 26–45 ppm) (25 mg/m^3; range, 18–32 mg/m^3) for 6 weeks (Diekman et al., 1993). A control group was not included in this study. The low concentration of ammonia was obtained by the flushing of manure pits weekly and the higher concentration of ammonia was maintained by adding anhydrous ammonia (purity not reported) to manure pits that were not flushed. After 6 weeks of exposure, 20 gilts from each group were sacrificed and sections of the lungs and snout were examined for gross lesions. In addition, the ovaries, uterus, and adrenal glands were weighed. The remaining 20 gilts/group were mated with mature boars and continued being exposed to ammonia until gestation day 30, at which time they were sacrificed. Fetuses were examined for viability, weight, and length and the number of copora lutea were counted.

Gilts exposed to 35 ppm (25 mg/m^3) ammonia gained less weight than gilts exposed to 7 ppm (5 mg/m^3) during the first 2 weeks of exposure (7% decrease, $p < 0.01$), but growth rate recovered thereafter. Mean scores for lesions in the lungs and snout were not statistically different between the two exposure groups and there were no differences in the weight of the ovaries, uterus, and adrenals. Age at puberty did not differ significantly between the two groups, but gilts exposed to 35 ppm (25 mg/m^3) ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 7 ppm (5 mg/m^3). In gilts that were mated, conception rates were similar between the two groups (94.1 vs. 100% in low vs. high exposure). At sacrifice on day 30 of gestation, body weights were not significantly different between the two groups. In addition, there were no significant differences between the two groups regarding percentage lung tissue with lesions and mean snout grade. Number of corpora lutea, number of live fetuses, and weight and length of the fetuses on day 30 of gestation were not significantly different between treatment groups. Diekman et al. (1993) did not identify NOAEL or LOAEL concentrations for

maternal or fetal effects in this study. The EPA did not identify NOAEL or LOAEL values from this study due to the absence of a control group and due to confounding by exposure to bacterial and mycoplasm pathogens.

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute Oral Studies

Two acute oral studies have been conducted using ammonia. Gastrointestinal effects were observed in rats following gavage with ammonium hydroxide (Nagy et al., 1996; Takeuchi et al., 1995). Takeuchi et al. (1995) reported hemorrhagic necrosis of the gastric mucosa in male Sprague-Dawley rats administered ammonium hydroxide once (by gavage) at ≥1% ammonium hydroxide (dose could not be estimated because gavage volume was not reported). Nagy et al. (1996) observed severe hemorrhagic mucosal lesions in female Sprague-Dawley rats (6/group) 15 minutes after exposure to 48 mg/kg ammonium hydroxide via gavage (dose estimated by EPA assuming an average body weight of 0.185 kg).

Leakage of cysteine proteases into the gastric lumen occurred 15 minutes after exposure and gastrointestinal effects were prevented by pretreatment with a sulfhydryl alkylating compound (N-ethylmaleimide) (Nagy et al., 1996).

4.4.2. Acute and Short-Term Inhalation Studies

Several acute and short-term inhalation studies (exposure duration of ≤30 days) have been conducted using ammonia. The lethality of ammonia inhalation has been investigated in rats and mice (rat LC_{50} values ≥11,590 mg/m^3; mouse LC_{50} values ≥2,990 mg/m^3) (Appleman et al., 1982; Kapeghian et al., 1982; Hilado et al., 1977; Silver and McGrath, 1948). Clinical signs of acute ammonia inhalation in rats and mice included eye and nose irritation, dyspnea, ataxia, seizures, coma, and death (Appleman et al., 1982; Kapeghian et al., 1982). Decreased body weight gain was observed in rats, mice, and pigs to ammonia concentrations as low as 400 mg/m^3 (500 ppm) for up to 30 days (Gustin et al., 1994; Urbain et al., 1994; Kapeghian et al., 1982; Richard et al., 1978a).

Ammonia inhalation produced changes in pulmonary function, with decreased respiratory rate observed in rats at concentrations ≥848 mg/m^3 (1,200 ppm) (Rejniuk et al., 2008, 2007), in mice at concentrations ≥214 mg/m^3 (303 ppm) (Kane et al., 1979), and in rabbits at concentrations ≥35 mg/m^3 (50 ppm) (Mayan and Merilan, 1972). Decreased airway pressure and pO_2 were also observed in rabbits exposed to ammonia concentrations ≥24,745 mg/m^3 (35,000 ppm) for 4 minutes (Sjöblom et al., 1999). Histopathological changes in the respiratory tract of ammonia-exposed rats (≥141 mg/m^3; 200 ppm) and mice (≥216 mg/m^3; 305 ppm) included alterations in the nasal and tracheal epithelium (i.e., irritation and inflammation) and pneumonitis, atelectasis, and intralveolar hemorrhage of the lower lung (Buckley et al., 1984; Kapeghian et al., 1982; Richard et al., 1978a; Gamble and Clough, 1976). Dodd and Gross

(1980) showed that pulmonary function deficits measured 1, 7, 21, and 35 days postexposure were correlated with lung histopathology in cats exposed to ammonia (707 mg/m^3 or 1,000 ppm for 10 minutes). Acute effects were followed by chronic respiratory dysfunction that was characterized by secondary bronchitis, bronchiolitis, and bronchopneumonia.

Several studies evaluated cardiovascular and/or metabolic effects of acute or short-term ammonia exposure. Bradycardia was observed in rabbits exposed to 1,768 mg/m^3 (2,500 ppm) for 180 minutes and arterial pressure variations were seen at 3,535 mg/m^3 (5,000 ppm) (Richard et al., 1978b). Acidosis, as evidenced by a decrease in blood pH and an increase in arterial blood pCO_2, occurred in rats exposed to 212 mg/m^3 (300 ppm) ammonia for 5, 10, or 15 days (Manninen et al., 1988) and in rabbits exposed to ≥3,535 mg/m^3 (5,000 ppm) for up to 180 minutes (Richard et al., 1978b). No effects on blood pH, pO_2, or pCO_2 were seen in rats exposed to ≤818 mg/m^3 (1,157 ppm) (Schaerdel et al., 1983) or cattle exposed to concentrations of <71 mg/m^3 (100 ppm) (Mayan and Merilan, 1976). Several studies also investigated amino acid levels and neurotransmitter metabolism in the brain following acute inhalation exposure to ammonia in rats and mice (Manninen and Savolainen, 1989; Manninen et al., 1988; Sadasivudu et al., 1979; Sadasivudu and Murthy, 1978). It has been suggested that glutamate and γ-amino butyric acid (GABA) play a role in ammonia-induced neurotoxicity. These acute and short-term studies are further described in Appendix C.5.

4.4.3. 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). However, there is no evidence that the decreased resistance to infection in these cases represents a primary impairment of the immune system in humans since necrosis of exposed tissues facilitates infection by pathogenic organisms. Nevertheless, animal studies have shown that exposure to airborne ammonia may impair immune function as described below (Gustin et al., 1994; Neumann et al., 1987; Targowski et al., 1984; Schoeb et al., 1982; Richard et al., 1978a; Broderson et al., 1976).

Broderson et al. (1976) exposed Sherman and F344 rats (11–12/sex/dose) continuously to ammonia concentrations of 108 or 212 ppm ammonia (76 or 150 mg/m^3), respectively (from soiled bedding), for 7 days prior to inoculation with *M. pulmonis* and for 30–35 days following inoculation. The matched control groups (bedding changed daily) were exposed to 11 ppm (8 mg/m^3, Sherman rats) or 2 ppm (1 mg/m^3, F344 rats). Additional groups of F344 rats (6/sex/group) were exposed continuously to 25 (two groups), 50, 100, or 250 ppm ammonia (18, 35, 71, or 177 mg/m^3, respectively) in an inhalation chamber for 7 days prior to inoculation with *M. pulmonis* and for up to 42 days following inoculation. Each treatment group had a corresponding control group that was inoculated with *M. pulmonis* in order to produce murine respiratory mycoplasmosis (MRM). Clinical observations were performed (frequency not

given). Rats were sacrificed at the end of the exposure period and tissues were prepared for histopathological examination of nasal passages, middle ear, trachea, lungs, liver, kidneys, adrenal, pancreas, testicle, spleen, mediastinal lymph nodes, and spleen.

Typical signs of MRM (i.e., snuffling, head shaking) began in all rat groups approximately 10 days after inoculation. Rats exposed to ammonia exhibited dyspnea and hyperpnea, had rough coats, and sat in a hunched posture. All ammonia concentrations significantly increased the severity of rhinitis, otitis media, tracheitis, and pneumonia characteristic of *M. pulmonis*. Furthermore, there was a significant concentration response between observed respiratory lesions and increasing environmental ammonia concentration for gross and microscopic lesions (see Table 4-9). All lesions observed were characteristic of MRM. Gross bronchiectasis and/or pulmonary abscesses and the extent of gross atelectasis and consolidation were consistently more prevalent in exposed animals at all ammonia concentrations than in their corresponding controls. Increasing ammonia concentration was associated with an increasing rate of isolating of *M. pulmonis* from the respiratory tract. EPA identified a LOAEL of 18 mg/m^3 (25 ppm) for increased severity of rhinitis, otitis media, tracheitis, and pneumonia, and increased incidence of respiratory lesions in F344 rats. A NOAEL was not identified in this study.

Table 4-9. Incidence of pulmonary lesions in rats inoculated with *M. pulmonis* and exposed to ammonia (7 days later for 28–42 days)

Ammonia concentration (mg/m^3)	Incidence of gross lesions (%)		Incidence of microscopic lesions (%)	
	Ammonia	Matched control	Ammonia	Matched control
Soiled bedding				
76	20/24 (83%)[a]	10/23 (43%)	23/24 (96%)[a]	14/23 (61%)
150	6/22 (27%)	2/24 (8%)	19/22 (86%)[a]	8/24 (33%)
Inhalation chamber[b]				
18	5/12 (42%)	1/12 (8%)	9/12 (75%)	6/12 (50%)
18	6/12 (50%)	4/12 (33%)	9/12 (75%)	7/12 (58%)
40	8/12 (66%)	2/12 (17%)	10/12 (83%)	9/12 (75%)
70	4/12 (33%)	1/12 (8%)	12/12 (100%)	6/12 (50%)
180	10/12 (83%)	2/12 (17%)	12/12 (100%)	2/12 (17%)

[a]$p \leq 0.01$, χ^2 test comparing ammonia-exposed group to matched controls.
[b]$p \leq 0.01$, χ^2 test comparing the sum of all ammonia group to the sum of matched controls. Regression analysis showed that increasing concentrations of ammonia were related to the increased incidence of gross and microscopic lesions.

Source: Broderson et al. (1976).

Richard et al. (1978a) exposed groups of 99 male OFI mice continuously to ammonia concentrations of 0 or 500 ppm (0 or 354 mg/m^3) for 8 hours and for 168 hours prior to infection

by aerosolized *P. multocida* (2-minute exposure to a predetermined 50% lethal concentration of bacteria). Mice were observed for 12 days for mortality. At 168 hours, a significant increase in mortality was observed among ammonia-exposed mice compared to controls (+36%) (Table 4-10). Based on supporting findings in rat studies conducted by these same researchers (summarized under Section 4.4.2), Richard et al. (1978) suggested that the irritating action of ammonia destroys the tracheobronchial mucosa and causes inflammatory lesions so that the sensitivity to respiratory infection increased with exposure to ammonia for 168 hours, while the same exposure for 8 hours was not sufficient to increase this sensitivity.

Table 4-10. Mortality in *P. multocida*-infected mice exposed to ammonia for 8 or 168 hours

Exposure duration	Controls	Exposed (400 mg/m^3)
8 h	23/49 (47%)[a]	21/49 (43%)
168 h	25/50 (50%)	42/49 (86%)[b]

[a]Number of deaths/total number of animals (percentage mortality).
[b]Significantly different from controls ($p < 0.05$, Student's t-test).

Source: Richard et al. (1978a).

Schoeb et al. (1982) exposed pathogen-free F344 rats (5–15 rats/dose) continuously to ammonia concentrations of ≤2 ppm (≤1 mg/m^3; controls, air concentration was usually not measureable and never exceeded 2 ppm) or 100 ppm (71 mg/m^3) for either 7 days prior to inoculation with *M. pulmonis*, or simultaneously with inoculation, for up to 28 days. Rats were sacrificed in groups of five at unspecified intervals up to 28 days following inoculation. At sacrifice, serum was collected from each rat for determination of IgG and IgM antibodies against *M. pulmonis* by enzyme-linked immunosorbent assay. Nasal passages, larynges, tracheas, and lungs were collected for quantitative assessment of *M. pulmonis* growth. Growth of *M. pulmonis* increased most rapidly in the nasal passages and subsequently in the larynx, trachea, and lungs in both control and exposed rats. However, overall growth of *M. pulmonis* was much greater in ammonia-exposed rats than controls. Serum immunoglobin antibody responses (IgG and IgM) were also greater in ammonia-exposed rats than in controls. Schoeb et al. (1982) also conducted two additional in vivo experiments to evaluate pulmonary absorption and clearance of ammonia from the respiratory tract and one in vitro experiment to evaluate whether increased populations of *M. pulmonis* in ammonia-exposed rats are the result of direct enhancement of growth of the organism or indirect effects on the host. In one in vivo experiment, 11 rats that were exposed in the first experiment to ammonia at 100 ppm (71 mg/m^3) for 1 week were exposed under anesthesia to ammonia via face mask at ≤500 ppm (354 mg/m^3) for an unspecified length of time. Tracheas were surgically exposed, cannulated, and attached to a gas detector for measurement of ammonia concentration in the trachea. Ammonia was not detected in the

tracheal air of any rats during exposure to <500 ppm (<354 mg/m^3), and measured 10–20 ppm (7–14 mg/m^3) in some rats (number not specified) at 500 ppm (354 mg/m^3). These results indicate that virtually all ammonia is absorbed in the nasal passages in rats exposed to <500 ppm (<354 mg/m^3) ammonia. In the other in vivo experiment, groups of 38 F344 rats were exposed to either 0 or 100 ppm (0 or 71 mg/m^3) ammonia for 1 week prior to inoculation with radiolabeled *Staphylococcus epidermis* (a bacterium commonly present on human skin) to measure pulmonary bacterial clearance. Following inoculation, half of the exposed animals and half of the controls were sacrificed immediately and the remaining rats were sacrificed 6 hours later. There were no significant differences between ammonia-exposed and control rats regarding the rate of pulmonary clearance (combined results showed 91 ± 6% clearance of bacteria for both groups in 6 hours). In vitro, growth of *M. pulmonis* was inhibited by 1 mM ammonium ion added to a culture medium as ammonium hydroxide. Lower concentrations (0.01, 0.1, and 0.5 mM) had no effect. The study authors suggest that these data indicate that ammonia increases growth of *M. pulmonis* indirectly through effects on the host.

Hartley guinea pigs (8/concentration, sex not specified) vaccinated with *Mycobacterium bovis* BCG and challenged intradermally with 2.5 µg of purified protein derivative (PPD) of tuberculin were continuously exposed to ammonia concentrations of 50 or 90 ppm (35 or 64 mg/m^3) for 3 weeks (Targowski et al., 1984). An additional eight PPD-positive guinea pigs exposed to a normal environment with ammonia concentrations <15 ppm (<11 mg/m^3) served as controls. Guinea pigs were again challenged intradermally with PPD following the exposure period. Clinical observations were recorded daily and body weights were measured at the beginning and end of the experiment. Blood samples collected at the beginning and end of the experiment were submitted for determination of ammonia content and hematology (red blood cells and white blood cells). Cultures of separated blood lymphocytes and bronchial leukocytes were evaluated for responsiveness to mitogens (1 µg/mL phytohemagglutin [PHA] or concanavalin A) or 24 µg/mL PPD. Cultures of alveolar and bronchial macrophages from control and exposed guinea pigs were measured for bactericidal and phagocytic capacities in the presence of *Staphylococcus aureus*. Targowski et al. (1984) also evaluated the effect of ammonia exposure on the in vitro immune response in lymphocytes and macrophages collected from five normal Hartley guinea pigs. The percentage of viable lymphocytes (unstained) was determined by trypan blue exclusion test at 0, 2, 4, 24, 48, and 72 hours following incubation in medium alone or medium containing ammonia concentrations ranging from 1 to 100 mg/L (ammonium hydroxide added to medium). Macrophages were incubated with concentrations of ammonia ranging from 0.1 to 50 mg/L for 1 hour prior to the addition of *S. aureus*.

Guinea pigs exposed to ammonia at 50 or 90 ppm (35 or 64 mg/m^3) did not exhibit any significant changes in body weight and did not show any notable signs of distress, conjunctivitis, or respiratory disease (Targowski et al., 1984). Dermal response to PPD measured as the diameter of erythema was significantly less at $p < 0.05$ (Student's t-test) in guinea pigs exposed

to 64 mg/m^3 at 24, 72, and 96 hours compared to controls (Table 4-11). No significant hematological changes were observed between exposed and control groups. The response to mitogens and PPD of blood lymphocytes and bronchial lymphocytes from animals exposed to 90 ppm (64 mg/m^3) ammonia was significantly less than the response from control animals ($p < 0.01$) (shown graphically by authors). The lymphocyte response for guinea pigs exposed to 50 ppm (35 mg/m^3) ammonia was similar to control. In vitro exposure of blood lymphocytes to ammonia at 10 or 100 mg/L resulted in decreased viability and reduced response to PHA stimulation (significantly different from controls at $p < 0.01$). Lower concentrations of ammonia (≤1 mg/L) did not significantly affect viability or suppress the responsiveness of lymphocytes in vitro. Bactericidal and phagocytic activity of alveolar macrophages from animals exposed to ammonia was not significantly affected. Similarly, phagocytic activity was not significantly affected in vitro following treatment with ammonia at 1–100 mg/L. However, a significant inhibition in the macrophage bactericidal activity was observed in vitro at 0.01 and 0.05 mg/L ($p < 0.01$). No significant changes in bactericidal activity were observed in cultures treated with ammonia at ≤1 mg/L.

Table 4-11. Dermal response to the injection of tuberculin in animals exposed to ammonia for 3 weeks (mean diameter of redness in mm)

Time of observation (hrs)	Ammonia concentration (mg/m^3)		
	Control	35	64
24	17.6 ± 1.7[a]	15.2 ± 2.23	14.4 ± 2.2[b]
48	15.5 ± 1.9	13.8 ± 1.6	14.7 ± 1.8
72	12.0 ± 1.1	12.6 ± 1.0	8.7 ± 1.4[b]
96	10.4 ± 1.5	12.6 ± 0.7	0.0[b]

[a]Mean ± standard deviation.
[b]Significantly different from controls ($p < 0.05$, Student's t-test).

Source: Targowski et al. (1984).

Neumann et al. (1987) conducted three experiments to evaluate the ability of unweaned piglets exposed to ammonia to fight off infection following an intranasal challenge with *P. multocida* (Carter type A). In the first experiment (Trial 1), unweaned piglets were exposed to ammonia at 50 ppm (35 mg/m^3) for 42 days either without additional treatment (22 piglets) or following intranasal challenge with *P. multocida* (10 piglets). Groups of 8–10 piglets were also exposed for 35 days during the second experiment (Trial 2) under these same conditions. A thermo-motor stress swim test (water temperature was 15°C) was also included as an additional condition during Trial 2. During the third experiment (Trial 3), all three conditions (i.e., ammonia inhalation, intranasal challenge with *P. multocida* and a thermo motor swim test) were tested in groups of 8–10 piglets; however, the piglets were exposed to 100 ppm (71 mg/m^3)

ammonia for 31 days. The test groups were compared to corresponding controls during each trial. Blood samples were collected on multiple days during each trial and analyzed for nucleolar activity of peripheral lymphocytes and determination of gamma globulins. Lavage fluid from the lungs was collected on multiple days to characterize differentiation of bronchoalveolar cells. Additionally, on the 23rd day of exposure, a small test group (five test and five control pigs from Trial 2) was exposed to a radioactive aerosol (Tc^{99m}-tagged sulfur colloid complex suspended in NaCl) through a breathing mask for 10 minutes to evaluate pulmonary aerosol distribution patterns. During this test, the dorsal pulmonary radioactivity was recorded using a gamma camera.

Lymphocyte nucleolar activity increased in a dose-related manner following ammonia exposure (data not shown) (Neumann et al., 1987). A more pronounced increase was observed in the presence of infection and was further intensified under additional thermo-motor stress. Neumann et al. (1987) suggested that ammonia concentrations of 35 and 71 mg/m^3 can cause activation of RNA synthesis in the cell nucleus of the peripheral lymphocytes of piglets, as evidenced by an increase in the percentage of lymphocytes with compact nucleoli. Gamma globulin concentrations were not significantly affected at 35 mg/m^3 ammonia, but in pigs inhaling 71 mg/m^3 ammonia, gamma globulin concentrations were significantly lower than controls ($\alpha = 0.01$, Wilcoxon, Mann, and Whitney U-test) as early as 8 days after starting the test. This effect was intensified in the presence of infection and additional thermo motor stress. No differences in bronchoalveolar cells were observed in pigs exposed to either 35 or 71 mg/m^3 compared to controls. However, a significantly higher proportion of neutrophils was detected in lavage fluid from infected pigs exposed to 35 mg/m^3 ammonia compared with infected controls at 7 days postinfection ($\alpha = 0.02$, Wilcoxon, Mann, and Whitney U-test). Under additional stress, the proportion of neutrophils at 7 days post infection was slightly less than in pigs that were not put under additional stress, but remained significantly elevated above controls ($\alpha = 0.10$, Wilcoxon, Mann, and Whitney U-test). In addition, the proportion of alveolar macrophages in the lavage fluid of pigs exposed to 35 mg/m^3 ammonia was significantly lower in infected pigs ($\alpha = 0.05$, Wilcoxon, Mann, and Whitney U-test) and in infected pigs subjected to the swimming test ($\alpha = 0.10$, Wilcoxon, Mann, and Whitney U-test) at postinfection day 7. Under these conditions, pigs exposed to 71 mg/m^3 demonstrated similar differentiation in bronchoalveolar cells with controls at postinfection day 7. However, significant differences from controls in the proportions of alveolar macrophages and neutrophils were observed at postinfection day 3 among these pigs. In summary, results of the experiments conducted by Neumann et al. (1987) suggested that ammonia exposure in young pigs has the potential to reduce systemic resistance to infection as evidenced by lower serum gamma globulin concentrations and elevated nucleolar activity of peripheral lymphocytes among exposed piglets. Local resistance to infection in the lungs measured by pulmonary accumulation of aerosol particles and quantitative cytology of the broncho-alveolar space was also reduced in piglets

exposed to ammonia. This study suggests that ammonia toxicity may play an important role in infectious respiratory diseases of swine.

F344/N rats (48/group, sex not specified) inoculated with *M. pulmonis* were exposed to ammonia concentrations of 0 or 100 ppm (0 or 71 mg/m^3) for 3, 5, 7, or 9 days (Pinson et al., 1986). Following the exposure periods, rats were killed and their respiratory organs were collected for histological examination. Ammonia-exposed rats exhibited hyperplastic and degenerative changes in the anterior nasal epithelium. Lesions of mycoplasmosis were more severe in ammonia-exposed rats than controls.

Gustin et al. (1994) injected endotoxin (*E. coli* O127:B8) into the ventilated perfused lungs collected from 7 to 23 male Belgian Landrace pigs exposed to ammonia concentrations of 0, 25, 50, or 100 ppm (0, 18, 35, or 71 mg/m^3) ammonia for 6 days. Lethargy and decreased body weight gain were observed at all ammonia concentrations. Blood samples taken on the first and last day of the exposure period revealed no changes in the level of cortisol, total leukocyte numbers, or in differential leukocyte percentages. No significant differences were observed on the baseline pulmonary hemodynamics and microvascular permeability in perfused lungs from any control or exposed pigs (see Section 4.4.2). However, the endotoxin-induced vascular reaction was strongly altered in lungs from pigs exposed to 100 ppm (71 mg/m^3) where the endotoxin-induced increase in total blood flow resistance was completely abolished. The vascular response to endotoxin was also reduced in pigs exposed to 50 ppm (35 mg/m^3). In summary, concentrations of ammonia >50 ppm (35 mg/m^3) were shown to modulate the pulmonary vascular response to endotoxins in pig lungs in vitro.

4.5. MECHANISTIC STUDIES

Studies have been conducted to investigate the mechanisms by which ammonia induces irritation and effects on the gastric mucosa. A limited number of studies have looked at the potential for ammonia to induce genetic changes, although the available studies of ammonia genotoxicity are inadequate to characterize the genotoxic potential of this compound.

Studies conducted to examine the mechanisms of ammonia-related effects on the central nervous system and kidney were largely conducted using ammonium salts (ATSDR, 2004); however, as discussed in Section 4, because it is unclear the extent to which the anion can influence the toxicity of the ammonium salt, these mechanistic studies are not further reviewed in this assessment. A more detailed review of mechanistic studies of ammonia is provided as supplemental information in Appendix C.6.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

The only available data for oral exposure to ammonia in humans consists of case reports of individuals involved in the ingestion of household cleaning solutions containing ammonia or

biting into capsules of ammonia smelling salts (Dworkin et al., 2004; Rosenbaum et al., 1998; Christesen, 1995; Wason et al., 1990; Lopez et al., 1988; Klein et al., 1985; Klendshoj and Rejent, 1966). Clinical signs reported in the case studies included headache, stomachache, nausea, dizziness, diarrhea, drooling, erythematous and edematous lips, reddened and blistered tongues, dysphagia, vomiting, oropharyngeal burns, laryngeal and epiglottal edema, erythmatous esophagus with severe corrosive injury, and hemorrhagic esophago-gastro-duodeno-enteritis.

Oral toxicity data in animals are limited to 4- and 8-week drinking water studies that examined the effects of ammonia on rat gastric mucosa (Tsujii et al., 1993; Kawano et al. 1991), a gavage study in rabbits exposed to ammonium hydroxide for up to 142 days (Fazekas, 1939), and a lifetime drinking water study in mice (Toth, 1972). Kawano et al. (1991) reported that ammonia administered to Sprague Dawley rats at concentrations of 0, 0.01 and 0.1% for two or four weeks resulted in a statistically significant decrease in mean antral mucosal thickness; the magnitude of the effect increased with dose and duration of exposure. In a follow-up study, Tsujii et al. (1993) reported that treatment with 0.01% ammonia for increased epithelial cell migration in the mucosa of the rat stomach, and in particular in the antrum, leading to a decrease in mucosal thickness and to mucosal atrophy. Fazekas (1939) reported initial decreases in body weights and blood pressure, and elevated adrenal weights in rabbits exposed to ammonium hydroxide at 0.5 or 1% in solution for up to 142 days. Histological changes in the adrenal cortex were also reported. Toth (1972) conducted the only chronic oral study of ammonia in experimental animals, but did not evaluate noncancer endpoints.

4.6.2. Inhalation

Human and animal data indicate that the primary effects of ammonia inhalation are irritation and burns and that the primary targets of ammonia toxicity are the respiratory tract, eyes, and skin (Kerstein et al., 2001; George et al., 2000; Latenser and Lucktong, 2000; Morgan, 1997; de la Hoz et al., 1996; Leduc et al., 1992; Holness et al., 1989; Millea et al., 1989; Weiser and Mackenroth, 1989; Burns et al., 1985; Flury et al., 1983; Price et al., 1983; Close et al., 1980; Hatton et al., 1979; Sobonya, 1977; Verberk, 1977; Taplin et al., 1976; Couturier et al., 1971; Heifer, 1971; Slot, 1938).

Studies documenting inhalation exposure to ammonia in humans include numerous case reports following acute exposures to high concentrations of ammonia (i.e., accidental spill or release), controlled exposure studies involving exposing volunteers to ammonia vapors to evaluate irritation effects and changes in pulmonary function, studies in livestock farmers, and studies of occupational exposures. Effects reported in case reports of human exposure included acute point-of-contact irritation effects (e.g., burns to body surfaces, mouth, and respiratory tract) and delayed effects including restricted pulmonary function (see Section 4.1.1 and Appendix C.2). Inhalation effects in these studies were often accompanied by dermal and ocular irritation. Primary effects reported in volunteer studies were eye, nose, and throat irritation (see Section

4.1.2 and Appendix C.3). Pulmonary function was not affected in volunteers acutely exposed to ammonia concentrations as high as 71 mg/m^3 (100 ppm). Studies comparing the pulmonary function of asthmatics and healthy volunteers exposed to ammonia do not suggest that asthmatics are more sensitive to the pulmonary effects of ammonia. Studies in livestock farmers demonstrated an association between ammonia exposure and impaired respiratory function, but ammonia exposures in these studies were generally confounded by concomitant exposures to airborne dust, bacteria, fungal spores, endotoxin, and mold (see Section 4.1.3 and Appendix C.4). Studies of industrial exposures in workers reported pulmonary effects including coughing, wheezing, phlegm, chest tightness, shortness of breath, and decreased respiratory function (Table 4-12) (Rahman et al., 2007; Ali, 2001; Ballal et al., 1998; Holness et al., 1989).

There are no chronic inhalation studies of ammonia in animals. The observed effects in subchronic animal studies in which ammonia was administered via inhalation included inflammatory changes in the nasal mucosa and tracheal epithelium of rats and pigs (Broderson et al., 1976; Doig and Willoughby, 1971), and histological changes in the livers, kidneys, lung, spleen, and adrenal gland of rats and guinea pigs (Coon et al., 1970; Weatherby, 1952) (Table 4-12). Exposure durations in these studies ranged from 5 to 18 weeks. NOAELs and LOAELs did not vary greatly across species and strains. LOAELs ranged from 18 to 127 mg/m^3 and NOAELs ranged from 7 to 53 mg/m^3. In three studies, effects were observed at the lowest dose, and NOAELs could not be identified. In the only available study of developmental toxicity of ammonia, no maternal or developmental effects were observed in pigs following gestational exposure to ammonia concentrations up to 25 mg/m^3 (Diekman et al., 1993).

Acute and short-term studies in animals following inhalation exposure demonstrate eye and nose irritation, dyspnea, ataxia, seizures, coma, and death in rats and mice (rat LC_{50} values $\geq$11,590 mg/m^3; mouse LC_{50} values $\geq$2,990 mg/m^3), decreased pulmonary function with decreased respiratory rate in rats at concentrations $\geq$848 mg/m^3, mice at concentrations $\geq$214 mg/m^3, and in rabbits at concentrations $\geq$35 mg/m^3. Histopathological changes in the respiratory tract following acute exposure to ammonia include irritation and inflammation of the nasal and tracheal epithelium, pneumonitis, atelectasis, and intralveolar hemorrhage of the lower lung.

Table 4-12. Summary of noncancer results of human occupational studies and repeat-dose studies in experimental animals involving inhalation exposure to ammonia

Exposed population	Exposure concentration (mg/m^3)/ duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effects at the LOAEL concentration	Comment	Reference
Occupational worker studies						
Industrial workers (58 male workers and 31 male controls from office area)	low (<4.4), medium (4.4–8.8), and high (>8.8) avg exposure: 12 y	8.8 (adjusted: 3.1)[a]	Not determined		Cross-sectional study in soda ash plant. No differences in pulmonary function or subjective symptomology relative to the control group	Holness et al., 1989
Industrial workers (63 ammonia plant and 77 urea plant workers; 25 controls from administration building)	ammonia plant (mean): 4.9 urea plant (mean): 18.5 mean employment duration: 16 y	4.9	18.5	Increased prevalence of respiratory symptoms and decrease in lung function	Cross-sectional study in urea fertilizer factory. Concentrations in this table were based on the PAC III method; concentrations measured using the Dräger tube method were 4-5 times higher.	Rahman et al., 2007
Experimental animal studies						
Rat (Sprague-Dawley and Longs-Evans) (15/sex/group)	0, 40, 127, 262, 455, or 470 for 90–114 d	40	127	Nonspecific inflammatory changes in the lungs and kidneys	Nasal discharge and nonspecific circulatory and degenerative changes in the lungs and kidneys were observed at 262 mg/m^3 (not further described, incidence not reported). Frank-effect-level of 455 mg/m^3 based on 90–98% mortality	Coon et al., 1970
Sherman rat (5/sex/group)	7 or 106 for 75 d	7	106	Nasal lesions		Broderson et al., 1976
F344 rat (6/sex/group)	0, 18, 35, 71, or 177 for up to 49 d	Not determined	18	Increased severity of rhinitis, otitis media, tracheitis, and pneumonia, and increased incidence of respiratory lesions	Rats were exposed continuously to ammonia for 7 days prior to inoculation with *M. pulmonis* and 28–42 days following inoculation	Broderson et al., 1976
Guinea pigs (12 guinea pigs, sex not specified)	0 or 120, 6 hrs/d, 5 d/wk for 18 wks	Not determined	120	Congestion of the spleen, liver, and kidneys and early degenerative changes in the adrenal gland		Weatherby, 1952

Table 4-12. Summary of noncancer results of human occupational studies and repeat-dose studies in experimental animals involving inhalation exposure to ammonia

Exposed population	Exposure concentration (mg/m^3)/ duration	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Effects at the LOAEL concentration	Comment	Reference
Yorkshire-Landrace pig (6/group, sex not specified)	0 or 71 for 6 wks	Not determined	71	Thickening of the tracheal epithelium with a concomitant decrease in the number of tracheal epithelial goblet cells	The mean concentration of ammonia in the control chamber was measured at 5.6 mg/m^3	Doig and Willoughby, 1971
Pig (4–8/group, sex not specified)	0, 35, or 53 for 109 d	53	Not identified			Curtis et al., 1975
Pig (24 pigs/group, several breeds, sex not reported)	0, 0.4, 7, 13.3, or 26 for 5 wks	26	Not identified		Exposures were to ammonia at concentrations of 0, 0.4, 7, 13.3, or 26 mg/m^3 and to inhalable dust at 1.2, 2.7, 5.1, or 9.9 mg/m^3; all pigs including controls demonstrated infections common to pigs on commercial farms	Done et al., 2005

[a]Adjusted to continuous exposure as follows: $NOAEL_{ADJ} = NOAEL \times VE_{ho}/VE_h \times 5 \text{ days}/7 \text{ days} = 8.8 \text{ mg/m}^3 \times 10 \text{ m}^3/20 \text{ m}^3 \times 5 \text{ days}/7 \text{ days} = 3.1 \text{ mg/m}^3$, where:

VE_{ho} = human occupational default minute volume and VE_h = human ambient default minute volume.

4.6.3. Mode-of-Action Information

Inhalation exposure to ammonia is associated with upper respiratory irritation in humans and animals. Ammonia interacts with moisture along the respiratory tract to form ammonium hydroxide, which causes necrosis of tissue through saponification leading to inflammation (Amshel et al., 2000; Jarudi and Golden, 1973).

Oral studies with *H. pylori* suggest possible mechanisms by which ammonia may cause gastric mucosal damage, namely, increased cell vacuolation and decreased viability of the cells (Mégraud et al., 1992), increased release of endothelin-1 and thyrotropin releasing hormone from the gastric mucosa (Mori et al., 1998), release of cysteine proteases in the stomach that contribute to the development of gastric hemorrhagic mucosal lesions (Nagy et al., 1996) and apoptosis directed by mitochondrial membrane destruction (Suzuki et al., 2000).

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of ammonia based on the absence of ammonia carcinogenicity studies in humans, and a single lifetime drinking water study of ammonia in mice that showed no evidence of carcinogenic potential.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Human data on the carcinogenic effects of ammonia are not available. Animal carcinogenicity data are limited. Toth (1972) did not observe any evidence for carcinogenicity of ammonium hydroxide administered orally to mice over a lifetime. Tsujii et al. (1995, 1992) suggest that ammonia administered in drinking water may act as a cancer promoter in *H. pylori* induced gastric cancer. In these studies (Tsujii et al., 1995, 1992), rats dosed with ammonia and pretreated with MNNG had a greater incidence of gastric cancer and number of tumors per tumor-bearing rat than rats receiving only MNNG and tap water (Tsujii et al., 1995, 1992), suggesting that ammonia can act as a tumor promoter. The available studies of ammonia genotoxicity are inadequate to characterize the genotoxic potential of this compound.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

There are no studies of the toxicity of ammonia in children comparing them to any other life stages that would support an evaluation of childhood susceptibility. Case reports of acute exposure of children (<18 years old) to ammonia are summarized in Table C-1 (Dilli et al., 2005; Dworkin et al., 2004; Rosenbaum et al., 1998; Wason et al., 1990; Millea et al., 1989; Lopez et al., 1988; Klein et al., 1985; Close et al., 1980; Hatton et al., 1979; Helmers et al., 1971; Levy et al., 1964); however, these studies do not provide information useful for evaluation of

susceptibility from chronic low-level exposure to ammonia. No experimental animal studies of ammonia were identified that compared effects in juvenile animals to adults that could be used to inform childhood susceptibility in humans.

4.8.2. Possible Gender Differences

There are no studies of gender differences in susceptibility to the toxic effects of ammonia; information from the available toxicity studies of ammonia provides no evidence of gender differences.

4.8.3. Other Susceptible Populations

Persons who suffer from severe liver or kidney disease (including cirrhosis, acute renal failure, or liver failure) may be more susceptible to ammonia intoxication (hyperammonemia), as it is chiefly by the actions of these organs that ammonia is biotransformed and excreted (Córdoba et al., 1998; Gilbert, 1988; Jeffers et al., 1988; Souba, 1987). Individuals with hereditary urea cycle disorders are also at risk (Schubiger et al., 1991). In these disease conditions, it is the brain where ammonia is most toxic, and the elevated ammonia levels that accompany human diseases such as acute liver or renal failure can predispose an individual to encephalopathy; these effects are especially marked in newborn infants (Miñana et al., 1995; Souba, 1987).

Individuals with respiratory disease (e.g., asthma) also could represent a susceptible population; however, controlled human studies that examined both healthy volunteers and volunteers with asthma, as well as cross sectional studies of livestock farmers, exposed to ammonia (Petrova et al., 2008; Sigurdarson et al., 2004; Vogelzang et al., 2000, 1998, 1997; Preller et al., 1995) generally did not observe a greater sensitivity to respiratory effects in populations with underlying respiratory disease. However, the findings from an epidemiological study of a group of workers chronically exposed to airborne ammonia indicated that ammonia inhalation can exacerbate existing symptoms, including cough, wheeze, nasal complaints, eye irritation, throat discomfort, and skin irritation (Holness et al., 1989).

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

An RfD for ammonia was not derived because the available oral data for ammonia were considered insufficient to support derivation of a chronic reference value. As discussed in Sections 4.1.1, 4.2.1, and 4.6.1, oral toxicity data in humans are limited to case reports of individuals suffering from acute neurological (e.g., headache, dizziness) and gastrointestinal (e.g., stomach ache, nausea, diarrhea, distress and burns along the digestive tract) effects from ingesting household cleaning solutions containing ammonia or biting into capsules of ammonia smelling salts. In animals, the only chronic toxicity study of ammonia is the lifetime carcinogenicity drinking water study in mice by Toth (1972) that reported tumor incidence only and did not provide noncancer data to support development of an RfD. A subchronic gavage study in rabbits exposed to ammonium hydroxide for up to 142 days (Fazekas, 1939) is available but was inadequate for deriving an oral RfD because of limited reporting of study details and results, as well as inadequate study design. The only remaining oral study is an eight-week drinking water study in Sprague Dawley rats (Tsujii et al., 1993) that examined the effects of ammonia on the gastric mucosa.

Tsujii et al. (1993) found that ammonia at a concentration of 0.01% (equivalent to a daily dose of 33 mg/kg-day) increased epithelial cell migration in the mucosa of the stomach (in particular the antrum) leading to a statistically significant decrease in the thickness of the antral mucosa at 4 and 8 weeks of treatment; there was no effect on the body mucosa. The authors reported that the gastric mucosal effects observed in rats resemble mucosal changes in human atrophic gastritis. EPA identified a LOAEL for the Tsujii et al. (1993) study of 33 mg/kg-day based on decreased gastric mucosal thickness, an effect considered by EPA to be adverse; a NOAEL was not identified. The Tsujii et al. (1993) study and decreased antral mucosal thickness were considered as a potential principal study and critical effect. EPA identified a potential point of departure (POD) based on the LOAEL of 33 mg/kg-day; BMD modeling was not utilized because this single-concentration study is not amenable to dose-response analysis.

In EPA's guidance document entitled, *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011a), the Agency endorses a hierarchy of approaches for converting doses administered orally to laboratory animal species to human equivalent oral exposures in deriving the RfD, with the preferred approach being physiologically-based toxicokinetic modeling. An alternate approach includes using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. In lieu of a toxicokinetic model or chemical-specific data to inform the generation of human equivalent oral exposures, EPA endorses body weight scaling to the ¾ power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from

laboratory animals to humans for the purpose of deriving an RfD. When $BW^{3/4}$ scaling is used in deriving the RfD, EPA also advocates a reduction in the interspecies uncertainty factor (UF_A) from 10 to 3, as $BW^{3/4}$ scaling addresses predominantly toxicokinetic (and some toxicodynamic) aspects of the UF_A.

The guidance raises some important uncertainties in applying allometric (more specifically $BW^{3/4}$) scaling when the critical effect used to derive the reference value is a portal-of-entry effect. No physiologically-based pharmacokinetic model or chemical-specific information exists to inform the generation of human equivalent oral exposures for ammonia. Furthermore, the potential critical effect (i.e., decreased gastric antral mucosal thickness) is considered a portal-of-entry effect based on the following. Tsujii et al. (1993) postulated that the difference in response of the mucosa in the stomach body versus the mucosa of the antrum relates to differences in pH in the two stomach regions. Most ammonia is transformed to ammonium ion in solution at physiological pH; the ratio of ammonia to ammonium ion increases 10-fold with each unit rise in pH. In the mucosa of the stomach body—an acid-secreting mucosa—ammonia is protonated to the ammonium ion, which reduces the cytotoxicity associated with nonionized ammonia. In the antral mucosa—a nonacid secreting area of the stomach—pH is higher, resulting in a relatively higher concentration of ammonia and thus enhanced cytotoxicity. Because ammonia toxicity appears to be a function of the physical/chemical environment at the mucosal surface (i.e., a portal-of-entry effect) and it is not clear if regions of the stomach scale allometically across species, a surface area adjustment would be the most relevant for interspecies extrapolation; however, a dose scaling approach involving mass per unit surface area has not been developed (U.S. EPA, 2011a). Therefore, because effects on the gastric antral mucosa are not expected to scale allometrically, a $BW^{3/4}$ scaling approach has not been applied as a default approach (in combination with a reduced default UF for interspecies extrapolation).

An RfD is derived by dividing the POD by a composite uncertainty factor (UF). The UFs, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty. Considering the available oral data, the composite UF for ammonia would be 10,000. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the RfD/RfC technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of uncertainty, or when the total uncertainty factor is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Therefore, consistent with the recommendations in U.S. EPA (2002), the available oral data for ammonia were considered insufficient to support reference value derivation and an RfD for ammonia was not derived.

Route-to-route extrapolation of the inhalation data was considered for deriving the oral RfD; however, in the absence of a PBPK model and because the critical effect from the

inhalation literature is a portal-of-entry effect (respiratory symptoms and lung function changes), route-to-route extrapolation is not supported.

5.1.1. Previous RfD Assessment

No RfD was derived in the previous IRIS assessment for ammonia.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Inhalation studies of ammonia exposure in humans include numerous case reports following acute exposures to high concentrations (e.g., accidental spills/releases), controlled exposure studies involving volunteers exposed to ammonia vapors for short periods of time to evaluate irritation effects and changes in pulmonary function, studies in livestock farmers, and studies of industrial occupational exposures comparing the prevalence of acute respiratory symptoms and changes in lung function between exposed and nonexposed worker populations. Studies in experimental animals (including rats, guinea pigs, and pigs) have also examined respiratory and other systemic effects of ammonia following subchronic inhalation exposures.

Acute exposure studies (i.e., case reports, controlled volunteer studies) involved exposures too brief in duration to be used for derivation of a chronic RfC. Further, case reports of acute exposure do not typically have the appropriate exposure information necessary for a valid evaluation of dose response. Studies of livestock farmers are also not suitable for dose-response assessment because of multiple and possibly confounding exposures to dusts, endotoxins, bacteria, fungi, molds, and other chemicals (including potentially volatile chemicals for which monitoring data have not been collected).

There are two occupational studies of ammonia exposure in workers for which quantitative dose-response information is available. Holness et al. (1989) reported similar odor detection thresholds, lung function, and prevalence in the reporting of acute respiratory symptoms between workers exposed to a mean TWA ammonia concentration of 9.2 ppm (6.5 mg/m^3) and a nonexposed worker population. When the exposed workers were grouped into three exposure categories (high: ≥12.5 ppm [8.8 mg/m^3], medium: 6.25–12.5 ppm [4.4–8.8 mg/m^3], and low: ≤6.25 ppm [4.4 mg/m^3]), no statistically significant differences ($p < 0.05$) in symptoms or changes in lung function between the groups were evident. Based on lack of symptomology and changes in lung function, EPA identified a NOAEL of 12.5 ppm (8.8 mg/m^3) for the Holness et al. (1989) study based on the grouping of workers into three exposure categories. In a more recent occupational study, Rahman et al. (2007) reported a significantly higher prevalence of acute respiratory symptoms among workers exposed to 26.1 ppm (18.5 mg/m^3) ammonia in the urea plant of a fertilizer factory than in workers exposed to 6.9 ppm (4.9 mg/m^3) ammonia in the ammonia plant or nonexposed workers in the administration building. Furthermore, workers in the urea plant demonstrated significant cross-

shift (comparing preshift measurements to postshift measurements) changes in lung function (i.e., FVC and FEV decreased significantly). A similar cross-shift change in lung function was not observed among workers in the ammonia plant. EPA identified a NOAEL (4.9 mg/m^3) and a LOAEL (18.5 mg/m^3), based on increased prevalence of respiratory symptoms and decrease in lung function from the Rahman et al. (2007) study. The findings from Holness et al. (1989) and Rahman et al. (2007) are supported by the results from the cross-sectional studies of fertilizer factory workers by Ballal et al. (1998) and Ali (2001), which suggest that occupational exposure to ammonia concentrations >18 mg/m^3 are associated with respiratory symptoms and altered pulmonary function.

Figure 5-1 compares effect levels from studies of workers occupationally exposed to ammonia with those from studies of human volunteers acutely exposed to ammonia. As shown in this figure, irritation effects in controlled human volunteer studies were generally observed at concentrations higher than those that induced respiratory symptoms in industrially exposed workers; however, Sundblad et al. (2004) reported eye irritation and other symptoms at a concentration below effect levels reported in occupationally-exposed populations. Effects in the Sundblad et al. study were transient, and the investigators noted a tendency towards adaptation. Nevertheless, it is interesting to note that effect levels in acute inhalation studies may occur in the same concentration range as those in occupational exposure settings.

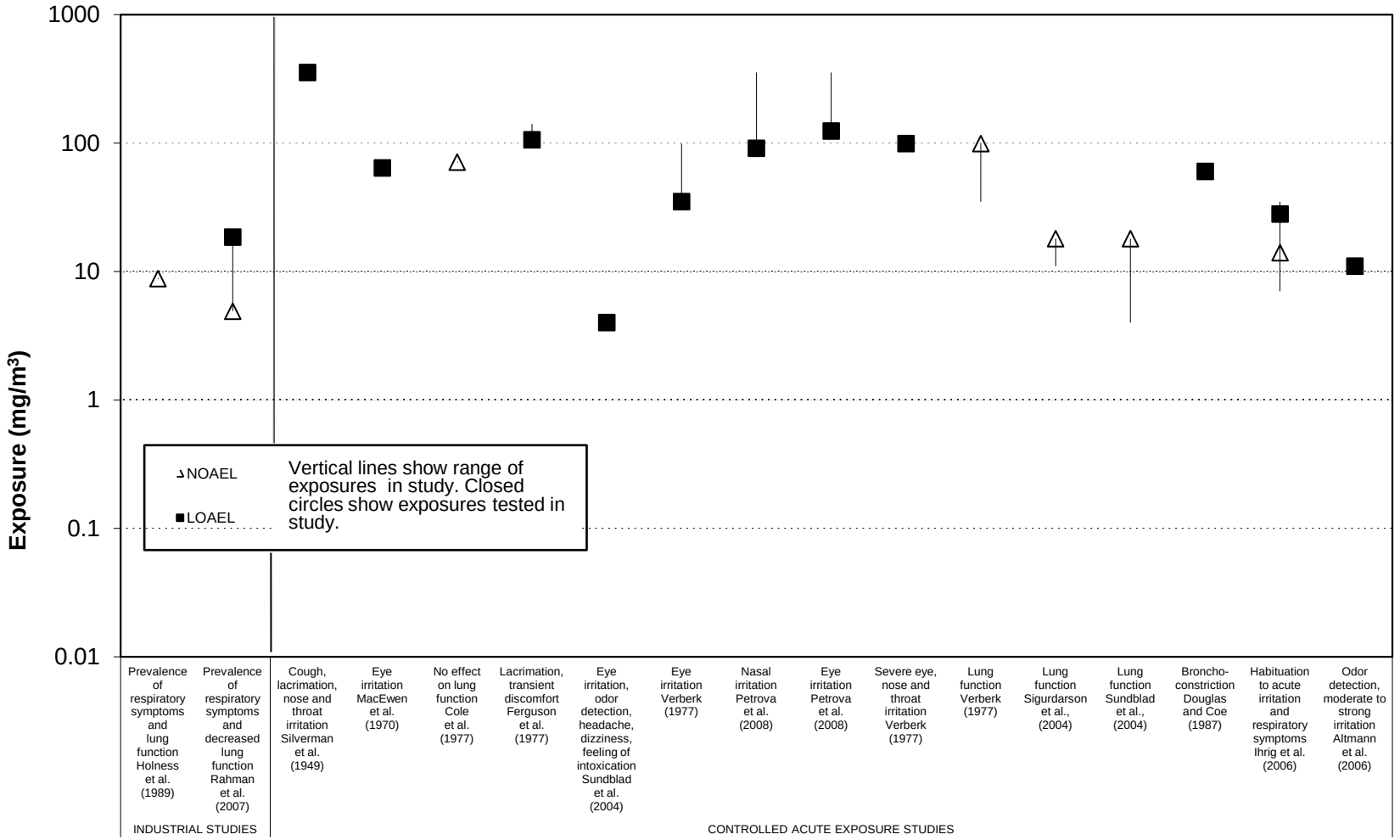


Figure 5-1. Exposure-response array comparing workers occupationally exposed and human volunteers acutely exposed to ammonia.

Animal studies of inhalation exposure to anhydrous ammonia include subchronic studies that reported inflammatory changes in nasal mucosa and tracheal epithelium (Gaafar et al., 1992; Broderson et al., 1976; Doig and Willoughby, 1971), and histological changes in liver and kidneys (Coon et al., 1970; Weatherby, 1952). NOAEL and LOAEL values could not be identified for a number of these studies. Studies for which effect levels could be derived are summarized in Table 4-12. Figure 5-2 is an exposure-response array comparing inhalation exposure to ammonia in humans (occupational exposure studies) and animals. As shown, effects in animals are observed near or above the LOAEL observed in the human studies.

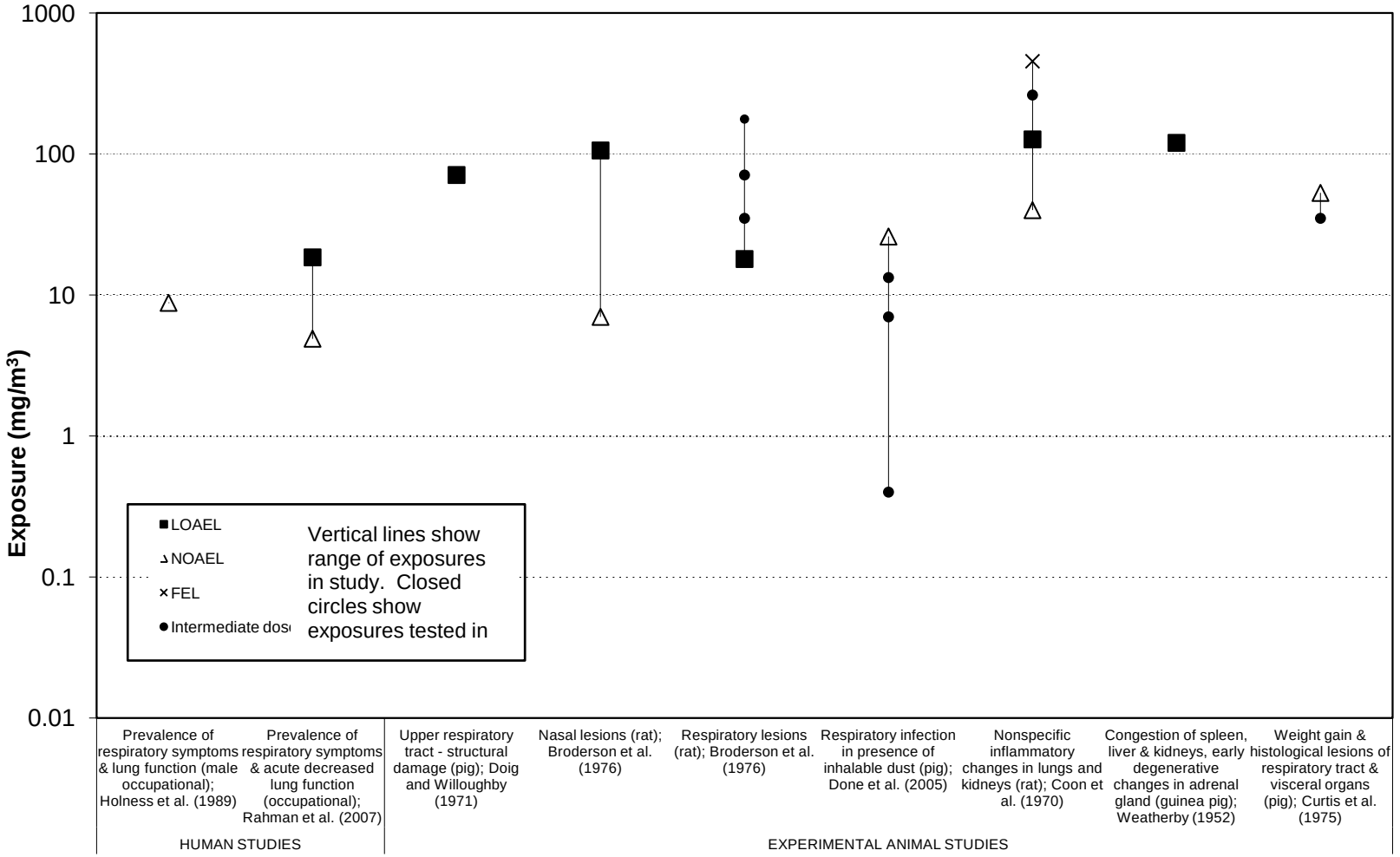


Figure 5-2. Exposure-response array comparing noncancer effects in occupationally exposed workers and experimental animals exposed to ammonia by inhalation.

As adequate data on the effects of inhalation exposure to ammonia are available in humans for deriving an RfC, animal data are considered in this assessment as supportive. Use of human data to derive the RfC also avoids the uncertainty associated with interspecies extrapolation introduced when animal data are used as the basis for the RfC. The two occupational exposure studies of ammonia by Holness et al. (1989) and Rahman et al. (2007) which examined respiratory symptoms and effects on lung function provide consistent estimates of the effect level for ammonia, with the NOAEL of 8.8 mg/m^3 identified from the Holness et al. (1989) study falling between the NOAEL and LOAEL values (4.9 mg/m^3 and 18.5 mg/m^3, respectively) from the Rahman et al. (2007) study. The Holness et al. (1989) study was selected as the principal study for deriving an RfC over the Rahman et al. (2007) study because it identified the higher NOAEL of these two occupational studies. Consideration of analytical methods also supports the selection of Holness et al. (1989) as the principal study. Rahman et al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air (Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by four- to five-fold, indicating some uncertainty in these measurements. In contrast, the Holness et al. (1989) study used an established analytical method of measuring exposure to ammonia recommended by NIOSH that involved the collection of air samples on ATSG absorption tubes.

Therefore, the Holness et al. (1989) study was selected as the principal study, and increased respiratory symptoms and decreased lung function, considered by EPA to be adverse, were selected as the critical effect.

5.2.2. Methods of Analysis

EPA identified a NOAEL of 8.8 mg/m^3, based on the lack of increased respiratory symptoms and decreased lung function in Holness et al. (1989) and selected this as the point of departure (POD) for RfC derivation. This NOAEL was identified as the POD in the context of the entire ammonia database, including other occupational studies that reported pulmonary effects at higher workplace ammonia concentrations (i.e., the LOAEL of 18.5 mg/m^3 identified by Rahman et al. (2007)). Additionally, effects in animals are observed near or above the LOAEL observed in human studies, thereby supporting the occupational epidemiology studies.

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The NOAEL of 8.8 mg/m^3 identified in the Holness et al. (2007) study is used as the POD for RfC derivation. Since this POD is based on occupational exposure, the value was first adjusted for continuous exposure as follows:

$$NOAEL_{ADJ} = NOAEL \times VE_{ho}/VE_h \times 5 \text{ days}/7 \text{ days}$$
$$= 8.8 \text{ mg/m}^3 \times 10 \text{ m}^3/20 \text{ m}^3 \times 5 \text{ days}/7 \text{ days}$$
$$= 3.1 \text{ mg/m}^3$$

Where:

VE_{ho} = human occupational default minute volume (10 m^3 breathed during the 8-hour workday, corresponding to a light to moderate activity level [U.S. EPA, 2011b])

VE_h = human ambient default minute volume (20 m^3 breathed during the entire day)

The UFs, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), address five areas of uncertainty resulting in a composite UF of 10. This composite uncertainty factor was applied to the selected POD to derive an RfC.

- An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhaled ammonia in the human population.
- An interspecies uncertainty factor, UF_A, of 1 was applied to account for uncertainty in extrapolating from laboratory animals to humans because the POD was based on human data from an occupational study.
- A subchronic to chronic uncertainty factor, UF_S, of 1 was applied because the occupational exposure period in the principal study (Holness et al., 1989), i.e., mean years at present job for exposed workers, of approximately 12 years was of chronic duration.
- A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because a NOAEL value was used as the POD.
- A database uncertainty factor, UF_D, of 1 was applied to account for deficiencies in the database. The ammonia inhalation database consists of a large number of case reports of acute exposure to high ammonia concentrations (e.g., accidental spills/releases), controlled exposure studies involving volunteers exposed to ammonia vapors for short periods of time to evaluate irritation effects and changes in pulmonary function, studies in livestock farmers, and studies of occupational exposure focused on effects of ammonia on respiratory symptoms and lung function. Studies of the toxicity of inhaled ammonia in experimental animals include subchronic studies in rats, guinea pigs, and pigs that examined respiratory and other systemic effects of ammonia and one limited, reproductive toxicity study in young female gilts pigs. The database lacks developmental and multigeneration reproductive toxicity studies.

  Ammonia is endogenously produced in humans and animals during fetal and adult life and concentrations in blood are homeostatically regulated to remain at low levels. Baseline blood levels in healthy individuals range from 0.1-1.0 µg/mL (Monsen, 1987; Conn, 1972; Brown et al., 1957). Evidence in animals (Manninen et al., 1988; Schaerdel et al., 1983) suggests that exposure to ammonia at concentrations up to 18 mg/m^3 does not alter blood ammonia levels (see Section 3). Therefore,

exposure at the POD (3.1 mg/m^3) associated with respiratory effects following inhalation exposure would not be expected to alter ammonia homeostasis or result in measureable increases in blood ammonia concentrations. Thus, the concentration of ammonia at the POD for the RfC would not be expected to result in systemic toxicity, including reproductive or developmental toxicity. The fact that the fetoplacental unit produces ammonia and that concentrations in human umbilical vein and artery blood (at term) have been shown to be higher than concentrations in maternal blood (see Section 3) also provides assurance that developmental toxicity would not be associated with concentrations of ammonia at or below the POD. As noted in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), "the size of the database factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical and, consequently, the POD." Because the lack of two-generation reproductive and developmental toxicity studies in the ammonia toxicity database should not impact the determination of ammonia toxicity at the POD, a database UF to account for the lack of these studies was not considered necessary.

The RfC for ammonia was calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{NOAEL}_{\text{ADJ}} \div \text{UF} \\ &= 3.1 \text{ mg/m}^3 \div 10 \\ &= 0.3 \text{ mg/m}^3 \end{aligned}$$

5.2.4. Previous RfC Assessment

The previous IRIS assessment for ammonia (posted to the database in 1991) presented an RfC of 0.1 mg/m^3 based on co-critical studies—the occupational exposure study of workers in a soda ash plant by Holness et al. (1989) and the subchronic study by Broderson et al. (1976) that examined the effects of ammonia exposure in F344 rats inoculated on day 7 of the study with *M. pulmonis*. The NOAEL of 6.4 mg/m^3 (mean concentration of the entire exposed group) from the Holness et al. (1989) study (duration adjusted: $\text{NOAEL}_{\text{ADJ}}$ = 2.3 mg/m^3) was used to derive the RfC by applying a composite UF of 30, 10 to account for the protection of sensitive individuals and 3 for database deficiencies to account for the lack of chronic data, the proximity of the LOAEL from the subchronic inhalation study in the rat (Broderson et al., 1976) to the NOAEL, and the lack of reproductive and developmental toxicology studies. A database UF larger than 3 was not applied because studies in rats (Schaerdel et al., 1983) that showed no increase in blood ammonia levels at an inhalation exposure to 32 ppm and only minimal increases at 300–1000 ppm suggested that no significant distribution is likely to occur at the human equivalent concentration.

5.3. CANCER ASSESSMENT

As discussed in Section 4.7, data are "inadequate for assessing the carcinogenic potential" of ammonia, so no quantitative cancer assessment was conducted. Also, no carcinogenicity assessment was presented in the previous IRIS assessment.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Ammonia is a colorless gas that occurs naturally in the environment. Ammonia is found in water, soil, and air, and is a source of nitrogen for plants and animals. Most of the ammonia in the environment comes from the natural breakdown of animal waste and dead plants and animals. Ammonia may also be present in the environment as the result of its use as a fertilizer, chemical intermediate, alkalizer, metal treating/extraction agent, water treatment chemical or as emissions from urea selective catalytic reduction aftertreatment systems in diesel vehicles. If compressed or in an aqueous solution, ammonia can occur as a liquid. Ammonia has a very sharp odor that is familiar to most people because of its use in smelling salts and household cleaners. In water, most of the ammonia is converted to the ionic form, ammonium ion. Ammonium ions are not gaseous and have no odor.

Ammonia can be absorbed by both 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. Both exogenous and endogenously produced ammonia are absorbed in the intestine. Ammonia that reaches the circulation is widely distributed to all body compartments, although substantial first-pass metabolism occurs in the liver, where biotransformation into urea and glutamine occur. In normal circumstances, ammonia exists in the blood as ammonium ion (NH_4^+). Ammonia or ammonium ion reaching the tissues is utilized for glutamate production, which participates in transamination and other reactions. The principal means of excretion of absorbed ammonia in mammals is as urinary urea; minimal amounts are excreted in the feces and in expired air.

As a liquid, ammonia is capable of burning the skin and causing permanent eye damage. Case reports in humans following intentional or accidental ingestion of household cleaning products or ammonia smelling salts observed neurological and gastrointestinal clinical symptoms and burns along the digestive tract. As a gas, ammonia is capable of causing severe eye damage and mild to severe pulmonary irritation and/or inflammation (see human case studies and case reports presented in Appendix C.2). Occupational exposure studies and controlled human exposure studies indicate respiratory symptoms (e.g., coughing, wheezing, etc.), altered lung function, and respiratory irritation following inhalation exposure to ammonia. Animal studies also reported respiratory irritation and inflammation following ammonia inhalation.

As discussed in Section 4.7, under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess the carcinogenic potential" of ammonia. A series of studies provided limited evidence that ammonia may act as a cancer

promoter, and positive responses in a few genotoxicity studies have been reported, including one study in fertilizer factory workers.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The available oral data for ammonia were considered insufficient to support reference value derivation. Therefore, an RfD for ammonia was not derived.

6.2.2. Noncancer/Inhalation

The RfC for ammonia of 0.3 mg/m^3 was derived using a duration-adjusted NOAEL of 3.1 mg/m^3 as the POD based on a lack of respiratory symptoms and the absence of changes in lung function at this concentration in occupationally exposed workers at a soda ash plant (Holness et al., 1989). To derive the RfC, the POD was divided by a total UF of 10 to account for human interindividual variability. The default human variability factor was applied because of the lack of quantitative information to assess toxicokinetic or toxicodynamic differences in the range of susceptibilities to ammonia in the human population.

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). The overall confidence in the RfC for ammonia is medium. Confidence in the principal study (Holness et al., 1989) is medium. The design, conduct, and reporting of this occupational exposure study were adequate, but the study was limited by a small sample size and by the fact that workplace ammonia concentrations to which the study population was exposed were below those associated with ammonia-related effects (i.e., only a NOAEL was identified). However, this study is supported in the context of the entire database, including the NOAEL and LOAEL values identified in the Rahman et al. (2007) occupational exposure study, multiple studies of acute ammonia exposure in volunteers, and the available subchronic and chronic inhalation data from animals. Confidence in the database is medium. The inhalation ammonia database includes limited studies of reproductive toxicity and no studies of developmental toxicity; however, reproductive, developmental, and other systemic effects are not expected at the RfC because it is well documented that ammonia is endogenously produced in humans and animals, because ammonia concentrations in blood are homeostatically regulated to remain at low levels, and because ammonia concentrations in air at the POD are not expected to alter homeostasis. Reflecting medium confidence in the principal study and medium confidence in the database, the overall confidence in the RfC is medium.

6.2.3. Cancer

No cancer assessment was conducted for ammonia due to the lack of adequate carcinogenicity data in humans or animals.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

[To be added]

APPENDIX B. SUMMARY OF REPEAT DOSE TOXICITY INFORMATION FOR SELECTED AMMONIUM SALTS

Table B-1. Summary of noncancer results of repeat dose studies of oral exposure of experimental animals to selected ammonium salts

Strain/ species/sex	Ammonia species	Dose (mg/kg-d)/ duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the LOAEL dose	Comment	Reference
Subchronic studies							
Wistar rat (10/sex/group)	Ammonium chloride	0, 1,590, or 3,050 (males); 0, 1,800, or 3,700 (females) for 13 wks (administered in diet)	Not determined	1,590 (males) 1,800 (females)	Decreased body weights (6-17% in males; 11-19% in females), changes in serum chemistry (increased plasma chloride and ALP activity), increased relative kidney weights (both dose levels, 7-28%) and adrenal weights (high-dose males, 18%), metabolic acidosis (males and females) and subsequent hypertrophy of the adrenal zona glomerulosa (males only)		Lina and Kuijpers, 2004
Wistar rat (5 females/ group)	Ammonium acetate in diet and drinking water	17.1 or 2,370 in diet; and 0 or 42.8 in water continuously for 90 d	Not determined	2,410 (combined dose)	Depression in body weight gain	Control diet contained 0.024% ammonia. Authors suggested that the transient effects on GFAP protein and comparable blood levels were indicative of an adaptive response.	Bodega et al., 1993

Table B-1. Summary of noncancer results of repeat dose studies of oral exposure of experimental animals to selected ammonium salts

Strain/ species/sex	Ammonia species	Dose (mg/kg-d)/ duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the LOAEL dose	Comment	Reference
Chronic studies							
Sprague-Dawley rat (11 males/group)	Ammonium chloride	0 or 1,800 for 330 d (administered in drinking water)	Not determined	1,800	Depression in body weights (13-20% with regular and low-calcium diets, respectively), metabolic acidosis, and loss of bone (femur) tissue	Metabolic acidosis (reduced blood pH and plasma carbon dioxide). Combined effects of calcium intake and observed that the loss of bone tissue was independent of the level of dietary calcium.	Barzel et al., 1969
Wistar rat (15/sex/group)	Ammonium chloride	0, 481, or 1,020 (males); 0, 610, or 1,370 (females) for 18 mo	Not determined	481 (males) 610 (females)	Metabolic acidosis	Femur weight significantly increased (~17%) in high-dose males. Adrenal and kidney weights elevated (<15%). Increased incidence of zona glomerulosa hypertrophy of the adrenal at high dose (not statistically significant). Severity of metabolic acidosis increased with dose.	Lina and Kuijpers, 2004
Wistar rat (50/sex/group)	Ammonium chloride	0, 455, or 1,000 (males); 0, 551, or 1,200 (females) for 30 mo	Not determined	455 (males) 551 (females)	Metabolic acidosis and an increased incidence of hypertrophy of the adrenal glomerulosa (males only)	Increased incidence of zona glomerulosa hypertrophy of the adrenal in high-dose females; attributed to chronic stimulation of the adrenal cortex by ammonium chloride induced acidosis. There was no evidence of a carcinogenic response.	Lina and Kuijpers, 2004

Table B-1. Summary of noncancer results of repeat dose studies of oral exposure of experimental animals to selected ammonium salts

Strain/ species/sex	Ammonia species	Dose (mg/kg-d)/ duration	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Effects at the LOAEL dose	Comment	Reference
F344 rat (10/sex/group)	Ammonium sulfate	0, 42, 256, or 1,527 (males); 0, 48, 284, or 1,490 (females) for 52 wks (administered in diet)	256 (males) 284 (females)	1,527 (males) 1,490 (females)	Elevated relative liver and kidney weights (7-10%)	No significant effects on hematology, serum chemistry, or histopathology.	Ota et al., 2006
F344 rat (50/sex/group)	Ammonium sulfate	0, 564, or 1,288 (males); 0, 650, or 1,371 (females) for 104 wks (administered in diet)	1,288 (males) 1,371 (females)	Not determined		Clinical chemistry and hematology not evaluated; organ weights not measured. Incidence of chronic nephropathy in male rats increased over control (1/48, 5/49, 3/48 in the control, mid and high dose); increase was statistically significant only at the mid-dose and not dose-related. There was no evidence of a carcinogenic response.	Ota et al., 2006
Developmental studies							
Wistar rat (group sizes of pregnant rats not reported)	Ammonium acetate	0 or 21,000 starting on d 1 of pregnancy through weaning to PND 21	Not determined (maternal or developmental)	Maternal effects not reported 21,000 (developmental)	Reduction in pup body weight gain	Pups exposed to ammonia during gestation and lactation only were resistant to acute ammonia toxicity induced by a single injection of 540 mg/kg ammonium acetate.	Minana et al., 1995

APPENDIX C. SUPPLEMENTAL INFORMATION ON AMMONIA

C.1. AMMONIA LEVELS MEASURED IN EXPIRED AIR IN HUMANS

Available data on ammonia levels in the expired air of volunteers are summaried in Table C-1.

Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples from the nose and trachea					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Three healthy male volunteers (>30 yrs of age)	Ammonia levels measured in nose-exhaled breath of test subjects each morning about 2 hrs after eating a regular breakfast; samples collected daily over a 4-wk period	Volunteer A = 103 ± 1.2 ppb (0.0728 ± 0.000848 mg/m^3) Volunteer B = 110 ±1.3 ppb (0.0777 ± 0.000919 mg/m^3) Volunteer C = 83 ± 1.2 ppb (0.0587 ± 0.000848 mg/m^3) (median ammonia levels estimated as geometric mean ± geometric standard deviation)	SIFT-MS analysis	Mean ambient air level of ammonia was 80 ± 10 ppb (0.056 ± 0.0071 mg/m^3) The authors indicated that ammonia measured in mouth-exhaled breath may be generated in the oral cavity and suggested that concentrations in nose-exhaled breath may better represent systemic conditions (such as metabolic disease)	Smith et al., 2008
Sixteen healthy subjects (9 males aged 25–63 yrs and 7 females aged 23–41 yrs); subgroups tested were all male	Breath samples collected during quiet nose breathing, and direct sampling during a deep inspiration followed by breath-holding with the glottis closed	Ammonia concentrations ranged from 0.013 to 0.046 mg/m^3 during nose breathing (median 0.025 mg/m^3) (5 male subjects), and 0.029 mg/m^3 from an air sampled collected from the trachea (collected from a tube inserted into one male subject's nose and into the trachea)	Chemiluminescence		Larson et al., 1977

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Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples from the mouth and oral cavity					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Three healthy male volunteers (>30 yrs of age)	Ammonia levels measured in mouth-exhaled breath and in the closed mouth cavity of test subjects each morning about 2 hrs after eating a regular breakfast; samples collected daily over a 4-wk period	via Mouth: Volunteer A = 1,088 ± 1.3 ppb (0.769 ± 0.000919 mg/m^3) Volunteer B = 885 ± 1.3 ppb (0.626 ± 0.000919 mg/m^3) Volunteer C = 855 ± 1.3 ppb (0.604 ± 0.000919 mg/m^3) via Oral Cavity: Volunteer A = 1,465 ± 1.4 ppb (1.04 ± 0.000990 mg/m^3) Volunteer B = 2,146 ± 1.5 ppb (1.52 ± 0.00106 mg/m^3) Volunteer C = 1,859 ± 1.3 ppb (1.31 ± 0.000919 mg/m^3) (median ammonia levels estimated as geometric mean ± geometric standard deviation)	SIFT-MS analysis	Mean ambient air level of ammonia was 80 ± 10 ppb (0.056 ± 0.0071 mg/m^3) The authors indicated that ammonia measured in mouth-exhaled breath may be generated in the oral cavity and suggested that concentrations in nose-exhaled breath may better represent systemic conditions (such as metabolic disease)	Smith et al., 2008
Twenty-six secondary school students (10 males and 16 females, 17–18 yrs old and one 19 yr old)	Three sequential breath exhalations collected over 5 min following the students listening to a 1-hr presentation (at least 1 hr following breakfast and before lunch); alveolar portion measured (identified using humidity)	Median values reported for: 17 yr olds = 233 ppb (0.165 mg/m^3) 18 yr olds = 346 ppb (0.245 mg/m^3)	SIFT-MS analysis	Significant differences in ammonia levels in exhaled breath between 17 and 18 yr olds ($p < 10^{-8}$) were reported	Spanel et al., 2007a

Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples from the mouth and oral cavity					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Four healthy children (2 males and 2 females, 4–6 yrs old) Thirteen senior volunteers (11 males and 2 females, 60–83 yrs old); four had type-2 diabetes mellitus with onset at ages between 50 and 70 yrs, and controlled by diet All subjects had their regular breakfast without any specific restrictions	Breath samples collected in morning at least 1 hr after breakfast and at least 1 hr prior to lunch; each volunteer performed two exhalation/inhalation cycles (both about 5–10 sec in duration)	Children = range 223–643 ppb (0.157–0.454 mg/m^3) Seniors = 317–2,091 ppb (0.224–1.48 mg/m^3)	SIFT-MS analysis	Ammonia breath levels significantly increased with age Some seniors reported diabetes Measured ammonia level in breath reported for each subject	Spanel et al., 2007b
Thirty healthy volunteers (19 males and 11 females, 24–59 yrs, 28 Caucasian, 1 African, and 1 mixed race); volunteers were instructed to maintain their normal daily routines and to not rinse out their mouths prior to providing a breath sample	Breath samples collected in the morning prior to lunch at approximately weekly intervals for about 6 mo; some volunteers provided samples more frequently than others; 480 samples collected and analyzed for ammonia	Geometric mean and geometric standard deviation = 833 ± 1.62 ppb (0.589 ± 0.00114 mg/m^3) Median = 842 ppb (0.595 mg/m^3) Range = 248–2,935 ppb (0.175–2.08 mg/m^3)	SIFT-MS analysis	Ammonia breath levels were shown to increase with age Background levels in the testing laboratory were typically around 400 ppb (0.28 mg/m^3)	Turner et al., 2006
Five subjects (2 females, 3 males; age range 27–65 yrs)	Breath samples collected between 8 and 9 am in three sequential breath exhalations on multiple days (12–30 d) over the course of a month	Ammonia concentrations ranged from 422 to 2,389 ppb (0.298–1.69 mg/m^3)	SIFT-MS analysis	Differences in ammonia breath levels between individuals were significant ($p < 0.001$)	Diskin et al., 2003

Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples from the mouth and oral cavity					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Six normal nonsmoking male volunteers (24–61 yrs old), fasted for 12 hrs prior to testing	Baseline breath sample obtained; breath samples collected 20, 40, and 60 min and 5 hrs following the ingestion of a liquid protein-calorie meal	Premeal levels ranged from 300 to 600 ppb (0.2–0.4 mg/m^3); Postmeal levels at 30 min were 200 ppb (0.1 mg/m^3) increasing to maximum values at 5 hrs of 600–1,800 ppb (0.4–1.3 mg/m^3)	SIFT-MS analysis	A biphasic response in breath ammonia concentration was observed after eating	Smith et al., 1999
Fourteen healthy, nonsmoking subjects (age range 21–54 yrs) performed one or more of the following hygiene maneuvers: (1) acidic oral rinse (pH 2.5), (2) tooth brushing followed by acidic oral rinse, (3) tooth brushing followed by distilled water rinse, and (4) distilled water rinse	Subjects fasted for 8 hrs prior to baseline measurement, refrained from oral hygiene after their most recent meal, refrained from heavy exercise for 12 hrs, and had no liquid intake for several hours; initial breath ammonia was measured between 8 and 10 am, then subjects performed one or more of the hygiene measures listed (at 30-min intervals for a total 90-min period; samples collected over 5 min)	Baseline levels varied from 120 to 1,280 ppb (0.085–0.905 mg/m^3)	Nitrogen oxide analyzer with an ammonia conversion channel (similar to chemiluminescence)	An 80–90% depletion of volatile ammonia emissions was seen within 10 min of acid rinsing; less than a 50% depletion of ammonia was seen following tooth brushing or distilled water rinse; gaseous ammonia levels increased after all rinse procedures over time	Norwood et al., 1992
Sixteen healthy subjects (9 males aged 25–63 yrs and 7 females aged 23–41 yrs); subgroups tested were all male	Breath samples collected during quiet mouth breathing	Ammonia concentrations ranged from 0.029 to 0.52 mg/m^3 during mouth breathing (median of 0.17 mg/m^3)	Chemiluminescence	The oral cavity appears to be a source of breath ammonia; no attempt was made to control the diet of subjects or standardize the interval between the last meal and the measurement	Larson et al., 1977

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Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples: source (nose/mouth/oral cavity) not specified					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Sixteen healthy, nonsmoking subjects (4 females and 12 males, 29 ± 7 yrs); no significant differences in mean age, height, weight, body mass index (BMI), or time since last oral intake; 10 subjects tested in each experiment	Experiment 1: single whole-breath samples collected from each subject (same samples immediately reanalyzed within <10 sec to assess instrument specific variability) Experiment 2: three repeat breath samples collected from each subject (to evaluate intra-subject differences); this experiment evaluated differences based on standardization of expiratory pressure and flow Experiment 3: two mixed breath samples and two bag alveolar breath samples collected in short succession from each subject	Experiment 1: 1,192 ± 85 ppb (0.843 ± 0.0601 mg/m^3; median ± measurement error) Experiment 2: Nonstandardized = 1,007 ± 184 ppb (0.712 ± 0.130 mg/m^3; median ± standard deviation) Standardized = 1,433 ± 160 ppb (1.01 ± 0.113 mg/m^3; median ± standard deviation) Experiment 3: Mixed = 1,216 ± 827 ppb (0.860 ± 0.585 mg/m^3; median ± standard deviation) Alveolar = 1,301 ± 791 ppb (0.920 ± 0.559 mg/m^3; median ± standard deviation)	SIFT-MS analysis This study establishes that SIFT-MS analysis is reliable and repeatable	Relatively small number of healthy subjects used Does not address the breath of those with disease Intra-day and inter-day repeatability were not investigated	Boshier et al., 2010

Table C-1. Ammonia levels in exhaled breath of volunteers

Breath samples: source (nose/mouth/oral cavity) not specified					
Test subjects	**Breath samples**	**Levels of ammonia in exhaled breath**	**Methods**	**Comments**	**Reference**
Eight healthy subjects (average age 39.8 ± 9.6 yrs)	Subjects fasted for 6 hrs prior to samples being collected. Subjects breathed normally into collection device for 5 min	Mean breath ammonia = 0.49 ± 0.24 ppm (0.35 ± 0.17 mg/m^3)	Fiber optic sensor	This study measured ammonia levels in healthy volunteers compared to *Helicobacter pylori* positive individuals (five subjects) (data not shown); the experiment also included a challenge with a 300 mg urea capsule to evaluate the urease activity of healthy vs. infected individuals (data not shown); the authors concluded that breath ammonia measurement may be feasible as a diagnostic test for *H. pylori*	Kearney et al., 2002
Three groups of children were used as test subjects: (1) 68 asthmatic children residing in a National Park in the mountains (mean age 10 yrs, 48 boys, 20 girls), (2) 52 asthmatic children in an urban area (mean age 9 yrs, 35 boys, 17 girls), and (3) 20 healthy children from the same urban area as a control group (mean age 10, 12 boys, 8 girls)	Subjects performed a 5-sec breath-hold and exhaled slowly into collection device	Asthmatic children from National Park = 5.6 ± 4.7 ppb (0.0040 ± 0.0033 mg/m^3) Asthmatic urban children: Mean NH_3 = 14.3 ± 10.2 ppb (0.0101 ± 0.00721 mg/m^3) Urban control group: Mean NH_3 = 14.8 ± 10.3 ppb (0.0105 ± 0.00728 mg/m^3)	Chemiluminescence	Both groups of asthmatic children had some subjects on glucocorticoids, often combined with histamine antagonists and/or b2 agonists, while others were left untreated; ammonia concentrations in exhaled breath appeared to be correlated with exposure to urban air	Giroux et al., 2002

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C.2. HUMAN CASE STUDIES AND REPORTS OF HUMAN EXPOSURE TO AMMONIA

Case report findings of injury in adults and children due to exposure to ammonia via inhalation of vapors, dermal contact, or ingestion of household cleaning solutions or ammonia inhalant capsules are presented in Table C-2 below and are organized by exposure route.

Oral exposure to ammonia most commonly involved ingestion of household cleaning solutions or biting into the capsules of ammonia smelling salts, which are commonly found in first aid kits. Young children, generally <4 years old, have been reported as "biting into" or ingesting smelling salts capsules. The acute effects included drooling, erythematous and edematous lips, reddened and blistered tongues, dysphagia, vomiting, and oropharyngeal burns (Rosenbaum et al., 1998; Wason et al., 1990; Lopez et al., 1988). Delayed effects were not noted in these cases. Gilbert (1988) reported ammonia intoxication characterized by lethargy, restlessness, irritability, and confusion in a 37-year-old man following surgery. Most other cases of ammonia ingestion involved household cleaning solutions and detergents. Many cases were intentional; however, not all were fatal. Klein et al. (1985) described two cases of ingestion of approximately 30 mL and "two gulps" of Parson's sudsy ammonia (ammonia 3.6%; pH 11.5), respectively. The first case resulted in a white and blistered tongue and pharynx, and esophageal burns with friable, boggy mucosa; and in the second case, several small esophageal lesions with mild to moderate ulceration and some bleeding were reported. There were no oropharyngeal burns in the second case and no delayed complications in either case. Christesen (1995) reported that of 11 cases involving accidental or intentional ingestion of ammonia water by adults (15 years or older), 2 cases exhibited acute respiratory obstruction, and 1 case developed an esophageal stricture 3 months postinjury. In cases involving fatalities, evidence of laryngeal and epiglottal edema, erythmatous esophagus with severe corrosive injury, and hemorrhagic esophago-gastro-duodeno-enteritis was noted (Klein et al., 1985; Klendshoj and Rejent, 1966). Dworkin et al. (2004) reported a case of ingestion of contaminated chicken tenders, prepared and served in a school cafeteria, by approximately 157 students and 6 teachers. The onset of acute symptoms occurred within an hour of ingestion, and included headache, nausea, vomiting, dizziness, diarrhea, and burning mouth. In a case of forced ingestion of an unknown quantity of dilute ammonia, a 14-year-old boy presented with difficulty speaking, ataxic gait, isochoric pupils, and evidence of brain edema. There were no burns to the eyes or mouth and no indication of gastric pathology. It was only after the patient was able to communicate that ammonia was involved that appropriate treatment, followed by a satisfactory outcome, was achieved.

Inhalation is the most frequently reported route of exposure and cause of morbidity and fatality, and often occurs in conjunction with dermal and ocular exposures. Acute effects from

inhalation have been reported to range from mild to severe, with mild symptoms consisting of nasal and throat irritation, sometimes with perceived tightness in the throat (Price and Watts, 2008; Prudhomme et al., 1998; Weiser and Mackenroth, 1989; Yang, 1987; O'Kane, 1983; Ward et al., 1983; Caplin, 1941). Moderate effects are described as moderate to severe pharyngitis, tachycardia, frothy, often blood-stained sputum, moderate dyspnea, rapid, shallow breathing, cyanosis, some vomiting, transient bronchospasm, edema and some evidence of burns to the lips and oral mucosa, and localized to general rhonchi in the lungs (Weiser and Mackenroth, 1989; Yang, 1987; O'Kane, 1983; Ward et al., 1983; Counturier et al., 1971; Caplin, 1941). Severe effects include second- and third-degree burns to the nasal passages, soft palate, posterior pharyngeal wall, and larynx; upper airway obstruction, loss of consciousness, bronchospasm, dyspnea, persistent, productive cough, bilateral diffuse rales and rhonchi, production of large amounts of mucous, pulmonary edema, marked hypoxemia, local necrosis of the lung, deterioration of the whole lung, and fatality. Delayed effects of acute exposure to high concentrations of ammonia include bronchiectasis, bronchitis, bronchospasm/asthma, dyspnea upon exertion and chronic productive cough, bronchiolitis/, severe pulmonary insufficiency, and chronic obstructive pulmonary disease (Lalic et al., 2009; Leduc et al., 1992; Bernstein and Bernstein, 1989; Flury et al., 1983; Ward et al., 1983; Stroud, 1981; Close, 1980; Taplin, et al., 1976; Walton, 1973; Kass et al., 1972; Slot, 1938).

Respiratory effects were also observed following chronic occupational exposure to ammonia. After 18 months and 1 year on the job, respectively, both men developed cough, chest tightness, and wheezing, typically after 2–6 hours from the beginning of each work day, but not on weekends or holidays. In another case, progressive deterioration of the clinical condition of a 68-year-old male was documented for 4 years and development of diffuse interstitial and severe restrictive lung disease was reported following long-term repetitive occupational exposure to ammonia at or above the odor recognition level (Brautbar et al., 2003). Lee et al. (1993) report a case of a 39-year-old man who developed occupational asthma 5 months after beginning a job requiring the polishing of silverware. The room in which he worked was poorly ventilated. The product used contained ammonia and isopropyl alcohol and the measured ammonia concentration in the breathing zone when using this product was found to be 8–15 ppm (6–11 mg/m^3).

Acute dermal exposure to anhydrous (liquid) ammonia and ammonia vapor has resulted in caustic burns of varying degrees to the skin and eyes. There are numerous reports of exposures from direct contact with anhydrous ammonia in which first-, second-, and third-degree burns occurred over as much as 50% of the total body surface (Lalic et al., 2009; Pirjavec et al., 2009; Arwood et al., 1985). Frostbite injury has also been reported in conjunction with exposure to sudden decompression of liquefied ammonia, which is typically stored at -33°F (George et al.,

2000; Sotiropoulos et al., 1998; Arwood et al., 1985). However, direct contact is not a prerequisite for burn injury. Several reports have indicated that burns to the skin occurred with exposure to ammonia gas or vapor. Kass et al. (1972) reported one woman with chemical burns to her abdomen, left knee, and forearm and another with burns to the feet when exposed to anhydrous ammonia gas released from a derailed train in the vicinity. Several victims at or near the scene of an overturned truck that had been carrying 8,000 gallons of anhydrous ammonia were reported as having second- and third-degree burns over exposed portions of the body (Burns et al., 1985; Close et al., 1980; Hatton et al., 1979). In a case involving a refrigeration leak in a poorly ventilated room, workers located in an adjacent room reported a "burning skin" sensation (de la Hoz et al., 1996) while, in another case involving the sudden release of ammonia from a pressure valve in a refrigeration unit, one victim received burns to the leg and genitalia (O'Kane, 1983).

In addition to the skin, the eyes are particularly vulnerable to ammonia burns due to the highly water-soluble nature of the chemical and the ready dissociation of ammonium hydroxide to release hydroxyl ions. When ammonia or ammonia in solution has been splashed or sprayed into the face (accidently or intentionally), immediate effects include temporary blindness, blepharospasm, conjunctivitis, corneal burns, ulceration, edema, chemosis, and loss of corneal epithelium (George et al., 2000; Helmers et al., 1971; Highman, 1969; McGuinness, 1969; Levy et al., 1964; Abramovicz, 1924). The long-term effects included photophobia, progressive loss of sensation, formation of bilateral corneal opacities and cataracts, recurrent corneal ulcerations, nonreactive pupil, and gradual loss of vision (Yang, 1987; Kass et al., 1972; Helmers et al., 1971; Highman, 1969; Osmond and Tallents, 1968; Levy et al., 1964; Abramovicz, 1924). White et al. (2007) reported a case with acute bilateral corneal injury that developed into bilateral uveitis with stromal vascularization and stromal haze and scarring, and pigmented keratic precipitates that resulted in legal blindness. An increase in intraocular pressure, resembling acute-angle closure glaucoma, was reported by Highman (1969) following ammonia intentionally sprayed into the eyes during robbery attempts.

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Oral exposure				
57-yr-old male	Ingested unknown quantity of dilute ammonium hydroxide (2.4% ammonia)	Hemorrhagic esophago-gastro-duodeno-enteritis, death	Not applicable	Klendshoj and Rejent, 1966

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
15-yr-old male	Ingestion of about 30 mL Parson's sudsy ammonia (ammonia 3.6%; pH 11.5)	White and blistered tongue and pharynx, esophageal burns with friable, boggy mucosa; no other complications	Not applicable	Klein et al., 1985
Middle-aged female	Ingested "two gulps" Parson's sudsy detergent ammonia	Several small esophageal lesions with mild to moderate ulceration and some bleeding; no oropharyngeal burns	Not applicable	Klein et al., 1985
69-yr-old female	Ingested an unknown quantity of Albertson's lemon ammonia (ammonia concentration 3%)	Lethargy, gurgling respiratory sounds, laryngeal, and epiglottal edema, and a friable, erythmatous esophagus with severe corrosive injury	Renal failure, death	Klein et al., 1985
Eight young children (ages not given)	Biting into an unbroken capsule of aromatic ammonia inhalant	Vomiting, drooling, dysphagia, cough, and oropharyngeal burns	Not applicable	Lopez et al., 1988
Three children, <4 yrs old	Ingestion of aromatic ammonia "smelling salt" capsules that contained 0.33 mL of a mixture of 18% ammonia and 36% alcohol	Pain swallowing, reddened and blistered tongues, drooling, erythema, and swelling of lower lip	Not applicable	Wason et al., 1990
Eleven adults, ≥15 yrs	Intentional or accidental ingestion of ammonia water	Two patients exhibited acute respiratory obstruction, no additional details were provided.	One patient developed esophageal stricture 3 mo postinjury	Christesen, 1995
3-yr-old female	Biting into an ammonia inhalant capsule	Drooling with multiple 1–2 cm white, ulcerative lesions on the mid-posterior upper surface of the tongue, bilaterally on the buccal mucosa, and on the posterior esophageal wall at the junction of the middle and upper thirds of the esophagus	Not applicable	Rosenbaum et al., 1998

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
2-yr-old female	Biting into an ammonia inhalant capsule	Drooling, edema, and erythema on the upper and lower lips with areas of desquamation, superficially ulcerative lesions on the anterior dorsum of the tongue; edema and erythema of the supraglottic structures and upper trachea	Not applicable	Rosenbaum et al., 1998
One hundred fifty seven students (median age 10 yrs) and six teachers	Ingestion of ammonia-contaminated chicken tenders (522–2,468 ppm [369–1,749 mg/m^3]) ammonia in uncooked chicken tenders)	Stomach ache, headache, nausea, vomiting, dizziness, diarrhea, and mouth burning	Not applicable	Dworkin et al., 2004
14-yr-old male	Ingestion of an unknown quantity of dilute ammonia	Difficulty articulating, ataxic gait, isochoric pupils, cranial CT indicating possible brain edema; hematology, and routine biochemical analysis normal, no burns of the eyes or mouth, and no pathology of the respiratory or gastrointestinal tract	Not applicable	Dilli et al., 2005
Inhalation-only exposure				
39-yr-old male	Fumes created during use of a silver polishing product containing isopropyl alcohol and ammonia (~12 ppm [~8 mg/m^3]) in a poorly ventilated basement	Cough, breathlessness, and wheezing, rhinitis, and tearing	Occupational asthma	Lee et al., 1993
68-yr-old male	Long-term, repetitive occupational exposure to anhydrous ammonia at or above odor recognition threshold for 15–20 yrs	Examination found persistent pulmonary infiltrates, mainly in upper portion of left chest, diagnosed as diffuse interstitial lung disease	Progressive deterioration of clinical condition over a 4-yr period, development of diffuse interstitial lung disease, and severe restrictive lung disease, with pulmonary function tests indicating reduced diffusion capacity at 47%	Brautbar et al., 2003

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Inhalation/dermal/ocular exposure				
Dental patient (age not given)	A 10% ammonia solution spilled into eyes while trying to revive patient from a faint	Both eyes red, swollen, with mucopurulent secretions, swollen conjunctiva; the lower left cornea and sclera showed loss of epithelium and the entire left cornea was dull	Increase in corneal ulceration with hypopyon and perforation was seen after 8 d; severe conjunctivitis still evident 4 mo after exposure	Abramovicz, 1924
21-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Shock, second-degree burns of both feet, the right leg, and small area of the right cheek	Residual bronchitis (not further described)	Slot, 1938
21-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Shock, conjunctivitis of the right eye, severe tracheitis	Not applicable	Slot, 1938
23-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Severe shock, persistent, blood-stained vomiting, confusion	Not applicable	Slot, 1938
21-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Shock, hemicranial headache with nausea	Residual bronchitis, “fullness of the head”, and body pains; congestion of both lungs	Slot, 1938
36-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Burns and shock; existing bronchitis greatly aggravated	Anxiety symptoms, insomnia, painful scar	Slot, 1938
25-yr-old female	Explosion in an ice cream factory; ammonia pipe burst	Extreme shock, grey pallor, burns to the face, eyes, neck, and both arms, difficulty/inability to swallow, labored breathing	Pulse often imperceptible, Cheyne-Stokes respiration, fatality; left lung was congested, edematous, and a small hemorrhage at the base, hemorrhagic bronchitis, desquamation, and small epithelial ulcers of the bronchi	Slot, 1938
Nine shelter occupants (ages not given)	Ammonia condenser leak in an air-raid shelter (low exposure group)	Eye and mouth irritation, pain on swallowing and hoarseness, suffused conjunctivae, swollen eyelids; lips, mouth, and tongue were erythematous with edema in the back of the throat, and a strong smell of ammonia on the breath	Not applicable	Caplin, 1941

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Twenty seven shelter occupants (ages not given)	Ammonia condenser leak in an air-raid shelter (moderate exposure group; victims in closer proximity to leak)	Cough, blood-stained sputum, hoarseness, chest tightness, increased respiration, hyperaemic conjunctiva, lacrimation; moist sounds in the chest; lips, mouth, tongue, and soft palate showed erythema and edema, with areas of denuded epithelium scattered over the buccal mucosa; three cases showed no improvement and fatality occurred within 36 hrs	Nine cases showed initial improvement, with signs of bronchopneumonia appearing on d 2 and 3 postinjury; six recovered while three died within 2 d of onset of secondary infection; autopsy on two males revealed intensely inflamed fauces, pharynx, and larynx; trachea, and bronchi denuded of epithelium or inflamed and filled with purulent exudate; lungs deeply congested with bronchopneumonia	Caplin, 1941
Eleven shelter occupants (ages not given)	Ammonia condenser leak in an air-raid shelter (high exposure group, victims closest to leak source with longest exposure time)	Slight cyanosis, intense dyspnea, and persistent cough with foamy sputum; weak and rapid pulse, generalized rales, and rhonchi; seven cases showed no improvement, becoming increasingly cyanotic and dysphonic and died within 48 hrs of exposure	The remaining four cases improved without incident	Caplin, 1941
17-yr-old male	Struck by a jet of anhydrous ammonia during crop dusting operations	Second-degree burns to the face, marked edema of the eyelids, and unable to open eyes; lips, tongue, and buccal mucosa were hyperemic and burned, swollen uvula (twice normal size), and edematous larynx	Permanent loss of vision, with the exception of sustained light perception	Levy et al., 1964
17-yr-old male	Sprayed with anhydrous ammonia during crop fertilizing operations	First- and second-degree burns to the left arm, anterior chest, face, and neck, burning and edema around the mouth and eyes, conjunctiva appeared inflamed and the mouth was edematous with the uvula swollen to twice the normal size, respiration was labored and rhonchi were present throughout the lungs	Chronic persistent cough with a small amount of mucoid sputum, no chronic visual effects	Levy et al., 1964

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
61-yr-old male	Struck in the face with a blast of anhydrous ammonia while fertilizing crops	First-degree burns of the arms and axillae, dyspnea, respiratory distress, red and edematous eyes, unable to see clearly, moderate edema of the mouth and throat with no clear view of the larynx, large amounts of bronchial secretions	No chronic impairment of pulmonary function or of vision occurred	Levy et al., 1964
28-yr-old male	Struck in the face by a spray of anhydrous ammonia while working on a refrigeration unit	First- and second-degree burns to the eyelids, face, anterior chest, ears, forehead, and upper arms, edema and erythema of the pharynx, and inspiratory and expiratory rales could be heard over both lung fields	Pulmonary function tests 3 yrs later were unremarkable	Levy et al., 1964
Male employee (age not given)	Exposure for an unspecified length of time to ammonia fumes created when a tanker overflowed during a filling operation with 25% ammonia water	Vomiting and coughing initially, with some difficulty breathing; after 3-hr delay in seeking medical attention, exhibited red and swollen face, conjunctivitis, red and raw mouth and throat with a swollen tongue, loss of speech, dyspnea, with a weak rhonchi in left lung, cardiac arrest and fatality; autopsy findings included necrosis of the lung, inflammation of the bronchioli; the epithelial layer of the trachea and bronchial tubes were denuded, with an absence of secretions	Not applicable	Mulder et al., 1967
Male victim at a bank robbery (age not given)	Ammonia thrown into face and forced down throat and up the nose	Burns to the face, eyes, and mouth, with severe edema of the nasopharynx and glottis	The right eye recovered within 4 d; the left eye showed gross chemosis, corneal staining, nonreactive pupil, intense uveitis with aqueous flare and cells	Osmond and Tallents, 1968

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
57-yr-old male	Assaulted with ammonia squirted into the eyes during a robbery	Decreased visual acuity in the left eye, conjunctivitis, necrosis over the tarsus of the lower lid; the pupil was semi-dilated, oval, and fixed, increased interocular pressure	Following initial improvement, the left eye became red, inflamed, rising interocular pressure on several occasions, each time responding to surgery and treatment, only to recur after 1 or 2 mo of recovery	Highman, 1969
46-yr-old male	Assaulted with ammonia squirted into the eyes during a robbery	Conjunctivitis in the right eye, the cornea was hazy with edema	Four d following discharge, severe anterior uveitis developed, along with recurrent corneal ulceration and diminished corneal sensation	Highman, 1969
Female (age not given)	Ammonia was thrown into eyes in the course of a robbery	Two hrs postexposure, loss of corneal epithelium, conjunctivitis, stromal haze, fibrinous material in the anterior chamber, iris atrophy, vertical, oval-shaped pupil, and subcapsular lens opacities	Band-shaped corneal degeneration and heterochromia of the iris, but no progression of lens opacities (5 mo following exposure)	McGuinness, 1969
40-yr-old male	Sprayed on face and chest during a transfer operation involving anhydrous ammonia	Facial burns (not extremely serious), pulmonary edema, and pneumonitis with inflammation and edema of the upper airways	No residual lung damage	Helmers et al., 1971
17-yr-old farmer	Sprayed with several gallons of 25% ammonia in water during transfer operation	Throat tightness during first few minutes following exposure; second-degree burns to the buttocks	No residual effects	Helmers et al., 1971
36-yr-old male	Sprayed in the face during field repairs to fertilization equipment	Immediate blepharo-spasm, second-degree facial burns, irritative conjunctivitis, superficial corneal ulceration, and palpebral edema of the left eye	No known sequelae	Helmers et al., 1971
45-yr-old farmer	Sprayed on the left side of face during a transfer operation	Immediate blepharo-spasm (30-min delay in irrigating the exposed areas with water); minor skin burns and irritation to the right eye; severe damage to the left eye (no details provided)	Diminished vision in left eye at 3 d; loss of sensation resulting in accidental scratching of the sclera at 3 mo; 1 yr following exposure, the left eye perceived only light and the cornea was vascularized and opaque	Helmers et al., 1971

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
20-yr-old male	Safety valve release in a refrigeration unit due to accidental heating of the ammonia line	Loss of consciousness, pinkish foaming at the nose and mouth, cyanotic, first- and second-degree burns to the neck, eyes, left arm, glans penis, scrotum, and both lower legs, spastic extremities, both lungs with fine and harsh rales, constant and profuse pulmonary excretions	Six-mo followup: normal vision, mild cough	White, 1971
20-yr-old female	Exposure (approximately 30 min) to anhydrous ammonia following train derailment	Chemical burns to the soft palate, oropharynx, and feet, unable to speak, blood-streaked sputum	Widespread infiltration consistent with bronchopneumonitis developed shortly after exposure; approximately 1.5–2 yrs following exposure, patient developed a productive cough and was increasingly short-of-breath, bronchiectatic changes involving entire left lung, mild changes in right lung	Kass et al., 1972
22-yr-old female	Exposure (approximately 90 min) to anhydrous ammonia following train derailment	Loss of consciousness, convulsions, chemical burns over the abdomen, left knee and forearm, both arms, soft palate, and oropharynx, damage to both corneas, respiratory distress, multiple areas of alveolar type of infiltrate in both lungs	Re-hospitalized 1 yr later with bilateral pneumonia, peripheral edema, and acute right heart failure; marked deterioration of vision in the left eye, bilateral corneal opacities and early cataract changes were present; 2 yrs later, progressive development of infiltrations in basilar section of the lower left lung, generalized varicose bronchiectatic changes in lung segments, hypoxemia, biopsies revealed some mucosal surface erosion, areas of atelectasis, emphysema, alveolar walls thickened with monocellular infiltrates	Kass et al., 1972

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
24-yr-old male, instrument artificer, smoker	Vent stack overflow from 100 feet above work area, resulting in complete drenching	Dyspnea, chest pain, blepharospasm, deep cyanosis, burning throat, blisters in the mouth and throat, and congested eyes, sloughs of oral mucosa and exposed skin, conjunctivitis, bronchitis	26 mo following exposure, dyspnea on exertion, excessive collagenization in the bronchial submucosal layer	Walton, 1973
39-yr-old male, smoker	Vent stack overflow from 100 feet above work area, resulting in complete drenching	Dyspnea, chest pain, blepharospasm, burning throat, extensive blistering, tachycardia, moist sounds from both lung bases, corneal burns, aphonia (lasting about 10 d)	Continued to smoke at follow up; lung function tests (FVC and expiratory volume in 1 sec) well below normal after 2 yrs	Walton, 1973
42-yr-old male, maintenance fitter, smoker	Exposure due to the inadvertent resumption of ammonia gas in a high pressure line during valve maintenance	Burns to the left eye (no further details)	Five-yr followup indicated normal lung function	Walton, 1973
39-yr-old male, assistant process foreman	Doused with ammonia liquid from approximately 130 feet above	Pink frothy sputum, cyanosis, bronchospasm, severe burns and blistering on the face, mouth and hands	Recurring bronchitis, dyspnea on exertion; in lung function tests, FVC reached near normal 1 yr following exposure; FEV_1 test indicated moderate obstructive airways disease and the gas transfer figure remained below normal	Walton, 1973
39-yr-old male, process foreman, smoker	Doused with ammonia liquid from approximately 130 feet above	Pain in eyes and throat, blepharospasm, burns to the eyes, mouth, face, and throat	Continued to smoke at follow up; lung function tests showed progressive improvement in ventilation but consistent depression of gas transfer factor	Walton, 1973
47-yr-old male, process worker	In vicinity of a high pressure compressor pump burst	Pain and tightness of the chest, blepharospasm, bloodstained sputum, burns and blistering of the face, mouth, eyes, throat, and left heel	Lung function tests indicate gradual improvement in ventilatory ability, but below normal in gas transfer factor	Walton, 1973

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Male, process worker (age not given)	In vicinity of a high pressure compressor pump burst	Immediate fatality; autopsy results indicate extensive edema and burns on the mouth, face, trunk, arms, and upper back; airway at the larynx almost completely blocked, greatly distended and congested lungs	Not applicable	Walton, 1973
28-yr-old male	Sprayed in the face when a hose coupling came loose	First- and second-degree burns to the face, nose, and oropharynx mucosa inflamed and sloughing, rhonchi throughout both lung fields, lung congestion, and segmental atelectasis	Reassessment at 6 mo revealed normal chest x-ray films but abnormal pulmonary arterial perfusion and indication of partial airway obstruction in the left basal segment; within 2 wks, this increased to total obstruction of the airways to the left basal segment	Taplin et al., 1976
25-yr-old male	Tank explosion	Initial effects: mild bilateral conjunctival edema, diffuse bilateral wheezing, rhonchi, and rales, burns to an estimated 30% of the skin surface area of the extremities, chest, and genitalia, pulmonary edema; shortly after admission, development of severe respiratory distress with production of large amounts of mucopurulent exudate	Arterial hypoxemia persisted despite respirator inspired oxygen at 50–60% and positive end expiratory pressure; wheezing and bronchospasm, bilateral infiltrates, fatality occurred at 60 d following exposure with evidence of purulent cavitary pneumonia	Sobonya, 1977
6-mo-old male; two 12-yr-old males; 17-yr-old female	Road accident involving a tank truck rolling from an overpass to the road below, resulting in a thick cloud of ammonia vapor	All four victims suffered varying degrees of burns to the face and body, eyes, mouth, oral mucosa, erythema, and edema of the epiglottis and larynx; two victims required intubation for upper airway obstruction	Measurement of several urinary metabolites of hydroxylysine indicated that considerable collagen degradation occurred after inhaling concentrated ammonia vapors	Hatton et al., 1979

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
17-yr-old female	Direct exposure to high concentrations resulting from a tanker truck explosion	Second-degree burns of the forehead and upper extremities, total epithelial loss of the corneas, second- and third-degree burns of the nasal passages, soft palate, posterior pharyngeal wall and full-thickness burns of the larynx, diffuse parenchymal densities in both lungs, inspiratory wheezing, rhonchi, and rales were present	Pulmonary function studies (1 yr following discharge) indicated moderate obstructive and restrictive abnormalities	Close et al., 1980
6-mo-old male	Direct exposure to high concentrations resulting from a tanker truck explosion	Second- and third-degree burns on the face, scalp, upper extremities, and buttocks, epithelial defects in both corneas, upper airway obstruction (requiring intubation), diffuse erythema and edema of the lips, soft palate, posterior pharyngeal wall, and epiglottis	No remarkable findings in 2-mo followup	Close et al., 1980
26-yr-old male	Direct exposure (approximately 20 min) to high concentrations resulting from a tanker truck explosion	Upper airway obstruction (requiring mechanical ventilation), extensive second-degree burns of the head, neck, and chest, total loss of corneal epithelium of both eyes, extensive second- and third-degree burns of the oropharynx, hypopharynx, and larynx, diffuse rales, rhonchi, and expiratory wheezing, bilateral patchy pulmonary infiltrates	Rapid degeneration and fatality followed a massive hemorrhage around the tracheostomy tube (innominate artery erosion); autopsy revealed full-thickness burns to the entire respiratory tract, acute necrotizing trachea-bronchitis, and bilateral bronchopneumonia	Close et al., 1980

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
23-yr-old female	Within a few hundred feet of the tanker truck explosion, exposure time >30 min	Mild facial erythema and bilateral conjunctival irritation, first- and second-degree burns of the oral cavity, oropharynx, and larynx, bilateral parenchymal infiltrate, mild hypoxemia	After a period of stability, progressive bilateral pneumonia with progressive dyspnea, and increased secretions; subsequent followup examinations revealed accentuated interstitial markings, profuse, diffuse combined bronchoalveolar and perfusion abnormalities, development of large pneumatoceles, and bullous changes to the right side of the chest with herniation to the left, facial edema, and anasarca; ventilator assistance through a tracheostomy required	Close et al., 1980
30-yr-old female	In the vicinity of the tanker truck explosion, exposure time approximately 30 min	Mild facial erythema, conjunctival irritation of the eyes, diffuse bilateral rales and rhonchi, bilateral infiltrates, hypoxemia (requiring mechanical ventilation)	After initial improvement, pneumonia developed in the lower left lung, accompanied by severe progressive hoarseness, moderate hypoxemia and development of a combined obstructive and restrictive pulmonary disorder; additional 2-yr followup indicated scattered parenchymal scarring, bleb formation in the left lung base and mild to moderate hypoxemia	Close et al., 1980

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Fourteen male fishermen, 18–39 yrs old	Refrigeration system leak below decks	Inflammation of the pharynx and conjunctiva present in 12–14 victims, with 2 showing corneal burns; tachypnea was present in 10 fisherman; tachycardia was seen in 4 fishermen; 5 victims had unremarkable chest examinations upon admission, while 9 were found to have rales, rhonchi, and wheezing, productive cough; 1 of the 9 moderately affected victims developed laryngeal edema requiring tracheostomy, while another had persistent and progressive airway obstruction for 48 hrs	Not applicable	Montague and Macneil, 1980
58-yr-old female	Leaking pipe near the offices for a grocery distributor	Loss of consciousness, severe eye irritation with bilateral corneal burns, dyspnea, pharyngeal edema, bilateral diffuse rhonchi, and rales	Two yrs following exposure, patient developed severe pulmonary insufficiency requiring a 1-yr hospitalization with a permanent tracheostomy and continuous oxygen	Stroud, 1981
30-yr-old female	Exposure to fumes released when a tanker truck overturned and exploded	Initial intense eye and nasopharyngeal irritation developing to breathlessness and nonproductive cough, initial transient infiltrate of pulmonary edema	Following apparent recovery from initial acute effects, development of purulent cough with progressive hypoxemia and hypercapnia requiring tracheotomy and mechanically assisted respiration for the next 3 yrs; development of bilateral, severe, cylindrical, and saccular bronchiectasis with obliterative fibrous adhesions of the right pleural space, broncho-pneumonia; fatality	Hoeffler et al., 1982

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
50-yr-old male	Splashed with liquid ammonia when a refrigeration coolant tank exploded	Burns to the right foot and second-degree burns to the thigh and groin, progressive hypoxemia and inspiratory stridor, hyperemic, edematous pharynx and vocal chords, with the posterior pharyngeal wall and the entire tracheal wall below the vocal chords covered with a yellow purulent pseudomembrane	Progressively worsening respiratory distress (mechanical ventilation was required for 10 d), extensive pseudomembrane formation throughout the trachea-bronchial tree, gastro-intestinal bleeding, persistent tachypnea and expiratory wheezing (following removal of tracheostomy tube), pneumonitis complications; 5-yr followup: asympto-matic except during very strenuous activity	Flury et al., 1983
Two male factory workers, smokers, 24 and 40 yrs old	Sudden release of ammonia gas to the factory floor when the pressure valve on a refrigeration unit released	Eye and throat irritation, slightly red throat; one patient had burns on the legs and genitals		O'Kane, 1983
Three male factory workers, smokers, 20–30 yrs old, with histories of asthma, smoker's cough, and chronic bronchitis	Sudden release of ammonia gas to the factory floor when the pressure valve on a refrigeration unit released	Moderate dyspnea, labored breathing, sore eyes, moderate to severe throat pain, cough, nausea		O'Kane, 1983
30-yr-old male factory worker, smoker, no history of lung problems	Sudden release of ammonia gas to the factory floor when the pressure valve on a refrigeration unit released; exposure time of 2–3 min with full inhalation	Severe cough, massive nasal discharge, very sore throat, constant retching, development of inspiratory and expiratory rhonchi	Hoarseness and productive cough persisting for several months	O'Kane, 1983
28-yr-old male factory worker, smoker, no history of lung problems	Sudden release of ammonia gas to the factory floor when the pressure valve on a refrigeration unit released; directly in line with escaping gas	Severely inflamed eyes, lips, tongue, edematous pharynx and hoarseness, high-pitched inspiratory and expiratory rhonchi	Not applicable	O'Kane, 1983

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
44-yr-old male factory worker, nonsmoker, no history of lung problems	Sudden release of ammonia gas to the factory floor when the pressure valve on a refrigeration unit released; 4–5-min exposure	Cyanosed, respiratory distress, bilateral inspiratory and expiratory rhonchi, inflamed eyes, tongue, pharynx; 2 hrs following admission, developed severe dyspnea, central cyanosis, and an audible gurgle from bronchial secretions	Following initial improvement, the patient began deteriorating the 13th d following exposure, lungs developed multiple cavitating lesions, secondary infection, and finally necrotizing pneumonia; patient survived with chronic infective lung disease	O'Kane, 1983
25-yr-old male refrigeration technician	Sudden, massive leak from the refrigeration plant	Loss of consciousness, severe burns of the face, eyes, mouth, and throat; development of clinical and radiologic features of pulmonary edema with audible crackles over the lung fields and production of large amounts of mucous	Respiratory failure with tachypnea and arterial hypoxemia developed; death from respiratory failure 12 wks following exposure; intense congestion of the mucosal surfaces of the trachea and major bronchi; the cut surface of the left lung was crepitant, and cylindrical bronchiectasis was found in the middle and lower lungs	Price, 1983
Two males, 26 and 40 yrs old	Pipe valve failure allowing ammonia gas to fill a factory area	Mild pharyngitis and conjunctivitis; the 40-yr-old male also had burns to legs and genitals	Followup every 6 mo for up to 2 yrs: no additional findings	Ward et al., 1983
Three males, 26, 36, and 45 yrs old	Pipe valve failure allowing ammonia gas to fill a factory area	Moderate conjunctivitis, moderate to severe pharyngitis, tachycardia, and tachypnea, frothy sputum, moderate dyspnea, nausea	Followup every 6 mo for up to 2 yrs: impaired pulmonary function at discharge (all three men); two men showed improvement while the third did not; all three men had preexisting lung disease	Ward et al., 1983
30-yr-old male	Pipe valve failure allowing ammonia gas to fill a factory area	Severe dyspnea, sore eyes, nausea, generalized rhonchi, leukocytosis, and marked hypoxemia	Improvement in lung function tests was seen during followup; however, patient experienced dyspnea upon exertion and chronic productive cough	Ward et al., 1983
27-yr-old male	Pipe valve failure allowing ammonia gas to fill a factory area	Severe dyspnea, sore eyes, nausea, generalized rhonchi, leukocytosis, and marked hypoxemia	A right basal consolidation developed requiring prolonged treatment with oxygen; improvement in lung function was seen after 30 d; however, patient experienced dyspnea upon exertion and chronic productive cough	Ward et al., 1983

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
44-yr-old male	Pipe valve failure allowing ammonia gas to fill a factory area	Severe dyspnea, sore eyes, nausea, generalized rhonchi, leukocytosis, and marked hypoxemia	Following initial improvement from admission, a relapse occurred, coincident with development of pneumonia, requiring artificial respiration and tracheostomy; during 2-yr followup, patient remained severely impaired in pulmonary function	Ward et al., 1983
33-yr-old male	Explosion while transferring ammonia from one tank to another	Burns over 12% of total body surface area and oropharynx, bilateral corneal clouding, severe respiratory distress	Fatality due to cardiac arrest; autopsy findings: tracheobronchial ulcerations, denuded epithelium, congested lungs, and necrotizing bronchitis with ulceration and membrane formation, micro abscesses of the kidneys	Arwood et al., 1985
51-yr-old male smoker	Explosion while transferring ammonia from one tank to another	Burns over 50% total body surface area (eyes, back, legs, and oropharynx), severe respiratory distress (bilateral wheezing and bilateral lower lobe infiltrates)	Progressive decline/respiratory distress following initial improvement, fatality due to cardiac arrest; autopsy findings: tracheobronchial ulcerations, denuded epithelium, congested lungs, and necrotizing bronchitis with ulceration and membrane formation, medullary fibrosis and tubal necrosis of the kidneys	Arwood et al., 1985
Five males, 26–48 yrs old	An overturned tanker truck released a dense cloud of ammonia gas	Four immediate fatalities; autopsy findings: second-degree burns on exposed skin, hyperemic tracheobronchial mucosal surfaces, edematous and congested parenchyma, sloughing of the tracheobronchial epithelium and extensive edema and congestion of the lung parenchyma	One delayed fatality (2.5 wks after exposure) due to bronchopneumonia and necrotizing tracheobronchitis	Burns et al., 1985

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
Twenty two seafood processing plant workers	Liquefied ammonia tank explosion within an enclosed area	Fourteen cases with irritative symptoms only; six workers exhibited transient bronchospasm and edema of the lips and oral mucosa	Five workers exhibited eye pain and photophobia, four of which developed corneal damage; one fatality due to respiratory failure (2 wks following exposure); one worker developed restrictive lung disease and chronic bronchitis (6-mo followup)	Yang, 1987
37-yr-old male with myotonic dystrophy	Ammonia intoxication 37 yrs after ureterosigmoidostomy	Lethargy, restlessness, irritability, and confusion, both lungs were underinflated due to high diaphragm, severe metabolic encephalo-pathy, right-sided hydronephrosis (left kidney absent), blood ammonia levels were markedly elevated.	None	Gilbert, 1988
Four males, all smokers (ages not given)	Acute exposure (no additional details provided)	Skin blisters, ocular and nasal burning, dyspnea, chest pain, chest tightness, nonproductive cough, and hemoptysis	Wheeze, productive cough, dyspnea persisting for 12–32 mo; pulmonary function tests indicated reversible airway obstruction in one patient with persistent hemoptysis; submucosal inflammation, denuded epithelium, and thickening of the basement membrane	Bernstein and Bernstein, 1989
28-yr-old male	Equipment failure while spraying anhydrous ammonia on farmland	Skin burns of 12% total body surface area including face, chest, neck, and right upper extremity; periorbital edema with the right eye swollen shut; edema of the uvula, soft palate, tonsilar pillars, and epiglottis with laryngeal burns, thick mucoid rhinorrhea, and bilateral rhonchi	Thirteen mo following exposure, patient presented with perihilar adenopathy and a right paratracheal mass, as well as mild obstructive disease; at 4 yrs postexposure, there was no evidence of paratracheal or hilar masses	Millea et al., 1989

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
17-yr-old male, farm supply store employee	A connector valve opened on a hose carrying ammonia	Partial thickness burns on 18% of total body surface area including the face, chest, and lower extremities; full thickness burns on approximately 3% of total body surface area, corneal burns to the right eye, hoarseness, erythema and edema of the oral mucosa and uvula	On postexposure d 5, a pneumomediastinum and subcutaneous emphysema were noted, as well as vocal cord and tracheobronchial edema	Millea et al., 1989
22-yr-old male farmer	Sprayed while applying anhydrous ammonia to a field	Partial thickness burns over 12% total body surface area of the chest, abdomen, and upper extremities; no pulmonary effects	Patient was readmitted 6 d following discharge for an infected burn wound on the right arm	Millea et al., 1989
21-yr-old male refrigeration plant worker	Directly below refrigeration pipe rupture	Cardiorespiratory arrest; third-degree burns to 20% of the body surface, conjunctivitis, burns of the oral mucosa with swelling of the epiglottis, pulmonary edema and bronchial inflammation	Fatality 13 d after exposure due to development of treatment-resistant bronchopneumonia	Weiser and Mackenroth, 1989
35-yr-old male refrigeration plant worker	In vicinity of refrigeration pipe rupture	Second-degree burns over 10% of total body surface area, dyspnea, nausea, severe eye irritation, nose and throat irritation, pulmonary edema	Not applicable	Weiser and Mackenroth, 1989
Six refrigeration plant workers, male and female, 19–24 yrs old	In vicinity of refrigeration pipe rupture	Erythema, small burn areas, irritation of the eyes, nose, and throat, dyspnea and nausea to varying degrees	Not applicable	Weiser and Mackenroth, 1989
Workers at a fertilizer plant	Leak and explosion of an ammonia tank at a fertilizer plant	Seven fatalities, 57 injuries (no further details provided)	Not applicable	Andersson, 1991

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
28-yr-old male	Tank explosion in an industrial butter plant	Bilateral corneal scarring; first- and second-degree burns of the chest and hands, pharyngeal and laryngeal edema with exudative lesions, crackles and wheezes at the lung bases; production of copious bronchial secretions	At 1 wk followup: severe tracheobronchial damage with diffuse erythema, inflammation of the airway walls, hemorrhagic areas and abundant purulent secretions, with severe airflow blockage; during 10 yrs of followup, severe fixed airway obstruction remained, with coexisting bronchiectasis	Leduc et al., 1992
30-yr-old male, smoker	Occupational exposure to a refrigeration gas leak in adjacent room; approximately 15-min exposure	Immediate burning of the eyes, upper airways, and skin, with cough and pleuritic chest pain, conjunctivitis and rhinopharyngitis	Following discharge from hospital, persistent and progressively worse dyspnea, wheezing on exertion, and atypical chest pain; clinical diagnosis of restrictive lung disease with symptoms ongoing at 2 yrs and beyond	de la Hoz et al., 1996
27-yr-old male, smoker	Occupational exposure to a refrigeration gas leak in a poorly ventilated room; approximately 1.5–2 min of additional exposure after detecting a strong odor	Difficulty breathing; irritation of the eyes, nose, throat, burning sensation on the skin; respiratory failure requiring mechanical ventilation	At 26 mo following exposure, victim had persistent productive cough, dyspnea on exertion and expiratory wheezing; pulmonary function testing showed severe obstructive impairment unresponsive to bronchodilation	de la Hoz et al., 1996
46-yr-old male	Sprayed while unloading tanks of ammonium hydroxide (29.4% ammonia)	Mucocutaneous burns (unspecified), productive cough, dyspnea and wheezing	During 2 yrs following initial treatment and discharge, persistent dyspnea at rest and exertion, frequent episodes of dry cough, wheezing, increasing dyspnea and parethesia of the hands and feet; tests indicated a diagnosis of laryngotracheobronchitis and possible pneumonitis, with evidence of nonspecific bronchial hyper-reactivity and small airway disease	de la Hoz et al., 1996

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
41-yr-old male, fish-processing plant	Several hours spent in the vicinity of an ongoing ammonia leak from a refrigeration unit, without respiratory protection	Eye and nasal irritation and mild facial burning	Nasal congestion and intermittent epistaxis, which resolved in about 2 wks, hyposmia (decreased sense of smell), which persisted without additional abnormal findings	Prudhomme et al., 1998
51-yr-old maintenance man	Pressurized liquid ammonia explosion during inspection for maintenance and repair on a refrigerator coolant storage system	Examination revealed 28% total body surface area burns to the extremities with some torso involvement; there were no facial burns, erythema, mucosal swelling, or signs of respiratory distress	None	Sotiropoulos et al., 1998
57-yr-old male, supervisor to above	Pressurized liquid ammonia explosion during inspection for maintenance and repair on a refrigerator coolant storage system	Pain in the genital area was first noted an hour after explosion, with significant blistering on the scrotum and distal penis	Healing without incident or sequelae	Sotiropoulos et al., 1998
28-yr-old male	Anhydrous ammonia explosion at a fertilizer factory	Second- and third-degree burns (45% total body surface area) to posterior trunk, buttocks, and bilateral lower extremities, as well as inhalation injury (no additional details provided)	No effects observed at 18-mo followup	Amshel et al., 2000
47-yr-old male	Burst pipe in a liquefied ammonia production plant, with over 45 min of exposure	Mixed burns on the face, neck, chest, genitalia, both lower limbs, and full thickness burns of the back (38% total body surface area), frostbite injury to the back, the eyes showed chemosis and abrasions, congestion and corneal edema increasing with subconjunctival hemorrhaging, harsh breathing sounds with crepitations, intermittent bradycardia	Eschar necrosis down to the muscles on the back, persistent intermittent bradycardia and low blood pressure, unusual muscle weakness in all four limbs, fatality due to severe hemorrhage from the endotracheal tube and cardiac arrest	George et al., 2000

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
25-yr-old male	Liquid ammonia spill during transfer from a barge tank to a dockside tank	Facial and neck hyperemia, slightly injected sclera, erythematous petechiae on the right ear, edematous and peeling lips, and bilateral corneal abrasions; vision was blurred and had difficulty breathing	Not applicable	Latenser and Lucktong, 2000
32-yr-old male	Liquid ammonia spill during transfer from a barge tank to a dockside tank	Asymptomatic at the scene; within 90 min developed swollen face and lips, edematous pharynx and vocal compromising 50% of the airway, partial thickness burns on the face, neck, anterior chest, left medial upper arm, both hands, and left anterior thigh, 8–14% total body surface area with full thickness burns on the anterior thigh	Not applicable	Latenser and Lucktong, 2000
40-yr-old male	Exposed to liquid ammonia while emptying a refrigeration unit	Skin burns of 15% total body surface area to the right neck, torso, arm, and axilla, scattered bilateral rhonchi but no wheezing or stridor, minimal edema and erythema of the larynx and proximal trachea	Bilateral infrahilar consolidations and apical infiltrates were noted in postburn d 3; there was no long-term followup	Kerstein et al., 2001
Two food processing plant workers, ages not specified	Discharge of pressurized ammonia during maintenance	One immediate fatality (pulmonary edema, focal lung hemorrhage); one worker permanently blinded (no additional details)	Not applicable	Morton, 2005

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
32-yr-old male	Warehouse explosion resulting in an ammonia tank rupture	Skin burn of 2% total body surface area to the face and scrotum; perforated left tympanic membrane and right corneal injury; initial artificial respiration reinstated within 24 hrs for hypoxia and aspiration	Airway sloughing with large amounts of debris requiring tracheostomy; after removal of tracheostomy, patient exhibited dysphagia and hoarseness, evidence of supraglottic and aryenoid edema and left true vocal cord paresis; pulmonary function test at 8 mo indicated obstructive pulmonary disease	White et al., 2007
22-yr-old male	Warehouse explosion resulting in an ammonia tank rupture	0.5% total body surface area burns to the scrotum, bilateral corneal injury, tracheobronchitis	At 4-wk followup, bilateral uveitis with stromal vascularization, haze and scarring, and pigmented keratic precipitates resulting in legal blindness (after unsuccessful attempt at corneal transplants)	White et al., 2007
19-yr-old male	Warehouse explosion resulting in an ammonia tank rupture	0.5% total body surface area burns to the face and scrotum; bilateral corneal injury; tracheobronchitis	Peribronchiolar edema was noted 5 d after injury, stridor developed and the patient was reintubated and given artificial respiration; pulmonary function testing at 6 mo showed a fixed obstructive pattern	White et al., 2007
21-yr-old male	Warehouse explosion resulting in an ammonia tank rupture	1% total body surface area burns to the face, left axilla, and scrotum, bilateral corneal injuries, airway sloughing with large amounts of debris required performing a tracheostomy and mechanical ventilation	Extensive peribronchiolar edema, necrosis and sloughing were observed down to the tertiary bronchioles; fatality at 100 d postinjury	White et al., 2007
33-yr-old male	Warehouse explosion resulting in an ammonia tank rupture	Respiratory difficulty, but no burns to the skin and eyes; peribronchiolar edema limited to the right lung	Not applicable	White et al., 2007
Patients and hospital staff in the critical care unit	Ammonia leak from a refrigerator	Respiratory and ocular irritation	Not applicable	Price and Watts, 2008

Table C-2. Human case studies and reports of human exposure to ammonia

Case(s)	Exposure conditions	Immediate effects[a]	Delayed effects[b]	Reference
57-yr-old male, nonsmoker	At age 25, massive accidental exposure to anhydrous ammonia while working in a refrigeration unit	Loss of consciousness, corneal scarring, and burns to the face and chest	Chronic hypoxemic respiratory failure, bilateral inspiratory crackles, and prolonged expiration, central cylindrical, varicose and cystic bronchiectasis with mild interstitial fibrosis	Tonelli and Pham, 2009
20-yr-old male	Forklift caught an ammonia pipe causing a sudden burst of a large quantity of ammonia	Loss of consciousness, acute respiratory distress syndrome (which required percutaneous tracheotomy), second and third degree burns of his face, anterior neck, left axilla, left arm, parts of anterior and posterior chest wall, genitalia, and both lower limbs covering 50% total body surface area, symblepharon of both eyes	Continued hospital care for respiratory support until transplantation of both lungs performed 6 mo after exposure	Lalic et al., 2009; Pirjavec et al., 2009
53-yr-old male	Forklift caught an ammonia pipe causing a sudden burst of a large quantity of ammonia	Loss of consciousness, acute respiratory distress syndrome	Developed obliterating bronchiolitis and restrictive and obstructive ventilation disturbances, unable to work, breathless at rest, lung transplant candidate	Lalic et al., 2009
Fifteen males, 31.1 ± 8.8 yrs old	Resulted from events during illicit methamphetamine production, 14/15 due to explosions	Burns on body, respiratory distress	One fatality, ventilator assistance and skin grafting required	Bloom et al., 2008
Five males, 49.4 ± 23.0 yrs old	Resulted from farming accidents, 2/5 due to explosions	Burns on body, respiratory distress	Ventilator assistance and skin grafting required	Bloom et al., 2008

[a]Effects occurring within 48 hrs of exposure.
[b]Effects occurring longer than 48 hrs after exposure.

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C.3. CONTROLLED HUMAN EXPOSURE STUDIES OF AMMONIA INHALATION

Controlled human exposure studies of inhaled ammonia are summarized in Table C-3.

Table C-3. Controlled human exposure studies of ammonia inhalation

Subjects	Exposure conditions	Results	Reference
Seven male volunteers	500 ppm (354 mg/m^3) for 30 min from masked breathing apparatus for nose and throat inhalation. There was no mention of preexposure examinations	Hyperventilation (50–250% increase above controls) characterized by increased breathing rate and expiratory minute volume (i.e., volume of air exhaled in 1 min); no coughing was induced, excessive lacrimation occurred in two subjects; two subjects reported nose and throat irritation that lasted 24 hrs after exposure; no changes were reported in nitrogen metabolism or in blood or urine urea, ammonia, or nonprotein nitrogen.	Silverman et al., 1949
Seven male volunteers with an average age of 31 yrs	30, 50, and 90 ppm (21, 35, and 64 mg/m^3) for 10 min in an inhalation chamber Physical and neurological examinations were conducted prior to exposure	Increased eye erythema at 90 ppm (64 mg/m^3) compared to 30 and 50 ppm (21 and 35 mg/m^3) exposure; 90 ppm (64 mg/m^3) did not produce significant bronchiospasm or severe lacrimation; intensity of odor perception was reported as higher at 30 and 50 ppm (21 and 35 mg/m^3) than at 90 ppm (64 mg/m^3)	MacEwen et al., 1970
18 healthy servicemen volunteers, 18–39 yrs old	50–344 mg/m^3 (70–486 ppm) for a half-day (session day 2); sessions on days 1 and 3 acted as controls All volunteers underwent a preliminary examination prior to exposure	No effect at concentrations of 71 mg/m^3 (100 ppm); reduced expiratory minute volume at concentrations ranging from 106 to 235 mg/m^3 (150–332 ppm) compared to controls (not dose dependent); exercise tidal volume was increased at 106 mg/m^3 (150 ppm), but reduced at higher concentrations in a dose-dependent manner	Cole et al., 1977
Six male and female volunteers, 24–46 yrs old	25, 50, and 100 ppm (18, 35, and 71 mg/m^3) ammonia for 6 hrs/d 1 time/wk over 6 wks; occasional brief exposure to 150–200 ppm (106–141 mg/m^3) There was no mention of preexposure examinations	Habituation to eye, nose, and throat irritation after 2–3 wks with short-term adaption; there were no significant differences for common biological indicators, physical exams, or in normal job performance when compared to control subjects; continuous exposure to 100 ppm (71 mg/m^3) became easily tolerated and had no effect on general health after acclimation occurred; brief exposure to 150–200 ppm (106–141 mg/m^3) produced lacrimation and transient discomfort	Ferguson et al., 1977
15 volunteers, 18–53 yrs old	50, 80, 110, and 140 ppm (35, 57, 78, and 99 mg/m^3) for 2 hrs in an exposure chamber There was no mention of preexposure examinations.	No effect on vital capacity, FEV_1 or forced expiratory volume; 140 ppm (99 mg/m^3) caused severe irritation and could not be tolerated; reported eye irritation increased with concentration	Verberk, 1977

Table C-3. Controlled human exposure studies of ammonia inhalation

Subjects	Exposure conditions	Results	Reference
20 male volunteers; groups of four were exposed to ammonia at various concentrations and durations	Group 1: exposed to 2 mg/m^3 (3.0 ppm) for 37 d; Group 2: exposed to 5 mg/m^3 (7.2 ppm) for 17 d; Group 3: exposed to 2 mg/m^3 (3.0 ppm) for 35 d with short-term increases to 10 mg/m^3 (14 ppm); Groups 4 and 5: exposed to 2 and 5 mg/m^3 (3.0 and 7.2 ppm), respectively, for 20 d with variations in temperature and humidity; exposure duration each day was not specified ; there was no mention of preexposure examinations	Significantly elevated adrenalin levels in urine at 2.1 mg/m^3 (3.0 ppm); dopamine and DOPA levels in urine were not significantly affected at any concentration; significant increase of adrenalin and 7-oxy-corticosteroids in urine, and 11-oxycorticosteroids free fractions in plasma at 5.1 mg/m^3 (7.2 ppm); increased temperature and humidity resulted in increased urine adrenalin, urine 7-oxycorticosteroids and free 11-oxycortico-steroid levels in plasma at 5.1 mg/m^3 (7.2 ppm)	Kalandarov et al., 1984
Unspecified number of volunteer subjects	Acute exposure up to 15 sec, 1 time/d at unspecified concentrations; also a separate exposure of 10 inhaled breaths via mouthpiece at unspecified concentrations; there was no mention of preexposure examinations	The lachrymatory threshold was 55 ppm (39 mg/m^3) and bronchoconstriction was seen at 85 ppm (60.1 mg/m^3)	Douglas and Coe, 1987
Six healthy volunteers (two males and four females, 25–45 yrs old) and eight volunteers with mild asthma (four males and four females, 18–52 yrs old)	16–25 ppm (11–18 mg/m^3) for 30-min sessions with 1 wk between sessions Pulmonary function was measured before and after exposure	No significant changes in pulmonary function in healthy subjects at any concentration; a decrease in FEV_1 and increased bronchial hyperreactivity was reported in asthmatics exposed to dust and ammonia, but not to ammonia alone; exposure to dust alone caused similar effects, suggesting that dust was responsible for the effects.	Sigurdarson et al., 2004
12 healthy volunteers (7 females, 5 males) 21–28 yrs old	5 and 25 ppm (4 and 18 mg/m^3) for three separate exposures in inhalation chamber for 1.5 hrs resting and 1.5 hrs exercising on a stationary bike; 1–4 volunteers were exposed on each occasion Lung function and nasal lavage were performed before and after exposure	Reported discomfort in eyes, detection of solvent smell, headache, dizziness, and feeling of intoxication were significantly increased at 5 ppm (4 mg/m^3); there were no changes in lung function or exhaled nitric oxide levels in exposed individuals; exposure did not result in upper-airway inflammation or bronchial responsiveness	Sundblad et al., 2004

Table C-3. Controlled human exposure studies of ammonia inhalation

Subjects	Exposure conditions	Results	Reference
Healthy male and female volunteers grouped by age, 18–35 and 45–65 yrs old	Repeated 2-sec exposures at increasing concentrations ranging from 0.9 to 228 ppm (0.6–161 mg/m^3) by dynamic olfactometry There was no mention of preexposure examinations	Mean odor detection threshold <20 ppm (14 mg/m^3), mean irritation (lateralization) threshold well above 20 ppm (14 mg/m^3), dose-response for odor annoyance and irritation; strong olfactory and moderate to strong irritating sensations at >15 ppm (11 mg/m^3)	Altmann et al., 2006
43 healthy male volunteers age 21–47 yrs; one group of 30 men not familiar with the smell of ammonia and 10 men exposed to ammonia regularly at the workplace	0, 10, 20, 20 + 2 peak exposures at 40, and 50 ppm (0, 7, 14, 14 + 2 peak exposures at 28, and 35 mg/m^3) on 5 consecutive days for 4 hrs/d in an exposure chamber	Subjects familiar to ammonia reported fewer symptoms than naïve subjects; at concentrations ≤20 ppm (14 mg/m^3), there were no significant differences in symptoms reported between the groups; the perceived intensity of symptoms was concentration-dependent in both groups	Ihrig et al., 2006
25 healthy volunteers (mean age 29.7 yrs), and 15 mild/moderate persistent asthmatic volunteers (mean age 29.1 yrs)	2–500 ppm (1–354 mg/m^3) (ocular and nasal exposure) for various durations lasting up to 2.5 hrs Baseline lung function was recorded prior to exposure	Irritation threshold, odor intensity and annoyance were not significantly different between healthy volunteers and asthmatics; nasal irritation threshold = 129 ppm (91 mg/m^3); ocular irritation threshold = 175 ppm (124 mg/m^3); there were no changes in lung function (FEV_1) for subjects in either group	Petrova et al., 2008
24 healthy female volunteers age 18–45 yrs (mean age 29.9 yrs)	0.03–615.38 ppm (0.02–435 mg/m^3) (nasal exposure) for a maximum of 2 sec Preexposure measurements included rhinoscopic exam, screening for chemical sensitivities, allergies, respiratory disease, general health, and prior chemical exposure by personal interview	Both the static and dynamic methods showed similar averages for detection thresholds for the odor and irritancy of ammonia; mean odor detection threshold of 2.6 ppm (2 mg/m^3) (both static and dynamic) and mean irritation thresholds of 31.7 or 60.9 ppm (22 or 43 mg/m^3) for static and dynamic methods, respectively	Smeets et al., 2007

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C.4. CROSS SECTIONAL STUDIES OF LIVESTOCK FARMERS EXPOSED TO AMMONIA

Cross sectional studies of livestock farmers exposed to ammonia are summarized in Table C-4.

Table C-4. Cross sectional studies of livestock farmers exposed to ammonia

Subjects	Methods	Exposure conditions	Results	Reference
27 pig farmers (mean age of 29 yrs)	Environmental and personal exposures were analyzed; lung function was measured on Monday, Tuesday, and Friday	Mean exposure to dust = 1.57 mg/m^3; endotoxin = 24 ng/m^3, and ammonia = 5.60 mg/m^3	There was no significant correlation with lung function and exposure to dust or endotoxins; there was a correlation with decreased lung function (5–10%) and exposure to ammonia on the Tuesday testing, but not the Monday or Friday testing; reported respiratory symptoms included cough, phlegm, and wheezing	Heederik et al., 1990
29 farm workers; 48 electronic factory workers (controls)	20 pig houses were monitored for dust and ammonia concentrations; respiratory symptoms were determined by questionnaire; lung function tests were performed; 24 subjects provided blood samples to determine IgE and IgG antibody levels	Mean airborne ammonia concentrations ranged from 1.5 to 13.23 ppm (1–9 mg/m^3) and mean dust concentrations ranged from approximately 2 to 21 mg/m^3	Respiratory symptoms included chest tightness, wheeze, nasal and eye irritation (23/29 farm workers); 3/29 farm workers had impaired lung function (decreased FEV_1 and FVC); 3 farmers had IgE antibodies to pig squames or urine; specific IgG antibodies were found in 14 workers to pig squames, and 9 to pig urine, suggesting an allergic response	Crook et al., 1991

Table C-4. Cross sectional studies of livestock farmers exposed to ammonia

Subjects	Methods	Exposure conditions	Results	Reference
102 pig farmers (mean age 39.7 yrs; mean duration of employment of 15.7 yrs) who worked at least half time in a swine confinement building; 51 male dairy farmers (mean age 40.1 yrs; mean duration of employment of 20.3 yrs) and 81 male dairy industry workers (controls; mean age 38.5 yrs; mean duration of employment of 15.7 yrs)	Pulmonary function tests were given to subjects before and after a methacholine challenge; respiratory symptoms were determined by questionnaire	Mean total dust level of 2.41 mg/m^3; mean airborne ammonia concentration of 8.5 mg/m^3; mean personal ammonia exposure of 3.23 mg/m^3	Pig and dairy farmers had higher prevalence of reported cough and morning phlegm; bronchial hyperreactivity to methacholine was higher for pig and dairy farmers compared to controls	Choudat et al., 1994
54 male swine producers (mean age = 36.3 yrs; mean duration of employment = 10.7 yrs)	Assessment of respiratory symptoms with questionnaire and lung function tests	Mean contaminant levels: carbon dioxide = 2,632 ppm (1,861 mg/m^3); ammonia = 11.3 ppm (8 mg/m^3); total dust = 2.93 mg/m^3; respirable dust = 0.13 mg/m^3; endotoxin = 11,332 units/m^3	Exposure to high concentrations of ammonia was associated with chronic cough and bronchitis; incidence of chronic cough was dependent on interaction of ammonia with endotoxin, and respirable dust; ammonia concentrations were not correlated with changes in lung function parameters	Zejda et al., 1994
207 males ≥18 yrs of age employed at swine farms and spent time in swine confinement buildings (mean years of employment = 9.6); a farm comparison group (nonconfinement production) was included (number not given)	Pulmonary function tests were performed before shift (baseline) and then after a minimum of 2 hrs of exposure; environmental and personal air samples were made for ammonia, carbon dioxide, hydrogen sulfide, carbon monoxide, total and respirable dust	Mean personal air exposure for all subjects: total dust = 4.53 mg/m^3; respirable dust = 0.23 mg/m^3; total endotoxin = 202.35 EU/m^3; respirable endotoxin = 16.59 EU/m^3; ammonia = 5.64 ppm (4 mg/m^3)	Positive correlations were associated with pulmonary function and exposure to total dust, respirable dust, respirable endotoxin, and ammonia; exposure to ammonia concentrations of ≥7.5 ppm (5 mg/m^3) were predictive of a ≥3% decrease in FEV_1; the correlation between exposure and decreased pulmonary function was stronger after 6 yrs of exposure	Donham et al., 1995

Table C-4. Cross sectional studies of livestock farmers exposed to ammonia

Subjects	Methods	Exposure conditions	Results	Reference
194 Dutch pig farmers (94 with chronic respiratory symptoms, 100 without symptoms)	Cross-sectional study evaluating exposure response relations of exposures to dust, endotoxins, ammonia, and disinfection procedures	Estimates of long-term exposure based on two personal exposure samples (one winter sample, one summer sample); Mean estimated exposure to dust = 2.7 mg/m^3, endotoxin = 112 ng/m^3, ammonia = 2 mg/m^3	Chronic respiratory symptoms included cough, phlegm, chest tightness, and wheezing; exposure to dust, endotoxins, and ammonia were not correlated to chronic respiratory symptoms; ammonia exposure and duration of disinfection were correlated with impairment of baseline lung function (decreased FEV_1, MMEF, and PEF)	Preller et al., 1995
151 males ≥18 yrs of age employed at swine farms and spent time in swine confinement buildings (mean years of employment = 12.4); a farm comparison group (nonconfinement production) was included (number not given)	Followup study from Donham et al. (1995) previously described; followup measurements taken 48 mo from the initial measurements	Mean personal air exposure for all subjects: total dust = 3.45 mg/m^3; respirable dust = 0.26 mg/m^3; total endotoxin = 176.12 EU/m^3; respirable endotoxin = 11.86 EU/m^3; ammonia = 5.15 ppm (4 mg/m^3)	Swine workers had a mean cross-shift 2% decrease in FEV_1 that was correlated with personal exposure to total dust, total endotoxin, respirable endotoxin, and ammonia	Reynolds et al., 1996
196 pig farmers (96 with chronic respiratory symptoms, 100 without symptoms)	Pig farmers tested for lung function and bronchial responsiveness to histamine challenge	Estimates of long-term exposure based on two personal exposure samples (one winter sample, one summer sample); Mean estimated exposure to respirable dust = 2.7 mg/m^3, endotoxin = 111 ng/m^3, ammonia = 2 mg/m^3	No association between bronchial responsiveness and exposure to respirable dust, endotoxins, or ammonia; mild bronchial responsiveness was associated with the disinfectant use of quaternary ammonia	Vogelzang et al., 1997
171 pig farmers (82 with chronic respiratory symptoms, 89 without)	Longitudinal study for cohort of pig farmers observed over 3 yrs; subjects examined for lung function and tested for bronchial responsiveness to histamine challenge	Estimates of long-term exposure based on two personal exposure samples (one winter sample, one summer sample); Mean estimated exposure to respirable dust = 2.63 mg/m^3, endotoxin = 105 ng/m^3, ammonia = 2 mg/m^3	Decreased lung function (FEV_1 and FVC) was observed over time; long-term exposure to ammonia was associated with increased bronchial responsiveness to histamine; exposure to respirable dust also caused increased bronchial responsiveness to histamine	Vogelzang et al., 2000, 1998

Table C-4. Cross sectional studies of livestock farmers exposed to ammonia

Subjects	Methods	Exposure conditions	Results	Reference
Eight healthy male volunteers (23–28 yrs old)	Exposed for 4 hrs at 1-wk intervals to swine confinement buildings	Mean airborne ammonia concentration of 20.7 ppm (15 mg/m^3); also exposed to airborne dust, bacteria, endotoxin, and molds	Decreased expiratory flows (FEV_1), increased neutrophils in the nasal wash and increased white blood cell count	Cormier et al., 2000
257 poultry workers (30% women, 70% men); 63 women and 87 men nonexposed blue-collar workers served as control subjects	Personal sampling conducted for total and respirable dust, total and respirable endotoxin, and ammonia; medical evaluations included pulmonary function tests given before and after a work period	Mean exposure levels of poultry workers: ammonia = 18.4 ppm (13 mg/m^3); total dust = 6.5 mg/m^3; respirable dust = 0.63 mg/m^3; total endotoxin 1,589 EU/m^3 (0.16 μg/m^3); respirable endotoxin = 58.9 EU/m^3 (0.006 μg/m^3)	Significant cross-shift declines in pulmonary function were reported for poultry workers; concentrations associated with significant pulmonary function deficits were 12 ppm ammonia (8 mg/m^3), 2.4 mg/m^3 total dust, 0.16 mg/m^3 respirable dust, and 614 EU/m^3 endotoxin (0.614 μg/m^3)	Donham et al., 2000
Survey of 8,482 farmers and spouses; exposure study conducted in 102 farmers	Exposure study with survey of respiratory symptoms; personal exposure to total dust, fungal spores, bacteria, endotoxin, and ammonia in 12 tasks were measured in 102 farmers	Ammonia concentrations ranged from 0 to 8.2 ppm (0–6 mg/m^3) over the 12 tasks; total dust (0.4–5.1 mg/m^3), fungal spores (0.02–2.0 10^6/m^3), bacteria (0.2–48 10^6/m^3), endotoxin (0.5–28/10^3 EU/m^3 [0.05–2.8 μg/m^3)	There was a significant positive correlation between task mean exposures to total dust, fungal spores, and endotoxins and task-specific symptoms; there was no association between exposures to bacteria and ammonia and task specific symptoms; symptoms included eye, nose, and throat irritation, cough, chest tightness, and wheezing	Melbostad and Eduard, 2001
13 stable workers (6 males, 7 females)	Stable workers were tested for lung function and nasal lavage was performed to analyze for inflammation markers; tests were performed during two consecutive winters and the interjacent summer	Ammonia concentration was 20–27 ppm (14–19 mg/m^3) in late summer, but was not detected in winter; levels of endotoxin were highest during late summer (15 ng/m^3) while levels of 1,3-β-glucan (85 ng/m^3) and horse allergen (18,300 U/m^3) were highest during the winter	Increased PEF-variability in 2/13 workers; eosinophil cationic protein in 3/13 (indicative of bronchial obstruction and allergic inflammation equivalent to allergic asthma); increased myeloperoxidase and lysozyme levels in 9/13 (indicating enhanced activity of neutrophil granulocytes in the airways and enhanced mucosal secretion)	Elfman et al., 2009

EU = endotoxin unit (10 EU/ng); MMEF = mean midexpiratory flow

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C.5. ACUTE AND SHORT-TERM INHALATION TOXICITY STUDIES OF AMMONIA IN EXPERIMENTAL ANIMALS

Acute and short-term inhalation studies (exposure duration of ≤30 days) of ammonia in experimental animals are summarized in Table C-5. These studies demonstrate that ammonia inhalation can produce changes in pulmonary function and histopathological changes in the respiratory tract. Acute effects may be followed by chronic respiratory dysfunction characterized by secondary bronchitis, bronchiolitis, and bronchopneumonia. In studies of cardiovascular and/or metabolic effects of acute or short-term ammonia exposure, ammonia exposure was associated with bradycardia, arterial pressure variations, and acidosis (as evidenced by a decrease in blood pH and an increase in arterial blood pCO_2). Several studies have investigated amino acid levels and neurotransmitter metabolism in the brain of rats and mice following acute inhalation exposure to ammonia. It has been suggested that glutamate and γ-amino butyric acid (GABA) play a role in ammonia-induced neurotoxicity.

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Rats					
Female Porton rats (16/group)	0 or 141 (0 or 200 ppm)	Continuous exposure for 4, 8, or 12 d	Histology of the trachea	4 d: transitional-stratified appearance of the epithelium; 8 d: gross change with disappearance of cilia and stratification on luminal surface; 12 d: increased epithelial thickness	Gamble and Clough, 1976
Male OFA rats (27/group)	0 or 354 (0 or 500 ppm)	Continuous exposure for 1–8 wks	Body weight, organ weights, airway structure, cell population, alveolar macrophages	No deaths occurred; decreased food consumption and body weight gain; increased lung and kidney weights; at 3 wks nasal irritation and upper respiratory tract inflammation, but no effect on lower airways; slight decrease in alveolar macrophages; no histopathological effects seen at 8 wks, suggesting adaptation to exposure	Richard et al., 1978a
Male and female Wistar rats (5/sex/group)	9,898–37,825 (14,000–53,500 ppm) (no mention of control group)	10, 20, 40, or 60 min	Clinical signs, pathology, LC_{50}	Eye irritation, eye and nasal discharge, dyspnea; hemorrhagic lungs on necropsy; 10-min LC_{50} = 28,492 mg/m^3 (40,300 ppm) 20-min LC_{50} = 20,217 mg/m^3 (28, 595 ppm) 40-min LC_{50} = 14,352 mg/m^3 (20,300 ppm) 60-min LC_{50} = 11,736 mg/m^3 (16,600 ppm)	Appelman et al., 1982
Male Crl:COBS CD (Sprague-Dawley) rats (8/group)	11, 23, 219, and 818 (15, 32, 310, and 1,157 ppm); arterial blood collected prior to exposure served as control	24 hrs	Clinical signs, histology, blood pH, blood gas measurement	No clinical signs of toxicity, no histologic differences in tracheal or lung sections, no change in blood pH or pCO_2, minor changes in pO_2	Schaerdel et al., 1983
Male Crl:COBS CD (Sprague-Dawley) rats (14/group)	3, 17, 31, 117, and 505 (4, 24, 44, 165, and 714 ppm); arterial blood collected prior to exposure served as control	3 and 7 d	Hepatic cytochrome P450 content and ethylmorphine–N-demethylase activity	No dose-related change in P450 content or enzyme activity	Schaerdel et al., 1983

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Male Long Evans rats (4/group)	70 and 212 (100 and 300 ppm); results were compared to "control", but it was not clear if the authors were referring to historical or concurrent controls	6 hrs	Clinical signs, behavioral observation	Decreased running, decreased activity	Tepper et al., 1985
Female Wistar rats (5/group)	0, 18, or 212 (0, 25, or 300 ppm)	6 hrs/d for 5, 10, or 15 d	Blood ammonia, urea, glutamine, and pH; brain ammonia, glutamine; histopathology of lungs, heart, liver, and kidneys (light and electron microscopy)	Brain and blood glutamine increased; slight acidosis (i.e., decreased blood pH) 212 mg/m^3 (300 ppm); lung hemorrhage observed in some exposed rats	Manninen et al., 1988
Female Wistar rats (5/group)	0, 18, or 212 (0, 25, or 300 ppm)	6 hrs/d for 5 d	Plasma and brain ammonia and amino acid analysis	Increase in brain and plasma glutamine concentrations, increased brain/plasma ratio of threonine	Manninen and Savolainen, 1989
Female albino rats (8/group)	0, 848–1,068 (1,200–1,510 ppm)	3 hrs	Mortality, respiratory movement and O_2 consumption	No deaths reported; inhibition of external respiration and decreased O_2 consumption	Rejniuk et al., 2007
Male Sprague-Dawley rats (number/group not given)	Air concentration not given; ammonia vapor added to inspiratory line of ventilator; controls exposed to same volume of room air	20 sec	Activity of upper thoracic spinal neurons	Lower airway irritation, activation of vagal pulmonary afferents and upper thoracic spinal neurons receiving pulmonary sympathetic input	Qin et al., 2007a, b

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Male Wistar rats (4/group)	0, 92–1,243 (130–1,758 ppm); the preexposure period was used as the control for each animal	45 min	Airway reflexes by the changes in respiratory patterns elicited by ammonia in either dry, steam-humidified or aqueous aerosol containing atmospheres	Ammonia-induced upper respiratory tract sensory irritation is not affected to any appreciable extent by wet atmospheres (with or without aerosol) up to 1,243 mg/m^3	Li and Pahluhn, 2010
Male rats (10/group)	0, 848–1,068 (0, 1,200–1,510 ppm) at the beginning and end of the exposure period)	3 hrs	Oxygen consumption	Decreased O_2 consumption	Rejniuk et al., 2008
Mice					
Mice (20/group, species, sex not specified)	6,080–7,070 (8,600–10,000 ppm); no controls	10 min	LC_{50}	LC_{50} = 7,056 mg/m^3 (9,980 ppm)	Silver and McGrath, 1948
Male Swiss albino mice (4/group)	5,050–20,199 (7,143–28,571 ppm); no controls	30–120 min	LC_{50}	LC_{50} (30 min) = 15,151 mg/m^3 (21,430 ppm)	Hilado et al., 1977
Albino mice (sex not specified; 6/dose)	Air concentration not measured; results were compared to "control", but it was not clear if the authors were referring to historical or concurrent controls	Continuously for 2 or 5 d	Regional brain metabolism (cerebral cortex, cerebellum, brainstem); MAO, enzymes of glutamate and ã-amino butyric acid (GABA) metabolism, and (Na^+-K^+)-ATPase; amino acid levels in the brain	Altered activities of MAO, glutamate decarboxylase, alanine amino transferase, GABA-transaminase, and (Na^+-K^+)-ATPase; increased alanine and decreased glutamate	Sadasivudu et al., 1979; Sadasivudu and Murthy, 1978

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Male Swiss-Webster mice (4/group)	Concentrations not given; baseline levels established prior to exposure	10 min	Reflex decrease in respiratory rate was used as an index of sensory irritation; RD_{50} = the concentration associated with a 50% decrease in the respiratory rate	RD_{50} = 214 mg/m^3 (303 ppm)	Kane et al., 1979
Male albino ICR mice (12/dose)	0–3,436 (0–4,860 ppm)	1 hr (14-d follow up)	Clinical signs, body weight, organ weight, histopathology, LC_{50}	Eye and nose irritation, dyspnea, ataxia, seizures, coma, and death; decreased body weight and increased liver to body weight ratio in mice surviving to 14 d; effects in the lung included focal pneumonitis, atelectasis, and intralveolar hemorrhage; liver effects included hepatocellular swelling and necrosis, vascular congestion; LC_{50} = 2,990 mg/m^3 (4,230 ppm)	Kapeghian et al., 1982
Male Swiss-Webster mice (16–24/group)	0 or 216 (0 or 305 ppm)	6 hrs/d for 5 d	Respiratory tract histopathology	Lesions in the nasal respiratory epithelium (moderate inflammation, minimal necrosis, exfoliation, erosion, or ulceration); no lesions in trachea or lungs	Buckley et al., 1984
Male albino ICR mice (12/dose)	0, 954, 3,097, or 3,323 (0, 1,350, 4,380, or 4,700 ppm)	4 hrs	Hexobarbitol sleeping time, microsomal protein content, liver microsomal enzyme activity	Increased hexobarbitol sleeping time (3,097 mg/m^3), increased microsomal protein content, aminopyrene-N-deethylase and aniline hydroxylase activities (3,323 mg/m^3)	Kapeghian et al., 1985
Male albino ICR mice (12/dose)	0, 81, or 233 (0, 115, or 330 ppm)	4 hrs/d for 4 d	Microsomal protein content, liver microsomal enzyme activity	No dose-dependent effects on microsomal enzymes	Kapeghian et al., 1985
Male Swiss mice (6/dose)	71 and 212 (100 and 300 ppm); data collected during the 2 d separating each ammonia exposure served as the control baseline	6 hrs	Clinical signs, behavioral observation	Decreased running, decreased activity	Tepper et al., 1985

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Mice (4/group)	3, 21, 40, or 78 (4, 30, 56, or 110 ppm), lowest measured concentration was the nominal control group	2 d	Responses to atmospheric ammonia in an environmental preference chamber with four chambers of different concentrations of ammonia	No distinguishable preference for, or aversion to, different NH_3 concentrations	Green et al., 2008
Male OF1 mice (4/group)	0, 92–1,243 (130–1,758 ppm); the preexposure period was used as the control for each animal	45 min	Airway reflexes by the changes in respiratory patterns elicited by ammonia in either dry, steam-humidified, or aqueous aerosol containing atmospheres	Ammonia-induced upper respiratory tract sensory irritation is not affected to any appreciable extent by wet atmospheres (with or without aerosol) up to 1,243 mg/m^3	Li and Pahluhn, 2010
Rabbits					
Female New Zealand White rabbits (7–9/dose)	0, 35, or 71 (0, 50, or 100 ppm)	2.5–3.0 hrs	Pulmonary function	Decreased respiratory rate at both concentrations	Mayan and Merilan, 1972
Rabbits (species, sex, number/dose not specified)	0, 707–14,140 (0, 1,000–20,000 ppm)	15–180 min	Pulmonary function, death	Bradycardia at 1,768 mg/m^3 (2,500 ppm); arterial pressure variations and blood gas modifications (acidosis indicated by decreased pH and increased pCO_2) at 3,535 mg/m^3 (5,000 ppm); death occurred at 4,242 mg/m^3 (6,000 ppm)	Richard et al., 1978b
New Zealand White rabbits (16 total; 8/dose)	Peak concentrations: 24,745–27,573 mg/m^3 (35,000–39,000 ppm); concurrent controls tested	4 min	Pulmonary function, heart rate, blood pressure, blood gases	Lung injury was evident after 2–3 min (decreased pO_2, increased airway pressure)	Sjöblom et al., 1999

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Cats					
Mixed breed stray cats (5/group)	0 or 707 (0 or 1,000 ppm)	10 min	Pulmonary function, lung histopathology on 1, 7, 21, and 35 d postexposure	Pulmonary function deficits were correlated with lung histopathology; acute effects are followed by chronic respiratory dysfunction (secondary bronchitis, bronchiolitis, and bronchopneumonia)	Dodd and Gross, 1980
Pigs					
Young pigs (2/group)	0, 35, 71, or 106 (0, 50, 100, or 150 ppm)	Continuous exposure for 4 wks	Clinical signs, food consumption, body weight, gross necropsy, organ weight, histopathology	Lethargy and histopathological alterations in the tracheal and nasal epithelium were observed at 71 and 106 mg/m^3; decreased body weight occurred at all concentrations (7–19% decrease from control)	Drummond et al., 1980
Male and Female Belgian Landrace pigs (4/group)	0, 18, 35, or 71 (0, 25, 50, or 100 ppm)	6 d	Clinical signs, body weight, pulmonary function	Lethargy and decreased body weight gain (all concentrations); no effect on pulmonary microvascular hemodynamics and permeability	Gustin et al., 1994
Belgian Landrace pigs (4/group)	0, 18, 35, or 71 (0, 25, 50, or 100 ppm)	6 d	Clinical signs, body weight, neutrophil count, and albumin in nasal lavage fluid	Nasal irritation (increased neutrophils in nasal lavage fluid) and decreased body weight gain at all concentrations	Urbain et al., 1994
Landrace-Yorkshire pigs (4/group)	0 or 42 (0 or 60 ppm)	15 min/d for 8 wks	Thromboxane A2 (TXA2), leukotriene C4 (LTC4), and prostaglandin (PGI2) production	Significant increases in TXA2 and LTC4, no significant effect on PGI2 production	Chaung et al., 2008
Hybrid gilts (White synthetic Pietrain, white Duroc, Landrace, Large White) (14 pigs/group)	<4 (control) or 14 (<5 or 20 ppm)	15 wks	Salivary cortisol, adrenal morphometry, body weight, food conversion efficiency, general health scores, play behavior; reaction to light and noise intensity tested concurrently	Decreased salivary cortisol, larger adrenal cortices, less play behavior, no measurable impact on productivity or physiological parameters	O'Connor et al., 2010

Table C-5. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Concentration (mg/m^3)	Duration	Parameter examined	Results	Reference
Cattle					
Male Holestein calves (number/group not specified)	0, 35, or 71 (0, 50, or 100 ppm)	2.5 hrs	Respiration rate, clinical chemistry	No significant effect on respiration, BUN, pH, pO_2, or pCO_2	Mayan and Merilan, 1976
Brahman/Charolais (group size not reported)	<6 (control), 11, 23, or 34 (<8 [control], 16, 32, or 48 ppm)	12 d	Behavioral activity, body weight, analysis of bronchioalveolar lavage (BAL) fluid, hematological variables (hemoglobin, mean cell volume, platelet volume, eosinophils, neutrophils, total white cell count, monocytes)	Increased lacrimation, nasal secretions, coughing, increased standing (as opposed to lying down), dose related increases in macrophage activity and neutrophil percentage in BAL fluid indicating pulmonary inflammation, no effect on hematological variables or body weight	Phillips et al., 2010
Holstein Friesian and Brown Swiss (10 of each breed)	~0, 4, and 15, (0.3×10^{-6}, 6, and 21 ppm)	10 d at each concentration	Respiration and pulse rate, blood gas parameters	Respiration and pulse rates were higher in inadequately ventilated barns (elevated ammonia and CO_2)	Sabuncuoglu et al., 2008

C.6. MECHANISTIC STUDIES

Portions of this appendix were adapted from the Mechanisms of Action and Genotoxicity sections (Sections 3.3 and 3.5) of the Toxicological Profile for Ammonia (ATSDR, 2004) under a Memorandum of Understanding with ATSDR.

C.6.1. Irritation

As described in Section 4.1, ammonia is an irritant in humans where the primary and most immediate effect of ammonia exposure is burns to the skin, eyes, gastrointestinal tract, and respiratory tract. Due to its high water solubility, ammonia interacts immediately upon contact with available moisture in the skin, eyes, oral cavity, respiratory tract, and mucous membranes to form ammonium hydroxide, which is a weak base. Ammonium hydroxide causes the necrosis of tissues through disruption of cell membrane lipids (saponification) leading to cellular destruction (Jarudi and Golden, 1973). As cell proteins break down, water is extracted, resulting in an inflammatory response, which further damages the surrounding tissues (Amshel et al., 2000). The severity of tissue damage is related to the concentration of the hydroxyl ions and the duration of exposure (White et al., 2007; Welch, 2006; Millea et al., 1989).

C.6.2. Gastric Mucosal Damage

Ammonium ion may also contribute to adverse effects of *H. 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 decreased viability of the cells compared to a urease negative variant of the same cells (Mégraud et al., 1992). An in vivo study suggested that ammonia also causes macroscopic gastric lesions and increases the release of endothelin-1 and thyrotropin releasing hormone from the gastric mucosa, probably via an endothelin-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). Suzuki et al. (2000) reported findings of an in vitro assay with gastric surface mucous cells from mice that demonstrated apoptosis directed by mitochondrial membrane destruction and enhancing activities of caspase-3 and caspase-9 at concentrations of ammonia detected in *H. pylori* infected patients. Neutrophils that migrate to the gastric mucosa in response to the presence of *H. pylori* may release hypochlorous acid, which can interact with ammonium ion to produce the powerful cytotoxic oxidizing agent monochloramine (Murakami et al., 1995). Igarashi et al. (2001) suggest that ammonia accelerates cytokine-induced apoptosis in gastric epithelial cells.

Tsujii et el. (1992b) studied the mechanism by which ammonia damages the gastric mucosa by measuring the following: gastric mucosal damage, gastric mucosal hemodynamics, the viability and oxygen consumption of cells separated from the gastric mucosa, and the effect of ammonia on gastric mucosa mitochondrial oxygen consumption. For the measurement of gastric mucosal damage, ammonia solutions (2 mL; 0, 125, 187.5, and 150 mM) were administered to the stomach of male Sprague-Dawley rats (6 rats/group; 220-250 g). Thirty minutes later, the ulcer index (defined as the ratio of the ulcerated area to that of the whole stomach as measured using an image-analyzer system) was measured. Solutions ≥187.5 mM ammonia induced significantly larger ulcers and the increase was dose dependent. The effect of pH of the initial ammonia solution was measured by repeating the experiment with 250 mM glycine-NaOH/250 mM sodium phosphate solution (pH 10.3); this solution did not cause any significant damage to the mucosa suggesting that the effect was due to ammonia and not to elevated pH.

The effects of ammonia on gastric mucosal hemodynamics were measured (5 rats/group) after the surgical placement of an optical fiber bundle of a reflectance spectrometer in the forestomach. Rats were administered 2 ml 250 mM ammonia/250 mM sodium phosphate solution and their hemoglobin concentration and hemoglobin oxygen saturation were measured. There was no significant effect of ammonia administration on indices of gastric hemoglobin concentration or hemoglobin oxygen saturation.

Cells from the gastric mucosa were separated and isolated. Ammonia (0, 1, 5, or 10 mM ammonia in sodium phosphate buffer, pH 7.4) was added to the cell cultures and incubated for 2 hours at 37 ºC. Cell viability was measured using trypan blue exclusion. Cell viability and oxygen consumption were significantly lower at 5 and 10 mM ammonia in sodium phosphate buffer.

Mitochondria were obtained from the gastric mucosa of five rabbits (2 kg, the strain was not specified) after removal of the stomach. Mitochondrial suspensions were treated with 0, 0.01, 0.1, 1, 5, or 10 mM ammonia/sodium phosphate in Tris buffer. Oxygen consumption was measured after the addition of α-ketoglutaric acid followed by adenosine diphosphate. There was significant inhibition of oxygen consumption at the lowest ammonia concentration tested (0.01%) without the addition of α-ketoglutaric acid or adenosine diphosphate. Oxygen consumption was significantly lower with α-ketoglutaric acid as the substrate at ≥1 mM ammonia concentrations; with adenosine diphosphate as the substrate, ammonia concentrations ≥0.1 mM were inhibitory.

In summary, Tsujii et al. (1992b) showed that millimolar concentrations of ammonia inhibit cellular respiration and was cytotoxic to gastric mucosal cells. The effects were not due

to the elevated pH of the ammonia solutions. The authors concluded that ammonia-induced gastric mucosal damage may be due to impairment of cellular energy metabolism.

C.6.3. Genotoxicity Studies

A limited number of genotoxicity studies are available for ammonia vapor; four of the available studies were published between 1932 and 1951. Studies examining in vivo genotoxicity are described in Table C-6. Yadav and Kaushik (1997) examined the genotoxic effects of ammonia exposure in 22 fertilizer factory workers exposed to ammonia at ambient concentrations of 88.2 $\mu g/m^3$; 42 nonexposed workers served as control subjects. Increased frequencies of chromosomal aberrations and sister chromatid exchanges in lymphocytes were observed in exposed workers compared to control subjects. Frequencies of chromosomal aberrations, sister chromatid exchanges, and mitotic index all increased with increased duration of exposure. This study is difficult to interpret because of small samples sizes and confounding by smoking and alcohol consumption. In addition, the levels of ammonia in the plant seemed low compared to other fertilizer plant studies (see for example Section 4.1.4; Rahman et al., 2007; Ali, 2001; Ballal et al., 1998); the accuracy and reliability of the sampling and measurement could not be determined.

Table C-6. In vivo genotoxicity studies of ammonia

Test system	Endpoint	Test conditions	Results[a]	Dose or concentration[b]	Reference
Human lymphocytes	Chromosome aberrations	22 healthy workers occupationally exposed to ammonia in fertilizer factory (ambient concentration of 88.28 µg/m^3); nonexposed factory staff served as control subjects	+	88.28 µg/m^3	Yadav and Kaushik, 1997
Human lymphocytes	Sister chromatid exchanges		+	88.28 µg/m^3	Yadav and Kaushik, 1997
Swiss albino mice	Micronucleus	Intraperitoneal injections for 24–48 hr expression times	+	12.5-50 mg/kg	Yadav and Kaushik, 1997
Drosophila melanogaster	Dominant lethal mutations	Dominant lethal assay; inhalation exposure up to 450 ppm (318 mg/m^3) ammonia, 6 hrs/d for 5 d	+ (T)	NA	Lobasov and Smirnov, 1934
D. melanogaster	Sex-linked recessive lethal mutations	Inhalation exposure to ammonia as vapor at a concentration killing the majority of flies	– (T)	NA	Auerbach and Robson, 1947
D. melanogaster	Dominant lethal mutations	Inhalation exposure to ammonia as vapor at a concentration killing the majority of flies	– (T)	NA	Auerbach and Robson, 1947

[a]+ = positive; − = negative; (T) = toxicity reported; NA = not available.
[b]Lowest effective dose for positive results, highest dose tested for negative or equivocal results.

Positive results were obtained in a single micronucleus assay with mice (Yadav and Kaushik, 1997). One study in Drosophila exposed to ammonia gas was positive for mutagenicity; however, survival after exposure was <2% (Lobasov and Smirnov, 1934). Results from another study in Drosophila were negative for sex-linked recessive lethal and dominant lethal mutagenicity; however, the majority of Drosophila was killed by ammonia treatment (Auerbach and Robson, 1947).

In vitro tests of the genotoxic effects of ammonia have been performed in bacteria and in chick fibroblasts (Table C-7). Ammonia (administered as vapor) did not induce reverse mutations in *Salmonella typhimurium* or *E. coli* either with or without metabolic activation (Shimizu et al., 1985). Demerec et al. (1951) reported an increase in reverse mutations in *E. coli*; however, positive findings were only reported for levels of ammonia that were toxic, with 98% lethality. Chick fibroblasts immersed in buffered ammonia solution were found to have increased frequencies of chromosomal aberrations (Rosenfeld, 1932).

Table C-7. In vitro genotoxicity studies of ammonia

Test system	Endpoint	Test conditions	Results[a]		Dose[c]	Reference
			Without activation	With activation[b]		
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1538); *E. coli* (WP2 uvrA)	Reverse mutation	Plate incorporation assay with ammonia vapor	–	–	25,000 ppm (17,675 mg/m^3) ammonia vapor	Shimizu et al., 1985
E. coli (B/SD-4 strains)	Reverse mutation, streptomycin resistance	Plate incorporation assay	+ (T)	ND	0.25% ammonia[d]	Demerec et al., 1951
Chick fibroblasts	Chromosomal aberrations	Cultures immersed in buffered ammonia solution	+	ND	NA	Rosenfeld, 1932

[a]+ = positive; − = negative; (T) = toxicity reported; NA = not available; ND = no data.
[b]Exogenous metabolic activation used; S9 liver fractions from male Sprague-Dawley rats pretreated with pentachlorobiphenyl (KC500).
[c]Lowest effective dose for positive results, highest dose tested for negative or equivocal results.
[d]Only positive in treatments using toxic levels of NH_3 (98% lethality).

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