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Toxicological Review of Ammonia

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

Supplemental Information

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TABLES AND FIGURES

ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BrDU	bromodeoxyuridine
BUN	blood urea nitrogen
CAC	cumulative ammonia concentration
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
IgE	immunoglobulin E
IgG	immunoglobulin G
IRIS	Integrated Risk Information System
LC_{50}	50% lethal concentration
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
NCEA	National Center for Environmental Assessment
NH_3	ammonia
NH_4^+	ammonium ion
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
ORD	EPA's Office of Research and Development
PEF	peak expirartory flow
PEFR	peak expiratory flow rate
RD_{50}	50% response dose
TLV	threshold limit value
UF	uncertainty factor

APPENDIX A. CHEMICAL AND PHYSICAL INFORMATION FOR AMMONIA

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 (Read, 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 A-1.

Table A-1. Chemical and physical properties of ammonia

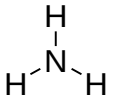
Chemical name	Ammonia[a]	
Synonym(s)	AM-Fol; anhydrous ammonia; ammonia gas; Nitro-sil; R 717; Spirit of hartshorn	ChemIDPlus (2012)
Structure		ChemIDPlus (2012)
Chemical formula	NH_3	ChemIDPlus (2012)
CASRN	7664-41-7[a]	ChemIDPlus (2012)
Molecular weight	17.031	Lide (2008, pp. 4.46-4.48, 8.40)
Form	Colorless gas; corrosive	O'Neil et al. (2006)
Melting point	-77.73°C	Lide (2008, pp. 4.46-4.48, 8.40)
Boiling point	-33.33°C	Lide (2008, pp. 4.46-4.48, 8.40)
Odor threshold	53 ppm (37 mg/m^3) 2.6 ppm (2 mg/m^3)	O'Neil et al. (2006) Smeets et al. (2007)
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, pp. 4.46-4.48, 8.40)
Solubility: Water Organic solvents	 4.82×10^5 mg/L at 24°C Soluble in ethanol, chloroform, and ether	 Dean (1985, pp. 10-3, 10-23); Lide (2008, pp. 4.46-4.48, 8.40); O'Neil et al. (2006)
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)

Table A-1. Chemical and physical properties of ammonia

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)

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

APPENDIX B. TOXICITY INFORMATION FOR SELECTED AMMONIUM SALTS

Due to uncertainty concerning the possible influence of anions on the toxicity of ammonium, information on ammonium salts was not used to characterize the effects or to derive reference values for ammonia or ammonium hydroxide. A summary of the subchronic and chronic toxicity of selected ammonium salts is presented here as supplemental information.

The toxicology literature for ammonium salts includes 13-, 78-, and 130-week ammonium chloride dietary studies in male and female Wistar rats (Lina and Kuijpers, 2004), a 47-week ammonium chloride drinking water study in Sprague-Dawley rats (Barzel and Jowsey, 1969), and 52- and 104-week ammonium sulfate dietary studies in male and female F344 rats (Ota et al., 2006). No inhalation toxicity studies of ammonium salts were found.

Ammonium chloride in the diet or drinking water of rats consistently altered the acid-base balance in the body (Lina and Kuijpers, 2004; Barzel and Jowsey, 1969) causing a dose-related hyperchloremic metabolic acidosis in rats as evidenced by increased plasma chloride levels and decreases in blood pH, base excess, and bicarbonate concentration. Ammonium chloride administered in the diet for 130-weeks was also associated with zona glomerulosa hypertrophy of the adrenal gland (Lina and Kuijpers, 2004). Kidney weights were not significantly affected by exposure to ammonium chloride for 78 or 130 weeks (Lina and Kuijpers, 2004); liver weights were not reported in this study.

Dietary administration of ammonium sulfate to rats has not been associated with metabolic acidosis, but this endpoint was not specifically evaluated in the 52- or 104-week studies by (Ota et al., 2006). Unlike ammonium chloride, no histopathologic changes in the adrenal gland were observed following ammonium sulfate exposure (Ota et al., 2006). The dose-related effects in male and female rats associated with 52-week exposure to ammonium sulfate were increased liver and kidney weights (Ota et al., 2006)). See Table B-1 for study details.

Table B-1. Summary of repeat dose studies of selected ammonium salts following oral exposure

Study design and reference	Results
Ammonium chloride	
Wistar rat (10/sex/group) 0, 1,590, or 3,050 mg/kg-d (males); 0, 1,800, or 3,700 mg/kg-d (females) administered in diet for 13 wks Lina and Kuijpers, 2004; Barzel and Jowsey, 1969	*Body weight*: ↓ (6–17% in males; 11–19% in females) *Liver weight*: not reported *Kidney weight (relative)*: ↑ (both dose levels, both sexes, 7–28%) *Adrenal weight (relative)*: ↑ (high-dose males, 18%) *Metabolic acidosis*[a]: observed in males and females; severity increased with dose *ALP activity*: ↑ at high dose, no change at lower doses
Wistar rat (15/sex/group) 0, 481, or 1,020 mg/kg-d (males); 0, 610, or 1,370 mg/kg-d (females) administered in diet for 78 wks Lina and Kuijpers, 2004; Barzel and Jowsey, 1969	*Body weight*: no significant change *Liver weight*: not reported *Kidney weight (relative)*: no significant change *Adrenal weight (relative)*: no significant change *Metabolic acidosis*[a]: observed in males and females; severity increased with dose *ALP activity*: not measured
Wistar rat (50/sex/group) 0, 455, or 1,000 mg/kg-d (males); 0, 551, or 1,200 mg/kg-d (females) administered in diet for 130 wks Lina and Kuijpers, 2004; Barzel and Jowsey, 1969	*Body weight*: no significant change *Liver weight*: not reported *Kidney weight (relative)*: no significant change *Adrenal weight (relative)*: no significant change *Metabolic acidosis*[a]: observed in males and females; severity increased with dose *ALP activity*: not measured *Hypertrophy of the adrenal glomerulosa*: ↑ incidence (both doses in males, high dose only in females) *Chronic progressive nephrosis*: ↓ incidence in males at the highest dose
Sprague-Dawley rat (11 males/group) 0 or 1,800 mg/kg-d administered in drinking water for 47 wks Lina and Kuijpers, 2004; Barzel and Jowsey, 1969	*Body weight*: ↓ (13–20% with regular and low-calcium diets, respectively) *Kidney weight (relative)*: not measured *Kidney weight (absolute)*: no change *Adrenal weight (relative)*: not measured *Femur weight (relative)*: ↓ *Femur calcium*: ↓ *Metabolic acidosis*: was inferred from measurements of reduced blood pH and plasma carbon dioxide *ALP activity*: not measured
Ammonium sulfate	
F344 rat (10/sex/group) 0, 42, 256, or 1,527 mg/kg-d (males); 0, 48, 284, or 1,490 mg/kg-d (females) administered in diet for 52 wks Ota et al., 2006	*Body weight*: no significant change in males and females *Liver weight (relative)*: ↑ in males (7%); ↑ in females (7%) *Kidney weight (relative)*: ↑ in males (10%); ↑ in females (10%) *Adrenal weight (relative)*: no significant change in males and females *Metabolic acidosis*[a]: not measured *ALP activity*: not significantly changed (except in females at intermediate dose, 284 mg/kg, % change compared to control ALP activity was -19%)

Table B-1. Summary of repeat dose studies of selected ammonium salts following oral exposure

Study design and reference	Results
F344 rat (50/sex/group) 0, 564, or 1,288 mg/kg-d (males); 0, 650, or 1,371 mg/kg-d (females) administered in diet for 104 wks Ota et al., 2006	*Body weight*: not measured *Liver weight (relative)*: not measured *Kidney weight (relative)*: not measured *Adrenal weight (relative)*: not measured *Metabolic acidosis*[a]: not measured *ALP activity*: not measured *Hypertrophy of the adrenal glomerulosa*: no change in incidence *Chronic nephropathy*: ↑ incidence in male rats over control (1/48, 5/49, 3/48 in the control, mid and high dose); increase was statistically significant only at the mid-dose.

[a]Metabolic acidosis was assessed as decreased base excess in blood, decreased urinary pH, and increased urinary net acid excretion.

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APPENDIX C. HEALTH ASSESSMENTS AND REGULATORY LIMITS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Toxicity values and other health-related regulatory limits for ammonia that have been developed by other national and international health agencies are summarized in Table C-1.

Table C-1. Other national and international health agency assessments for ammonia

Organization	Toxicity value
Agency for Toxic Substances and Disease Registry (ATSDR, 2004)	Chronic inhalation MRL = 0.1 ppm (0.07 mg/m^3) ***Basis***: Lack of significant alterations in lung function in chronically exposed workers (Holness et al., 1989) and a composite UF of 30 (10 for human variability and a modifying factor of 3 for the lack of reproductive and developmental studies).
National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NRC, 2008)	**AEGL-1** (nondisabling) = 30 ppm (21 mg/m^3) for exposures ranging from 10 mins to 8 hrs to protect against mild irritation ***Basis***: mild irritation in human subjects (MacEwen et al., 1970) **AEGL-2** (disabling) = 220 ppm (154 mg/m^3) for a 10-min exposure to 110 ppm (77 mg/m^3) for an 8-hr exposure ***Basis***: irritation (eyes and throat; urge to cough) in human subjects (Verberk, 1977) **AEGL-3** (lethal) = 2,700 ppm (1,888 mg/m^3) for a 10-min exposure to 390 ppm (273 mg/m^3) for an 8-hr exposure ***Basis***: lethality in the mouse (Kapeghian et al., 1982; MacEwen and Vernot, 1972)
American Conference of Governmental Industrial Hygienists (ACGIH, 2001) TLV established in 1973	TLV = 25 ppm (17 mg/m^3)[a] TWA for an 8-hr workday and a 40-hr work week ***Basis***: To protect against irritation to eyes and the respiratory tract. ACGIH stated that irritation is the prime hazard to workers, but that systemic effects cannot be ruled out based on the findings of reduced feed consumption and body weight loss in pigs exposed to 103 and 145 ppm ammonia. References cited in support of the TLV included papers from the primary literature for the years up to 1973; no specific reference served as the basis for the TLV.
National Institute for Occupational Safety and Health (NIOSH, 2010) REL established in 1992	REL = 25 ppm (18 mg/m^3)[a] TWA for up to a 10-hr workday and a 40-hr work week ***Basis***: To project against respiratory and eye irritation. References cited in support of the REL included review documents for the years up to 1992; no specific reference served as the basis for the REL.

Table C-1. Other national and international health agency assessments for ammonia

Organization	Toxicity value
Occupational Safety and Health Administration (OSHA, 2006) PEL established in early 1970s	PEL for general industry = 50 ppm (35 mg/m^3) TWA for an 8-hr work day ***Basis***: The 1968 ACGIH TLV was promulgated as the OSHA PEL soon after adoption of the Occupational Safety and Health Act in 1970. The ACGIH TLV from 1968 was intended to protect against irritation of ammonia in humans; no specific reference served as the basis for the 1968 TLV.
Food and Drug Admistration (FDA, 2011a, b)	Ammonium hydroxide: direct food substance affirmed as generally recognized as safe (21 CFR 184.1139); substance generally recognized as safe when used in accordance with good manufacturing or feeding practices (21 CFR 582.1139)

[a]ACGIH andr NIOSH used different ppm to mg/m^3 conversion factors.

AEGL = Acute Exposure Guideline Level; MRL = minimal risk level; PEL = Permissible Exposure Limit; REL = Recommended Exposure Limit; TWA = time weighted average; UF = uncertainty factor.

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APPENDIX D. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-REPONSE ANALYSIS

D.1. TOXICOKINETICS

Overview

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 occurs. 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 is an integral part of nitrogen homeostasis. Given its important metabolic role, ammonia exists in a homeostatically regulated equilibrium in the body.

Absorption

Inhalation Exposure

Experiments with volunteers[1] show that ammonia, regardless of its tested concentration in air (range, 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 Herrmann, 1950). However, longer-term acute exposure (10–27 minutes) to a concentration of 354 mg/m^3 resulted in lower

[1]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.

retention (4–30%), with expired breath concentrations of 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. 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 (18 mg/m^3), assuming 30% uptake into blood, would yield an increase in blood ammonia concentration of 0.09 µg/mL (calculated by IPCS, 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 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 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).

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 and 33 µg/mL, respectively (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. Approximately 4,200 mg of ammonia are produced each day with >70% of that amount liberated from fecal contents within the colon (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 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.

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). IPCS (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.

Distribution

The range of mean ammonia concentrations in humans as a result of endogenous production was reported as 0.1–0.6 µg/mL in arterial blood and 0.2–1.7 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-Wollheim, 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; Hauguel et al., 1983; Meschia et al., 1980; Remesar et al., 1980; Holzman et al., 1979; Holzman et al., 1977; Rubaltelli and Formentin, 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 that 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 approximately 0.39 to 0.60 µg/mL (Jóźwik et al., 2005; Jóźwik et al., 1999).

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

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 212 mg/m^3 ammonia 6 hours/day for 15 days exhibited increased blood ammonia (200%) and brain glutamine (28%) 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).

Oral Exposure

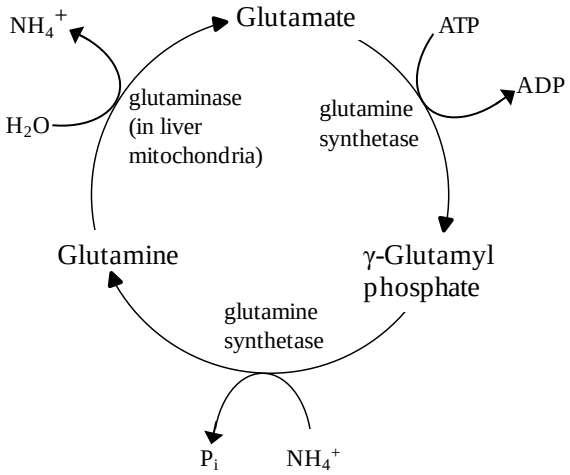
Human oral exposure data indicate that ammonia readily enters the portal circulation and is delivered to the liver, as has been shown to be the case for endogenously produced ammonia (Pitts, 1971; Summerskill and Wolpert, 1970). Un-ionized ammonia is freely diffusible, whereas the ammonium ion is less so, and is relatively confined to the extracellular compartment (Stabenau et al., 1959).

Dermal Exposure

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

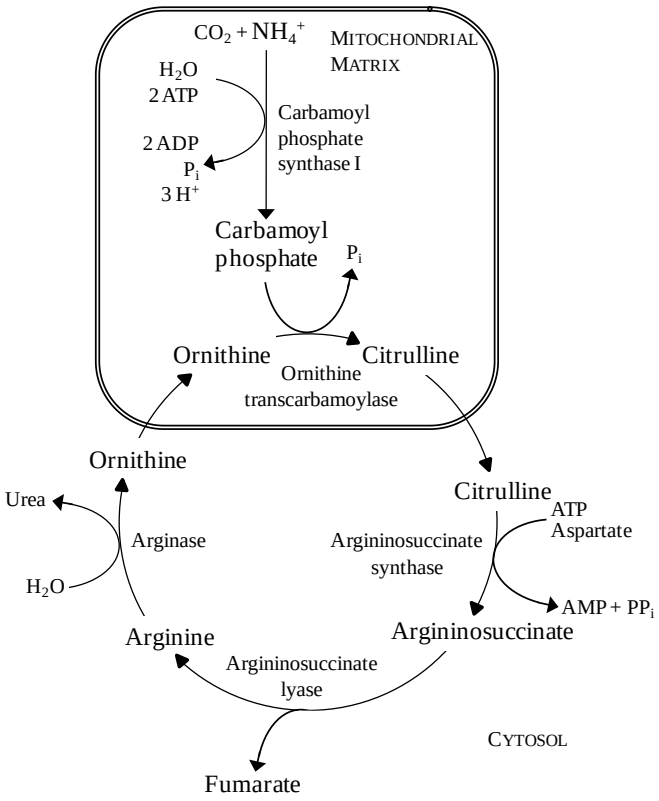
Metabolism

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 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 D-1), or incorporated into urea as part of the urea cycle as observed in the hepatic mitochondria and cytosol (Figure D-2) (Nelson and Cox, 2008). Ammonia can be rapidly converted to glutamine in the brain as well (Takagaki et al., 1961). van de Poll et al. (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 D-1. Glutamine cycle.



Adapted from: Nelson and Cox (2008).

Figure D-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-Wollheim, 1995; Souba, 1987). Two studies in rats (Manninen et al., 1988; Schaerdel et al., 1983) provide evidence that ammonia concentrations in air below 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 11–818 mg/m^3. Exposure to 11 mg/m^3 ammonia did not increase blood ammonia concentrations after 24 hours; concentrations of ≥23 mg/m^3 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 18 mg/m^3 ammonia 6 hours/day for 5 days did not exhibit blood or brain ammonia or glutamine levels that were different from controls; however, rats inhaling 212 mg/m^3 for the same daily exposure 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 of ammonia permeability across the blood-brain barrier relative to healthy controls (Keiding et al., 2010; Keiding et al., 2006). Furthermore, Sørensen 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.

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). 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; Lee et al., 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 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 of 0.059–0.078 mg/m^3 in exhaled breath from the nose of three healthy volunteers (with samples collected daily over a 4-week period); these concentrations were similar to or slightly higher than the mean laboratory air level of ammonia reported in this study of 0.056 mg/m^3. 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/m^3—a concentration within the range of that found 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 breath exhaled from the nose. In studies that reported ammonia in breath samples from the mouth or oral cavity, ammonia concentrations were commonly found in the range of 0.085 to 2.1 mg/m^3 (Smith et al., 2008; Spanel et al., 2007a, b; 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 gastrointestinal 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 six 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, b; Turner et al., 2006; Diskin et al., 2003), with ammonia concentrations increasing on average about 0.1 mg/m^3 for each 10 years of life (Spanel et al., 2007a). Turner et al. (2006) reported that the age of the individual accounts for about 25% of the variation observed in 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., 2007a; Davies et al., 1997). These studies are further described below in Table D-1.

Because ammonia measured in samples of breath exhaled from the mouth or oral cavity can be generated in the oral cavity and may thus 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 0.007–0.250 mg/m^3 (mean = 0.06 mg/m^3) (Barrow and Steinhagen, 1980). Larson et al. (1980) reported ammonia concentrations measured in the larynx of dogs exposed to sulfuric acid ranging between 0.02 and 0.16 mg/m^3 following mouth breathing and between 0.04 and 0.16 mg/m^3 following nose breathing.

Physiologically Based Pharmacokinetic Models

No physiologically based pharmacokinetic 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.

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

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Breath samples from the nose and trachea					
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 = 0.0728 ± 0.000848 mg/m^3 Volunteer B = 0.0777 ± 0.000919 mg/m^3 Volunteer C = 0.0587 ± 0.000848 mg/m^3 (median ammonia levels estimated as geometric mean ± geometric SD)	SIFT-MS analysis	Mean ambient air level of ammonia was 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 sample collected from the trachea (collected from a tube inserted into one male subject's nose and into the trachea)	Chemiluminescence		Larson et al. (1977)

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

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Breath samples from the mouth and oral cavity					
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 = 0.769 ± 0.000919 mg/m^3 Volunteer B = 0.626 ± 0.000919 mg/m^3 Volunteer C = 0.604 ± 0.000919 mg/m^3 Via oral cavity: Volunteer A = 1.04 ± 0.000990 mg/m^3 Volunteer B = 1.52 ± 0.00106 mg/m^3 Volunteer C = 1.31 ± 0.000919 mg/m^3 (median ammonia levels estimated as geometric mean ± geometric SD)	SIFT-MS analysis	Mean ambient air level of ammonia was 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)
Four healthy children (two males and two 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 0.157–0.454 mg/m^3 Seniors = 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. (2007a)

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

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
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 = 0.165 mg/m^3 18-yr-olds = 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. (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 SD = 0.589 ± 0.00114 mg/m^3 Median = 0.595 mg/m^3 Range = 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 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 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)
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 0.2–0.4 mg/m^3; Postmeal levels at 30 min were 0.1 mg/m^3 increasing to maximum values at 5 hrs of 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)

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

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
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 (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 0.085–0.905 mg/m^3	Nitrogen oxide analyzer with an ammonia conversion channel (similar to chemi-luminescence)	An 80–90% depletion of volatile ammonia emissions was seen within 10 min of acid rinsing; <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)

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

Test subjects	Breath samples	Levels of ammonia in exhaled breath	Methods	Comments	Reference
Breath samples: source (nose/mouth/oral cavity) not specified					
Sixteen healthy, nonsmoking subjects (4 females and 12 males, 29 ± 7 yrs); no significant differences in mean age, height, weight, 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: 0.843 ± 0.0601 mg/m^3 (median ± measurement error) Experiment 2: Nonstandardized = 0.712 ± 0.130 mg/m^3 (median ± SD) Standardized = 1.01 ± 0.113 mg/m^3 (median ± SD) Experiment 3: Mixed = 0.860 ± 0.585 mg/m^3 (median ± SD) Alveolar = 0.920 ± 0.559 mg/m^3 (median ± SD)	SIFT-MS analysis This study established that SIFT-MS analysis is reliable and repeatable	Relatively small number of healthy subjects used Did not address the breath of those with disease Intra- and inter-day repeatability were not investigated	Boshier et al. (2010)

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

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 = 0.35 ± 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 versus 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) (3) 20 healthy children from the same urban area as a control group (mean age 10 yrs, 12 boys, 8 girls)	Subjects performed a 5-sec breath-hold and exhaled slowly into collection device	Asthmatic children from National Park = 0.0040 ± 0.0033 mg/m^3 Asthmatic urban children: Mean NH_3 = 0.0101 ± 0.00721 mg/m^3 Urban children control group: Mean NH_3 = 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)

BMI = body mass index; SD = standard deviation; SIFT-MS = selected ion flow tube mass spectrometry.

D.2. HUMAN STUDIES

Occupational Studies in Industrial Worker Populations

Holness et al. (1989)

Holness et al. (1989) conducted a cross-sectional study of workers in a soda ash (sodium carbonate) plant[2] 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 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 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 lung 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 (forced vital capacity [FVC], forced expiratory volume in 1 second [FEV_1], FEV_1/FVC, forced expiratory flow [FEF_{50}], and FEF_{75}). No statistical differences in the prevalence of respiratory irritation or eye irritation were evident between the exposed and control groups (Table D-2).

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

[2]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 recovered in the process and reused.

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 as the no-observed-adverse-effect level (NOAEL). A lowest-observed-adverse-effect level (LOAEL) was not identified for this study.

Table D-2. Symptoms and lung function results of workers exposed to different levels of TWA ammonia concentrations

Parameter	Ammonia concentration			
	Control 0.2 mg/m^3	Exposed <4.4 mg/m^3	Exposed 4.4–8.8 mg/m^3	Exposed >8.8 mg/m^3
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* (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

[a]Number affected/number examined. The percentage of workers reporting symptoms is indicated in parentheses.

*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)

Ballal et al. (1998) conducted a cross-sectional study of male workers at two urea fertilizer factories in Saudi Arabia[3]. 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, and workers in factory B were exposed to levels of 0.02–7 mg/m^3. 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 [TLV] of 18 mg/m^3) showed that those exposed to ammonia concentrations higher than the TLV had 2.2- to fourfold higher relative risks for cough, phlegm, wheezing, dyspnea, and asthma than workers exposed to levels below the TLV (Table D-3). 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 2- to 4.8-fold higher relative risk for all of the above symptoms among those with higher CAC (Table D-3). 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 D-4).

[3]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.

Table D-3. The prevalence of respiratory symptoms and disease in urea fertilizer workers exposed to ammonia

Respiratory symptom/disease	Relative risk (95% CI)			
	Exposure category		CAC[a] (mg/m^3-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)

[a] = one missing value

CI = confidence interval.

Source: Ballal et al. (1998).

Table D-4. 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)*
Phlegm	1.36 (1.10–1.67)*
Shortness of breath with wheezing	1.26 (1.04–1.54)*
Wheezing alone	1.55 (1.17–2.06)*
Dyspnea on effort	0.83 (0.68–1.02)
Diagnosis of asthma	1.33 (1.07–1.65)*

*$p \leq 0.05$.

OR = odds ratio.

Source: Ballal et al. (1998).

Ali et al. (2001)

Results from limited spirometry testing of workers from factory A were reported in a followup study (Ali et al., 2001). The lung 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 et al. (2001) suggest that exposure to ammonia concentrations >18 mg/m^3 (50 mg/m^3-years) is associated with respiratory irritation and altered lung 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)

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 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 5-day period. Questionnaires were administered to inquire about demographics, past chronic respiratory disease, past and present occupational history, smoking status, 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 had planned to have less than a 4-hour working day; lung function was not tested in the control group. Personal ammonia exposure and lung 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 levels about 4–5 times higher than levels measured 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 2.5–200 ppm [1.8–141 mg/m^3]). The study authors 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. Based on an evaluation of the two monitoring methods and communication with technical support at Dräger Safety Inc.[4], 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 prevalence of respiratory irritation and decreased lung function 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 D-5). 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 4.9 mg/m^3 and a LOAEL of 18.5 mg/m^3 in the Rahman et al. (2007) study based on increased prevalence of respiratory symptoms and a decrease in lung function.

[4]Telephone conversations and e-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, SRC, Inc. (contractor to National Center for Environmental Assessment, Office of Research and Development, U.S. EPA).

Table D-5. Prevalence of 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%)*	2/25 (8%)
Chest tightness	4/24 (17%)	21/64 (33%)*	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)[d,e]			
FVC	0.2 ± 9.3	-2.3 ± 8.8	No data
FEV_1	3.4 ± 13.3	-1.4 ± 8.9	No data
PEFR	2.9 ± 11.1	-1.0 ± 16.2	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]Calculated as ([postshift - preshift]/preshift) × 100.

[e]Values are presented as mean ± standard deviation (SD).

*Statistically significant ($p \leq 0.05$) by Fisher's exact test, comparing exposed workers to administrators.

Source: Rahman et al. (2007).

Hamid and El-Gazzar (1996)

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 was reported on exposure levels. Blood samples were collected from each subject and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), hemoglobin, and blood urea levels, and for monoamine oxidase (MAO) and catalase activity. Table D-6 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 positive correlations between catalase activity and levels of

hemoglobin, AST, and ALT, and between catalase activities and MAO activities. Hamid and El-Gazzar (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 D-6. 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*
AST (U/mL)	14.5 ± 4.67	17.9 ± 4.14*
Hemoglobin (%)	14.8 ± 2.62	12.2 ± 2.29**
Blood urea (mg/mL)	0.203 ± 0.0512	0.319 ± 0.0755**
MAO (units)	31.9 ± 10.1	20.8 ± 4.30**
Catalase (IU/mL)	119.3 ± 4.76	80.9 ± 9.31**

[a]Mean ± SD.

*Significantly different from controls ($p < 0.05$).
**Significantly different from controls ($p < 0.01$).

Source: Hamid and El-Gazzar (1996).

Cross-Sectional Studies in Farmers Exposed to Inhaled Ammonia

Several studies have evaluated respiratory symptoms and changes in lung function in livestock farmers and stable workers exposed to ammonia (see Table D-7). 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, in general, 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 (e.g., decreased FEV_1, FVC) in farmers was associated with ammonia exposure in several studies (Cormier et al., 2000; Donham et al., 2000; Vogelzang et al., 1998; Reynolds et al., 1996; Donham et al., 1995; 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; Vogelzang et al., 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). Monsó et al. (2004) showed that indoor dust was the main determinant of chronic obstructive pulmonary disease (COPD) in never-smoking animal farmers by demonstrating a statistically significant dose response association between dust and COPD and suggested that ammonia was not a major determinant of COPD in these animal farmers. In summary, several studies have demonstrated an association between ammonia exposure in livestock farmers and respiratory symptoms or 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.

Table D-7. 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 D-7. 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)	Lung 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)	Lung 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, and 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 lung 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 lung function was stronger after 6 yrs of exposure	Donham et al. (1995)

Table D-7. 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 was 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 symptoms)	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); Vogelzang et al. (1998)

Table D-7. 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 female and 87 male 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 lung 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 lung function were reported for poultry workers; concentrations associated with significant lung 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 exposures 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)
105 never-smoking farmers (84 males, 21 females) working inside animal confinement buildings; sampled from the European Farmers' Study; mean age 45	Cross-sectional study assessing lung function and indoor air contaminants in animal confinement buildings to determine characteristics and risk factors for COPD; questionnaires determined respiratory symptoms; lung function measured by spirometry	Mean (range) of air concentrations: ammonia = 10 (5–17) ppm [7 (4–12) mg/m^3] total dust = 5.6 (2.3–9.4) mg/m^3 endotoxin = 687.1 (282–2203) units/m^3	COPD was found in 18 of the 105 farmers (17%); dust and endotoxin showed a dose response relationship with COPD that was statistically significant for dust only	Monsó et al. (2004)

Table D-7. Cross sectional studies of livestock farmers exposed to ammonia

Subjects	Methods	Exposure conditions	Results	Reference
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; COPD = chronic obstructive pulmonary disease.

Controlled Human Inhalation Exposure Studies

Controlled exposure studies conducted with volunteers to evaluate irritation effects and changes in lung function following acute inhalation exposure to ammonia are summarized in Table D-8.

Table D-8. 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)[a]
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 64 mg/m^3 compared to 21 and 35 mg/m^3 exposure; 64 mg/m^3 did not produce significant bronchiospasm or severe lacrimation; intensity of odor perception was reported as higher at 21 and 35 mg/m^3 than at 64 mg/m^3	MacEwen et al. (1970)[b]

Table D-8. Controlled human exposure studies of ammonia inhalation

Subjects	Exposure conditions	Results	Reference
18 healthy servicemen volunteers, 18–39 yrs old	50–344 mg/m^3 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; reduced expiratory minute volume at concentrations ranging from 106 to 235 mg/m^3 compared to controls (not dose-dependent); exercise tidal volume was increased at 106 mg/m^3, but reduced at higher concentrations in a dose-dependent manner	Cole et al. (1977)[b]
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 71 mg/m^3 became easily tolerated and had no effect on general health after acclimation occurred; brief exposure to 106–141 mg/m^3 produced lacrimation and transient discomfort	Ferguson et al. (1977)[a]
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 or FEV_1; 99 mg/m^3 caused severe irritation and could not be tolerated; reported eye irritation increased with concentration	Verberk (1977)[a]
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)[a]
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; lung function was measured before and after exposure	No significant changes in lung function in healthy subjects at any concentration; decreased FEV_1 and increased bronchial hyperreactivity were 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)[b]

Table D-8. Controlled human exposure studies of ammonia inhalation

Subjects	Exposure conditions	Results	Reference
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 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)[b]
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 ≤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)[b]
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)[b]
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)[b]

[a]This controlled-exposure study did not provide information on the human subjects research ethics procedures undertaken in the study; 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.

[b]Investigators reported the use of ethical standards involving informed consent by volunteers and/or study approval by an Institutional Review Board or other ethics committee.

Twelve healthy volunteers exposed to 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 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 35, 57, 78, and 99 mg/m^3; ammonia concentrations of 99 mg/m^3 caused severe and intolerable irritation Verberk (1977). The lachrymatory threshold was determined to be 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 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 354 mg/m^3 of ammonia gas for 30 minutes through a masked nose and throat inhalation apparatus resulted in two of seven volunteers reporting lacrimation, and two of seven 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 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 91 mg/m^3 and 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 0.02–435 mg/m^3. This study found a mean odor detection threshold of 2 mg/m^3 and a mean irritation threshold of 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, 7, 14, and 35 mg/m^3 on 5 consecutive days (4 hours/day) in an exposure chamber; an additional group was exposed to 14 mg/m^3 plus two peak exposures to 28 mg/m^3 for 30 minutes. Volunteers in the group familiar to the smell of ammonia reported fewer symptoms than the nonhabituated group, but at a concentration of 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 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.

Several studies evaluated lung 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 60 mg/m^3 (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in seven volunteers exposed to ammonia at concentrations of 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 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 occurred on the second 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. Exercise tidal volume was increased at 106 mg/m^3, but then decreased at higher concentrations in a concentration-dependent manner (Cole et al., 1977). Decreased FEV_1 and FVC were reported in eight healthy male volunteers exposed to a mean airborne ammonia concentration of 15 mg/m^3 in swine confinement buildings for 4 hours at 1-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 lung 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 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 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 lung 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 to 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 lung function (Sigurdarson et al., 2004).

In summary, volunteer studies demonstrate that eye irritation can occur following acute exposure to ammonia at concentrations as low as 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. Lung function was not affected in workers acutely exposed to ammonia concentrations as high as 71 mg/m^3. Studies comparing the lung function of asthmatics and healthy volunteers exposed to ammonia do not suggest that asthmatics are more sensitive to the lung effects of ammonia.

Case Reports of Human Exposure to Ammonia

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 (Robertson et al.,

2010; 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 the 11 cases involving accidental or intentional ingestion of ammonia water by adults (≥15 years old), 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 (Dilli et al., 2005), 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 et al., 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 et al., 1987; O'Kane, 1983; Ward et al., 1983; Couturier 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 (Lalić et al., 2009; Leduc et al., 1992; Bernstein and Bernstein, 1989; Flury et al., 1983; Ward et al., 1983; Stroud, 1981; Close et al., 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, two 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 of 3–50 ppm (Brautbar et al., 2003). Lee et al. (1993) reported 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 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 (Lalić 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; McGuiness, 1969; Levy et al., 1964; Abramovicz, 1925). 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 et al., 1987; Kass et al., 1972; Helmers et al., 1971; Highman, 1969; Osmond and Tallents, 1968; Levy et al., 1964; Abramovicz, 1925). 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.

D.3. ANIMAL STUDIES

Oral Exposure

Hata et al. (1994)

In a study designed to look at the effects of ammonia on gastric mucosa histology and cell kinetics, Hata et al. (1994) exposed groups of male Donryu rats (6 rats/group/time interval) to drinking water containing 0, 0.02, or 0.1% ammonia for durations up to 24 weeks. Based on an assumed body weight of 267 g and daily water intake of 37 mL (subchronic values for male Sprague-Dawley rat; U.S. EPA, 1988), the doses were estimated to be 0, 28, or 140 mg/kg-day. After 1, 3, and 5 days and 1, 4, 8, 12, and 24 weeks from the start of exposure, the gastric mucosa in the fundic gland region and the antrum was examined histologically. In addition, the labeling index of gastric mucosal tissue was measured using either a double labeling technique with bromodeoxyuridine (BrDU) and ^{3}H-thymidine (weeks 8 and 24) or the flash labeling technique with BrDU (other weeks).

A dose-related decrease in the height of the glandular ducts of the gastric mucosa was observed in the fundic region (by week 4) and in the pyloric region (by week 8). There was a decrease in periodic acid-Schiff (PAS)-positive mucus only in the early stages of ammonia exposure (through day 3 of exposure). Labeling index in gastric mucosa glands was increased at earlier time points (up to week 1 for fundic glands and to week 4 for pyloric glands), indicating enhanced cell cycling subsequent to repeated erosion and repair; however, at later time points up to 24 weeks of exposure, the labeling index was decreased, consistent with reduced capability of the generative cell zone of the mucosal region. The authors reported that there was no ammonia-induced gastritis or ulceration. Based on histological changes in the gastic mucosa, EPA identified a LOAEL of 0.02% ammonia in drinking water; a NOAEL was not identified.

Kawano et al. (1991); Tsujii et al. (1993)

Kawano et al. (1991) investigated the hypothesis that the bacterium *Helicobacter pylori*, which produces a potent urease that increases ammonia production, plays a significant role in the etiology of chronic atrophic gastritis. Male Sprague-Dawley rats (6/group) were given tap water or 0.01 or 0.1% ammonia ad libitum for 2 or 4 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 μm 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 D-9). Parietal cell number per oxyntic gland decreased in a statistically significant dose- and time-dependent fashion. The index of PAS 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 D-9. Effect of ammonia in drinking water on the thickness of the gastric antral and body mucosa of the rat stomach

Length of treatment	Thickness of mucosa (µm); mean ± standard error of the mean		
	Control (tap water)	Percent ammonia in drinking water	
		0.01%	0.1%
Antral mucosa			
2 wks	270 ± 18	258 ± 22	217 ± 40*
4 wks	276 ± 39	171 ± 22*	109 ± 12**,***
Body mucosa			
2 wks	574 ± 116	568 ± 159	591 ± 183
4 wks	618 ± 154	484 ± 123	440 ± 80*,***

*Statistically significant ($p < 0.05$) versus control group.
**Statistically significant ($p < 0.01$) versus control group.
***Statistically significant ($p < 0.01$) 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 male Sprague-Dawley 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 8 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 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).

As in Kawano et al. (1991), no mucosal lesions were found macroscopically or microscopically. The antral mucosal thickness decreased significantly at 4 and 8 weeks of treatment (Table D-10), 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 1 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. EPA identified a LOAEL of 33 mg/kg-day based on decreased thickness of the gastric antrum; a NOAEL was not identified.

Table D-10. 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)[a]	
	Antral mucosa	Body mucosa
Control (tap water only)	283 ± 26	534 ± 27
3 d	305 ± 45	559 ± 50
1 wk	272 ± 31	542 ± 28
2 wks	299 ± 26	555 ± 37
4 wks	159 ± 29*	531 ± 32
8 wks	168 ± 26*	508 ± 29

[a]Extracted from Figure 3 of Tsujii et al. (1993); mean ± SD.

*Statistically significant ($p < 0.05$) versus control (tap water only) group.

Source: Tsujii et al. (1993).

Fazekas (1939)

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. 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. 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 a daily gavage volume of 50–80 mL, daily doses for the rabbits receiving 0.5 and 1.0% ammonia solutions were approximately 61–110 and 120–230 mg/kg-day, respectively. 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 mm Hg. 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 65–90 mm Hg 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. EPA did not identify a NOAEL or LOAEL from this study.

Toth (1972)

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 for the 0.1, 0.2, and 0.3% groups as 9.2, 8.2, and 6.5 mL/day for males, respectively, and 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 C3H 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 (males), 4/50 (females). Tumor incidence at the 0.2% ammonium hydroxide concentration was: malignant lymphomas: 7/50 (males), 10/50 (females); lung adenoma or adenocarcinoma: 5/50 (males), 8/50 (females); 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 (males), 12/50 (females); 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 (C3H) 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 mice, 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 C3H female mice, the incidence of tumors in treated mice cannot be independently compared to control tumor incidence.

Tsujii et al. (1992b; 1995)

Tsujii et al. (1995; 1992b) 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; 1992b) 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; 1992b) 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., 1992b). 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 compared to 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 12% of controls). 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.

Inhalation Exposure

Anderson et al. (1964)

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 14 mg/m^3 for 6 weeks caused

darkening, edema, congestion, and hemorrhage in the lung. Exposure of guinea pigs to 35 mg/m^3 ammonia for 6 weeks caused grossly enlarged and congested spleens, congested livers and lungs, and pulmonary edema.

Coon et al. (1970)

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 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) that were more extensive than in control animals (incidence not reported) were observed in the lungs from rats and guinea pigs in the high-dose group.

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 for 90–114 days. Fifteen guinea pigs, three rabbits, two dogs, and three monkeys were also exposed continuously under similar conditions to ammonia at either 40 mg/m^3 or 470 mg/m^3. No significant effects were reported in any animals exposed to 40 mg/m^3 ammonia. Exposure of rats to 262 mg/m^3 ammonia caused nasal discharge in 25%—nonspecific circulatory and degenerative changes in the lungs and kidneys were also demonstrated (not further described, incidence not reported) that the authors stated were difficult to relate to ammonia inhalation. A frank effect level at 455 mg/m^3 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 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

ammonia. Histopathological evaluation of animals exposed to 470 mg/m^3 consistently showed focal or diffuse interstitial pneumonitis in all animals and 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 262 mg/m^3 and a LOAEL of 455 mg/m^3 based on nonspecific inflammatory changes in the lungs and kidneys in rats exposed to ammonia for 90 days.

Stombaugh et al. (1969)

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 because a control group was not used, it could not be determined whether this reduction was statistically significant. Food efficiency (food consumed/kg body weight gain) was not affected. Exposure to ≥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)

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 D-11. 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 D-11. Summary of histological changes observed in pigs exposed to ammonia for 6 weeks

	Thickness of tracheal epithelium (µm)		Number of tracheal goblet cells (per 500 µm)	
Duration of exposure (wks)	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 ± SD	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 based on damage to the upper respiratory epithelium. A NOAEL could not be identified from this single-concentration study.

Broderson et al. (1976)

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 7 mg/m^3 exposure level represented the background ammonia concentration resulting from cage bedding that was changed 3 times/week. The 106 mg/m^3 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 106 mg/m^3 for 75 days (from bedding) or 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 (7 mg/m^3 from bedding or 0 mg/m^3 from the inhalation chamber). Broderson et al. (1976) did not identify a NOAEL or LOAEL from this study. EPA identified a NOAEL of 7 mg/m^3 and a LOAEL of 106 mg/m^3 based on nasal lesions in rats exposed to ammonia (from bedding) for 75 days.

Gaafar et al. (1992)

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) for 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 5 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 of 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 of rats sacrificed at 8 weeks. Neoplastic changes included a carcinoma in situ in the nostril of one rat sacrificed at 7 weeks, 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. The presence of ALP was greatest 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)

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.

$$2 \times [4 \text{ dust concentrations} \times 4 \text{ ammonia concentrations} + 4 \text{ controls}] = 40 \text{ lots total}$$

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 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, based on the lack of respiratory or other disease following exposure to ammonia in the presence of respirable dust.

Weatherby (1952)

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 ppm (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 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)

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 53 mg/m^3 based on the absence of effects occurring in pigs exposed to ammonia; a LOAEL was not identified from this study.

Reproductive/Developmental Studies

Diekman et al. (1993)

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 25 mg/m^3 ammonia gained less weight than gilts exposed to 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 25 mg/m^3 ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m^3. In gilts that were mated, conception rates were similar between the two groups (94.1 versus 100% in low versus high exposure, respectively). 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 of 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. EPA did not identify NOAEL or LOAEL values from this study due to the absence of a control group and due to confounding exposures to bacterial and mycoplasm pathogens.

Acute and Short-term Inhalation Toxicity Studies

See Table D-12.

Table D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Rats					
Female Porton rats (16/group)	0 or 141	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	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; 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 20-min LC_{50} = 20,217 mg/m^3 40-min LC_{50} = 14,352 mg/m^3 60-min LC_{50} = 11,736 mg/m^3	Appelman et al. (1982)
Male Crl:COBS CD (Sprague-Dawley) rats (8/group)	11, 23, 219, and 818; 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; 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 D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male Long-Evans rats (4/group)	70 and 212; 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	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) at 212 mg/m^3; lung hemorrhage observed in some exposed rats	Manninen et al. (1988)
Female Wistar rats (5/group)	0, 18, or 212	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	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 D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male Wistar rats (4/group)	0, 92–1,243; 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 Pauluhn (2010)
Male rats (10/group)	0, 848–1,068 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; no controls	10 min	LC_{50}	LC_{50} = 7,056 mg/m^3	Silver and McGrath (1948)
Male Swiss albino mice (4/group)	5,050–20,199; no controls	30–120 min	LC_{50}	LC_{50} (30 min) = 15,151 mg/m^3	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 gamma aminobutyric acid (GABA) metabolism, and (Na^+-K^+)-ATPase; amino acid levels in the brain	Altered activities of MAO, glutamate decarboxylase, ALT, GABA-transaminase, and (Na^+-K^+)-ATPase; increased alanine and decreased glutamate	Sadasivudu et al. (1979); Sadasivudu and Radha Krishna Murthy (1978)
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	Kane et al. (1979)

Table D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male albino ICR mice (12/dose)	0–3,436	1 hr (14-d followup)	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	Kapeghian et al. (1982)
Male Swiss-Webster mice (16–24/group)	0 or 216	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	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 and 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	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; 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)
Mice (sex not specified; 4/group)	3, 21, 40, or 78, 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)

Table D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Male OF1 mice (4/group)	0, 92–1,243; 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 Pauluhn (2010)
Rabbits					
Female New Zealand White rabbits (7–9/dose)	0, 35, or 71	2.5–3.0 hrs	Lung function	Decreased respiratory rate at both concentrations	Mayan and Merilan (1972)
Rabbits (species, sex, number/dose not specified)	0, 707–14,140	15–180 min	Lung function, death	Bradycardia at 1,768 mg/m^3; arterial pressure variations and blood gas modifications (acidosis indicated by decreased pH and increased pCO_2) at 3,535 mg/m^3; death occurred at 4,242 mg/m^3	Richard et al. (1978b)
New Zealand White rabbits (sex not specified ; 16 total; 8/dose)	Peak concentrations: 24,745–27,573; concurrent controls tested	4 min	Lung 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)
Cats					
Mixed breed stray cats (sex not specified; 5/group)	0 or 707	10 min	Lung function, lung histopathology on 1, 7, 21, and 35 d postexposure	Lung function deficits were correlated with lung histopathology; acute effects were followed by chronic respiratory dysfunction (secondary bronchitis, bronchiolitis, and bronchopneumonia)	Dodd and Gross (1980)

Table D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Pigs					
Young pigs (sex not specified; 2/group)	0, 35, 71, or 106	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	6 d	Clinical signs, body weight, lung function	Lethargy and decreased body weight gain (all concentrations); no effect on lung microvascular hemodynamics and permeability	Gustin et al. (1994)
Belgian Landrace pigs (sex not specified; 4/group)	0, 18, 35, or 71	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 (sex not specified; 4/group)	0 or 42	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	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 D-12. Acute and short-term inhalation toxicity studies of ammonia in animals

Animal	Ammonia concentration (mg/m^3)	Duration	Parameters examined	Results	Reference
Cattle					
Male Holestein calves (number/group not specified)	0, 35, or 71	2.5 hrs	Respiration rate, clinical chemistry	No significant effect on respiration, BUN, pH, pO_2, or pCO_2	Mayan and Merilan (1976)
Male Brahman/ Charolais steer (group size not reported)	<6 (control), 11, 23, or 34	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 lung inflammation, no effect on hematological variables or body weight	Phillips et al. (2010)
Holstein Friesian and Brown Swiss cows (10 of each breed)	~0, 4, and 15	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)

D.4. OTHER PERTINENT TOXICITY INFORMATION

Genotoxicity Studies

Table D-13. Summary of in vitro studies of ammonia genotoxicity

Endpoint	Test system	Concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538); *Escherichia coli* (WP2 uvrA)	25,000 ppm (17,675 mg/m^3) ammonia vapor	–	–[c]	Plate incorporation assay with ammonia vapor	Shimizu et al. (1985)
Reverse mutation, streptomycin resistance	*E. coli* (B/SD-4 strains)	0.25% ammonia	+ (T)[d]	No data	Plate incorporation assay	Demerec et al. (1951)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Chromosomal aberrations	Chick fibroblasts	Not available	+	No data	Cultures immersed in buffered ammonia solution	Rosenfeld (1932)
Genotoxicity studies in mammalian systems						
DNA double strand breaks	Rabbit gastric mucosal or KATO III cells	0.1 mM NH_3 in solution	No data	–	15-minute incubation with 0.1 mM NH_3	Suzuki et al. (1997)
DNA fragmentation	Rabbit gastric mucosal cells	0.1 mM NH_3 in solution	No data	–		Suzuki et al. (1997)
Chromatin condensation	Rabbit gastric mucosal or KATO III cells	0.1 mM NH_3 in solution	No data	–	15-minute incubation with 0.1 mM NH_3	Suzuki et al. (1997)
DNA fragmentation	Gastric epithelial cell line MKN45	0.001 mM NH_3 in solution	No data	–[e]	5-hour incubation; cytoplasmic levels of mono- and oligonucleosomes measured	Suzuki et al. (1998)

[a]Lowest effective dose for positive results; highest dose tested for negative or equivocal results.

[b]+ = positive; – = negative; (T) = toxicity reported.

[c]Exogenous metabolic activation used; S9 liver fractions from male Sprague-Dawley rats pretreated with pentachlorobiphenyl (KC500).

[d]Only positive in treatments using toxic levels of NH_3 (98% lethality).

[e]Comparison was to elevated mono- and oligonucleosomes levels associated with monochloramine (NH_2Cl); control (untreated) value not reported.

Table D-14. Summary of in vivo studies of ammonia genotoxicity

Endpoint	Test system	Dose/ concentration[a]	Results[b]	Comments	Reference
Genotoxicity studies in mammalian systems					
Chromosomal aberrations	Human lymphocytes	88.28 µg/m^3	+[c]	22 healthy workers occupationally exposed to ammonia in an Indian fertilizer factory (ambient concentration of 0.0883 mg/m^3); 42 nonexposed factory staff served as control subjects	Yadav and Kaushik (1997)
Sister chromatid exchange	Human lymphocytes	88.28 µg/m^3	+[c]		Yadav and Kaushik (1997)
Micronucleus formation	Swiss albino mice	12.5–50 mg/kg	+	Intraperitoneal injections for 24–48 hr expression times	Yadav and Kaushik (1997)
Sex-linked recessive lethal mutations	*Drosophila melanogaster*	Not available	– (T)	Inhalation exposure to ammonia as vapor at a concentration killing the majority of flies	Auerbach and Robson (1947)
Dominant lethal mutations	*D. melanogaster*	Not available	– (T)	Inhalation exposure to ammonia as vapor at a concentration killing the majority of flies	Auerbach and Robson (1947)
Dominant lethal mutations	*D. melanogaster*	Not available	+ (T)[d]	Dominant lethal assay; inhalation exposure up to 318 mg/m^3 ammonia, 6 hrs/d for 5 d	Lobasov and Smirnov (1934)

[a]Lowest effective dose for positive results; highest dose tested for negative or equivocal results.

[b]+ = positive; – = negative; (T) = toxicity reported.

[c]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 factors of 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 1.1; Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998); the accuracy and reliability of the sampling and measurement could not be determined.

[d]Survival after exposure was <2%.

APPENDIX E. DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

(see next page)

Documentation of the IRIS Program's Implementation of the 2011 NRC Recommendations in the External Peer Review Draft Toxicological Review of Ammonia (June 2012)

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[5]. The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde[6]. The report language included the following:

"The Agency shall incorporate, as appropriate, based on chemical-specific datasets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process…For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated."

The NRC's recommendations, provided in Chapter 7 of their review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the table below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS ammonia assessment is the first in Phase 2 of implementation, which addresses all of the short-term recommendations from Table 1. The Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table 2, including the development of a standardized approach to describe the strength of evidence for

[5]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.
[6]National Research Council, 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

noncancer effects . On May 16, 2012, EPA announced[7] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

[7]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

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Table 1. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
General recommendations for completing the IRIS formaldehyde assessment that EPA will adopt for all IRIS assessments (p. 152 of the NRC report)	
To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Implemented.** The overall document structure has been revised in consideration of this NRC recommendation. The new structure includes a concise Executive Summary and an explanation of the literature review search strategy, study selection criteria, and methods used to develop the assessment. The main body of the assessment has been reorganized into two sections, Hazard Identification and Dose-Response Analysis, to help reduce the volume of text and redundancies that were a part of the previous document structure. Section 1 provides evidence tables and a concise synthesis of hazard information organized by health effect, More detailed summaries of the most pertinent epidemiology and experimental animal studies are provided in Appendix D. Information on chemical and physical properties and toxicokinetics is now provided in Appendices C and D1, respectively. The main text of the Toxicological Review is approximately 50 pages, which is a major reduction from previous IRIS assessments. Technical and scientific edits were performed to eliminate any redundancies or inconsistencies.
Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the RfCs and unit risk estimates.	**Implemented.** Chapter 1 has been replaced with a Preamble that describes the application of existing EPA guidance and the methods and criteria used in developing the assessment. The term "Preamble" was chosen to emphasize that these methods and criteria are being applied consistently across IRIS assessments. The new Preamble includes information on identifying and selecting pertinent studies, evaluating the quality of individual studies, weighing the overall evidence of each effect, selecting studies for derivation of toxicity values, and deriving toxicity values. These topics correspond directly to the five steps that the NRC identified in Figure 7-2 of their 2011 report. A new section, Literature Search Strategy and Study Selection, provides detailed information on the search strategy used to identify health effect studies, search outcomes, and selection of studies for hazard identification. This information is chemical-specific and has been designed to provide enough information that an independent literature search would be able to replicate the results. This section

Table 1. National Research Council recommendations that EPA is implementing in the short term	Implementation status
	also includes information on how studies were selected to be included in the document and provides a link to EPA's Health and Environmental Research Online (HERO) database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited.
Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix of deleted.	**Implemented.** In the new document template, standardized evidence tables that present key study findings that support how toxicological hazards are identified for all major health effects are provided in Section 1.1. More detailed summaries of the most pertinent epidemiology and experimental animal studies are provided in Appendix D.
All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research, for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Implemented.** Information in Section 4 of the Preamble provides an overview of the approach used to evaluate the quality of individual studies. Critical evaluation of the epidemiologic and experimental animal studies and is included in the evidence tables in Section 1.1. Additional information on study characteristics is found in Appendix D. The study information for ammonia is presented in text format only. EPA is developing standardized study summary tables that will replace written study summaries to clearly present more detailed study summary information and key study characteristics.
The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	**Implemented.** The Dose-Response Analysis section of the new document structure provides a clear explanation of the rationale used to select and advance studies that were considered for calculating toxicity values. Rationales for the selection of studies advanced for reference value derivation are informed by the weight-of-evidence for hazard identification as discussed in Section 1.2. In support of the RfC derivation for ammonia, an exposure-response array was included that compares effect levels for several toxicological effects (Section 2.2, Table 2-1). The exposure response array provides a visual representation of points of departure for various effects resulting from exposure to ammonia. The array informs the identification of doses associated with specific effects, and the choice of principal study and critical effects. In the case of ammonia, the database did not support development of multiple candidate RfC's. Such values have been developed previously and will be developed in future assessments, where the data allow.

Table 1. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
Strengthened, more integrative and more transparent discussions of weight-of-evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight-of-evidence, such as consistency.	**Partially implemented.** The new Hazard Identification (Section 1) provides a more strengthened, integrated and transparent discussion of the weight of the available evidence. This section includes standardized evidence tables to present the key study findings that support how potential toxicological hazards are identified and exposure-response arrays for each potential toxicological effect. Weight-of-evidence discussions are provided for each major effect (Section 1.1.1.2--respiratory, Section 1.1.2.2-- gastrointestinal, Section 1.1.3.1--reproductive/ developmental, Section 1.1.4.1—immune, and Section 1.1.5.1--other systemic effects). A more rigorous and formalized approach for characterizing the weight-of-evidence will be developed as a part of Phase 3 of the implementation process.
Other specific recommendations (p. # in NRC report)	
General Guidance for the Overall Process (p. 164) Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Implemented.** EPA has created Chemical Assessment Support Teams to formalize an internal process to provide additional overall quality control for the development of IRIS assessments. This initiative uses a team approach to making timely, consistent decisions about the development of IRIS assessments across the Program. This team approach has been utilized for the development of the ammonia assessment. Additional objectives of the teams is to help ensure that the necessary disciplinary expertise is available for assessment development and review, to provide a forum for identifying and addressing key issues prior to external peer review, and to monitor progress in implementing the NRC recommendations.
Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
Assess disciplinary structure of teams needed to conduct the assessments.	
Evidence Identification: Literature Collection and Collation Phase (p. 164) Select outcomes on the basis of available evidence and understanding of mode of action.	**Implemented.** A new section, Literature Search Strategy and Study Selection, contains detailed information on the search strategy used for the ammonia assessment, including key words used to identify relevant health effect studies. Figure LS-1 depicts the study selection strategy and the number of references obtained at each stage of literature screening. This section also includes information on how studies were selected to be included in the document and provides a link to an external database (www.epa.gov/hero) that contains the references that were cited in the document, along with those that were considered but not cited. Each citation in the Toxicological Review is linked to
Establish standard protocols for evidence identification.	
Develop a template for description of the search approach.	
Use a database, such as the Health and Environmental Research Online	

Table 1. National Research Council recommendations that EPA is implementing in the short term	**Implementation status**
(HERO) database, to capture study information and relevant quantitative data.	HERO such that the public can access the references and abstracts to the scientific studies used in the assessment. Section 3 of the Preamble summarizes the standard protocols for evidence identification that are provided in EPA guidance. For each potential toxicological effect identified for ammonia, the available evidence is informed by the mode of action information as discussed in Section 1.1.
Evidence Evaluation: Hazard Identification and Dose-Response Modeling (p. 165) Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight-of- evidence, and utility as a basis for deriving reference values and unit risks.	**Implemented.** Standardized tables have been developed that provide summaries of key study design information and results by health effect. The inclusion of all positive and negative findings in each health effect-specific evidence table supports a weight-of-evidence analysis. In addition, exposure-response arrays are utilized in the assessment to provide a graphical representation of points of departure for various effects resulting from exposure to ammonia. The exposure-response arrays inform the identification of doses associated with specific effects and the weight-of- evidence for those effects.
Develop templates for evidence tables, forest plots, or other displays.	**Implemented.** Templates for evidence tables and exposure-response arrays have been developed and are utilized in Section 1.1.
Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Implemented.** General principles for reviewing epidemiologic and experimental animal studies are described in Section 4 of the Preamble.
Selection of Studies for Derivation of Reference Values and Unit Risks (p. 165) Establish clear guidelines for study selection.	**Implemented.** EPA guidelines for study selection, including balancing strengths and weaknesses and weighing human vs. experimental evidence are described in the Preamble (Sections 3-6). These guidelines have been applied in Section 2 of the ammonia assessment to inform the evaluation of the weight-of-evidence across health effects and the strengths and weaknesses of individual studies considered for reference value derivation. In the case of ammonia, the database did not support the combination of estimates across studies. In future assessments, combining estimates across studies will be routinely considered.
Balance strengths and weaknesses.	
Weigh human vs. experimental evidence.	
Determine whether combining estimates among studies is warranted.	

Table 1. National Research Council recommendations that EPA is implementing in the short term	Implementation status
Calculation of Reference Values and Unit Risks (pp. 165-166) Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented as applicable.** The rationale for the selection of the point of departure (a no-observed-adverse-effect level; NOAEL) for the derivation of the inhalation reference value for ammonia is transparently described in Section 2. No modeling was applied in the derivation of the reference value. An oral reference value was not derived.
Provide explanation of the risk-estimation modeling processes (for example, a statistical or biologic model fit to the data) that are used to develop a unit risk estimate.	**Not applicable.** The ammonia assessment concludes that there is inadequate information to assess the carcinogenic potential. Therefore, a unit risk estimate for cancer was not derived.
Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report, sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided when appropriate.	**Implemented.** The new template structure that has been developed in response to the NRC recommendations provides a clear explanation of the literature search strategy, study selection criteria, and methods used to develop the ammonia assessment. It provides for a clear description of the decisions made in developing the hazard identification and dose-response analysis. Information contained in the Preamble and throughout the document reflects the guidance that has been utilized in developing the assessment. As recommended, supplementary information is provided in the accompanying appendices.

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Table 2. National Research Council recommendations that EPA is implementing in the long-term (p. # in NRC report)	**Implementation status**
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (p. 165) Review use of existing weight-of-evidence guidelines. Standardize approach to using weight-of-evidence guidelines. Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. Develop uniform language to describe strength of evidence on noncancer effects. Expand and harmonize the approach for characterizing uncertainty and variability. To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for noncancer effects. On May 16, 2012, EPA announced[8] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA may hold additional workshops on issues related to weight-of-evidence.
Calculation of Reference Values and Unit Risks (pp. 165-166) Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the estimates.	As discussed in Section 1.2, the respiratory system is the primary and most sensitive target of inhaled ammonia toxicity. There is some evidence that inhaled ammonia may be associated with toxicity to target organs other than the respiratory system, but the evidence for these associations is weak. Therefore, these endpoints were not considered appropriate for the development of candidate or alternative reference values. In addition, no modeling was performed in this assessment. Assessing the sensitivity of the inhalation reference value to model assumptions and endpoint selection was not possible.

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[8]EPA Announces NAS' Review of IRIS Assessment Development Process (www.epa.gov/iris)

APPENDIX F. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND EPA's DISPOSITION

To be added

REFERENCES FOR APPENDICES

Multiple references published in the same year by the same author(s) have been assigned a letter (e.g., 1986a, 1986b) based on order of appearance in the text of the document. Those same letters have been retained for the appendices.

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