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Toxicological Review of Ammonia
Noncancer Inhalation

(CASRN 7664-41-7)

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Integrated Risk Information System
National Center for Environmental Assessment
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Washington, DC

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ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BCG	bacillus Calmette-Guérin
BMCL	95% lower bound on the benchmark concentration
BMDL	95% lower bound on the benchmark dose
CAC	cumulative ammonia concentration
CCRIS	Chemical Carcinogenesis Research Information System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFU	colony forming unit
CI	confidence interval
DAP	diammonium phosphate
EPA	Environmental Protection Agency
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
HERO	Health and Environmental Research Online
HSDB	Hazardous Substances Data Bank
IgE	immunoglobulin E
IgG	immunoglobulin G
IRIS	Integrated Risk Information System
LD_{50}	50% lethal dose
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRM	murine respiratory mycoplasmosis
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
PEFR	peak expiratory flow rate
pO_2	oxygen partial pressure
POD	point of departure
PPD	purified protein derivative
RfC	reference concentration
RfD	reference dose
RTECS	Registry of Toxic Effects of Chemical Substances
TSCATS	Toxic Substance Control Act Test Submission Database
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
VEh	human occupational default minute volume
VEho	human ambient default minute volume

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This assessment was provided for review to other federal agencies and the Executive Office of the President. Comments were submitted by:

Department of Agriculture/Food Safety and Inspection Service
Department of Health and Human Services/Agency for Toxic Substances and Disease Registry
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This assessment was released for public comment on June 8, 2012 and comments were due on August 7, 2012. A summary and EPA's disposition of the comments received from the public is included in Appendix G of the Supplemental Information to the Revised External Review draft of the Toxicological Review. Comments were received from the following entities:

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This assessment was peer reviewed by independent expert scientists external to EPA convened by EPA's Science Advisory Board (SAB), the Chemical Assessment Advisory Committee Augmented for the IRIS Ammonia Assessment. A peer review meeting was held on July 14 to 16, 2014. The report of the SAB's review of EPA's Draft Toxicological Review of Ammonia, dated August 6, 2015, is available on the IRIS website.

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PREFACE

This Toxicological Review critically reviews the publicly available studies on ammonia in order to identify its adverse health effects and to characterize exposure-response relationships. The assessment covers gaseous ammonia (NH_3) and ammonia dissolved in water (ammonium hydroxide, NH_4OH). It was prepared under the auspices of the Environmental Protection Agency's (EPA's) Integrated Risk Information System (IRIS) program.

Ammonia and ammonium hydroxide are listed as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA). Ammonia is subject to reporting requirements for the Toxics Release Inventory under the Emergency Planning and Community Right-to-Know Act of 1986 and to emergency planning requirements under section 112(r) of the Clean Air Act.

This assessment updates a previous IRIS assessment of ammonia that was developed in 1991. The previous assessment included only an inhalation reference concentration (RfC) for effects other than cancer. This assessment provides an updated review of information on all noncancer health effects by the inhalation route only.

This assessment was conducted in accordance with EPA guidance; relevant EPA guidance documents can be found on the IRIS website (http://www.epa.gov/iris/). The findings of this assessment and related documents produced during its development are also available on the IRIS website (http://www.epa.gov/iris/). Appendices for other health toxicity values, details of the literature search strategy and study selection and evaluation, supporting information for hazard identification and dose response, and other information are provided as Supplemental Information to this assessment (see Appendices A to C).

Portions of this Toxicological Review were adapted from the Toxicological Profile for Ammonia developed by the Agency for Toxic Substances and Disease Registry (ATSDR, 2004) under a Memorandum of Understanding that encourages interagency collaboration, sharing of scientific information, and more efficient use of resources.

The IRIS program released this assessment for public comment and peer review in June 2012, as it was beginning to implement systematic review. The approach to implementation is to use procedures and tools available at the time, without holding assessments until new methods become available. Accordingly, the IRIS program edited this assessment to increase transparency and clarity and to use more tables and figures. It conducted literature searches and evaluated studies using tools and documentation standards then available. Problem formulation materials and protocol development began with assessments started in 2015, after this assessment was well

into peer review. This assessment addresses peer review comments and retains the structure of the peer review draft, to maintain fidelity with what the peer reviewers saw. Implementation of systematic review is a process of continuous improvement subject to periodic review by the Chemical Assessment Advisory Committee of the U.S. EPA's Science Advisory Board. This assessment represents a step in the evolution of the IRIS program.

Assessments by Other National and International Health Agencies

Toxicity information on ammonia has been evaluated by ATSDR, the National Research Council (NRC), the National Institute for Occupational Safety and Health, and the Food and Drug Administration. The results of these assessments are presented in Appendix A of the Supplemental Information. It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

Overview of Uses, Sources, and Environmental Exposure

About 80% of commercially produced ammonia is used in agricultural fertilizers. Ammonia is also used as a corrosion inhibitor, in water purification, as a household cleaner, as an antimicrobial agent in food products, as a refrigerant, as a stabilizer in the rubber industry, in the pulp and paper and metallurgy industries, as a source of hydrogen in the hydrogenation of fats and oils, and as a chemical intermediate in the production of pharmaceuticals, explosives, and other chemicals. Ammonia is also used to reduce nitrogen oxide emissions from combustion sources such as industrial and municipal boilers, power generators, and diesel engines (HSDB, 2012; Johnson et al., 2009; Eggeman, 2001).

Major sources of ammonia gas include leaks and spills during commercial synthesis, production, storage, processing, or transporting of ammonia; refrigeration equipment failure; decaying manure from livestock; application of fertilizers; sewage or wastewater effluent; burning of coal, wood or other natural products; volcanic eruptions, forest fires and the decomposition of nitrogenous compounds. Ammonia from agricultural and other sources, along with sulfate and nitrate salts, is an important contributor to fine inorganic particulate matter ($PM_{2.5}$) mass (e.g., see Paulot and Jacob (2014)). This literature on airborne particular matter is reviewed and evaluated in EPA's Integrated Science Assessment for Particulate Matter (PM ISA) (U.S. EPA, 2009b).

Environmental exposures to ammonia in the air vary widely. Average ambient concentrations of ammonia in the United States range from 0.28–15 $\mu g/m^3$, as measured in 2012 by the National Atmospheric Deposition Program's Ammonia Monitoring Network (AMoN, 2012). Indoor residential ammonia concentrations can vary widely; one survey reported ammonia concentrations in homes in Connecticut and southwest and central Virginia ranging from 0.09–

166 μg/m^3, depending on the season, use of air conditioning, type of heating, and other factors (Leaderer et al., 1999).

Ammonia is found naturally in the environment and is a component of the global nitrogen cycle; it is essential to many biological processes. Nitrogen-fixing bacteria convert atmospheric nitrogen to ammonia that is available for uptake into plants. Organic nitrogen released from biota can be converted to ammonia. Ammonia in water and soil can be converted to nitrite and nitrate through the process of nitrification. Ammonia is also endogenously produced in humans and other mammals, where it is an essential metabolite used in nucleic acid and protein synthesis, is necessary for maintaining acid-base balance, and is an integral part of nitrogen homeostasis (Nelson and Cox, 2008; Socolow, 1999; Rosswall, 1981).

Scope of this Assessment

This assessment presents an evaluation of the noncancer health effects of ammonia by the inhalation route of exposure. To address peer-review recommendations to expand the scope of the oral toxicity literature to include ammonium salts and to allow expeditious completion of the assessment of inhaled ammonia, ingested ammonia, including consideration of ammonium salts, will be the focus of a separate assessment. Because carcinogenicity studies of ammonia have been performed by the oral route of exposure only, the cancer assessment will be moved into the separate oral assessment.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

The Preamble summarizes the objectives and scope of the IRIS program, general principles and systematic review procedures used in developing IRIS assessments, and the overall development process and document structure.

1. Objectives and Scope of the IRIS Program

Soon after the EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in support of actions to protect human health and the environment. The EPA's IRIS program[1] contributes to this endeavor by identifying adverse health effects of chemicals in the environment and characterizing exposure–response relationships. IRIS assessments cover the hazard identification and dose–response steps of risk assessment. Exposure assessment and risk characterization are outside the scope of IRIS assessments, as are political, economic, and technical aspects of risk management.

An IRIS assessment may cover one chemical, a group of structurally or toxicologically related chemicals, or a chemical mixture. Exceptions outside the scope of the IRIS program are radionuclides, chemicals used only as pesticides, and the "criteria air pollutants" (particulate matter, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead).

Enhancements to the IRIS program are improving its science, transparency, and productivity. To improve the science, the IRIS program is adapting and implementing principles of systematic review (i.e., using explicit methods to identify, evaluate, and synthesize study findings). To increase transparency, the IRIS program releases a problem formulation and other materials during draft development and discusses key science questions with the scientific community and the public. External peer review, independently managed and in public, improves both science and transparency. Increased productivity requires that assessments be concise, focused on EPA's needs, and completed without undue delay.

This assessment was conducted in accordance with EPA guidance.[2] This Preamble summarizes and does not change IRIS operating procedures or EPA guidance.

Periodically, the IRIS program asks for nomination of agents for future assessment or reassessment. Selection depends on EPA's priorities, relevance to public health, and availability of pertinent studies. The IRIS multiyear agenda[3] lists upcoming assessments. The IRIS program may also assess other agents in anticipation of public health needs.

2. Planning an Assessment: Scoping, Problem Formulation, and Protocols

Early attention to planning ensures that IRIS assessments meet EPA's needs and properly frame science questions.

Scoping refers to the first step of planning, where the IRIS program consults with EPA's program and regional offices to ascertain their needs. Scoping specifies the agents an assessment will address, routes and durations of exposure, susceptible populations and lifestages, and other questions of interest to the EPA.

Problem formulation refers to the science questions an assessment will address and includes input from the scientific community and the public. A preliminary survey of secondary sources

[1] IRIS program website: http://www.epa.gov/iris/
[2] EPA guidance documents: http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance/
[3] IRIS multiyear agenda: https://www.epa.gov/iris/iris-agenda

(e.g., assessments by national and international health agencies and comprehensive review articles) identifies potential health outcomes and science questions. It also identifies related chemicals (e.g., toxicologically active metabolites and compounds that metabolize to the chemical of interest).

Each IRIS assessment comprises multiple systematic reviews for multiple health outcomes. It also evaluates hypothesized mechanistic pathways and characterizes exposure–response relationships. An assessment may focus on important health outcomes and analyses rather than expand beyond what is necessary to support EPA's needs.

Protocols refer to the systematic review procedures planned for use in an assessment. They include strategies for literature searches, criteria for study inclusion or exclusion, considerations for evaluating study methods and quality, and approaches to extracting data. As an assessment progresses, additional science questions may emerge and protocols may change.

3. Identifying and Selecting Pertinent Studies

IRIS assessments conduct systematic literature searches with criteria for inclusion and exclusion. The objective is to retrieve the pertinent primary studies (i.e., studies with original data on health outcomes or their mechanisms). *PECO statements* (Populations, Exposures, Comparisons, Outcomes) govern the literature searches and screening criteria. "Populations" and animal species generally have no restrictions. "Exposures" refers to the agent and related chemicals identified during scoping and problem formulation and may consider route, duration, or timing of exposure. "Comparisons" means studies that allow comparison of effects across different levels of exposure. "Outcomes" may become more specific (e.g., from "toxicity" to "developmental toxicity" to "hypospadias") as an assessment progresses.

For studies of absorption, distribution, metabolism, and elimination, the first objective is to create an inventory of pertinent studies. Subsequent sorting and analysis facilitates characterization and quantification of these processes.

Studies on mechanistic events can be numerous and diverse. Here, too, the objective is to create an inventory of studies for later sorting to support analyses of related data. The inventory also facilitates generation and evaluation of hypothesized mechanistic pathways.

IRIS assessments go beyond standard practices of systematic review in including pertinent studies. After posting search strategies on its website and adding search results to the EPA's HERO database,[4] the IRIS program encourages the scientific community and the public to provide information on additional studies and ongoing research. Assessments also consider data submitted under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Even during the review process, IRIS assessments consider late-breaking studies that would impact the credibility of the conclusions.[5]

4. Evaluating Study Methods and Quality

IRIS assessments evaluate study methods and quality, using uniform approaches for each group of similar studies. The objective is that subsequent syntheses can weigh study results on their merits. Key concerns are *bias* (factors that affect the magnitude or direction of an effect) and *sensitivity* (factors that limit the ability of a study to detect a true effect).

For human and animal studies, the evaluation of study methods and quality considers study design, exposure characterization, outcome assessment, data analysis, and selective reporting. For human studies, this evaluation also considers selection of participant and referent groups and potential confounding. Emphasis is on discerning bias that would substantively change an effect estimate, considering also the expected direction of the bias. Low sensitivity is a bias towards the null.

[4] Health and Environmental Research Online: https://hero.epa.gov/hero/

[5] IRIS "stopping rules": https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf

Study-evaluation considerations are specific to each study design, agent, and health effect. Subject-matter experts evaluate each group of studies to identify characteristics that would make results more or less informative. For carcinogenicity, neurotoxicity, reproductive toxicity, and developmental toxicity, there is EPA guidance for study evaluation. As subject-matter experts examine a group of studies, additional methodologic concerns may emerge and a second pass become necessary.

Assessments use evidence tables to summarize the design and results of pertinent studies. If tables become too numerous or unwieldy, they may focus on effects that are more important or studies that are more informative.

The IRIS program posts on its website the study-evaluation considerations and table entries for illustrative studies, then considers public input on these approaches as it completes study evaluation and data extraction.

5. Integrating the Evidence of Causation for Each Health Outcome

Synthesis within lines of evidence. For each health outcome, IRIS assessments synthesize the human evidence and the animal evidence, augmenting each with informative subsets of mechanistic data. Each synthesis considers aspects of an association that may suggest causation: consistency, exposure–response relationship, strength of association, temporal relationship, biological plausibility, coherence, and "natural experiments" in humans.

Each synthesis seeks to reconcile ostensible inconsistencies between studies, taking into account differences in study methods and quality. This leads to a distinction between *conflicting evidence* (unexplained positive and negative results in similarly exposed human populations or in similar animal models) and *differing results* (mixed results attributable to differences between human populations, animal models, or exposure conditions).

Each synthesis of human evidence explores alternative explanations (e.g., chance, bias, or confounding) and determines whether they satisfactorily explain the results. Each synthesis of animal evidence explores the potential for analogous results in humans. Coherent results across multiple species increase confidence that the animal results are relevant to humans.

Mechanistic data are useful to augment the human or animal evidence with information on precursor events, to evaluate the human relevance of animal results, or to identify susceptible populations and lifestages. An agent may operate through multiple mechanistic pathways, even if one hypothesis dominates the literature.

Integration across lines of evidence. For each health outcome, IRIS assessments integrate the human, animal, and mechanistic evidence to answer the question: *What is the nature of the association between exposure to the agent and the health outcome?*

For cancer, the EPA includes a standardized hazard descriptor in characterizing the strength of the evidence of causation. The objective is to promote clarity and consistency of conclusions across assessments.

Carcinogenic to humans: convincing epidemiologic evidence of a causal association; or strong human evidence of cancer or its key precursors, extensive animal evidence, identification of mode-of-action and its key precursors in animals, and strong evidence that they are anticipated in humans.

Likely to be carcinogenic to humans: evidence that demonstrates a potential hazard to humans. Examples include a plausible association in humans with supporting experimental evidence, multiple positive results in animals, a rare animal response, or a positive study strengthened by other lines of evidence.

Suggestive evidence of carcinogenic potential: evidence that raises a concern for humans. Examples include a positive result in the only study, or a single positive result in an extensive database.

Inadequate information to assess carcinogenic potential: no other descriptors apply. Examples include little or no pertinent information, *conflicting evidence*, or negative results not sufficiently robust for *not likely*.

Not likely to be carcinogenic to humans: robust evidence to conclude that there is no basis for concern. Examples include no effects in well-

conducted studies in both sexes of multiple animal species, extensive evidence showing that effects in animals arise through modes-of-action that do not operate in humans, or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

If there is credible evidence of carcinogenicity, an assessment determines whether the mode-of-action involves mutagenicity, because this influences the approach to dose–response assessment and subsequent application of adjustment factors for exposures early in life.

The EPA is discussing the potential use of hazard descriptors for noncancer outcomes in IRIS assessments.

6. Selecting Studies for Derivation of Toxicity Values

The purpose of toxicity values (i.e., slope factors, unit risks, reference doses, reference concentrations; see section 7) is to estimate exposure levels likely to be without appreciable risk of adverse health effects. The EPA uses these values to support its actions to protect human health.

The health outcomes considered for derivation of toxicity values may depend on the hazard descriptors. For example, IRIS assessments generally derive cancer values for agents that are *carcinogenic* or *likely to be carcinogenic*, and sometimes for agents with *suggestive evidence*.

Derivation of toxicity values begins with a new evaluation of studies, as some studies used qualitatively for hazard identification may not be useful quantitatively for exposure–response assessment. Quantitative analyses require quantitative measures of exposure and response. An assessment weighs the merits of the human and animal studies, of various animal models, and of different routes and durations of exposure. Study selection is not reducible to a formula, and each assessment explains its approach.

Other biological determinants of study quality include appropriate measures of exposure and response, investigation of early effects that precede overt toxicity, and appropriate reporting of related effects (e.g., combining effects that comprise a syndrome, or benign and malignant tumors in a specific tissue).

Statistical determinants of study quality include multiple levels of exposure (to characterize the shape of the exposure–response curve) and adequate exposure range and sample sizes (to minimize extrapolation and maximize precision).

Studies of low sensitivity tend to underestimate toxicity and may be less useful.

7. Deriving Toxicity Values

General approach. EPA guidance describes a two-step approach to dose–response assessment: analysis in the range of observation, then extrapolation to lower levels. The analysis considers studies by the exposure route of interest and may include studies by other routes if dose conversion is possible.

IRIS assessments derive a candidate value from each suitable data set. Consideration of candidate values yields a toxicity value for each organ or system. Consideration of the organ/system-specific values results in the selection of an overall toxicity value to cover all health outcomes. The organ/system-specific values are useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common anatomical site.

Analysis in the range of observation. Within the observed range, the preferred approach is modeling to incorporate a wide range of data. Toxicokinetic modeling has become increasingly common for its ability to support target-dose estimation, cross-species adjustment, or exposure-route conversion. If data are too limited to support toxicokinetic modeling, there are standardized approaches to estimate daily exposures and scale them from animals to humans.

For human studies, an assessment may develop exposure–response models that reflect the structure of the available data. For animal studies, the EPA has developed a set of empirical ("curve-fitting") models[6] that can fit typical data sets. Such modeling yields a *point of departure*, defined as a dose near the lower end of the

[6] Benchmark Dose Software: http://www.epa.gov/bmds/

observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower bounds).

With complex data, an assessment may develop specialized exposure–response models if compatible with the scope of the assessment. Toxicodynamic ("biologically based") modeling is possible if data are sufficient to ascertain the key events of a mode-of-action and to estimate their parameters. For a group of agents that act at a common site or through common mechanisms, an assessment may derive relative potency factors based on relative toxicity, rates of absorption or metabolism, quantitative structure–activity relationships, or receptor-binding characteristics.

Extrapolation: slope factors and unit risks. An *oral slope factor* or an *inhalation unit risk* facilitates subsequent estimation of human cancer risks at low levels of exposure. They presuppose a linear component to the dose–response curve below the point of departure (e.g., if the mode-of-action involves mutagenicity), or there may be no established mode-of-action. Extrapolation proceeds linearly (i.e., risk proportional to dose) from the point of departure to the levels of interest.

Differences in susceptibility may warrant derivation of multiple slope factors or unit risks. For early-life exposure to known or likely carcinogens whose mode-of-action involves mutagenicity, the EPA has developed default *age-dependent adjustment factors* for agents without chemical-specific susceptibility data.

If data are sufficient to ascertain the key events of the mode-of-action and to conclude that they are not linear at low levels, extrapolation may use the reference-value approach.

Extrapolation: reference values. An *oral reference dose* or an *inhalation reference concentration* is an estimate of human exposure (including in susceptible populations) likely to be without appreciable risk of adverse health effects over a lifetime. Reference values generally cover effects other than cancer. They are also appropriate for cancer if a well-characterized mode-of-action indicates that a necessary key event does not occur below a specific dose.

Calculation of reference values starts with a point of departure, generally for an early effect that precedes overt toxicity. To account for different sources of uncertainty and variability, an assessment applies *uncertainty factors* (each typically 1, 3, or 10) to the point of departure.

Human variation: An uncertainty factor covers susceptible populations and lifestages that may respond at lower levels, unless the data originate from a susceptible study population.

Animal-to-human extrapolation: For reference values based on animal results, an uncertainty factor reflects cross-species differences, which may cause humans to respond at lower levels.

Subchronic-to-chronic exposure: For reference values based on subchronic studies, an uncertainty factor reflects the likelihood that a lower level over a longer duration may induce a similar response. This factor may not be necessary for reference values of shorter duration.

Adverse-effect level to no-observed-adverse-effect level: For reference values based on a lowest-observed-adverse-effect level, an uncertainty factor reflects a level judged to have no observable adverse effects.

Database deficiencies: If there is concern that additional studies may identify a more sensitive effect, target organ, population, or lifestage, a *database uncertainty factor* reflects the nature of the database deficiency.

8. Process for Developing and Peer-Reviewing IRIS Assessments

The IRIS process (revised in 2009 and enhanced in 2013) involves extensive public engagement and multiple levels of scientific review.

Step 1: Draft development. As outlined in section 2 of this Preamble, IRIS program scientists specify the scope of an assessment and formulate science questions for discussion with the scientific community and the public. Next, they release protocols for the systematic review procedures planned for use in the assessment. IRIS program scientists then develop a first draft, using structured approaches to identify pertinent studies,

evaluate study methods and quality, integrate the evidence of causation for each health outcome, select studies for derivation of toxicity values, and derive toxicity values, as outlined in Preamble sections 3–7.

Step 2: Agency review. Health scientists across the EPA review the draft assessment.

Step 3: Interagency science consultation. Other federal agencies and the Executive Office of the President review the draft assessment.

Step 4: Public comment, followed by external peer review. The public reviews the draft assessment. IRIS program scientists address the public comments, then release a revised draft for independent external peer review. The peer reviewers consider whether the draft assessment assembled and evaluated the evidence according to EPA guidance and whether the evidence justifies the conclusions.

Step 5: Revise assessment. IRIS program scientists revise the assessment to address the comments from the peer review.

Step 6: Final agency review and interagency science discussion. The IRIS program discusses the revised assessment with EPA's program and regional offices and with other federal agencies and the Executive Office of the President.

Step 7: Post final assessment. The IRIS program posts the completed assessment and a summary on its website.

9. General Structure of IRIS Assessments

Main text. IRIS assessments generally comprise two major sections: (1) Hazard Identification and (2) Dose–Response Assessment. Section 1.1 briefly reviews chemical properties and toxicokinetics to describe the disposition of the agent in the body. This section identifies related chemicals and summarizes their health outcomes, citing authoritative reviews. If an assessment covers a chemical mixture, this section discusses environmental processes that alter the mixtures humans encounter and compares them to mixtures studied experimentally.

Section 1.2 includes a subsection for each major health outcome. Each subsection discusses the respective literature searches and study considerations, as outlined in Preamble sections 3 and 4, unless covered in the front matter. Each subsection concludes with evidence synthesis and integration, as outlined in Preamble section 5.

Section 1.3 links health hazard information to dose–response analyses for each health outcome. One subsection identifies susceptible populations and lifestages, as observed in human or animal studies or inferred from mechanistic data. These may warrant further analysis to quantify differences in susceptibility. Another subsection identifies biological considerations for selecting health outcomes, studies, or data sets, as outlined in Preamble section 6.

Section 2 includes a subsection for each toxicity value. Each subsection discusses study selection, methods of analysis, and derivation of a toxicity value, as outlined in Preamble sections 6 and 7.

Front matter. The Executive Summary provides information historically included in IRIS summaries on the IRIS program website. Its structure reflects the needs and expectations of EPA's program and regional offices.

A section on systematic review methods summarizes key elements of the protocols, including methods to identify and evaluate pertinent studies. The final protocols appear as an appendix.

The Preface specifies the scope of an assessment and its relation to prior assessments. It discusses issues that arose during assessment development and emerging areas of concern. The Preface also identifies assessment-specific approaches that may differ from the general approaches outlined in this Preamble.

May 2016

EXECUTIVE SUMMARY

Occurrence and Health Effects

Ammonia occurs naturally in air, soil, and water. Ammonia is also produced by humans and other animals as part of normal biological processes.

Ammonia is used as an agricultural fertilizer and in many cleaning products. Exposure to ammonia occurs primarily through breathing air containing ammonia gas, and may also occur via diet, drinking water, or direct skin contact. Concentrations of ammonia measured in ambient outdoor air range from 0.28–15 µg/m^3 and in indoor air from 0.09–166 µg/m^3.

Health effects of inhaled ammonia observed at levels exceeding naturally-occurring concentrations are generally limited to the respiratory tract, the site of direct contact with ammonia. Short-term inhalation exposure to high levels of ammonia in humans can cause irritation and serious burns in the mouth, lungs, and eyes. Chronic exposure to airborne ammonia can increase the risk of respiratory irritation, cough, wheezing, tightness in the chest, and reduction in the normal function of the lung in humans. Studies in experimental animals similarly indicate that breathing ammonia at sufficiently high concentrations can result in effects on the respiratory system. Animal studies also suggest that exposure to high levels of ammonia in air may adversely affect other organs, such as the liver, kidney, and spleen.

Chemical Properties

Ammonia (NH_3) is a colorless alkaline gas with a pungent odor. In solution, ammonia exists as ammonium hydroxide, a weak base that is only partially ionized in water according to the following equilibrium (ATSDR, 2004): $NH_3 + H_20 \rightleftarrows NH_4^+ + OH^-$. A decrease in pH results in an increase in the concentration of ammonium ion (NH_4^+) and a decrease in the concentration of the un-ionized form (NH_3). At physiological pH (7.4), this equilibrium favors the formation of NH_4^+.

Toxicokinetics

Inhaled ammonia is almost completely retained in the upper respiratory tract. Ammonia produced endogenously in the intestines through the use of amino acids as an energy source and by bacterial degradation of nitrogenous compounds from ingested food is largely absorbed. At physiological pH, 98.3% of ammonia is present in the blood as the ammonium ion (NH_4^+). Given its importance in amino acid metabolism, the urea cycle, and acid-base balance, ammonia is homeostatically regulated to remain at low concentrations in the blood. Ammonia is present in fetal circulation and in human breast milk as a source of nonprotein nitrogen. Ammonia production

occurs endogenously by catabolism of amino acids by glutamate dehydrogenase or glutaminase primarily in the liver, renal cortex and intestines, but also in the brain and heart. Ammonia is metabolized to glutamine via glutamine synthetase in the glutamine cycle or incorporated into urea as part of the urea cycle. The principal means of excretion of ammonia is as urinary urea; lesser amounts are eliminated in the feces, through sweat production, and in expired air.

Effects Other Than Cancer Observed Following Inhalation Exposure

Respiratory effects have been identified as a human health hazard following inhalation exposure to ammonia. This hazard determination is based on findings from multiple epidemiology studies in human populations exposed to ammonia in different settings (workers in industrial, cleaning and agricultural settings, volunteers exposed for up to 6 hours under controlled conditions, and case reports) and animals (short-term and subchronic studies in several species and across different exposure regimes).

Cross-sectional occupational studies involving chronic exposure to ammonia in industrial settings provide evidence of an increased prevalence of respiratory symptoms (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Bhat and Ramaswamy, 1993). Other studies of exposure to ammonia when used as a disinfectant or cleaning product, for example in health care workers, provide additional evidence of effects on asthma, asthma symptoms, and pulmonary function, using a variety of study designs (Casas et al., 2013; Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005). Additional evidence of respiratory effects of ammonia is seen in studies of pulmonary function in an agricultural setting, specifically in the studies that accounted for effects of co-exposures to other agents such as endotoxin and dust (Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Heederik et al., 1990) and in one study that did not control for co-exposures (Loftus et al., 2015). Despite the variation in population characteristics, level and pattern of exposure, and potential confounders across these three settings of epidemiology studies, respiratory effects were consistently observed in these studies. Further, but more limited, support for the respiratory system as a target of ammonia toxicity comes from controlled human exposure studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia. Additionally, respiratory effects were observed in several animal species following short-term and subchronic inhalation exposures to ammonia.

Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on organs distal from the portal of entry, but there is inadequate information to draw conclusions about the liver, kidney, spleen, or heart as sensitive targets of ammonia toxicity.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

Table ES-1. Summary of reference concentration (RfC) derivation

Critical effect	Point of departure[a]	UF	Chronic RfC
Decreased lung function and respiratory symptoms Occupational epidemiology studies Holness et al. (1989), supported by Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001)	$NOAEL_{ADJ}$: 4.9 mg/m^3	10	0.5 mg/m^3

[a] An estimate of the 95% lower confidence bound of the mean exposure concentration in the high-exposure group of the Holness et al. (1989) study was used as the NOAEL. Because the study involved workplace exposure conditions, the NOAEL of 13.6 mg/m^3 was adjusted for continuous exposure based on the ratio of VEho (human occupational default minute volume of 10 m^3 breathed during an 8-hour workday) to VEh (human ambient default minute volume of 20 m^3 breathed during the entire day) and an exposure of 5 days out of 7 days.

NOAEL = no-observed-adverse-effect level; UF = uncertainty factor

The study of ammonia exposure in workers in a soda ash plant by Holness et al. (1989), with support from three studies in urea fertilizer plants by Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001), was identified as the principal study for RfC derivation. Respiratory effects, characterized as increased respiratory symptoms based on self-report (including cough, wheezing, and other asthma-related symptoms) and decreased lung function in workers exposed to ammonia, were selected as the critical effect. Holness et al. (1989) found no differences in the prevalence of respiratory symptoms or lung function between workers (mean exposure 6.5 mg/m^3) and the control group, and no differences when stratified by exposure level (highest exposure group, >8.8 mg/m^3). Rahman et al. (2007) observed an increased prevalence of respiratory symptoms and decreased lung function in workers exposed in a plant with a mean ammonia concentration of 18.5 mg/m^3, but not in workers in a second plant exposed to a mean concentration of 4.9 mg/m^3. Ballal et al. (1998) observed an increased prevalence of respiratory symptoms among workers in one factory with exposures ranging from 2 to 27.1 mg/m^3,[7] but no increase in another factory with exposures ranging from 0.02–7 mg/m^3. A companion study by Ali et al. (2001) also observed decreased lung function among workers exposed to higher cumulative ammonia levels (>50 mg/m^3-years), with an approximate 5–7% decrease in FVC % predicted and FEV_1% predicted.

These four studies addressed smoking by a variety of methods (e.g., adjustment for smoking, exclusion of smokers, or stratification of the results by smoking status). Two of the studies—Rahman et al. (2007) and Holness et al. (1989)—addressed other potential confounders as appropriate. In particular, a high level of control of exposures in the facility studied by Holness

[7]This concentration range does not include exposures in the urea store (number of employees = 6; range of ammonia concentrations = 90–130.4 mg/m^3) because employees in this area were required to wear full protective clothing, thus minimizing potential exposure.

et al. (1989) was reported, suggesting a low potential for co-exposures. As discussed in more detail in the Literature Search Strategy/Study Selection and Evaluation section, confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation of these studies.

Considerations in selecting the principal study for RfC derivation include the higher confidence placed in the measures of ammonia exposure in Holness et al. (1989), evaluation of both respiratory symptoms and lung function parameters in this study, and the fact that the estimate of the no-observed-adverse-effect level (NOAEL) for respiratory effects of 13.6 mg/m^3 from Holness et al. (1989) was the highest of the studies with adequate exposure-response information. The synthesis of findings from the full body of evidence demonstrates that there is a relationship between ammonia exposure and respiratory effects. Although Holness et al. (1989) do not report associations between ammonia exposure and respiratory effects, it is included in the body of epidemiologic studies of industrial settings because it is informative of the levels below which ammonia causes effects. These epidemiology studies include those with higher workplace ammonia concentrations associated with respiratory effects (i.e., higher concentrations relative to those reported by Holness et al. (1989)) and for which LOAELs could be identified. The Holness et al. (1989) study is identified as the principal study for RfC derivation based on the quality of the exposure data and other factors, as stated above.

In summary, the study of ammonia exposure in workers in a soda ash plant by Holness et al. (1989) was identified as the principal study for RfC derivation, with support from Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001), and respiratory effects were identified as the critical effect. The NOAEL, represented by an estimate of the 95% lower confidence bound of the mean exposure concentration in the high-exposure group from the Holness et al. (1989) study, or 13.6 mg/m3, was used as the point of departure (POD) for RfC derivation. The NOAEL adjusted to continuous exposure ($NOAEL_{ADJ}$) was 4.9 mg/m3.

An RfC of 0.5 (rounded) mg/m^3 was calculated by dividing the POD (adjusted for continuous exposure, i.e., $NOAEL_{ADJ}$) by a composite uncertainty factor (UF) of 10 to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhaled ammonia in the human population.

Confidence in the Chronic Inhalation RfC

Study – medium
Database – medium
RfC – medium

Consistent with EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), the overall confidence in the RfC is medium and reflects medium confidence in the principal study (adequate design, conduct, and reporting of the principal study; limited by small sample size and identification of a NOAEL only) and medium

confidence in the database, which includes occupational, cleaner, agricultural, and human exposure studies and studies in animals that are mostly of subchronic duration. There are no studies of developmental toxicity, and studies of reproductive and other systemic endpoints are limited; however, the likelihood of reproductive, developmental, and other systemic effects at the RfC is considered small because it is well documented that ammonia is endogenously produced in humans and animals, and any changes in blood ammonia levels at the POD would be small relative to normal blood ammonia levels. Further, EPA is not aware of any mechanisms by which ammonia can exert effects at the point of contact (i.e., respiratory system) that could directly or indirectly impact tissues or organs distal to the point of contact.

Susceptible Populations and Lifestages

Studies of the toxicity of ammonia in children that would support an evaluation of childhood susceptibility are limited. Casas et al. (2013) and Loftus et al. (2015) reported evidence of an association between ammonia exposure and decrements in lung function in children; however these studies did not report information that would allow a comparison of children and adults.

A limited number of studies provides inconsistent evidence of greater respiratory sensitivity to ammonia exposure in asthmatics (Loftus et al., 2015; Petrova et al., 2008; Sigurdarson et al., 2004; Preller et al., 1995). Loftus et al. (2015) reported no increase in asthma symptoms and medication use in asthmatic children living near animal feeding operations; however, ammonia exposure was associated with lower FEV_1.

Hyperammonemia is a condition of elevated levels of circulating ammonia that can occur in individuals with severe diseases of the liver or kidney or with hereditary urea [$CO(NH_2)_2$] cycle disorders. These elevated ammonia levels can predispose an individual to encephalopathy due to the ability of ammonia to cross the blood-brain barrier; these effects are especially marked in newborn infants. Thus, individuals with disease conditions that lead to hyperammonemia may be more susceptible to the effects of ammonia from external sources, but there are no studies that specifically support this susceptibility.

Key Issues Addressed in This Assessment

Comparison of Exhaled Ammonia to the RfC

Ammonia is generated endogenously in multiple organs and plays central roles in nitrogen balance and acid-base homeostasis (Weiner et al., 2014; Weiner and Verlander, 2013). Given its important metabolic role, free ammonia is homeostatically regulated to remain at low concentrations in blood (Souba, 1987). Elimination of ammonia occurs primarily in urine and exhaled breath. Consideration was given to the presence of ammonia in exhaled air because the range of ammonia concentrations in exhaled breath (0.009–2 mg/m^3) overlaps the ammonia RfC (0.5 mg/m^3).

In general, the higher and more variable ammonia concentrations (0.03 to 2 mg/m^3) are reported in human breath exhaled from the mouth or oral cavity (Schmidt et al., 2013; Smith et al., 2008; Španěl et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Smith et al., 1999; Norwood et

al., 1992; Larson et al., 1977). Ammonia concentrations measured in breath derived from oral breathing largely reflect the production of ammonia via bacterial degradation of food protein in the oral cavity or gastrointestinal tract, and can be influenced by diet, oral hygiene, age, and saliva pH. In contrast, concentrations of ammonia in breath exhaled from the nose and trachea of humans (0.0092–0.1 mg/m^3) are lower than those in air exhaled from the mouth (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977), and are generally lower than the RfC by a factor of five or more. Concentrations in breath exhaled from the nose appear to better represent levels at the alveolar interface of the lung and are more relevant to understanding systemic levels of ammonia than breath exhaled from the mouth (Schmidt et al., 2013; Smith et al., 2008); however, neither concentrations in breath from the mouth or nose can be used to predict blood ammonia concentration or previous exposure to environmental (ambient) concentrations of ammonia.

Regardless of the source of expired ammonia (mouth or nose), the level of ammonia in breath, even at concentrations that exceed the RfC, does not necessarily raise questions about the appropriateness of the RfC. The exhalation of ammonia is a clearance mechanism for a product of metabolism that is otherwise toxic in the body at sufficiently high concentrations. Thus, ammonia concentrations in exhaled breath may be higher than inhaled concentrations. However, the presence of ammonia in exhaled breath is not considered an uncertainty in the RfC.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

Literature Search and Screening Strategy

The literature search for ammonia was conducted in six online scientific databases, including PubMed, Toxline, the Toxic Substances Control Act Test Submissions (TSCATS) database, Web of Science (WOS), HERO[8], and Toxcenter. The initial search was performed in March 2012 (PubMed, Toxline, TSCATS, HERO, and Toxcenter) and literature search updates were conducted in March 2013 (PubMed, Toxline, TSCATS, HERO, and WOS) and September 2015 (PubMed, Toxline, TSCATS, and WOS). Toxcenter is a database in which titles may be viewed for free after a fee-based search, but full citations and abstracts are purchased. The use of Toxcenter was discontinued in 2013. No unique relevant hits were returned in the 2013 update search of HERO; therefore, this search was not repeated in 2015. The detailed search approach, including the query strings, is presented in Appendix B, Table B-1. This search of online databases identified approximately ~28,000 unique citations (after electronically eliminating duplicates).

The core computerized database searches were supplemented by a review of citations in other national and international health agency documents (see Table B-2). The ATSDR (2004) *Toxicological Profile of Ammonia*[9] was used to identify toxicokinetic studies for ammonia. A search of online chemical assessment-related websites was performed in 2012 and 2015; links to the websites that were searched are provided in Table B-2. An additional focused search strategy was also employed to obtain studies of cleaning and hospital workers to address a new area of research identified during the 2013 literature search update. This strategy involved a manual reference list review of several seminal studies published in 2012 (see Appendix B, Table B-2). In addition, electronic forward searches were conducted in WOS in 2013 and 2015, using a methods paper describing the development of a job exposure matrix focusing on asthma as a health outcome

[8]Health and Environmental Research Online (HERO) is a database of scientific studies and other references used to develop EPA assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 1.6 million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO. For each IRIS assessment, a HERO project page is created that stores all citations identified from that chemical-specific literature search. These citations may be organized using various tags to indicate if the citations are used in the assessment and how they are categorized.

[9]Portions of this Toxicological Review were developed under a Memorandum of Understanding with the Agency for Toxic Substances and Disease Registry (ATSDR) and were adapted from the Toxicological Profile for Ammonia (_ENREF_12) and the references cited in that document, as part of a collaborative effort in the development of human health toxicological assessments for the purposes of making more efficient use of available resources and sharing scientific information.

(Kennedy et al., 2000). The disposition of studies obtained from the manual backward and electronic forward searches is presented in Table B-3.

In Federal Register notices announcing annual IRIS agendas and on the IRIS website, EPA encouraged the public to submit information on IRIS chemicals throughout the assessment development process, and specifically requested that the public submit additional data to support development of the ammonia assessment on December 21, 2007 and November 2, 2009 (U.S. EPA, 2009a, 2007). No public submissions were received in response to these calls for data.

Figure LS-1 depicts a summary of the literature search and screening process and the number of references included or excluded at each step. In 2012, the initial literature search was conducted in core computerized databases. These citations were electronically screened in an EndNote database using a set of terms intended to prioritize "on-topic" references for title and abstract review. The electronic screening process created two broad categories: one of all citations that contain (in title, abstract, or keywords) at least one inclusion term related to health outcomes, epidemiological or toxicological study design, absorption/distribution/metabolism/excretion (ADME) or toxicokinetics, or mechanistic information (see Appendix B, Table B-4), and one that did not contain any of the terms. Some of the electronic inclusion terms listed in Table B-4 are generic (i.e., not chemical specific) and are intended to capture health effect studies of any type. Other terms are specific to ammonia and are based on previous knowledge of health effects and possible mechanisms of toxicity summarized in other health agency review documents (see Appendix A). Citations that did not contain at least one inclusion term in Table B-4 (i.e., excluded by the electronic screening) were subjected to a quality control check to verify that relevant references were not missed. Specifically, a random sample (approximately 10%) of the electronically excluded citations were subjected to title and abstract review by a toxicologist to confirm that the electronic screening process produced acceptable results (i.e., no relevant citations were inadvertently missed). Relevant items were added to the HERO project page for ammonia and retrieved for full-text review. The results from the updated literature searches performed in March 2013 and September 2015 were not screened electronically in EndNote. All titles and abstracts obtained from these search updates were reviewed manually by a toxicologist.

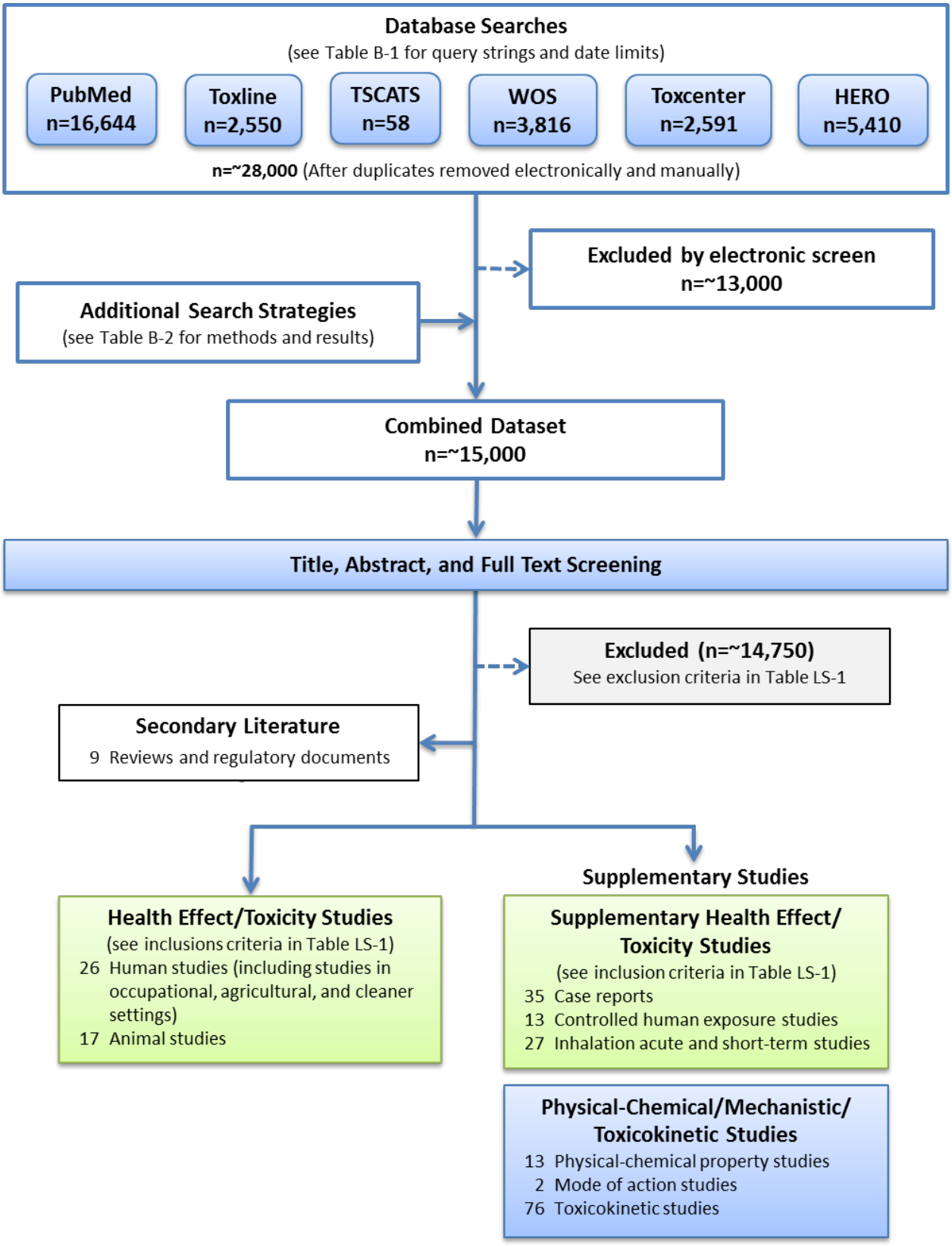


Figure LS-1. Summary of literature search and screening process for ammonia.

Manual screening of titles/abstracts and full text was accomplished using a set of inclusion and exclusion criteria to identify sources of primary human health effects data and sources of primary data that supplement the assessment of ammonia health effects (i.e., bottom boxes in Figure LS-1). The inclusion/exclusion criteria that were used prior to peer review are presented in Table LS-1. Manual screening of the post-peer review literature search update (i.e., September 2015) was performed using more stringent inclusion and exclusion criteria to capture studies that would impact the credibility of the assessment's conclusions consistent with EPA's IRIS Stopping Rules (http://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf). For ammonia, those references identified in the post-peer review literature search that were considered for inclusion in hazard identification were in vivo animal toxicity and epidemiology studies. No additional in vivo animal toxicity studies of ammonia were identified in the post-peer review search. The disposition of epidemiology studies obtained from the post-peer review literature search update (i.e., September 2015) is provided in Table B-5.

Specific inclusion/exclusion criteria were not applied in identifying sources of mechanistic and toxicokinetic data. Because ammonia is produced endogenously and serum ammonia levels are measured in certain disease states, the toxicokinetics literature is large and complex; relevant toxicokinetic studies for ammonia were initially identified using the ATSDR (2004) *Toxicological Profile of Ammonia* and supplemented by more recent studies identified in literature search updates. The number of mechanistic studies identified for ammonia was not large, and therefore all mechanistic studies were included.

Table LS-1. Inclusion/exclusion criteria for inhalation health effect/toxicity studies (pre-peer review)*

	Inclusion criteria	Exclusion criteria
Population	• Humans, including occupational workers, livestock workers and those in close proximity to agricultural operations, hospital workers/cleaners and volunteers • Standard mammalian animal models, including rat, mouse, hamster, rabbit, guinea pig, monkey, dog • Pigs	• Ecological species/ecosystem effects • Nonmammalian species • Agricultural species/livestock (except pigs)
Exposure	• Exposure is to ammonia by the inhalation route (any duration) • Exposure is measured as a concentration in air • Exposure is in vivo	• Not chemical specific (i.e., not ammonia-specific) • Animal studies: exposure is to a mixture only • Human studies: exposure is inferred but not measured (e.g., some cleaning and hospital worker studies) • Exposure by oral, dermal, injection or instillation routes • Studies of quaternary ammonia
Outcome	• One or more of the following health effect endpoints is evaluated: effects on the	• No health outcome evaluated • Pathogenic effects of *H. pylori* infection

	Inclusion criteria	Exclusion criteria
	cardiovascular, dermal/integumentary, endocrine, gastrointestinal, immune, musculoskeletal, nervous, reproductive, respiratory, hepatic, or renal (urinary) systems; effects on the eyes, survival, growth, or development	
Other		• Review article or abstract only (i.e., no primary data) • Environmental fate and transport of ammonia • Analytical methods for measuring ammonia in environmental media, and use in sample preparations and assays • Study of physical-chemical properties • Study of in vitro or in vivo toxicokinetics • Study of in vitro or in vivo mechanistic endpoints • Other studies not on topic and not captured by other exclusion criteria

* Reviews and regulatory documents were retained as Secondary Literature. Studies that provided primary information on the physical-chemical properties, mode of action, or toxicokinetics of ammonia were also retained, but were not screened as sources of health effect/toxicity information for ammonia.

The results of the pre- and post-peer review literature screening are described below and graphically in Figure LS-1:

- 43 references (including 26 human studies and 17 animal studies) were identified as studies with health effects data and were considered for data extraction to evidence tables and exposure-response arrays.
- Supplementary health effect/toxicity studies included 35 case reports, 13 acute-duration controlled human exposure studies, and 27 acute or short-term animal studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as supplementary studies for assessing ammonia health effects.
- 91 studies were identified as physical-chemical, mode of action, or toxicokinetic studies, including 13 studies of physical-chemical properties, 2 studies providing mode of action information, and 76 toxicokinetic studies. Information from these studies was not extracted into evidence tables; however, these studies were considered as supplementary studies for assessing ammonia health effects (e.g., consideration of toxicokinetic information in assessing the health effects literature).
- Nine reviews or regulatory/health assessment documents were identified as secondary literature. These references were retained as additional resources in developing the Toxicological Review.
- More than 27,000 references were identified as not pertinent to an evaluation of the inhalation health effects of ammonia. Approximately 13,000 were excluded by

electronic screening (see Table B-4) and approximately 14,750 were excluded by manual screening (see Table LS-1 for exclusion criteria).

Study Selection and Evaluation

Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment and general study quality considerations. In general, the relevance and scientific quality of the available studies was evaluated as outlined in the Preamble and in EPA guidance (i.e., *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* (U.S. EPA, 1994)). The scientific considerations used to evaluate and select studies and the relevance of these studies to the assessment are described in the section below.

Considerations for evaluation of epidemiology studies

Case reports are often anecdotal and describe unusual or extreme exposure situations, providing little information that would be useful for characterizing chronic health hazards. Ammonia case studies were only briefly reviewed; representative citations from the collection of case reports are provided as supplemental information in Appendix C, Section C.2.4. Similarly, acute controlled human exposure studies would not be useful for characterizing chronic health effects; these studies were therefore briefly reviewed and are provided as supplemental information in Appendix C, Section C.2.3.

Epidemiology studies of chronic exposure to ammonia have primarily focused on industrial worker populations, workers exposed to ammonia as a cleaning or disinfectant product, and those exposed in an agricultural setting. There is considerable variation in population characteristics, level and pattern of exposure, and potential confounders across the three categories of studies. Evaluations of the observational epidemiology studies of industrial worker populations and workers exposed to ammonia as a cleaning or disinfectant product identified in Figure LS-1 (i.e., the studies considered most informative for evaluating ammonia toxicity from chronic exposure) are provided in Appendix B (Tables B-6 to B-8). The process used to evaluate these studies addressed aspects relating to the selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis. As discussed below, studies of populations exposed in agricultural settings were considered to be supporting material because of the variety of potential co-exposures in these studies (including dust, endotoxin, mold, and disinfectant products). The process for evaluating studies in an agricultural setting considered the same five aspects (selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis); however, specific study evaluation tables were not provided in Appendix B for this set of studies.

For study evaluation purposes, EPA differentiated between "major" limitations, defined as biases or deficiencies that could materially affect the interpretation of the study, and "minor" limitations, defined as limitations that are not likely to be severe or to have a substantive impact on

the results. These categories are similar to the "serious risk of bias" and "moderate risk of bias" categories, respectively, described by Stearne et al. (2014) in the Cochrane Collaborative Assessment Tool for non-randomized studies of clinical interventions. Identification of major limitations in the epidemiology studies of populations exposed in industrial, cleaning, and agricultural settings is included in the broader evaluation of study quality below. Uninformative studies are also noted.

Studies of Industrial Settings

Selection of study participants

All of the studies were cross-sectional analyses in occupational settings. The workers were healthy enough to remain in the work area for a considerable time; with one exception, mean duration ranged from 52 months to 16 years. One study (Bhat and Ramaswamy, 1993) grouped workers into those exposed for up to 10 years and those with more than 10 years of exposure; a minimum exposure duration was not provided. As in inherent property of occupational studies, these designs may result in a "healthy worker" bias. In addition, the workers in these studies are not representative of the general population, as they do not include children and only one study of ammonia exposure in hair salons included women (Nemer et al., 2015). These aspects of the study design may result in an underestimate of the risk of health effects of ammonia exposure, as the worker population may not exhibit health effects (such as decreased lung function or increased prevalence of respiratory symptoms) to the same degree that would be seen in the general population under the same conditions. In addition to the "healthy worker" effect, the Nemer et al. (2015) study exhibited a potential selection bias in the controls due to differences in recruitment (self-selected based on interest) or workload.

Exposure parameters

Exposure methods differ across these occupational studies, which makes comparison of ammonia measurements among the studies difficult. Spectrophotometric absorption measures of areas samples (Ali et al., 2001; Ballal et al., 1998) are not directly comparable to direct-reading diffusion methods Rahman et al. (2007) or electrochemical sensors methods (Nemer et al., 2015) used to analyze personal samples. Nor are they comparable to the NIOSH-recommended protocol for personal sampling and analysis of airborne contaminants (Holness et al., 1989). In the study by Rahman et al. (2007), exposure concentrations were determined by both the Dräger tube and Dräger PAC III methods. The Dräger tube method yielded concentrations of ammonia in the two plants studied that were approximately fourfold higher than the concentrations obtained by the Dräger PAC III method; a strong correlation between measurements by the two methods was reported. Rahman et al. (2007) stated that their measurements indicated only relative differences in exposures between workers and production areas, and did not identify one analytical measure as the more valid of the two. Based on communication with technical support at Dräger Safety Inc. (Bacom and Yanosky, 2010), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Ammonia concentrations based on the PAC III

method were also in line with concentrations reported in other studies. Therefore, exposure levels based on PAC III air measurements of ammonia were used in the current health assessment to characterize the exposure-response relationship in the Rahman et al. (2007) study.

In the Abdel Hamid and El-Gazzar (1996) study, no direct measurement of ammonia exposure was made; blood urea was used as a surrogate measure of ammonia exposure. The correlation of blood urea with ammonia is not reported by the authors. EPA considered this a major limitation of this study, based on other data indicating no correlation between ammonia levels in air and serum urea levels in a study of six groups of workers with varying types of exposure (Giroux and Ferrières, 1998). No exposure measurements of ammonia were used in the study by Bhat and Ramaswamy (1993). EPA considered the lack of exposure measure in this study to be a major limitation. In the Nemer et al. (2015) study, the measurement device had limited specificity for measuring ammonia relative to other gases and therefore could have produced false positive results in the presence of other gases. In addition, few exposure measurements were made in the Nemer et al. (2015) study. EPA considered the limited specificity for measuring ammonia, the limited number of exposure measurements, as well as possible misclassification of exposure in the Nemer et al. (2015) study to be major limitations.

Outcome measurement

Assessment of respiratory symptoms in Rahman et al. (2007), Ballal et al. (1998), Holness et al. (1989), and Nemer et al. (2015) was based on four different questionnaires; each of these, however, is a standardized, validated questionnaire. Self-reporting of types and severity of respiratory symptoms could be biased by the knowledge of exposure, for example, in studies comparing factory workers to office workers. EPA evaluated this non-blinded outcome assessment as a potential bias. In each of these studies, comparisons were made across exposure categories among the exposed; EPA concluded that the non-blinded outcome assessment as a potential bias is unlikely in these types of comparisons. One study also compared exposed to nonexposed, and observed little differences in symptom prevalence between these groups (Holness et al., 1989). Thus, EPA concluded that the non-blinded outcome assessment was not a major bias in this analysis either. Assessment of lung function was performed by standard spirometry protocols in five studies (Nemer et al., 2015; Rahman et al., 2007; Ali et al., 2001; Bhat and Ramaswamy, 1993; Holness et al., 1989). EPA did not consider any of these procedures for assessing lung function to be a source of bias.

Confounding

Co-exposures to other ambient chemicals in urea fertilizer factories included inorganic gases (nitrogen dioxide and sulfur dioxide) and dust. In one of these studies (Rahman et al., 2007), nitrogen dioxide was measured concurrently with ammonia and found to be below detection limits for all areas (urea plant, ammonia plant, and administration area). The other urea fertilizer studies (Ali et al., 2001; Ballal et al., 1998; Abdel Hamid and El-Gazzar, 1996) did not describe potential co-exposures. [It appears from the exposure measurements that the plant in Ali et al. (2001) is

"Factory A" in Ballal et al. (1998)]. In the fertilizer plant in Bhat and Ramaswamy (1993), co-exposures are not discussed, but the workers are grouped based on different parts of the plant (ammonia, urea, and diammonium phosphate); effects observed with respect to lung function tests were similar in magnitude, albeit slightly stronger, in the ammonia plant workers compared with the urea plant workers. One study was conducted in a soda ash production plant (Holness et al., 1989). No measurements of co-exposures were described in this study, but the authors note the high level of control of exposures (resulting in low ammonia levels) in this facility. Because of the lack of demonstration of co-exposures correlated with ammonia levels in these studies, and lack of demonstration of stronger associations between potential co-exposures and respiratory outcomes, EPA concluded that confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation for the urea plant and soda ash plant studies. However, in a study of ammonia exposure among hairdressers (Nemer et al., 2015), co-exposures to other workplace contaminants (such as persulfates and paraphenylenediamine) were not measured or controlled for in the analysis; therefore, possible confounding is considered to be a limitation in this study.

The analyses of respiratory symptoms and lung function may also be confounded by smoking. In six studies, analyses accounted for smoking as follows: the analysis included either an adjustment for smoking (Rahman et al., 2007; Holness et al., 1989), the exclusion of smokers (Nemer et al., 2015; Bhat and Ramaswamy, 1993), or stratification of the results by smoking status (Ali et al., 2001; Ballal et al., 1998). Thus, EPA did not consider potential confounding by smoking to be a major limitation of these studies.

Ammonia is present in both tobacco and cigarette smoke (Callicutt et al., 2006). Typical concentrations of ammonia in commercial U.S. tobacco blends range from 0.02–0.4% (Seeman and Carchman, 2008). Thus, there is some potential for additional exposure to ammonia associated with use of ammonia-containing tobacco products and/or inhalation of tobacco smoke. This finding reinforces the importance of controlling for smoking in the analyses of the respiratory symptoms and lung function. EPA did not consider potential confounding by smoking of ammonia-containing tobacco or by inhaling tobacco smoke to be a major limitation of these studies because smoking as a potential confounder was adequately addressed in the studies that examined effects on the respiratory system.

Information on smoking habits and use of alcohol (an exposure potentially affecting liver function tests) was not documented in the study of liver function by Abdel Hamid and El-Gazzar (1996). The lack of information and potential failure to control for these confounders is considered a major limitation.

Statistical analysis

EPA considered the statistical analysis in the epidemiological studies (Nemer et al., 2015; Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Abdel Hamid and El-Gazzar, 1996; Bhat and Ramaswamy, 1993; Holness et al., 1989) to be adequate and appropriate. Although the type of statistical testing was not specified in Abdel Hamid and El-Gazzar (1996), the results were presented in sufficient detail to allow interpretation of the data and analysis. Sample size, an

important consideration with respect to statistical power, was also considered. EPA noted the small number of exposed workers and low levels of exposure in the study by Holness et al. (1989) as limitations that could result in "false negative" results (i.e., a test result indicating a lack of association, whereas a positive association between exposure and a health effect exists).

Identification of uninformative studies

The study by Abdel Hamid and El-Gazzar (1996) was determined to have major limitations. Air concentrations of ammonia were not directly measured, and the use of blood urea has not been established as a reliable surrogate of ammonia exposure. Further, the lack of information on smoking and alcohol use, factors that could affect liver function, in a study intended to examine the association between liver function and ammonia exposure, was considered a significant flaw. Therefore, Abdel Hamid and El-Gazzar (1996) was not further considered in this assessment.

Major limitations were also identified in the Nemer et al. (2015) study: potential selection bias in the control group due to differences in recruitment (self-selected based on interest in the study) or workload; limited specificity of the analytical method used to measure ammonia (i.e., potential for false positives from other gases); and failure to control for confounders. In addition, the study used small sample sizes and only a single measurement of ammonia for each location (which may not have been representative of workplace exposures). Therefore, the Nemer et al. (2015) study was deemed to be uninformative and was not further considered in this assessment.

Studies of Health Care and Cleaning Settings

Selection of study participants

EPA also evaluated the studies that examined exposure to ammonia when used as a cleaning or disinfectant product. EPA noted the potential for the "healthy worker" bias arising from movement out of jobs by affected individuals in most of these studies (Le Moual et al., 2008). This issue was less of a concern in the study by Zock et al. (2007), which was conducted in a general (non-occupational) population sample, focusing on cleaning activities in the home. In a birth cohort that evaluated the association between exposure to cleaning products and children's respiratory health (Casas et al., 2013), 35% of the recruited population were excluded because information on the use of cleaning products and/or respiratory tests was not available, representing a potential study limitation. However, the authors of this study noted that the children included were not different from those excluded regarding most study characteristics (sex atopy, asthma, parental asthma and parental smoking).

Exposure parameters

None of these studies used a direct measure of ammonia exposure in the analysis, precluding interpretation of the results in relation to an absolute level of exposure. The limited data available concerning exposure levels in cleaning scenarios found median exposures of 0.6 to 5.4 ppm (0.4 to 3.8 mg/m^3), with peaks exceeding 50 ppm (35 mg/m^3), in a small study (n = 9) using personal samples during a domestic cleaning session (Medina-Ramón et al., 2005). Although

an absolute level of exposure is not available, the relative ranking of exposure used in these studies does allow examination of risk by relative levels of exposure.

Key considerations regarding the validity of the exposure measures are the specificity of the classification and the extent to which classification could be influenced by knowledge of the disease or symptoms under study. Methodological research has reported underestimation of self-reported exposure to specific products by health care workers, and differential reporting by disease status (i.e., asthma) for self-reported use of cleaning products in patient care, but not in instrument cleaning or building materials (Donnay et al., 2011; Delclos et al., 2009; Kennedy et al., 2000). Two of these studies used an exposure assessment protocol that incorporated an independent, expert review, blinded to disease status (Dumas et al., 2012; Lemiere et al., 2012); one study collected exposure information using a 2-week daily diary (Medina-Ramón et al., 2006) and one study (Casas et al., 2013) developed a composite exposure score based on an interviewer-led questionnaire concerned with the frequency of use and number of products used. EPA considered these to be the strongest of the exposure protocols used within this set of studies.

Outcome measures

Six of the studies in this set of studies used standard protocols for the assessment of respiratory symptoms in epidemiological studies (Casas et al., 2013; Arif and Delclos, 2012; Dumas et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2005), and one study included a clinical assessment protocol designed specifically for the assessment of occupational asthma (Lemiere et al., 2012). Details of the specific questions were provided, and EPA did not consider any of these methods to be a limitation in terms of specificity of the outcome. The study by Medina-Ramón et al. (2006) collected information on daily respiratory symptoms in a two-week diary, and also trained the participants to measure peak expiratory flow three times daily. A potential limitation in the Casas et al. (2013) study was the lack of information about the reliability of the pulmonary function measures.

Confounding

All of these studies addressed the potential for smoking to act as a confounder in the analysis. Two of the studies reported relatively weak correlations between ammonia and other products assessed (Zock et al., 2007; Medina-Ramón et al., 2005) and one study reported stronger associations with ammonia than with bleach (Dumas et al., 2012). Based on this information, EPA did not consider potential confounding to be a major limitation of this set of studies.

Statistical analysis

EPA considered the statistical analysis in this set of studies to be appropriate. One study, however, was limited in terms of the level of detail provided pertaining to the results for ammonia from multivariate models (Medina-Ramón et al., 2005).

Studies of Agricultural Settings

Selection of study participants

EPA also evaluated a set of studies conducted among livestock farmers and one study of asthmatic children in close proximity to animal feeding operations (Loftus et al., 2015). As with the other occupational studies discussed above, the selection of sensitive individuals out of the workforce ("healthy worker bias") would be a potential bias in cross-sectional studies of livestock farmers.

Exposure parameters

Among the studies examining pulmonary function, one study collected 24-hour air sampling from 14 ammonia monitoring devices located outside the home of a subset of the participants every 6 days for at least 3 months during the air monitoring period (Loftus et al., 2015), two studies used area-based exposure sampling in animal confinement buildings (Monsó et al., 2004; Zejda et al., 1994), one study used area samples taken in conjunction with specific tasks and calculated a personal exposure measure taking into account duration spent in specific locations and tasks (Heederik et al., 1990), four studies collected personal samples over a workshift (Donham et al., 2000; Reynolds et al., 1996; Preller et al., 1995), or an unspecified time period (Donham et al., 1995), and two studies used colorimetric tubes, which are generally less precise, to measure ammonia exposure (Monsó et al., 2004; Zejda et al., 1994) . EPA considered the use of the area-based samples without consideration of exposure duration to be limitations of the studies by Zejda et al. (1994) and Monsó et al. (2004).

Outcome measures

All of the studies reported using a standard spirometric technique; one study (Loftus et al., 2015) used twice daily home lung function measurements taken by the test subject; four studies compared two measures per individual (i.e., pre- and post-shift) (Monsó et al., 2004; Donham et al., 2000; Reynolds et al., 1996; Heederik et al., 1990); and two studies used a single pulmonary function measure, adjusted for height, age, and smoking variables (Preller et al., 1995; Zejda et al., 1994). EPA did not consider any of these outcome measures to be limitations in these studies, although the self-administered spirometry testing in the Loftus et al. (2015) study is a potential limitation.

Confounding

Six of these studies addressed confounding in some way. Four studies controlled for co-exposures (e.g., endotoxin, dust, disinfectants) (Melbostad and Eduard, 2001; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995), one study noted only weak correlations (i.e., Spearman $r < 0.20$) between ammonia and dust or endotoxin (Donham et al., 2000), and one study observed associations with ammonia but not with endotoxin or dust measures (Heederik et al., 1990). Three studies did not address confounding (Loftus et al., 2015; Monsó et al., 2004; Zejda et al., 1994).

Based on these considerations, EPA considered the studies by Reynolds et al. (1996), Preller et al. (1995), Donham et al. (2000), Donham et al. (1995), and Heederik et al. (1990) to be the methodologically strongest studies of this set.

Based on the evaluation of the epidemiology studies of ammonia in terms of selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis, the studies listed in Table LS-2 were selected for data extraction into evidence tables in Chapter 1.

Table LS-2. Summary of epidemiology database

Study setting	Reference
Industrial	Rahman et al. (2007) Ali et al. (2001) Ballal et al. (1998) Bhat and Ramaswamy (1993) Holness et al. (1989)
Cleaning	Casas et al. (2013) Arif and Delclos (2012) Dumas et al. (2012) Lemiere (2012) Vizcaya (2011) Zock (2007) Medina-Ramón et al. (2006) Medina-Ramón et al. (2005)
Agricultural	Loftus et al. (2015) Monsó et al. (2004) Melbostad and Eduard (2001) Donham et al. (2000) Reynolds et al. (1996) Donham et al. (1995) Preller et al. (1995) Choudat et al. (1994) Zejda et al. (1994) Crook et al. (1991) Heederik et al. (1990)

Considerations for evaluation of animal studies

Repeat-exposure toxicity studies of ammonia in experimental animals were evaluated using the study quality considerations outlined in the Preamble and discussed in various U.S. EPA guidance documents (U.S. EPA, 2005, 2002, 1994), including consideration of aspects of design,

conduct, or reporting that could affect the interpretation of results, overall contribution to the synthesis of evidence, and determination of hazard potential. The objective was to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

Additionally, a number of general questions, presented in Table LS-3, were considered in evaluating the animal studies. Much of the key information for conducting this evaluation can be determined based on study methods and how the study results were reported.

Table LS-3. Considerations and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of the species, strain, sex, and source of the test animals
Experimental design	Suitability of animal age/lifestage at exposure and endpoint testing; periodicity and duration of exposure (e.g., hrs/day, days/week); timing of endpoint evaluations; sample size and experimental unit (e.g., animals; dams; litters)
Exposure	Characterization of test article source, composition, purity, and stability; suitability of the control (e.g., vehicle control); documentation of exposure techniques (e.g., chamber type); verification of exposure levels (e.g., consideration of homogeneity, stability, analytical methods)
Endpoint evaluation	Suitability of specific methods for assessing the endpoint(s) of interest
Results presentation	Data presentation for endpoint(s) of interest (including measures of variability) and for other relevant endpoints needed for results interpretation (e.g., decrements in body weight in relation to organ weight)

Information relevant to study evaluation is reported in evidence tables and was considered in the synthesis of evidence. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant. The general finings of this evaluation are presented in the remainder of this section. Study evaluation considerations that are outcome specific are discussed in the relevant hazard section in Section 1.2.

Test animal

The ammonia database consists of toxicology studies conducted in rats (F344, Sprague-Dawley, Long-Evans, Sherman, Wistar), mice (OF1, Swiss albino), New Zealand white rabbits, guinea pig (Princeton-derived, Hartley), beagle dog, squirrel monkey, and pig (several strains). The species and strains of animals used are consistent with those typically used in laboratory studies, and all were considered relevant to assessing the potential human health effects of ammonia. The species, strain, and sex of the animals used in the experimental studies were recorded in the evidence tables. The Anderson et al. (1964a) and Weatherby (1952) guinea pig studies provided no information on the strain of the test animal; this is considered a minor limitation of these studies.

Experimental design

General aspects of study design and experimental design were evaluated to determine if they were appropriate for evaluation of specific endpoints. Key features of the experimental design, including the periodicity and duration of exposure and sample sizes, were summarized in the evidence tables in Chapter 1.

A single exposure group was used in a number of the general toxicity studies (Gaafar et al., 1992; Broderson et al., 1976; Doig and Willoughby, 1971; Anderson et al., 1964a; Weatherby, 1952), and in about half of the studies that examined immune endpoints (Hamilton et al., 1999; Hamilton et al., 1998; Schoeb et al., 1982; Richard et al., 1978). Use of a single exposure group limits the extent to which conclusions about a dose-response relationship can be drawn.

Sample size was not a basis for excluding a study from consideration, as studies with small numbers of animals can still inform the consistency of effects observed for a specific endpoint. Nevertheless, the following studies with small sample sizes were considered relatively less informative: Anderson et al. (1964a) studies in the mouse (4 animals/exposure interval) and guinea pigs (2 animals/exposure interval); the Weatherby (1952) study in guinea pigs (2 control and 4 exposed animals/exposure interval); and the Coon et al. (1970) studies in the rabbit (3 animals/group), monkey (3 animals/group), and dog (2 animals/group).

Exposure

Because inhalation toxicity studies can be technically difficult to perform, particular attention was paid to each study's exposure methods and documentation for assurance that the animals were properly exposed to gaseous ammonia. Exposure evaluation focused on those studies that reported effects on the respiratory system. Of the studies evaluated for exposure quality, six provided information on generation method, analytical method used to measure ammonia concentrations, analytical chamber concentrations, and chamber type; exposure characterization for these studies was considered robust (Broderson et al., 1976; Coon et al., 1970) (Done et al., 2005; Diekman et al., 1993; Doig and Willoughby, 1971; Stombaugh et al., 1969). Studies by Anderson et al. (1964a) and Curtis et al. (1975) failed to report analytical chamber concentrations, but otherwise exposures were considered to be adequately characterized. Exposure characterization in two studies (Gaafar et al., 1992; Weatherby, 1952) was considered poor because the studies failed to report analytical chamber concentrations, analytical method, and the type of inhalation chamber used. One of these two studies (Gaafar et al., 1992) also failed to describe how gaseous ammonia was generated from a 12% "ammonia solution."

Endpoint evaluation

Respiratory system and other noncancer effects were largely evaluated based on clinical signs (in the case of respiratory system effects) and histopathologic examination. All studies identified the tissues taken for histopathologic examination; however, the extent to which histopathologic methods were described varied across studies. Because histopathology is

considered a relatively routine measure, limited reporting of methodologic details was not considered a significant study deficiency.

Essentially all studies examined tissues from the lung and approximately half of the studies examined upper respiratory tissues. This is a concern because the highest exposure would have been to the upper respiratory tract due to the fact that ammonia is both water soluble and highly reactive. Gaafar et al. (1992) examined only the nasal mucosa. Tissues from other organs remote from the point of entry were inconsistently examined. Coon et al. (1970) examined sections from the heart, lung, liver, kidney, and spleen from all surviving monkeys, dogs, and rabbits, but from approximately half of the surviving guinea pigs and rats only; this incomplete histopathological investigation of guinea pigs and rats is considered a limitation. Anderson et al. (1964a) examined only the liver and spleen from exposed mice and guinea pigs. Broderson et al. (1976) examined sections from the liver, kidney, adrenal gland, pancreas, testicle, spleen, mediastinal nodes, and thymus. Curtis et al. (1975) noted that "visceral organs" were taken at necropsy for subsequent histopathologic examination, but provided no further details. Weatherby (1952) examined the heart, liver, stomach, small intestines, spleen, kidney, and suprarenal gland, but only reported limited incidence and severity information for the exposed and control guinea pigs. The extent of histopathological examination of the tissues was taken into consideration in evaluating animal findings.

Methodological considerations related to immune-specific endpoints are discussed in Section 1.2.2.

Results presentation

The majority of studies reported only limited qualitative results. With the exception of Broderson et al. (1976), none provided information on the incidence of histopathologic lesions.

In summary, relatively few repeat-dose toxicity studies of inhaled ammonia in experimental animals are available. The majority of these studies come from the older toxicological literature and were generally limited in terms of study design (e.g., small group sizes), documentation of methods, and reporting of results. Nevertheless, no study was considered sufficiently flawed as to be uninformative. Therefore, all in vivo animal toxicity studies, as listed in Table LS-4, were considered in hazard identification and data extraction to evidence tables.

Table LS-4. Summary of experimental animal database

Reference and study description (duration, route, species/strain)
Done et al. (2005) -- 5-week inhalation study in pigs (several breeds)
Andreasen et al. (2000b) -- 63-day inhalation study in Landrace X large white pigs
Hamilton et al. (1999) – 4-week inhalation study in large white pigs
Hamilton et al. (1998) – 14-day inhalation study in large white pigs
Diekman et al. (1993) – 6-week inhalation study in crossbred gilts (female pigs)

Reference and study description (duration, route, species/strain)
Gaafar et al. (1992) – 8-week inhalation study in white albino mice
Gustin (1994) – 6-day inhalation study in pigs
Manninen and Savolainen (1989) – 5-day inhalation study in Wistar rats*
Manninen et al. (1988) – 15-day inhalation study in Wistar rats*
Neumann (1987) – 35-day inhalation study in unweaned piglets
Targowski et al. (1984) – 3-week inhalation study in Hartley guinea pigs
Schaerdel et al. (1983a) -- 24-hour inhalation study in Crl:COBS CD(SD) rats *
Schoeb et al. (1982) – 35-day study in F344 rats
Richard (1978) – 7-day study in OF1 mice
Broderson et al. (1976) – 35- to 75-day inhalation studies in Sherman rats and F344 rats
Curtis et al. (1975) – 109-day inhalation study in crossbred pigs
Doig and Willoughby (1971) – 6-week inhalation study in Yorkshire-Landrace pigs
Coon et al. (1970) – 42- to 90-day inhalation studies in Sprague-Dawley and Long-Evans rats, New Zealand albino rabbits, Princeton-derived guinea pigs, squirrel monkeys, and beagle dogs
Stombaugh et al. (1969) – 5-week inhalation study in Duroc pigs
Anderson et al. (1964b) – 7- to 42-day inhalation studies in Swiss albino mice and guinea pigs (strain not specified)
Weatherby (1952) – 6- to 18-week inhalation study in guinea pigs (strain not provided)

*These studies were not identified as health effect/toxicity studies in Figure LS-1, but were included in Table 1-6 (evidence pertaining to other system effects in animals) as studies that provided useful quantitative information on the biochemical/metabolic effects of ammonia.

The references considered and cited in this document, including bibliographic information and abstracts, can be found on the Health and Environmental Research On-line (HERO) website (http://hero.epa.gov/ammonia).

1. HAZARD IDENTIFICATION

1.1. OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS

1.1.1. Chemical Properties

Ammonia (NH_3) is a colorless alkaline gas with a pungent odor. Ammonia is very soluble in water (NRC, 2008); in solution, it exists as ammonium hydroxide. Ammonium hydroxide is a weak base that is partially ionized in water according to the following equilibrium (ATSDR, 2004):

$$NH_3 + H_20 \rightleftarrows NH_4^+ + OH^-$$

Ammonium hydroxide ionizes with a dissociation constant of 1.77×10^{-5} at 25°C that increases slightly with increasing temperature (Read, 1982). A decrease in pH results in an increase in the concentration of ammonium ion (NH_4^+ or protonated form), a decrease in the concentration of the un-ionized form (NH_3), and an increase in solubility of ammonia in water. At pH 9.25, half of the ammonia will be ionized (NH_4^+) and half will be un-ionized (NH_3). At pH values of 8.25 and 7.25, 90% and 99%, respectively, of ammonia will be ionized (NH_4^+) (ATSDR, 2004). Thus, 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 1-1.

Table 1-1. Chemical and physical properties of ammonia

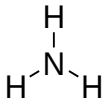
Parameter	Value	Reference
Chemical name	Ammonia[a]	
Synonym(s)	AM-Fol; anhydrous ammonia; ammonia gas; Nitro-sil; R 717; Spirit of hartshorn	NLM (2012)
Structure		NLM (2012)
Chemical formula	NH_3	NLM (2012)
CASRN	7664-41-7[a]	NLM (2012)
Molecular weight	17.031	Lide (2008), pp. 4.46-4.48, 8.40
Form	Colorless 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)

Table 1-1. Chemical and physical properties of ammonia

Parameter	Value	Reference
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	 Lange and 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)
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.

1.1.2. Toxicokinetics

Ammonia is absorbed by the inhalation route of exposure. Most inhaled ammonia is retained in the upper respiratory tract and is subsequently eliminated in expired air. Ammonia (as NH_4^+) is produced endogenously in the human intestines through the use of amino acids as an energy source (glutamine deamination) and by bacterial degradation of nitrogenous compounds from ingested food is largely absorbed. At physiological pH, 98.3% of ammonia is present in the blood as the ammonium ion (NH_4^+). Given its importance in amino acid metabolism, the urea cycle, and acid-base balance, ammonia is homeostatically regulated to remain at low concentrations in the blood. Ammonia is present in fetal, as well as adult, circulation, and is also present in human breast milk as one of the sources of nonprotein nitrogen. Ammonia is produced endogenously by catabolism of amino acids by glutamate dehydrogenase or glutaminase primarily in the liver, renal cortex and intestines, but also in the brain and heart. Ammonia is metabolized to glutamine via glutamine synthetase in the glutamine cycle or incorporated into urea as part of the urea cycle. The liver removes an amount of ammonia from circulation equal to the amount added by the intestines at metabolic steady state, such that the gut does not contribute significantly to systemic ammonia release under normal conditions. Renal elimination via the kidney is a major contributor to ammonia homeostasis; however, the kidneys are themselves a source of systemic ammonia. The principal means of excretion of ammonia is as urinary urea; lesser amounts are eliminated in the feces, through sweat production, and in expired air. A more detailed summary of ammonia toxicokinetics is provided in Appendix C, Section C.1.

1.2. SYNTHESIS OF EVIDENCE

Section 1.2 provides a synthesis and evaluation of the literature on the health effects of inhaled ammonia in humans and experimental animals organized by organ/system. Evidence for ammonia health effects is also summarized in organ/system-specific evidence tables, which present key study design information and results, and graphically in exposure-response arrays. More detailed study design information and results are provided in individual study summaries in Appendix C in the Supplemental Information.

1.2.1. Respiratory Effects

The respiratory system is the primary target of toxicity of inhaled ammonia in humans and experimental animals. Five cross-sectional occupational epidemiology studies in industrial settings (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Bhat and Ramaswamy, 1993; Holness et al., 1989) examined the association between inhaled ammonia and prevalence of respiratory symptoms or changes in lung function (Table 1-2). Another set of studies examined pulmonary function or asthma symptoms in relation to ammonia exposure in health care workers and domestic cleaners (Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005) (Table 1-3). The association between ammonia exposure and respiratory effects indicated by these studies is also informed by studies of pulmonary function in individuals in agricultural settings and subchronic inhalation toxicity studies in various experimental animal species (Table 1-4). The evidence of respiratory effects in humans and experimental animals exposed to ammonia is summarized in an exposure-response array in Figure 1-1 at the end of this section.

Respiratory Symptoms

Respiratory symptoms (including cough, wheezing, and other asthma-related symptoms) were reported in two cross-sectional studies of industrial worker populations exposed to ammonia at levels greater than or equal to approximately 18 mg/m^3 (Rahman et al., 2007; Ballal et al., 1998) (Table 1-2). One of these studies also examined frequency of respiratory symptoms by cumulative ammonia concentration (CAC, mg/m^3-years) and observed significantly higher relative risks (2.4–5.3) with higher CAC (>50 mg/m^3-years) compared to those with a lower CAC (≤50 mg/m^3-years) (Ballal et al., 1998). In three studies examining lower exposure settings (Rahman et al., 2007; Ballal et al., 1998; Holness et al., 1989) (Table 1-2), no differences were observed in the prevalence of respiratory symptoms between ammonia-exposed workers and controls. Ammonia concentrations reported in these lower exposure settings included a mean ammonia concentration of 6.5 mg/m^3 and a high-exposure group defined as >8.8 mg/m^3 in Holness et al. (1989), an exposure range of 0.2—7 mg/m^3 in "Factory B" of Ballal et al. (1998), and a mean concentration of 4.9 mg/m^3 in Rahman et al. (2007). The primary limitation noted in all of these studies was the potential under-ascertainment of effects inherent in the study of a long-term worker population (i.e., "healthy worker" effect) (see Literature Search Strategy | Study Selection and Evaluation section and Table B-6 in the Supplemental Information). Confounding by other workplace exposures, although a

potential concern, was unlikely to be a major limitation affecting the interpretation of the pattern of results seen in these studies, given the lack of nitrogen dioxide measurements above the detection limit in one study (Rahman et al., 2007) and the high level of control of exposures in another study (Holness et al., 1989).

Studies of health care workers or hospital workers (Arif and Delclos, 2012; Dumas et al., 2012) (Table 1-3) provide evidence that exposure to ammonia as a cleaning or disinfectant product is associated with increased risk of asthma or asthma symptoms. Use of ammonia as a cleaning product in other settings has also been associated with asthma and respiratory symptoms (Casas et al., 2013; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2005) (Table 1-3). Occupational exposure to ammonia was associated with work-exacerbated asthma (compared to non-work related asthma) in a study at two occupational asthma specialty clinics by Lemiere et al. (2012) (Table 1-3). Six studies, from Europe, Canada, and the United States, observed elevated odds ratios, generally between 1.5 and 2.0, with varying degrees of precision. These studies were conducted using a variety of designs, including a prospective study (Zock et al., 2007) and two nested case-control studies (Medina-Ramón et al., 2006; Medina-Ramón et al., 2005). Criteria used to define current asthma or asthma symptoms were generally well defined and based on validated methods. A major limitation of this collection of studies is the lack of direct measures of ammonia exposure. Two of the studies included expert assessment of exposure (blinded to case status); expert assessment improves reliance on self-reported exposure (Dumas et al., 2012; Lemiere et al., 2012). Confounding by other cleaning products is an unlikely explanation for these results, as two of the studies noted only weak correlations between ammonia and other product use (Zock et al., 2007; Medina-Ramón et al., 2005), and another study observed stronger associations with ammonia than with bleach (Dumas et al., 2012). All of the studies addressed smoking as a potential confounder.

Studies in populations exposed in agricultural settings, including swine and dairy farmers, that analyzed for prevalence of respiratory symptoms (including cough, phlegm, wheezing, chest tightness, and eye, nasal, and throat irritation) in relation to ammonia exposure provided generally negative results (Loftus et al., 2015; Melbostad and Eduard, 2001; Preller et al., 1995; Zejda et al., 1994) (Appendix C, Table C-7). Two other studies that measured ammonia, but did not present an analysis in relation to variability in ammonia levels, reported an increased prevalence of respiratory symptoms in pig farmers exposed to ammonia from animal waste (Choudat et al., 1994; Crook et al., 1991) (Appendix C, Table C-8). With the exception of the Loftus et al. (2015) study, all studies involving exposure in agricultural settings documented exposures to compounds in addition to ammonia, such as airborne dust, endotoxin, mold, and disinfectants: Loftus et al. (2015) did not analyze for other contaminants.

Reports of irritation and hyperventilation in volunteers acutely exposed to ammonia at concentrations ranging from 11 to 354 mg/m^3 ammonia for durations up to 4 hours under controlled exposure conditions (Petrova et al., 2008; Smeets et al., 2007; Ihrig et al., 2006; Verberk, 1977; Silverman et al., 1949) provide support for ammonia as a respiratory irritant (Appendix C, Section C.2.3 and Table C-9). Two controlled-exposure studies provide some evidence of

habituation to eye, nose, and throat irritation in volunteers after repeated ammonia exposure. Following exposure to ammonia at concentrations ranging from 7 to 35 mg/m^3 for 4 hours/day on five consecutive days, Ihrig et al. (2006) reported higher mean intensities for irritative, olfactory, and respiratory symptoms in male volunteers unfamiliar with ammonia when compared to male chemical company workers exposed to ammonia vapor for several years in a urea department; differences were statistically significant only for olfactory symptoms; however the sample size was small. In a more limited study with only four male volunteers each exposed to 18, 35, or 71 mg/m^3 ammonia (exposure to each concentration was for one week, 2–6 hour/day, 5 days/week), fewer occurrences of irritation occurred upon the second weekly exposure to the same concentration Ferguson et al. (1977).

Numerous case reports document the acute respiratory effects of inhaled ammonia, ranging from mild symptoms (including nasal and throat irritation and perceived tightness in the throat) to moderate effects (including pharyngitis, tachycardia, dyspnea, rapid and shallow breathing, cyanosis, transient bronchospasm, and rhonchi in the lungs) to severe effects (including burns of the nasal passages, soft palate, posterior pharyngeal wall, and larynx, upper airway obstruction, bronchospasm, persistent, productive cough, bilateral diffuse rales and rhonchi, mucous production, pulmonary edema, marked hypoxemia, and necrosis of the lung) (Appendix C, Section C.2.3).

Experimental studies in laboratory animals also provide consistent evidence that repeated exposure to ammonia can affect the respiratory system (Table 1-4 and Appendix C, Section C.3). The majority of available animal studies did not look at measures of respiratory irritation, in contrast to the majority of human studies, but rather examined histopathological changes of respiratory tract tissues. Histopathological changes in the nasal passages were observed in Sherman rats after 75 days of exposure to 106 mg/m^3 ammonia and in F344 rats after 35 days of exposure to 177 mg/m^3 ammonia, with respiratory and nasal epithelium thicknesses increased 3–4 times that of normal (Broderson et al., 1976). Thickening of nasal and tracheal epithelium (50–100%) was also observed in pigs exposed to 71 mg/m^3 ammonia continuously for 1–6 weeks (Doig and Willoughby, 1971). Nonspecific inflammatory changes (not further described) were reported in the lungs of Sprague-Dawley and Long-Evans rats and guinea pigs intermittently exposed to 770 mg/m^3 ammonia for 6 weeks; continuous exposure to 455 and 470 mg/m^3 ammonia increased mortality in rats (Coon et al., 1970). Focal or diffuse interstitial pneumonitis was observed in all Princeton-derived guinea pigs, New Zealand white rabbits, beagle dogs, and squirrel monkeys exposed to 470 mg/m^3 ammonia (Coon et al., 1970). Additionally, under these exposure conditions, dogs exhibited nasal discharge and other signs of irritation (marked eye irritation, heavy lacrimation). Nasal discharge was observed in 25% of rats exposed to 262 mg/m^3 ammonia for 90 days (Coon et al., 1970).

At lower concentrations, approximately 50 mg/m^3 and below, the majority of studies of inhaled ammonia did not identify respiratory effects in laboratory animals exposed to ammonia. No increase in the incidence of respiratory or other diseases common to young pigs was observed after continuous exposure to ammonia and inhalable dust at concentrations representative of those

found in commercial pig farms (≤26 mg/m^3 ammonia) for 5 weeks (Done et al., 2005). No gross or histopathological changes in the turbinates, trachea, and lungs of pigs were observed after continuous exposure to 35 or 53 mg/m^3 ammonia for up to 109 days (Curtis et al., 1975). No signs of toxicity in rats or dogs were observed after continuous exposure to 40 mg/m^3 ammonia for 114 days or after intermittent exposure (8 hours/day) to 155 mg/m^3 ammonia for 6 weeks (Coon et al., 1970). Only one study reported respiratory effects at concentrations <50 mg/m^3 (i.e., lung congestion, edema, and hemorrhage in guinea pigs and mice exposed to 14 mg/m^3 ammonia for up to 42 days; Anderson et al. (1964a)), but confidence in the findings from this study is limited by inadequate reporting and the small numbers of animals tested.

Lung Function

Decreased lung function in ammonia-exposed workers has been reported in three of the four studies examining this outcome measure (Rahman et al., 2007; Ali et al., 2001; Bhat and Ramaswamy, 1993); the exception is the study by Holness et al. (1989) (Table 1-2) in which no significant changes in lung function were observed in workers exposed to ammonia in an industrial setting with relatively low ammonia exposure levels (Table 1-2). These effects were observed in short-term scenarios (i.e., cross-work shift changes in lung function) in fertilizer factory workers (mean ammonia concentration of 18.5 mg/m^3) compared with administrative staff controls (Rahman et al., 2007), and in longer-term scenarios, in workers with a cumulative exposure of >50 mg/m^3-years when compared with workers with a lower cumulative exposure of ≤50 mg/m^3-years (with an approximate 5–7% decrease in FVC% predicted and FEV_1% predicted) (Ali et al., 2001). There were no decrements in the percent of predicted lung function values when comparing the total exposed group to a control group of office workers in the latter study, in the relatively low exposure scenario examined in Holness et al. (1989) (mean ammonia concentration of 6.5 mg/m^3 and high-exposure group defined as >8.8 mg/m^3), or in the low-exposure group (mean ammonia concentration of 4.9 mg/m^3) in Rahman et al. (2007). Another study of ammonia plant fertilizer workers reported statistically significant decreases in forced expiratory volume (FEV_1) and peak expiratory flow rate (PEFR/minute) in workers compared to controls (Bhat and Ramaswamy, 1993); however, measurements of ammonia levels were not included in this study. As discussed previously in the summary of respiratory symptoms studies, the primary limitation within this set of studies is the potential under-ascertainment of effects in these studies of long-term worker populations.

One of the studies of domestic cleaning workers described in Table 1-3 included a measure of pulmonary function (Medina-Ramón et al., 2006). Ammonia use was associated with a decrease in peak expiratory flow (PEF) (-9.4 [95% CI, -17, -2.3]). A limitation of this study was the use of lung function measurements conducted by the participant; the reliability of this procedure has not been established. In a study by Casas et al. (2013) on the effects of cleaning product use on the respiratory health of children, ammonia exposure was associated with decreased lung function (FEV_1: -28 [95% CI -131, 76]) (Table 1-3).

Impaired respiratory function (e.g., decreased FEV_1 and/or forced vital capacity [FVC]) in an agricultural setting was associated with ammonia exposure in six of the eight studies that included pulmonary function measures (Loftus et al., 2015; Monsó et al., 2004; Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Zejda et al., 1994; Heederik et al., 1990) (Appendix C, Table C-7). In general, EPA considered these eight studies to be the strongest with respect to methodology, based on considerations of exposure assessment and assessment of potential confounding (see Literature Search Strategy | Study Selection and Evaluation section).

Changes in lung function following acute exposure to ammonia have been observed in some, but not all, controlled human exposure studies conducted in volunteers (Appendix C, Section C.2.3 and Table C-9). Cole et al. (1977) reported reduced lung function as measured by reduced expiratory minute volume and changes in exercise tidal volume in volunteers exposed for a half-day in a chamber at ammonia concentrations ≥106 mg/m^3, but not at 71 mg/m^3. Bronchoconstriction was reported in volunteers exposed to ammonia through a mouthpiece for 10 inhaled breaths of ammonia gas at a concentration of 60 mg/m^3 (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in volunteers exposed to ammonia in an exposure chamber at concentrations of up to 35 mg/m^3 for 10 minutes (MacEwen et al., 1970). Similarly, no changes in bronchial responsiveness or lung function (as measured by FVC and FEV_1) were reported in healthy volunteers exposed to ammonia at concentrations up to 18 mg/m^3 for 1.5 hours during exercise (Sundblad et al., 2004). There were no changes in lung function as measured by FEV_1 in 25 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers exposed to ammonia concentrations up to 354 mg/m^3 ammonia for up to 2.5 hours (Petrova et al., 2008), or in 6 healthy volunteers and 8 mildly asthmatic volunteers exposed to 11–18 mg/m^3 ammonia for 30-minute sessions (Sigurdarson et al., 2004).

Lung function effects following ammonia exposure were not evaluated in the available animal studies.

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Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings

Study design and reference	Results
Respiratory symptoms	
Rahman et al. (2007) (Bangladesh) Urea fertilizer factory worker (all men); 24 ammonia plant workers, 64 urea plant workers, and 25 controls (staff from administration building). Mean employment duration: 16 years **Exposure:** Personal samples (2 methods[a]; correlation = 0.80) Low-exposure group (ammonia plant), mean: 6.9 ppm (4.9 mg/m^3); range: 2.8–11.1 ppm (2–8 mg/m^3) High-exposure group (urea plant), mean: 26.1 ppm (18.5 mg/m^3); range: 13.4–43.5 ppm (9–31 mg/m^3) **Outcome**: Respiratory symptoms (5 point scale for severity over last shift), based on Optimal Symptom Score Questionnaire	Percentage of workers reporting symptoms (*p*-value): Controls (n = 25); Low exposed (n = 24) (*p*-value)[1]; High exposed (n = 64) (*p*-value)[2]; (*p*-value)[3] Cough: 8; 17 (0.42); 28 (0.05); (0.41) Chest tightness: 8; 17 (0.42); 33 (0.02); (0.19) Stuffy nose: 4; 12 (0.35); 16 (0.17); (1.0) Runny nose: 4; 4 (1.0); 16 (0.17); (0.28) Sneeze: 8; 0 (0.49); 22 (0.22); (0.01) [1]*p*-value for ammonia plant compared to control [2]*p*-value for urea plant compared to control [3]*p*-value for urea plant compared to ammonia plant
Ballal et al. (1998) (Saudi Arabia) Urea fertilizer factory workers (two factories) (all men); 161 exposed workers and 355 unexposed controls[b]. Mean employment duration: 51.8 months (exposed workers) and 73.1 months (controls) **Exposure:** Area monitors (3 sets in each work section taken at least 3 months apart, mean 16 measures per set). Factory A (high-exposure factory): 2–130[1] mg/m^3 (mid-point = 66 mg/m^3); geometric mean <18 mg/m^3, except for urea packaging and store areas (geometric means = 18.6 and 115 mg/m^3, respectively) Factory B (low-exposure factory): 0.02–7 mg/m^3; geometric mean <18 mg/m^3 Cumulative exposure calculated based on exposure and duration; dichotomized to high and low at 50 mg/m^3-years **Outcome**: Respiratory symptoms based on British Medical Research Council questionnaire [1]The ammonia concentration range in Factory A is better represented as 2–27.1 mg/m^3. This range excludes the employees in the urea store (n = 6; range of ammonia concentrations = 90–130.4 mg/m^3) who were required to wear full protective clothing, thus minimizing potential exposure. Number of workers in Factory A excluding urea store workers = 78.	Relative risk (95% CI), compared with controls Factory B[2] (0.02–7 mg/m^3; n = 77); Factory A[2] (2–27.1 mg/m^3; n = 78)[1] Cough: No cases; 2.0 (0.38, 10.4) Phlegm: No cases; 2.0 (0.38, 10.4) Wheezing: 0.97 (0.21, 4.5); 3.4 (1.2, 9.5) Dyspnea: 0.45 (0.11, 1.9); 1.8 (0.81, 4.2) Relative risk (95% CI), compared with lower exposure setting (≤18 mg/m^3 [n = 138] or ≤50 mg/m^3-years [n = 130]) >18 mg/m^3 (n = 17); Cumulative >50 mg/m^3-years (n = 30) Cough: 3.5 (1.8, 6.6); 2.8 (1.6, 5.0) Phlegm: 3.8 (2.0. 7.1); 3.0 (1.7, 5.5) Wheezing: 5.0 (2.4, 10.6); 5.2 (2.9, 9.5) Dyspnea: 4.6 (2.4, 8.8); 2.6 (1.3, 5.4) Asthma: 4.3 (2.1, 9.0); 2.4 (1.1, 5.4) Chronic bronchitis: 2.3 (0.31, 17); 5.3 (1.7, 16) [2]Factory-specific analyses stratified by smoking status; results presented here are for non-smokers. Similar patterns seen in other smoking categories. Approximate 1.3–1.5 relative risk ($p < 0.05$) per unit increase in ammonia concentration for cough, phlegm, wheezing, and asthma, adjusting for duration of work, cumulative exposure, smoking, and age.

Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings

Study design and reference	Results
Holness et al. (1989) (Canada) Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant)[c]. Average exposure: 12.2 years **Exposure:** Personal samples, one work-shift per person, mean 8.4 hours Low: <6.25 ppm (<4.4 mg/m^3); n = 34 Medium: 6.25–12.5 ppm (4.4–8.8 mg/m^3); n = 12 High: >12.5 ppm (>8.8 mg/m^3); n = 12 All exposed workers (mean): 6.5 mg/m^3 **Outcome**: Respiratory symptoms based on American Thoracic Society questionnaire	Percentage of workers reporting symptoms (%): Cough: Control (n = 31) 10; Exposed (n = 58) 16; *p*-value 0.53 Sputum: 16; 22; 0.98 Bronchitis: 19; 22; 0.69 Wheeze: 10; 10; 0.91 Chest tightness: 6; 3; 0.62 Dyspnea (shortness of breath): 13; 7; 0.05 Chest pain: 6; 2; 0.16 Rhinitis (nasal complaints): 19; 10; 0.12 Throat irritation: 3; 7; 0.53 No increased risk seen in analyses stratified by exposure group.
Lung function	
Rahman et al. (2007) (Bangladesh) Urea fertilizer factory workers (all men); 24 ammonia plant workers, 64 urea plant workers, and 25 controls (staff from administration building). Mean employment duration: 16 years **Exposure:** Personal samples (2 methods[a]; correlation = 0.80) Low-exposure group (ammonia plant), mean: 6.9 ppm (4.9 mg/m^3); range: 2.8–11.1 ppm (2–8 mg/m^3) High-exposure group (urea plant), mean: 26.1 ppm (18.5 mg/m^3); range: 13.4–43.5 ppm (9–31 mg/m^3) **Outcome**: Lung function (standard spirometry)	Pre-shift / Post-shift / *p*-value Ammonia plant (low-exposure group, 4.9 mg/m^3); n = 24 ammonia plant workers FVC: 3.308; 3.332; 0.67 FEV_1: 2.627; 2.705; 0.24 PEFR: 8.081; 8.313; 0.22 Urea plant (high-exposure group, 18.5 mg/m^3); n = 64 urea plant workers FVC: 3.362; 3.258; 0.01 FEV_1: 2.701; 2.646; 0.05 PEFR: 7.805; 7.810; 0.97 *p*-value reflects the comparison of pre- and post-shift values. Multiple regression model (data from 23 ammonia and urea plant workers with concurrent measurements of ammonia exposure and lung function): -- Concentration of ammonia and exposure duration (yrs of employment as proxy for duration) were significantly correlated with percentage cross-shirt decrease in FEV_1% (ΔFEV_1%). -- Each year of work in a production section was associated with a decrease in ΔFEV_1% of 0.6%. [Limitation of analysis: failure to explore the age parameter; age and years of work were highly correlated (Pearson correlation coefficient 0.97).

Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings

<table>
<tr><th>Study design and reference</th><th>Results</th></tr>
<tr><td>Ali et al. (2001) (Saudi Arabia)
Urea fertilizer factory workers (all men)—(additional study of “Factory A” in Ballal et al. (1998)); 73 exposed workers and 348 unexposed controls.
Mean employment duration: not reported
Exposure: 4-hour measurements. Cumulative exposure calculated based on exposure and duration; dichotomized to high and low at 50 mg/m^3-years
Outcome: Lung function (standard spirometry; morning measurement)</td><td>
<table>
<tr><th></th><th>≤50 mg/m^3-y (n = 45)</th><th>>50 mg/m^3-y (n = 28)</th><th>p-value</th></tr>
<tr><td>FVC$_1$% predicted</td><td>100.7</td><td>93.4</td><td>0.006</td></tr>
<tr><td>FVC% predicted</td><td>105.6</td><td>100.2</td><td>0.03</td></tr>
<tr><td>FEV$_1$/FVC%</td><td>84.7</td><td>83.4</td><td>NS</td></tr>
</table>
NS = not significant (p-values not provided by study authors)
</td></tr>
<tr><td>Bhat and Ramaswamy (1993) (India)
Fertilizer chemical plant workers; 30 diammonium phospate (DAP) plant workers, 30 urea plant workers, 31 ammonia plant workers, and 68 controls (people with comparable body surface area chosen from the same socio-economic status and sex as exposed workers)
Exposure: Measurements not reported; duration dichotomized as ≤10 and >10 years
Outcome: Lung function (standard spirometry)</td><td>
<table>
<tr><th></th><th>Controls (n = 68)</th><th>DAP plant (n = 30)</th><th>Urea plant (n = 30)</th><th>Ammonia plant (n = 31)</th></tr>
<tr><td>FVC</td><td>3.4 ± 0.21</td><td>2.5 ± 0.06*</td><td>3.3 ± 0.11</td><td>3.2 ± 0.07</td></tr>
<tr><td>FEV$_1$</td><td>2.8 ± 0.10</td><td>2.1 ± 0.08*</td><td>2.7 ± 0.10</td><td>2.5 ± 0.1*</td></tr>
<tr><td>PEFR</td><td>383 ± 7.6</td><td>228 ± 18*</td><td>307 ± 19*</td><td>314 ± 20*</td></tr>
</table>
*p < 0.05
</td></tr>
<tr><td>Holness et al. (1989) (Canada)
Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant)[c]. Average exposure: 12.2 years
Exposure: Personal samples, one work-shift per person, mean 8.4 hours
Low: <6.25 ppm (<4.4 mg/m^3); n = 34
Medium: 6.25–12.5 ppm (4.4–8.8 mg/m^3); n = 12
High: >12.5 ppm (>8.8 mg/m^3); n = 12
All exposed workers (mean): 6.5 mg/m^3
Outcome: Lung function (standard spirometry; beginning and end of shift, at least two test days per worker)</td><td>
<table>
<tr><th></th><th>Control (n = 31)</th><th>Exposed (n = 58)</th><th>p-value[a]</th></tr>
<tr><td colspan="4">Lung function (% predicted values)[b]:</td></tr>
<tr><td>FVC</td><td>98.6 ± 11.3</td><td>96.8 ± 11.0</td><td>0.094</td></tr>
<tr><td>FEV$_1$</td><td>95.1 ± 12.5</td><td>94.1 ± 12.9</td><td>0.35</td></tr>
<tr><td>FEV$_1$/FVC</td><td>96.5 ± 6.1</td><td>97.1 ± 7.1</td><td>0.48</td></tr>
<tr><td colspan="4">Change in lung function over work shift:</td></tr>
<tr><td>FVC day1</td><td>-0.9</td><td>-0.8</td><td>0.99</td></tr>
<tr><td>day 2</td><td>+0.1</td><td>-0.0</td><td>0.84</td></tr>
<tr><td>FEV$_1$ day 1</td><td>-0.2</td><td>-0.2</td><td>0.94</td></tr>
<tr><td>day 2</td><td>+0.5</td><td>+0.7</td><td>0.86</td></tr>
</table>
[a]p-value for difference between exposed and control workers calculated by using actual baseline values and correcting for age, height, and pack-years smoked determined by multiple regression analysis.
[b]Percentage of the subject's predicted value (% predicted) has been widely adopted as follows: % predicted = recorded value x 100/predicted value); this value is now calculated on automated spirometers based on sex, race, age and height.
</td></tr>
</table>

Table 1-2. Evidence pertaining to respiratory effects in humans following inhalation exposure in industrial settings

Study design and reference	Results

FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEFR = peak expiratory flow rate.

[a]Exposure concentrations were determined by both the Dräger tube and Dräger PAC III methods. Using the Dräger tube method, concentrations of ammonia in the ammonia and urea plants were 17.7 and 88.1 mg/m^3, respectively; using the Dräger PAC III method, ammonia concentrations were 4.9 and 18.5 mg/m^3, respectively (Rahman et al. (2007). 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 communication with technical support at Dräger Safety Inc (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 NCEA, ORD, U.S. EPA), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, higher confidence is attributed to the PAC III air measurements of ammonia for the Rahman et al. (2007) study.

[b]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.

[c]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.

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Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study design and reference	Results
Asthma or asthma symptoms	
Dumas et al. (2012) (France) Hybrid design, hospital workers, drawn from population-based case-control study; 179 hospital workers (136 women), 545 other workers (333 women). **Exposure:** Asthma-specific job exposure matrix plus + expert review (blinded), ever exposed, 18 specific products, based on all jobs held at least 3 months; ammonia prevalence 23% in female hospital workers **Outcome**: Current asthma: Asthma attack, respiratory symptoms or asthma treatment in the last 12 months (based on standardized questionnaire)	Odds ratio (95% CI), current asthma Women: 3.05 (1.19, 7.82) Men: no associations with any specific products (prevalence low) Adjusted for age and smoking, and accounting for familial dependence (due to sampling of cases and first degree relatives)
Arif and Delclos (2012) (United States, Texas) Population survey of 3,650 health care workers (physicians, nurses, respiratory therapists, occupational therapists), (total n = 5,600, response rate 66%) **Exposure:** Structured questionnaire—frequency of use of products for longest job held; ever contact with list of 28 products; ammonia prevalence 23% **Outcome**: Structured questionnaire • Work-related asthma symptoms: wheezing/whistling at work or shortness of breath at works that gets better away from work or worse at work • Work-exacerbated asthma: onset before began work • Occupational asthma: onset after began work)	Odds ratio (95% CI) [n cases] Work-related asthma symptoms [n = 132] 2.45 (1.28, 4.69) Work-exacerbated asthma [n = 41] 1.58 (0.56, 4.43) Occupational asthma [n = 33] 1.86 (0.49, 7.13) Adjusted for age, sex, race/ethnicity, body mass index, seniority, atopy, and smoking status
Lemiere et al. (2012) (Quebec, Canada) Case-control study, workers seen at two tertiary care centers specializing in occupational asthma. Asthma (defined below) based on reversible airflow limitation or airway hyper-responsiveness tests; referent group = non-work related asthma (NWRA) seen at same clinics but symptoms did not worsen at work (n = 33). **Exposure:** Structured interview focusing on last/current job, combined with expert review (blinded); ammonia prevalence 19/153 = 12% **Outcome**: Diagnoses made based on reference tests • Occupational asthma if specific inhalation challenge test was positive • Work-exacerbated asthma if specific inhalation test was negative but symptoms worsened at work	Odds ratio (95% CI) [n cases] Work exacerbation [n = 53] 8.4 (1.1, 371.7) Occupational asthma [n = 67] 3.7 (0.4, 173.4) Age, smoking, occupational exposure to heat, cold, humidity, dryness, and physical strain assessed as confounders. [Wide confidence intervals reflect sparseness in referent group, with only 1 of the 33 classified as exposed to ammonia]

Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study design and reference	Results
Vizcaya et al. (2011) (Spain) Survey of cleaning service workers (n = 917) from 37 businesses (19% response rate to questionnaire distributed through the employers); 761 current cleaners, 86 former cleaners, 70 never cleaners; referent group = never cleaners and current cleaners who have not used any of the specified cleaning products in last year (n = 161) **Exposure:** Structured questionnaire, use of cleaning tasks and 12 products; ammonia prevalence 66% **Outcome:** Structured questionnaire • Current asthma: in past 12 months, woken by an attack of shortness of breath, had an attack of asthma or currently taking any asthma medications (including inhalers, aerosols or tablets) • Asthma score: Sum of "yes" answers to 5 symptoms in last 12 months (wheeze with breathlessness, woken up with chest tightness, attack of shortness of breath at rest, attack of shortness of breath after exercise, woken by attack of shortness of breath)	Odds ratio (95% CI) (among current cleaners) [n] Current asthma 1.4 (0.6, 3.2) [81] Wheeze without having a cold 2.1 (0.9, 4.7) [83] Chronic cough 1.6 (0.8, 3.3) [95] Asthma score 1.6 (1.0, 2.5) [mean 0.59, SD 1.12] Adjusted for age, country of birth (Spanish versus non-Spanish), sex, and smoking status
Zock et al. (2007) (Europe, 22 sites) Longitudinal study, n = 3,503, 9-year follow-up of European Community Respiratory Health Survey, population-based sample, ages 20-44 years. Excluded 764 individuals with asthma at baseline; limited to individuals reporting doing the cleaning or washing in their home. **Exposure:** Structured interview at follow-up; frequency of use of 15 products **Outcome:** Structured interview at follow-up • New onset (since baseline survey) current asthma, defined by asthma attack or nocturnal shortness of breath in the past 12 months or current use of medication for asthma • Current wheeze defined as wheezing or whistling in the chest in last 12 months when not having a cold • New onset physician-diagnosed asthma, asthma defined as above with confirmation by a physician and information on age or date of first attack	Odds ratio (95% CI) [n] Current asthma 1.4 (0.87, 2.23) [199] Current wheeze 1.3 (0.81, 2.13) [226] Physician-diagnosed asthma 0.92 (0.33, 2.59) [71] Adjusted for sex, age, smoking, employment in a cleaning job during follow-up, and study center; heterogeneity by center also assessed. Correlations among products generally weak (Spearman rho < 0.3)

Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study design and reference	Results
Medina-Ramón et al. (2005) (Spain) Nested case-control, cleaning workers; case (n = 40; 74% participation rate) based on asthma and/or bronchitis at both assessments. Controls (n = 155, 69% participation rate)—no history of respiratory symptoms in preceding year and no asthma at either assessment. **Exposure:** Structured interview; frequency of use of 22 products; ammonia prevalence 16% undiluted, 56% diluted **Outcome:** Asthma: asthma attack or being woken by attack or shortness of breath in past 12 months; Chronic bronchitis: regular cough or regular bringing up phlegm for at least 3 months each year	Odds ratio (95% CI) (unadjusted), ≥12 compared with <12 times per year Undiluted 3.1 (1.2, 8.0) Diluted 0.8 (0.4, 1.7)
FeNO and pulmonary function	
Casas et al. (2013) (Spain) Population based cross sectional birth cohort study; n = 432 infants enrolled; n = 295 total number of individuals recruited that completed the 10-year follow up visit and the cleaning products questionnaire and performed the FeNO and/or lung function test; 35% of recruited population were excluded because information on use of cleaning products and/or respiratory tests was not available; only 46 individuals reported use of ammonia **Exposure:** Interviewer-led questionnaire; frequency of use of 10 different cleaning products (bleach, ammonia, polishes or waxes, acids, solvents, furniture sprays, glass cleaning sprays, degreasing sprays, air freshening sprays, and air freshening plug- ins); exposure score developed based on frequency of use and number of products used **Outcome:** Questionnaires on wheezing asthma, treatment and allergies were administered by mother from birth to age 10; at age 10–13 FeNO and lung function tests were carried out	Adjusted[a] associations of FeNO, FVC and FEV_1[b] with weekly use of ammonia (n=46; 16%) FeNO[c]ppb: GM ratio (95% CI) 0.86 (0.66 to 1.12) FVC mL: β (95% CI) 3 (-127 to 133) FEV_1 mL: β (95% CI) -28 (-131 to 76) GM: geometric mean [a] adjusted for sex, age, asthma medication, season of respiratory measurement, maternal education and parental smoking; FVC and FEV_1 models were additionally adjusted for height and weight [b] change in FeNO, FVC and FEV_1 per interquartile range increase of the score (interquartile range = 6.5 d of product use per week). [c] FeNO (fraction of exhaled nitric oxide) is used to characterize asthma or other conditions associated with airway inflammation; it is measured in a breath test.
Medina-Ramón et al. (2006) (Spain) Panel study, sample selected from participants in nested case-control study by Medina-Ramón et al. (2005). Current asthma symptoms or chronic bronchitis in 2000–2001 survey; n = 51 of 80 (64%); 8 excluded for possible recording errors, outliers, learning effects **Exposure:** Daily diary of use of products **Outcome:** Respiratory symptoms based on 2-week daily diary (7 symptoms, 5 point intensity scale); summed score for upper respiratory symptoms (blocked nose, throat irritation, watery eyes) and lower respiratory symptoms (chest tightness, wheezing, shortness of breath, and cough); PEF measured with mini-Wright peak flow meter (with training and written	Diluted and undiluted / Diluted only OR (95% CI) Upper respiratory symptoms: 1.8 (0.7, 4.9) / 1.3 (0.3, 5.0) Lower respiratory symptoms: 1.6 (0.6, 4.4) / 3.0 (1.0, 9.1) Beta (95% CI) PEF at night: -9.4 (-17, -2.3) / -10.3 (-18, -2.7) PEF, following morning: -1.2 (-8.5, 6.2) / -2.9 (-11, 6.2)

Table 1-3. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study design and reference	Results
instructions); measured morning, lunchtime, night (3 measurements each; highest recorded)	Adjusted for respiratory infection, use of maintenance medication, and age; daily number of cigarettes smoked, years of employment in domestic cleaning, and/or weekly working hours in domestic cleaning also assessed as potential confounders

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Table 1-4. Evidence pertaining to respiratory effects in animals

Study design and reference	Results
Effects on the lungs	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*Saimiri sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	Gross necropsies were normal; focal pneumonitis in one of three monkeys at 155 mg/m^3. Nonspecific lung inflammation observed in guinea pigs and rats, but not in other species, at 770 mg/m^3.[a]
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	At 470 mg/m^3, focal or diffuse interstitial pneumonitis in all animals. Calcification of bronchial epithelium observed in several animals. Hemorrhagic lung lesion in one of two dogs; moderate lung congestion in two of three rabbits.[a] (This exposure was lethal to ~25% of the guinea pigs).
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262 or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 d	Focal or diffuse interstitial pneumonitis in all animals, and calcification of bronchial epithelium observed in several animals at 470 mg/m^3, an exposure that was lethal to most of the rats.[a]
Anderson et al. (1964a) Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d	Lung congestion, edema, and hemorrhage observed at 14 mg/m^3 after 42 d.[a]
Anderson et al. (1964a) Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m^3) for 42 d	Lung congestion, edema, and hemorrhage observed at 14 and 35 mg/m^3 after 42 d.[a]
Done et al. (2005) Pig (several breeds); sex not specified; 24/group 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 wks (Exposure to ammonia and inhalable dust at concentrations commonly found at pig farms)	No increase in the incidence of respiratory or other diseases.
Curtis et al. (1975) Pig (crossbred); sex not specified; 4–8/group 0, 50, or 75 ppm (0, 35, or 53 mg/m^3 for 109 d)	Turbinates, trachea, and lungs of all pigs were classified as normal.
Effects on the upper respiratory tract	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	Dyspnea in rabbits and dogs exposed to 770 mg/m^3 during wk 1 only; no indication of irritation after wk 1; nasal tissues not examined for gross or histopathologic changes.
Broderson et al. (1976)[b] Sherman rat; 5/sex/group 10 or 150 ppm (7 or 106 mg/m^3) from bedding for 75 d	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 106 mg/m^3.[a]

Table 1-4. Evidence pertaining to respiratory effects in animals

Study design and reference	Results
Broderson et al. (1976)[b] F344 rat; 6/sex/group 0 or 250 ppm (0 or 177 mg/m^3) in an inhalation chamber for 35 d	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 177 mg/m^3.[a]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262, or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 d	Nasal discharge at 262 mg/m^3 (25% of rats). Dyspnea and nasal irritation/discharge in all animals at 455 and 470 mg/m^3, an exposure that was lethal to the majority of the rats.[a]
Gaafar et al. (1992) White albino mouse; male; 50 Ammonia vapor of 0 or 12% ammonia solution for 15 min/d, 6 d/wk, for 8 wks	Histological changes in the nasal mucosa.[a]
Doig and Willoughby (1971) Yorkshire-Landrace pig; sex not specified; 6/group 0 or 100 ppm (0 or 71 mg/m^3) for 6 wks	↑ thickness of nasal and tracheal epithelium (50–100% increase).[a]
Stombaugh et al. (1969) Duroc pig; both sexes; 9/group 12, 61, 103, 145 ppm (8, 43, 73, or 103 mg/m^3) for 5 wks	Excessive nasal, lacrimal, and mouth secretions and ↑ frequency of cough at 73 and 103 mg/m^3.[a]
Coon et al. (1970) Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Nasal discharge at 470 mg/m^3.[a]

[a]Incidence data not provided.
[b]The Broderson et al. (1976) paper includes a number of experiments in rats designed to examine whether ammonia at concentrations commonly encountered in laboratory cage environments plays a role in the pathogenesis of murine respiratory mycoplasmosis caused by the bacterium *Mycoplasma pulmonis*. The experiments conducted without co-exposure to *M. pulmonis* are summarized in this table; the results of experiments involving co-exposure to *M. pulmonis* are discussed in Section 1.1.4, Immune System Effects.

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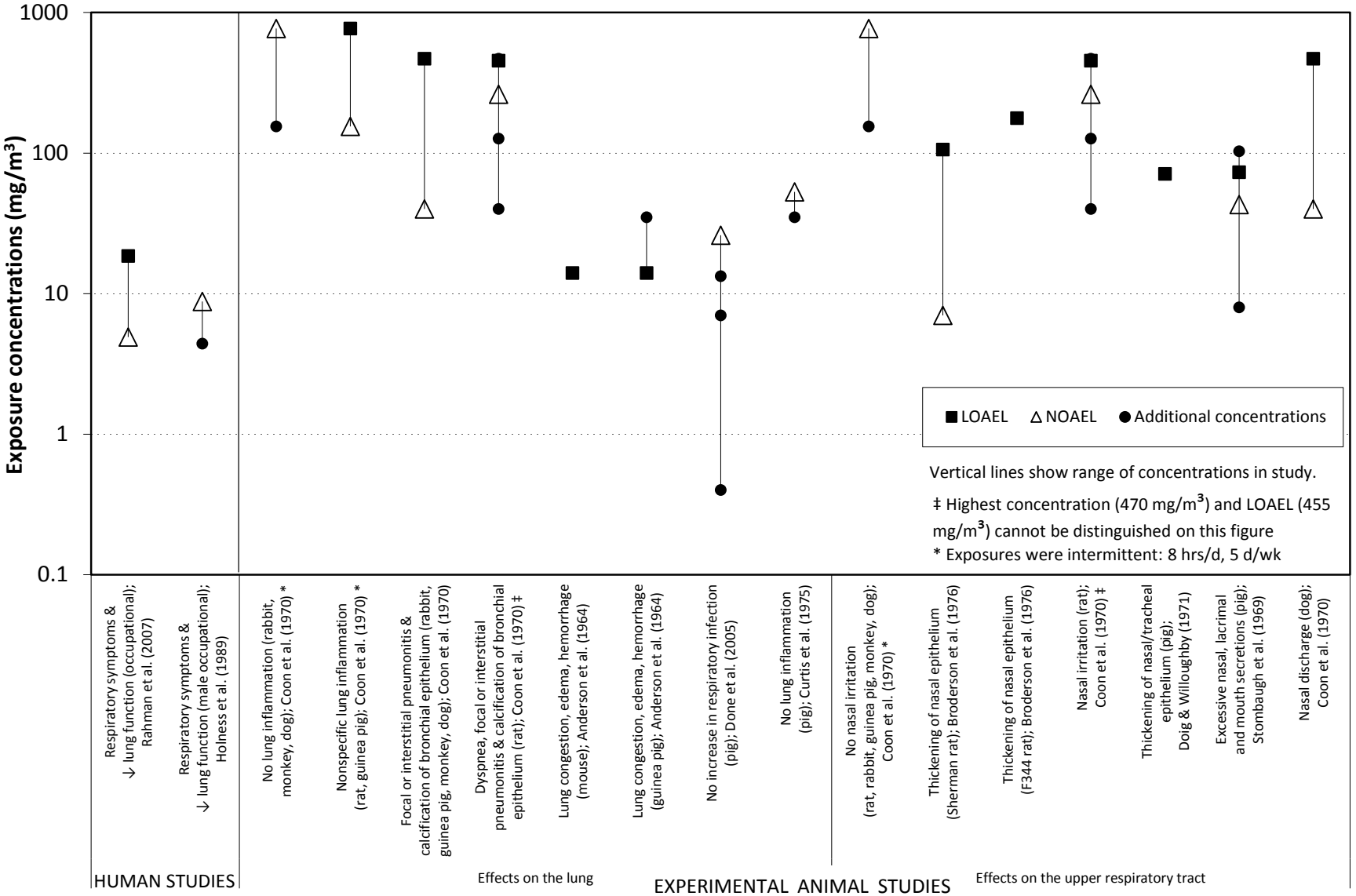

Figure 1-1. Exposure-response array of respiratory effects following inhalation exposure to ammonia.

Mode-of-Action Analysis—Respiratory Effects

Data on the potential mode of action for respiratory effects associated with chronic exposure to ammonia are limited. However, acute exposure data demonstrate that injury to respiratory tissues is primarily due to ammonia's alkaline (i.e., caustic) properties from the formation of hydroxide ion when it comes in contact with water and is solubilized. Ammonia readily dissolves in the moisture on the mucous membranes, forming ammonium hydroxide, which causes liquefactive necrosis of the tissues. Specifically, ammonia directly denatures tissue proteins and causes saponification of cell membrane lipids, which leads to cell disruption and death (necrosis). In addition, the cellular breakdown of proteins results in an inflammatory response, which further damages the surrounding tissues (Amshel et al., 2000; Millea et al., 1989; Jarudi and Golden, 1973).

Summary of Respiratory Effects

Evidence for respiratory toxicity associated with exposure to ammonia comes from studies in humans and animals. Multiple occupational studies involving chronic exposure to ammonia in industrial settings provide evidence of an increased prevalence of respiratory symptoms (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali et al., 2001; Bhat and Ramaswamy, 1993) (Table 1-2 and Appendix C, Section C.2.1). An increase in respiratory effects was reported both with higher workplace ammonia concentrations (Rahman et al., 2007; Ballal et al., 1998) and with greater cumulative ammonia concentration (expressed in mg/m^3-years) (Ali et al., 2001; Ballal et al., 1998). Evidence of respiratory effects is provided by studies of asthma, asthma symptoms, and pulmonary function in workers and others exposed to cleaning agents containing ammonia, in a variety of study designs and populations (Casas et al., 2013; Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005) (Table 1-3). Additional evidence of respiratory effects of ammonia is seen in studies of pulmonary function in an agricultural setting, specifically in livestock farmer studies that accounted for effects of co-exposures to other agents such as endotoxin and dust (Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Heederik et al., 1990), and in one study of asthmatic children that lived near animal feeding operations that did not control for co-exposures (Loftus et al., 2015) (Appendix C, Table C-7). The livestock farmer studies, however, do not provide evidence of associations between ammonia and respiratory symptoms. Controlled human exposure studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia provide additional support for the respiratory system as a target of ammonia toxicity when inhaled (Appendix C, Section C.2.3). Overall, the consistency of findings across three categories of epidemiological studies (industrial, cleaner, and agricultural settings) that differed in population characteristics, level and pattern of exposure, and potential confounders, and support from studies of acute exposures, adds strength to the evidence for an association between respiratory effects and ammonia exposure.

Evidence from animal studies supports an association between inhaled ammonia and respiratory effects. Short-term and subchronic animal studies show histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens (Gaafar et al., 1992; Broderson et al., 1976; Doig and Willoughby, 1971; Coon et al., 1970; Anderson et al., 1964a) (Table 1-4 and Appendix C, Section C.3). In general, responses in respiratory tissues increased with increasing ammonia exposure concentration. Based on evidence of respiratory effects in multiple human and animal studies (including epidemiological studies in different settings and populations), respiratory system effects are identified as a hazard associated with inhalation exposure to ammonia.

1.2.2. Immune System Effects

A limited number of studies have evaluated the immunotoxicity of ammonia in human populations and in experimental animal models. Immunological function was evaluated in two independent investigations of livestock farmers exposed to ammonia via inhalation. Immunoglobulin G- (IgG) and E-specific (IgE) antibodies for pig skin and urine (Crook et al., 1991), elevated neutrophils from nasal washes, and increased white blood cell counts (Cormier et al., 2000) were reported. These data on immunological function are suggestive of immunostimulatory effects; however, the test subjects were also exposed to a number of other respirable agents in addition to ammonia, such as endotoxin, bacteria, fungi, and mold that are known to stimulate immune responses. Data in humans following exposure to ammonia only are not available.

Animal studies that examined ammonia immunotoxicity were conducted using short-term inhalation exposures and were measured by three general types of immune assays: host resistance, T cell proliferation, and delayed-type hypersensitivity. Immunotoxicity studies of ammonia using measures of host resistance provide the most relevant data for assessing immune function since they directly measure the ability of the immune system to control microorganism growth. Other available studies of ammonia employed assays that evaluated immune function. Changes in immune cell populations without corresponding functional data are considered to be the least predictive, and studies that looked only at these endpoints (Gustin et al., 1994; Neumann et al., 1987) were considered less informative and not further considered in evaluating the immune system effects of ammonia.

Several host resistance studies utilized lung pathogens to assess bacterial clearance following ammonia exposure; however, these studies were not designed to discriminate between direct immunosuppression associated with ammonia exposure or immune effects secondary to damage to the protective mucosal epithelium of the respiratory tract. The available studies also do not correlate increased bacterial colonization with reduced immune function. Lung lesions, both gross and microscopic, were positively correlated with ammonia concentration in F344 rats continuously exposed to ammonia in an inhalation chamber for 7 days prior to inoculation with 10^8

colony forming units [CFU] of *Mycoplasma pulmonis* followed by up to 42 days of ammonia exposure post inoculation (Broderson et al., 1976). (Inoculation with the respiratory pathogen *M. pulmonis* causes murine respiratory mycoplasmosis [MRM] characterized by lung lesions.) The incidence of lung lesions was significantly increased at ammonia concentrations ≥35 mg/m^3, suggesting that ammonia exposure decreased bacterial clearance resulting in the development of *M. pulmonis*-induced MRM. However, increasing ammonia concentration was not associated with increased CFU of *M. pulmonis* isolated from the respiratory tract. The high number of inoculating CFU could have overwhelmed the innate immune response and elicited a maximal response that could not be further increased in immunocompromised animals.

Conversely, significantly increased CFU of *M. pulmonis* bacteria isolated in the trachea, nasal passages, lungs, and larynx were observed in F344 rats continuously exposed to 71 mg/m^3 ammonia for 7 days prior to *M. pulmonis* (10^4–10^6 CFU) inoculation and continued for 28 days post inoculation (Schoeb et al., 1982). This increase in bacterial colonization indicates a reduction in bacterial clearance following exposure to ammonia. Lesions were not assessed in this study.

OF1 mice exposed to 354 mg/m^3 ammonia for 7 days prior to inoculation with a 50% lethal dose (LD_{50}) of *Pasteurella multocida* exhibited significantly increased mortality compared to controls (86% versus 50%, respectively); however, an 8-hour exposure was insufficient to affect mortality (Richard et al., 1978). The authors suggested that the irritating action of ammonia destroyed the tracheobronchial mucosa and caused inflammatory lesions thereby increasing sensitivity to respiratory infection with prolonged ammonia exposure.

Pig studies support the findings observed in the rodent studies that ammonia exposure increases the colonization of respiratory pathogens. Andreasen et al. (2000a) demonstrated that 63 days of ammonia exposure increased the number of bacterial positive nasal swabs following inoculation with *P. multocida* and *Mycoplasma hyopneumoniae*; however, the effect was not dose responsive and did not result in an increase in lung lesions. Additional data obtained from pigs suggest that ammonia exposure eliminates the commensal flora of the nasal cavities, which allows for increased colonization of *P. multocida*; however, this effect abates following cessation of ammonia exposure (Hamilton et al., 1999; Hamilton et al., 1998).

Suppressed cell-mediated immunity and decreased T cell proliferation was observed following ammonia exposure. Using a delayed-type hypersensitivity test to evaluate cell-mediated immunity, Hartley guinea pigs were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to ammonia followed by intradermal challenge with a purified protein derivative (PPD). Dermal lesion size was reduced in animals exposed to 64 mg/m^3 ammonia, indicating immunosuppression (Targowski et al., 1984). Blood and bronchial lymphocytes harvested from naïve guinea pigs treated with the same 3-week ammonia exposure and stimulated with phytohaemagglutinin or concanavalin A demonstrated reduced T cell proliferation (Targowski et al., 1984). Bactericidal activity in alveolar macrophages isolated from ammonia-exposed guinea pigs was not affected. Lymphocytes and macrophages isolated from unexposed guinea pigs and treated with ammonia in vitro showed reduced proliferation and bactericidal capacity only at

concentrations that reduced viability, indicating nonspecific effects of ammonia-induced immunosuppression (Targowski et al., 1984). These data suggest that T cells may be the target of ammonia exposure since specific macrophage effects were not observed.

The evidence of immune system effects in experimental animals exposed to ammonia is summarized in Table 1-5 and as an exposure-response array in Figure 1-2.

Table 1-5. Evidence pertaining to immune system effects in animals

Study design and reference	Results
Host resistance	
Broderson et al. (1976) F344 rat; male and female; 11–12/sex/ group ≤5 (control), 25, 50, 100, or 250 ppm (≤3.5 [control], 18, 35, 71, or 177 mg/m^3), 7 d (continuous exposure) pre-inoculation/28–42 d post-inoculation with *M. pulmonis*	*% of animals with gross lung lesions:* 16, 46, 66*, 33, and 83% No effect on CFU.
Schoeb et al. (1982) F344 rat; 5-15/group (sex unknown) <2 or 100 ppm (<1.4 [control] or 71 mg/m^3), 7 d (continuous exposure) pre-inoculation/ 28 d post-inoculation with *M. pulmonis*	↑ bacterial colonization (as a result of reduced bacterial clearance).
Richard et al. (1978) OF1 mouse; male; 99/group 0 or 500 ppm (0 or 354 mg/m^3), 8 hrs or 7 d (continuous exposure), prior to infection with *P. multocida*	*% Mortality:* 50 and 86%*
Andreasen et al. (2000a) Landrace X large white pigs; 10/group (sex unknown) <5 (control), 50, or 100 ppm (3.5, 35, or 71 mg/m^3), 63 d (continuous exposure) inoculated with *M. hyopneumoniae* on day 9 and *P. multocida* on d 28, 42, and 56	*% of animals with positive day 49 nasal swab:* 24, 100*, and 90%*
Hamilton et al. (1998) Large white pigs; 4–7/group (sex unknown) 0 or 20 ppm (0 or 14 mg/m^3), 14 d (continuous exposure), inoculated with *P. multocida* on d 0	↑ bacterial colonization
Hamilton et al. (1999) Large white pigs; 5/group (sex unknown) 0 or 50 ppm (0 or 35 mg/m^3), 1 wk pre-inoculation with *P. multocida*, 3 wks post-inoculation	↑ bacterial colonization *Bacteria isolated from nasal cavities*: 3.18 and 4.30* CFU
T cell proliferation	
Targowski et al. (1984) Hartley guinea pig; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m^3), 3 wks (continuous exposure)	↓ proliferation in blood and bronchial T cells.

Table 1-5. Evidence pertaining to immune system effects in animals

Study design and reference	Results
Delayed-type hypersensitivity	
Targowski et al. (1984) Hartley guinea pig, BCG immunized; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m^3), 3 wks (continuous exposure) followed by PPD challenge	*Mean diameter of dermal lesion (mm):* 12, 12.6, and 8.7*

*Statistically significantly different from the control ($p < 0.05$).

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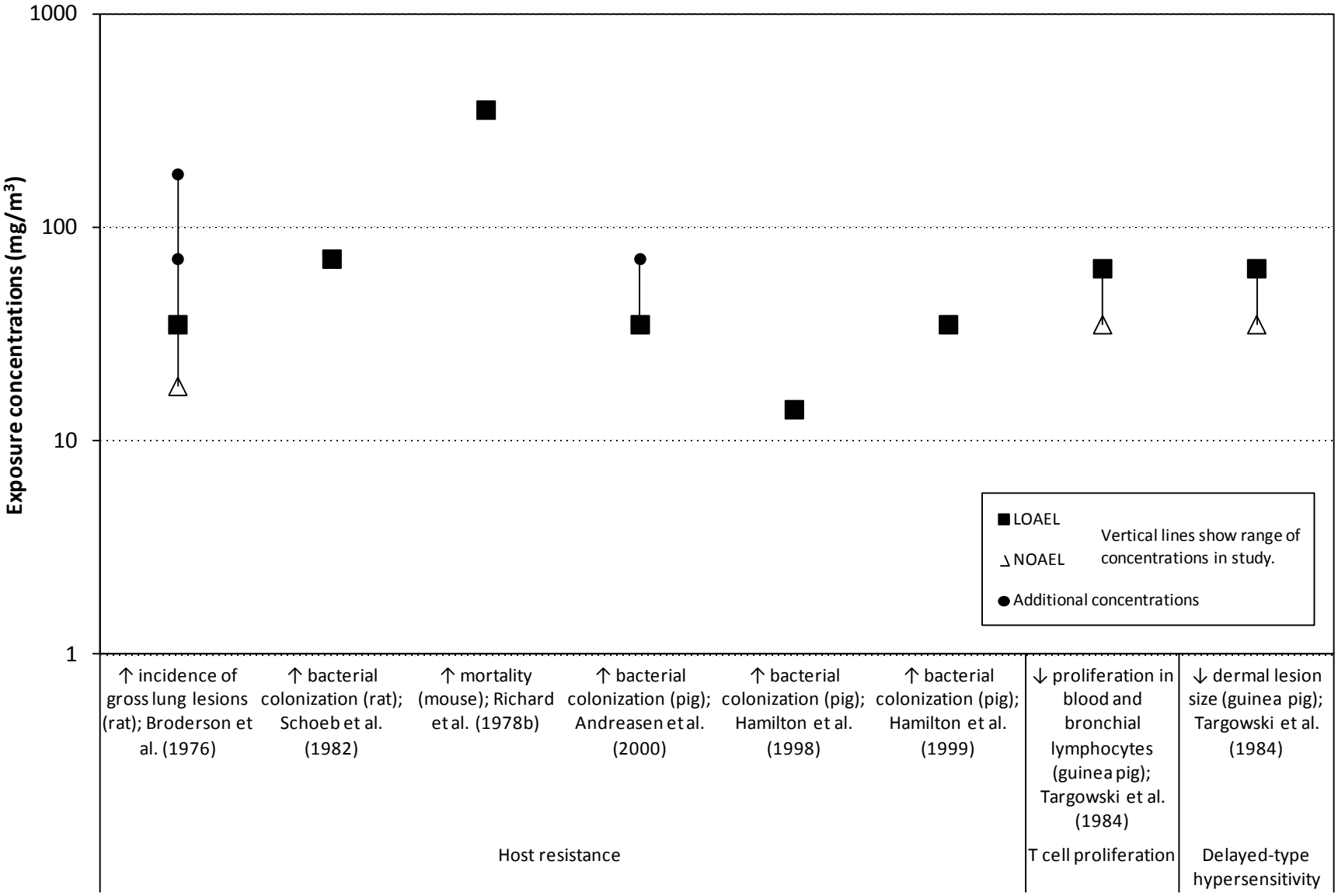

Figure 1-2. Exposure-response array of immune system effects following inhalation exposure to ammonia.

Summary of Immune System Effects

The evidence for ammonia immunotoxicity is based on epidemiological and animal studies. Available epidemiological studies that addressed immunological function are confounded by exposures to a number of other respirable agents that have been demonstrated to be immunostimulatory. Single-exposure human studies of ammonia evaluating immune endpoints are not available. Therefore, human studies are not particularly informative for evaluating whether ammonia has immunotoxic properties.

Animal studies provide consistent evidence of elevated bacterial growth following ammonia exposure. This is supported by observations of lung lesions (Broderson et al., 1976), elevated CFU (Schoeb et al., 1982), and increased mortality (Richard et al., 1978) in rats or mice exposed to ammonia; however, the findings from the Broderson et al. (1976) study (which described the percent of animals with gross lesions) were not dose-responsive, and the other studies used single concentrations of ammonia and therefore did not provide information on dose-response. A single study suggested that T cells are inhibited by ammonia (Targowski et al., 1984), but the data were not dose responsive.

Overall, there are suggestions that ammonia exposure may be associated with immunotoxicity, but it is unclear if elevated bacterial colonization is the result of damage to the protective mucosal epithelium of the respiratory tract or the result of suppressed immunity. Therefore, there is inadequate information to draw a conclusions about the immune system as a potential hazard of ammonia exposure.

1.2.3. Other Systemic Effects

The majority of information suggests that ammonia induces effects in and around the portal of entry. As discussed below, there is limited evidence from experimental animals that ammonia can produce effects on organs distal from the portal of entry, including the liver, kidney, spleen, and heart.

Evidence of liver toxicity in animals comes from observations of histopathological alterations in the liver. Histopathologic changes described as "fatty changes of the liver plate cells" were reported at an exposure concentration of 470 mg/m^3 ammonia in rats, guinea pigs, rabbits, dogs, and monkeys following the same subchronic inhalation exposure regimens (Coon et al., 1970); this concentration was lethal to approximately 25% of exposed guinea pigs and the majority of exposed rats. Congestion of the liver was reported in guinea pigs following inhalation exposure to 35 mg/m^3 for 42 days and 120 mg/m^3 18 weeks (Anderson et al., 1964a; Weatherby, 1952); no liver effects were observed in similarly exposed mice at 14 mg/m^3 (Anderson et al., 1964a).

Experimental animal studies provide some evidence that inhaled ammonia can affect the kidney and spleen. Alterations in the kidneys (calcification and proliferation of tubular epithelium) were reported in rats, rabbits, guinea pigs, monkeys, and dogs exposed to 470 mg/m^3, an ammonia concentration that was lethal to rats and guinea pigs (Coon et al., 1970). "Congestion" of the kidneys and spleen was reported in four guinea pigs exposed to 120 mg/m^3 ammonia for 18 weeks (but not 6 or 12 weeks) (Weatherby, 1952). Enlarged and "congested" spleens were reported in

guinea pigs exposed to 35 mg/m^3 ammonia for 6 weeks (Anderson et al., 1964a). None of these studies provided incidence of histopathologic lesions.

Myocardial fibrosis was observed in monkeys, dogs, rabbits, guinea pigs, and rats following subchronic inhalation exposure to 470 mg/m^3 ammonia, a concentration lethal to exposed guinea pigs and rats; no changes were observed at lower concentrations (Coon et al., 1970). At the same concentration, ocular irritation (characterized as heavy lacrimation, erythema, discharge, and ocular opacity of the cornea) was also reported by Coon et al. (1970) in small numbers of dogs and rabbits, but was not observed in similarly exposed monkeys or rats.

"Early degenerative changes" in the adrenal gland were reported in four guinea pigs exposed to 120 mg/m^3 ammonia by inhalation for 18 weeks, but not in guinea pigs exposed for 6 or 12 weeks (Weatherby, 1952). With the exception of Broderson et al. (1976), no other investigators examined effects on the adrenal gland following exposure to inhaled ammonia, and Broderson et al. (1976) did not describe effects on nonrespiratory tissues. These limited findings are insufficient to draw conclusions about possible effects of ammonia on the adrenal gland.

As discussed above, Coon et al. (1970) reported effects on the liver, kidney, and heart following continuous exposure to 470 mg/m^3; however, no histopathological changes were observed in rats, guinea pigs, rabbits, dogs, or monkeys when these animals were repeatedly, but not continuously, exposed to ammonia even at high concentrations (e.g., 770 mg/m^3 for 8 hours/day, 5 days/week; Table 1-6). These findings suggest that animals can recover from intermittent exposure to elevated ammonia levels (Coon et al., 1970), although the evidence to support this observation is limited.

Additionally, there is limited evidence of biochemical or metabolic effects of acute or short-term ammonia exposure. Evidence of slight acidosis, as indicated by a decrease in blood pH, was reported in rats exposed to 18 or 212 mg/m^3 ammonia for 5 days; the study authors stated that differences in pH leveled off at 10 and 15 days (Manninen et al., 1988). In another study, blood pH in rats was not affected by exposure to ammonia at concentrations up to 818 mg/m^3 for up to 24 hours (Schaerdel et al., 1983b).

Encephalopathy related to ammonia may occur in humans following disruption of the body's normal homeostatic regulation of the glutamine and urea cycles, e.g., due to severe liver disease resulting in elevated ammonia levels in blood (Minana et al., 1995; Souba, 1987). Acute inhalation exposure studies have identified alterations in amino acid levels and neurotransmitter metabolism (including glutamine concentrations) in the brain of rats and mice (Manninen and Savolainen, 1989; Manninen et al., 1988; Sadasivudu et al., 1979; Sadasivudu and Radha Krishna Murthy, 1978). It has been suggested that glutamate and γ-amino butyric acid play a role in ammonia-induced neurotoxicity (Jones, 2002). There is no evidence, however, that ammonia is neurotoxic in humans or animals following chronic inhalation exposure.

In the only study of the reproductive and developmental toxicity of ammonia, no changes in reproductive or developmental endpoints were found between two groups of female pigs (crossbred gilts) exposed to ammonia via inhalation for 6 weeks at mean concentrations of 5 or 25 mg/m^3 and then mated (Diekman et al., 1993). A control group without ammonia exposure was

not evaluated. Age at puberty did not differ significantly between the two groups. Gilts exposed to 25 mg/m^3 ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m^3; however, body weights of the two groups were similar at gestation day 30. Conception rates in the mated females were similar between the two groups (94.1 versus 100% in 5- versus 25-mg/m^3 groups). At sacrifice on day 30 of gestation, there were no significant differences between the two exposed groups in body weights of the pregnant gilts, number of corpora lutea, number of live fetuses, or weight and length of the fetuses. The strength of the findings from this study are limited by the absence of a control group with no ammonia exposure and possible confounding by exposures to bacterial and mycoplasm pathogens.

The evidence of systemic toxicity in experimental animals exposed to ammonia is summarized in Table 1-6 and as an exposure-response array in Figure 1-3.

Table 1-6. Evidence pertaining to other systemic effects in animals

Study design and reference	Results
Liver effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes observed.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	"Fatty changes of the liver plate cells" in several animals of each species at 470 mg/m^3.[a]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262, or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 days	"Fatty changes of the liver plate cells" in several rats at 470 mg/m^3, an exposure that was lethal to the majority of the rats.[a]
Anderson et al. (1964a) Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d	No visible signs of liver toxicity.
Weatherby (1952) Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 or 170 ppm (0 or 120 mg/m^3) for 6 hrs/d, 5 d/wk for 6, 12 or 18 wks	Congestion of the liver at 18 wks, not reported at earlier times.[a]
Anderson et al. (1964a) Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m^3) for 42 d	Congestion of the liver at 35 mg/m^3 for 42 d.[a]

Table 1-6. Evidence pertaining to other systemic effects in animals

Study design and reference	Results
Adrenal gland effects	
Weatherby (1952) Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 and 170 ppm (0 and 120 mg/m^3) 6 hrs/d, 5 d/wk for 6, 12, or 18 wks	"Early" degenerative changes in the adrenal gland (swelling of cells, degeneration of the cytoplasm with loss of normal granular structure) at 18 wks, not observed at earlier times.[a]
Kidney and spleen effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes reported.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3.[a] (This exposure was lethal to ~25% of guinea pigs.)
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262, or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3, an exposure that was lethal to the majority of the rats.[a]
Anderson et al. (1964a) Swiss albino mouse; male and female; 4/exposure interval 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d	No visible signs of toxicity.
Weatherby (1952) Guinea pig (strain not specified); male; 2 control and 4 exposed/exposure interval 0 or 170 ppm (0 or 120 mg/m^3) 6 hrs/d, 5 d/wk for 6, 12, or 18 wks	Congestion of the spleen and kidneys.[a]
Anderson et al. (1964a) Guinea pig (strain not specified); male and female; 2/exposure interval at 20 ppm, 6/exposure interval at 50 ppm 0 or 20 ppm (0 or 14 mg/m^3) for 7, 14, 21, 28, or 42 d or 50 ppm (35 mg/m^3) for 42 d	Enlarged and congested spleens at 35 mg/m^3.[a]

Table 1-6. Evidence pertaining to other systemic effects in animals

Study design and reference	Results
Myocardial effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes reported.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Myocardial fibrosis at 470 mg/m^3.[a] (This exposure was lethal to ~25% of guinea pigs.)
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262, or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 d	Myocardial fibrosis at 470 mg/m^3, an exposure that was lethal to the majority of the rats.[a]
Ocular effects	
Coon et al. (1970) Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	No ocular irritation reported.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 for 8 hrs/d, 5 d/wk for 6 wks	No ocular irritation reported.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d, 127, 262, or 470 mg/m^3 for 90 d, or 455 mg/m^3 for 65 d	No ocular irritation reported.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Erythema, discharge, and ocular opacity over ¼–½ of cornea at 470 mg/m^3.[a]
Coon et al. (1970) Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Heavy lacrimation at 470 mg/m^3.[a]

Table 1-6. Evidence pertaining to other systemic effects in animals

Study design and reference	Results
Blood pH changes	
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25 or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5, 10 or 15 d	↓ blood pH at 5 days; pH differences "leveled off at later time points (data not shown)". *Blood pH (day 5)*: 7.43, 7.34*, 7.36*
Schaerdel et al. (1983b) Crl:COBS CD(SD) rat; male; 8/group [blood pO_2 based on n = 5] 15, 32, 310, or 1,157 ppm (11, 23, 219, or 818 mg/m^3) for 0 (control), 8, 12, or 24 hrs	↑ blood pO_2 at 11 and 23 mg/m^3 at 8-, 12-, and 24-hr time points; no change at higher concentrations; no change in blood pH. *Percent change in pO_2 from time 0 (at 24 hours of exposure*[b]: 20*, 17*, 1, -2%
Amino acid levels and neurotransmitter metabolism in the brain	
Manninen and Savolainen (1989) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5 d	*% change compared to control*:[c] Brain glutamine: 42*, 40*%
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5, 10, or 15 d	*% change compared to control at 212 mg/m^3*:[c] Blood glutamine (5, 10, 15 d): 44*, 13, 14% Brain glutamine (5, 10, 15 d): 40*, 4, 2%
Reproductive and developmental effects	
Diekman et al. (1993) Crossbred gilt (female pig); 4.5 mo old; 40/group 7 ppm (5 mg/m^3), range 4–12 ppm (3–8.5 mg/m^3) or 35 ppm (25 mg/m^3), range 26–45 (18–32 mg/m^3) for 6 wks[d]	No change in any of the reproductive or developmental parameters measured (age at puberty, conception rates, body weight of pregnant gilts, number of corpora lutea, number of live fetuses, and weight or length of fetuses).

[a]Incidence data not provided.
[b]Measurements at time zero were used as a control; the study did not include an unexposed control group.
[c]Percent change compared to control calculated as: (treated value – control value)/control value x 100.
[d]A control group was not included. Prior to exposure to ammonia, pigs were also exposed naturally in conventional grower units to *Mycoplasma hypopneumoniae* and *Pasteurella multocida*, which cause pneumonia and atrophic rhinitis, respectively.

*Statistically significantly different from the control ($p < 0.05$).

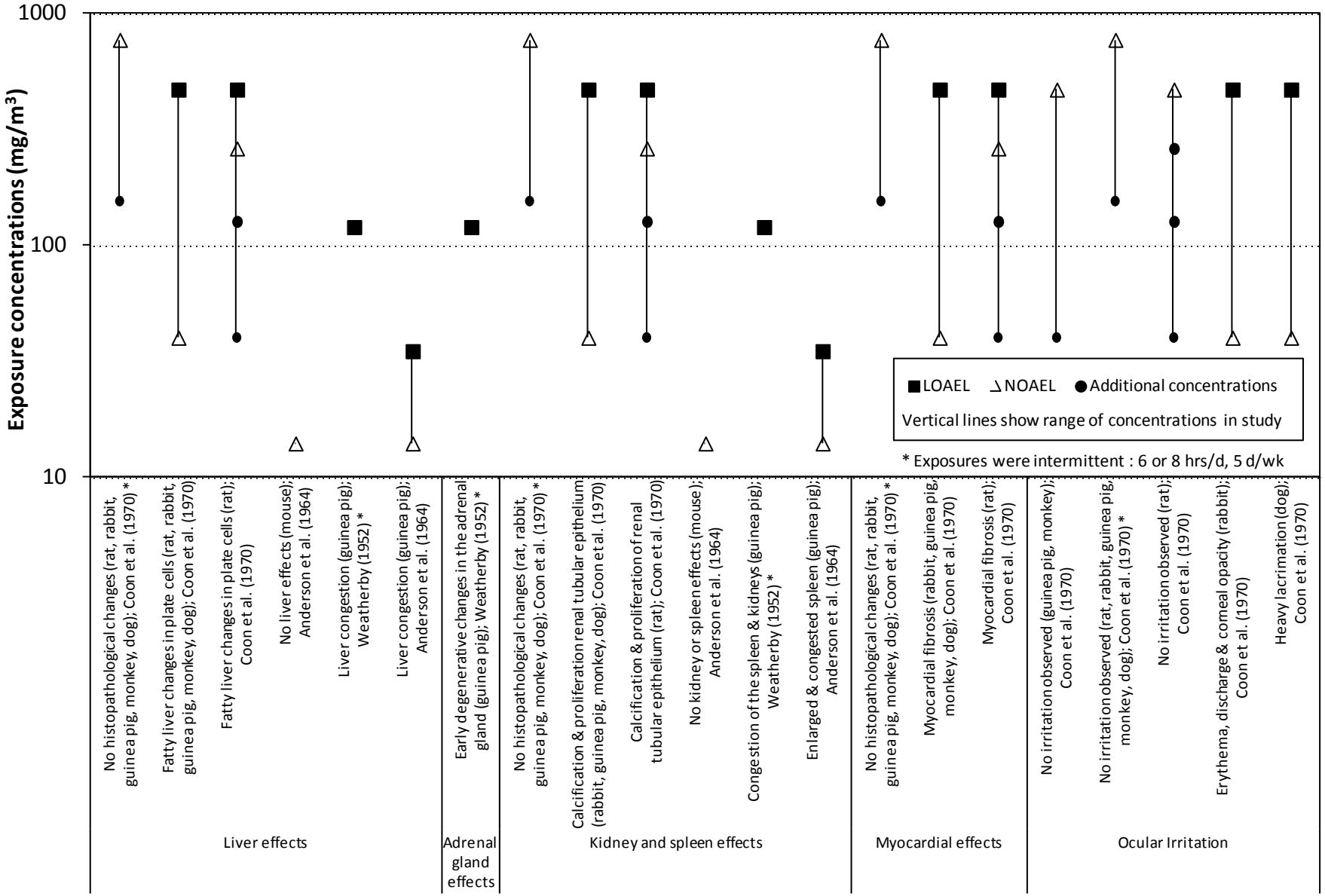

Figure 1-3. Exposure-response array of systemic effects following inhalation exposure to ammonia.

Summary of Other Systemic Effects

Effects of ammonia exposure on organs distal from the portal of entry (systemic effects) are based on evidence in animals. Effects on various organs, including liver, kidney, spleen, and heart, were observed in several studies that examined responses to ammonia exposure in a number of laboratory animal species. While effects on many of these organs were observed in multiple species, including monkey, dog, rabbit, guinea pig, and rat, effects were not consistent across exposure protocols. Evidence of ocular irritation in experimental animals was inconsistently observed, and then only at high ammonia concentrations (470 mg/m^3).

Studies of ammonia toxicity that examined other systemic effects were all published in the older toxicological literature. Three subchronic inhalation studies were published between 1952 and 1970 (Coon et al., 1970; Anderson et al., 1964a; Weatherby, 1952). In general, the information from these studies is limited by small group sizes, minimal characterization of reported histopathological changes (e.g., "congestion," "enlarged," "fatty liver"), insufficiently detailed reporting of study results, and incomplete, if any, incidence data. In addition, Weatherby (1952), Anderson et al. (1964a), and some of the experiments reported by Coon et al. (1970) used only one ammonia concentration in addition to the control, so no dose-response information is available from the majority of experimental studies to inform the evidence for systemic effects of ammonia. Finally, exposure characterization in Weatherby (1952) was considered poor.

Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on organs distal from the portal of entry, but there is inadequate information to draw conclusions about the liver, kidney, spleen, or heart as sensitive targets of ammonia toxicity.

Given the inadequacies of the available toxicology literature for other systemic effects, the potential toxicity of inhaled ammonia at sites distal from the respiratory system was evaluated by considering ammonia levels normally present in blood. As discussed in more detail in Appendix C, Section C.1.2, ammonia is produced endogenously in all human and animal tissues during fetal and adult life. In adults, the normal range of ammonia in venous blood is 0.1–0.8 µg/ml. Concentrations in fetal circulation are higher than maternal blood concentrations; two studies reported that mean umbilical concentrations of ammonia in venous blood at delivery were 50% to threefold higher than mean concentrations in maternal blood, with umbilical concentrations ranging from approximately 0.5–5 µg/ml (Jóźwik et al., 2005; DeSanto et al., 1993). Human fetal umbilical blood levels of ammonia at birth were not influenced by gestational age based on deliveries ranging from gestation week 25 to 43 (DeSanto et al., 1993).

At external concentrations that do not measurably change normal (baseline) levels of ammonia, the likelihood is low that exposures would pose a hazard for systemic effects. In rats, exposure to ammonia concentrations ≤18 mg/m^3 did not produce a statistically significant change in blood or brain ammonia concentrations(Manninen et al., 1988; Schaerdel et al., 1983b). Higher external ammonia concentrations (≥212 mg/m^3) were associated with elevated blood ammonia levels, but even at these relatively high concentrations, experimental findings in rats indicate that compensation readily occurs (Manninen et al., 1988). In a 24-hour exposure duration study, blood

ammonia concentrations at 12 hours of exposure to ≥219 mg/m^3 ammonia in air were lower than at 8 hours; in a second 15-day exposure duration study, blood ammonia concentrations that were elevated on day 5 of exposure to 212 mg/m^3 ammonia in air were not significantly different from control values on days 10 and 15 of exposure (Schaerdel et al., 1983a). See Appendix C, Section C.1.3, Metabolism/Endogenous Production of Ammonia, for a more detailed summary of the available literature that describes the relationship between environmental ammonia concentrations and blood ammonia levels. Therefore, the available experimental data suggest that any changes in blood ammonia at external concentrations ≤18 mg/m^3 would be small relative to levels normally present in blood. The potential for systemic effects (i.e., on tissues/organs distal from the respiratory system), including reproductive and developmental effects, at these concentrations cannot be ruled out, but the likelihood of such effects is considered small.

Because the health effects literature identified the respiratory system as the primary target of ammonia toxicity, EPA also considered the possibility that point of contact effects could translate into effects on tissues or organs distal from the respiratory system. EPA is not aware of any mechanisms by which point of contact effects could directly or indirectly impact distal tissues or organs.

1.3. SUMMARY AND EVALUATION

1.3.1. Weight of Evidence for Effects Other than Cancer

The respiratory system is the primary and most sensitive target of inhaled ammonia toxicity in humans and experimental animals. Evidence for respiratory system toxicity in humans comes from cross-sectional occupational studies in industrial settings that reported changes in lung function and an increased prevalence of respiratory symptoms. The findings of respiratory effects in workers exposed to ammonia as a disinfectant or cleaning product (primarily studies of asthma or asthma symptoms), studies in agricultural settings (primarily lung function studies), controlled human exposure studies, and case reports of injury following acute exposure provide additional evidence that the respiratory system is a target of inhaled ammonia. Short-term and subchronic animal studies show respiratory effects in several animal species across different dose regimens. Thus, the weight of evidence of observed respiratory effects observed across multiple human and animal studies identifies respiratory system effects as a hazard from ammonia exposure.

Evidence for an association between inhaled ammonia exposure and effects on other organ systems distal from the portal of entry is less compelling than for the respiratory system. Overall, there are suggestions in experimental animals that ammonia exposure may be associated with effects on the liver, kidney, spleen, or heart, but the available information is inadequate to draw conclusions. The two epidemiological studies that addressed immunological function are confounded by exposures to a number of other respirable agents that have been demonstrated to be immunostimulatory and provide little support for ammonia immunotoxicity. Animal studies provide consistent evidence of elevated bacterial growth following ammonia exposure. It is unclear, however, whether elevated bacterial colonization is the result of suppressed immunity or

damage to the barrier provided by the mucosal epithelium of the respiratory tract. Overall, the weight of evidence does not support the immune system as a target of ammonia toxicity.

Studies of the potential reproductive or developmental toxicity of ammonia in humans are not available. Reproductive effects were not associated with inhaled ammonia in the only animal study that examined the reproductive effects of ammonia (i.e., a limited-design inhalation study in the pig). As discussed in Section 1.2.3, ammonia is produced endogenously in human and animal tissues during fetal and adult life. Although the potential for effects on reproduction and the developing fetus cannot be ruled out at external concentrations that do not alter normal blood or tissue ammonia levels, there is no evidence that raises concerns for the developing fetus or reproduction or to other distal tissues/organs.

1.3.2. Susceptible Populations and Lifestages

Studies of the toxicity of ammonia in children or young animals that would support an evaluation of childhood susceptibility are limited. Casas et al. (2013) found evidence of airway inflammation (as indicated by increased exhaled nitric oxide) and decreased lung function in school-age children exposed to cleaning products.

Because the respiratory system is a target of ammonia toxicity, individuals with respiratory disease (e.g., asthmatics) might be expected to be a susceptible population. Loftus et al. (2015) reported no increase in asthma symptoms and medication use in asthmatic children living near animal feeding operations; however, ammonia exposure was associated with lower FEV_1. Controlled human exposure studies that examined both healthy adult volunteers and volunteers with asthma (Petrova et al., 2008; Sigurdarson et al., 2004) did not demonstrate greater respiratory sensitivity in asthmatics than healthy volunteers after acute exposure to ammonia. Under longer-term exposure conditions, however, as seen among livestock farmers, one study observed associations between ammonia exposure and decreased lung function among workers with chronic respiratory symptoms, but not among the asymptomatic workers (Preller et al., 1995). Additional research focusing on the question of susceptibility and variability in response to ammonia exposure in these populations is needed.

Individuals with disease conditions that lead to hyperammonemia, a condition of elevated levels of circulating ammonia, may be more susceptible to the effects of ammonia from external sources. Hyperammonemia can occur in individuals with severe diseases of the liver (e.g., cirrhosis) or kidney, organs that biotransform and excrete ammonia, urea cycle disorders, and other conditions such as fatty acid oxidation defects and Reye syndrome (Bürki et al., 2015; Auron and Brophy, 2012; Romero-Gómez et al., 2004; Córdoba et al., 1998; Davies et al., 1997; Schubiger et al., 1991; Gilbert, 1988; Jeffers et al., 1988; Souba, 1987). Elevated ammonia levels can predispose an individual to encephalopathy as a result of the ability of ammonia to cross the blood-brain barrier and subsequent disturbances in amino acid synthesis and alterations in neurotransmission systems. Neonates and infants are particularly susceptible to the neurological effects of elevated levels of ammonia; hyperammonemia can cause irreparable damage to the developing brain (Minana et al., 1995; Souba, 1987) (Auron and Brophy, 2012). While patients with

hyperammonemia could plausibly be considered a susceptible population, there are no studies that specifically support this hypothesized susceptibility.

2. DOSE-RESPONSE ANALYSIS

2.1. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The RfC (expressed in units of mg/m^3) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to these PODs to reflect limitations of the data used.

2.1.1. Identification of Studies and Effects for Dose-Response Analysis

As discussed in Section 1.2, the respiratory system is the primary and most sensitive target of inhaled ammonia in humans and experimental animals, and respiratory effects have been identified as a hazard following inhalation exposure to ammonia. The experimental toxicology literature for ammonia provides evidence that inhaled ammonia may be associated with toxicity to target organs other than the respiratory system, including the liver, kidney, spleen, heart, and immune system. Effects in these other (nonrespiratory) target organs were not considered as the basis for RfC derivation because the evidence for these associations is weak relative to that for respiratory effects.

Respiratory effects, characterized as increased prevalence of respiratory symptoms or decreased lung function, have been observed in worker populations exposed to ammonia concentrations ≥18.5 mg/m^3 (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998). Decrements in lung function parameters and increased prevalence of respiratory symptoms, such as wheezing, chest tightness, and cough/phlegm, have been identified as adverse respiratory health effects by the American Thoracic Society (ATS, 2000) and are similarly noted as adverse in the EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). At the population level, ATS (2000) stated that "any detectable level of permanent pulmonary function loss attributable to air pollution exposure should be considered as adverse" and that

> It should be emphasized that a small but significant reduction in a population mean FEV_1 or $FEV_{0.75}$ is probably medically significant, as such a difference may indicate an increase in the number of persons with respiratory impairment in the population. In other words, a small part of the population may manifest a marked change that is medically significant to them, but when diluted with the rest of the population the change appears to be small (ATS, 2000).

Thus, even small changes in the average (mean) of a distribution of pulmonary function parameters is considered adverse for purposes of deriving an RfC.

In general human data are preferred over animal data for deriving reference values because these data are more relevant for assessing human health effects than animal studies and avoid the uncertainty associated with interspecies extrapolation when animal data serve as the basis for the RfC. In the case of ammonia, the available occupational studies provide adequate data for the quantitative analysis of health outcomes considered relevant to potential general population exposures. Respiratory effects have also been observed in animals, but at ammonia concentrations higher than those associated with respiratory effects in humans and in studies involving exposure durations (up to 114 days) shorter than those in occupational studies (Section 1.2.1). Therefore, data on respiratory effects in humans were used for the derivation of the RfC and respiratory effects in animals were not further considered.

Of the available human data, associations between ammonia exposure and respiratory effects have been examined in epidemiology studies of industrial worker populations (Table 1-2), in studies of ammonia exposure in a cleaning setting (Table 1-3), and in studies of populations in agricultural settings. Studies using ammonia as a cleaning product provide evidence of an association between ammonia exposure and increased risk of asthma; however, these studies did not measure ammonia concentrations and thus are not useful for dose-response analysis. Studies in agricultural settings also support an association between ammonia exposure and decreased pulmonary function; however, because of co-exposures to other agents (including dust, endotoxin, mold, and disinfectant products) and the availability of studies with fewer co-exposures, studies in agricultural settings were considered to be supportive of the association between ammonia exposure and respiratory effects but were not carried forward for dose-response analysis. In addition, several controlled-exposure studies in volunteers evaluated the effects of ammonia on irritation and lung function following acute exposures. These human exposure studies have several methodological strengths compared to epidemiological studies of worker populations, including well characterized exposures and resistance to confounding; however, the short exposure durations used in these studies (i.e., 15 seconds to 6 hours) make them inappropriate for evaluating the effects of chronic exposure to ammonia.

Of the available studies of ammonia exposure in industrial settings, four cross-sectional epidemiology studies of industrial worker populations—three studies in urea fertilizer plants by Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001), and a study in a soda ash plant by Holness et al. (1989)—provide information useful for examining the relationship between chronic ammonia exposure and increased prevalence of respiratory symptoms and/or decreased lung function. Bhat and Ramaswamy (1993) evaluated lung function in ammonia plant workers, but did not measure ammonia concentrations in workplace air. Therefore, this study was not considered useful for RfC derivation.

In general, these four cross-sectional occupational studies provide a coherent set of estimated NOAELs and effect levels, and are considered candidate principal studies for RfC derivation. A brief description of these studies and the contribution of each to the understanding of

the dose-response relationship between ammonia exposure and respiratory effects follows. More study details are provided in the Supplemental Information, Section C.2.1 and in Table 1-2, and evaluation of the strengths and limitations are more fully considered in the Literature Search Strategy | Study Selection and Evaluation section.

- Rahman et al. (2007) observed an increased prevalence of respiratory symptoms (coughing, chest tightness) in urea fertilizer plant workers (mean employment duration: 16 years) exposed to a mean ammonia concentration of 18.5 mg/m^3 (range: 9–31 mg/m^3), but not in workers in a second plant exposed to a mean ammonia concentration of 4.9 mg/m^3 (range: 2–8 mg/m^3). Decrements in lung function (FVC and FEV_1) between pre- and post-shift in the high-exposure group (2–3%) were statistically significant. Exposure was measured by personal samples using two different analytical methods.

- Ballal et al. (1998) observed an increased prevalence of respiratory symptoms (cough, phlegm, wheezing, and dyspnea) among urea fertilizer factory workers (mean employment duration: 4.3 years) in one factory (Factory A) with ammonia exposures ranging from 2–27.1 mg/m^3,[10] but no increase in symptoms in another factory (Factory B) with exposures ranging from 0.02–7 mg/m^3. Lung function was not measured.

- A companion study by Ali et al. (2001) examined lung function among workers in Factory A from Ballal et al. (1998); respiratory symptoms were not evaluated. Workers with cumulative exposure >50 mg/m^3-years had significantly lower lung function values (declines of 5–7% in FVC% predicted and FEV_1% predicted) than workers with cumulative exposure ≤50 mg/m^3-years. In this and the Ballal et al. (1998) study, exposure was measured by air monitors.

- Holness et al. (1989) found no differences in the prevalence of respiratory symptoms or lung function between soda ash plant workers (mean exposure 6.5 mg/m^3; mean exposure duration of 12.2 years) and the control group, and also no differences in respiratory symptoms or lung function when workers were stratified by ammonia exposure level (lowest exposure group, <4.4 mg/m^3; middle exposure group, 4.4–8.8 mg/m^3; highest exposure group, >8.8 mg/m^3). Exposure was measured by personal samples. EPA identified the concentration range for the high-exposure group (i.e., >8.8 mg/m^3) as the NOAEL from this study. The authors stated that 3 of the 12 workers in the high-exposure group were exposed to concentrations >17.7 mg/m^3; therefore, the majority of workers in the high-exposure group (9 of 12) would have been exposed to ammonia concentrations in the range of 8.8–17.7 mg/m^3.

[10]This concentration range does not include exposures in the urea store (number of employees = 6; range of ammonia concentrations = 90–130.4 mg/m^3) because employees in this area were required to wear full protective clothing, thus minimizing potential exposure.

In selecting the principal study for RfC derivation, consideration was given to exposure measures, assessment of outcomes, potential for co-exposures, and the value of the NOAEL. Of the four candidate principal studies, higher confidence was associated with the exposure measures from Holness et al. (1989). Both Holness et al. (1989) and Rahman et al. (2007) collected personal air samples, but confidence in the analytical method used by Holness et al. (1989) is higher than that used by Rahman et al. (2007). Rahman et al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air (i.e., Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by four- to fivefold, indicating some uncertainty across the two measurement methods, although ammonia concentrations measured by the two methods were strongly correlated (correlation coefficient of 0.8). In contrast, the Holness et al. (1989) study used an established analytical method for measuring exposure to ammonia recommended by the National Institute for Occupational Safety and Health (NIOSH) that involved the collection of air samples on acid-treated silica gel absorption tubes. Ballal et al. (1998) used area monitors rather than personal air sampling methods; the latter method provides a better estimate of an individual's exposure.

As discussed in the Literature Search Strategy | Study Selection and Evaluation section, assessment of respiratory symptoms in all studies that measured this outcome was based on self-reporting by questionnaire, and assessment of lung function was performed using standard spirometry protocols. While considered unlikely, non-blinded outcome assessments of respiratory symptoms could introduce bias. Therefore, both Holness et al. (1989) and Rahman et al. (2007), the two studies of industrial populations that examined both respiratory symptoms and lung function, provide stronger evidence of respiratory effects than studies that evaluated symptoms data only (notably Ballal et al. (1998)).

Also as discussed in the Literature Search Strategy | Study Selection and Evaluation section, confounding by other workplace exposures is a potential concern, although not likely to be a major limitation of the studies considered for dose-response analysis. Only Rahman et al. (2007) measured another workplace chemical (nitrogen dioxide; below detection limits); other studies did not describe potential co-exposures. Therefore, a more rigorous examination of the potential for confounding by co-exposure to other workplace chemicals could not be performed. Holness et al. (1989) noted the high level of control of exposures in the facility used in their study, resulting in low ammonia levels.

Three of the four occupational studies supported the identification of a NOAEL (or, more correctly, an exposure range not associated with an increase in respiratory effects). Rahman et al. (2007) did not observe a change in respiratory effects in workers exposed to a mean ammonia concentration of 4.9 mg/m^3 (range: 2–8 mg/m^3). Holness et al. (1989) found no differences in respiratory effects in soda ash plant workers when compared to a control group or when workers were stratified by exposure level (low, medium, and high); the concentration range for the high-exposure group (i.e., >8.8 mg/m^3) was identified as the NOAEL. Ballal et al. (1998) reported no increase in respiratory symptoms in a factory with exposures ranging from 0.02–7 mg/m^3. Because Ali et al. (2001), the companion study to Ballal et al. (1998), evaluated only workers in a

second factory with higher exposures, study findings did not support identification of an estimated NOAEL.

In light of the above considerations, overall confidence in the Holness et al. (1989) study as the principal study for RfC derivation was higher than other candidate studies in terms of: measurement of ammonia exposure, evaluation of both respiratory symptoms and lung function parameters, smaller potential for co-exposures to other workplace chemicals, and the fact that the estimated NOAEL for respiratory effects of ≥8.8 mg/m^3 was the highest of the NOAELs estimated from the candidate principal studies. The Holness et al. (1989) study does not demonstrate a relationship between ammonia exposure and respiratory effects. The relationship between ammonia exposure and respiratory effects is based on the body of evidence, and the Holness et al. (1989) study is identified as the principal study for derivation of the RfC for the reasons given above.

In summary, the occupational study of ammonia exposure in workers in a soda ash plant by **Holness et al. (1989) was identified as the principal study for RfC derivation, with support from Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001), and respiratory effects were identified as the critical effect**.

2.1.2. Methods of Analysis

A **NOAEL of 13.6 mg/m^3, or an estimate of the lower confidence bound of the mean exposure concentration in the high-exposure group of the Holness (1989) study, was used as the point of departure (POD) for RfC derivation.** The point of departure (POD) for respiratory effects was based on the NOAEL representing the high-exposure group in Holness et al. (1989). The individual subject data from this study were no longer available (call from S. Rieth, U.S. EPA, to C. Clayton, administrative assistant to Dr. Holness, St. Michael's Hospital, Center for Research Expertise in Occupational Health, Toronto, Canada, February 11, 2015), so that the mean exposure in the high-exposure group could not be calculated precisely based on the data. Therefore, the mean was estimated assuming that the data in the study followed a skewed probability distribution, specifically the lognormal distribution. The frequency distribution provided in Holness et al. (1989) (see Table 2-1) was used to estimate the parameters (log-scale mean and standard deviation) of the lognormal distribution that best fit the data.

Table 2-1. Frequency distribution of ammonia exposure from Holness (1989)

Exposure group	Interval of exposures (mg/m^3)	Interval of exposures (ppm)	Number of exposed workers
Low	0–4.4	0–6.25	34
Medium	4.4–8.8	6.25–12.5	12
High[a]	8.8–17.7	12.5–25	9
	>17.7	>25	3

[a]EPA divided the high-exposure group into two subgroups based on the statement in Holness et al. (1989): "Three workers were exposed to TWA concentrations of ammonia in excess of 25 ppm, the current exposure guideline."

Lognormal parameter estimates were obtained by applying the maximum likelihood method to this frequency distribution. Using the estimated distribution defined by these parameter estimates, the estimated mean exposure in the high-exposure group and 95% lower confidence bound on this mean were calculated as follows. See Appendix C, Section C.4 for detailed documentation of this calculation.

mean exposure estimate (high-exposure group) = 17.9 mg/m^3

95% lower confidence bound on this mean (high-exposure group) = 13.6 mg/m^3

The lower confidence bound of 13.6 mg/m^3 was used as the POD for respiratory effects.

Because the RfC assumes continuous human exposure over a lifetime, the POD was adjusted to account for the noncontinuous exposure associated with occupational exposure (i.e., 8-hour workday and 5-day workweek). Cross-shift data for FVC and FEV_1 from the Rahman et al. (2007) study provide some evidence of an immediate effect of ammonia exposure on lung function[11], which could argue against adjustment from noncontinuous to continuous exposure; however, Rahman et al. (2007) also reported that duration of exposure (using years of employment as a proxy for exposure duration) was significantly associated with percentage cross-shift decrease in FEV_1%. In addition, Ballal et al. (1998) found a significant correlation between respiratory symptoms (cough, phlegm, and wheezing) and duration of service (a proxy for exposure duration). In the absence of clear evidence that respiratory effects in occupationally-exposed populations are an acute response, and given evidence for contributions of exposure duration (cumulative exposure) to the respiratory effects of ammonia, the standard adjustment to continuous exposure was applied. The duration-adjusted POD was calculated as follows:

[11]Rahman et al. (2007) reported that mean preshift FVC and FEV_1 values in ammonia and urea plants workers were similar, suggesting similar lung function in low- and high-exposure workers upon arrival at work. Cross-shift changes in FVC and FEV_1 were statistically significant decreased in the urea plant (more highly-exposed) workers only.

$NOAEL_{ADJ}$ = NOAEL × VEho/VEh × 5 days/7 days

= 13.6 mg/m³ × 10 m³/20 m³ × 5 days/7 days

= 4.9 mg/m³ or 5 mg/m³ (rounded)

Where:

VEho = human occupational default minute volume (10 m³ breathed during an 8-hour workday) (U.S. EPA, 1994). This inhalation rate corresponds to more current inhalation rates for light to moderate activity levels from U.S. EPA (2009c), as cited in U.S. EPA (2011). An occupational inhalation rate of 10.8 m³ for an 8-hour workday, similar to the default value from U.S. EPA (1994), can be derived as an average of activity-specific inhalation rates for males, in age groups from 21–60 years, for combined light and moderate activity from Table 6-17 of U.S. EPA (2011). The average inhalation rate of 1.3 m³/hour (0.022 m³/min) can be multiplied by 8 hours to obtain an inhalation rate of 10.8 m³/8-hour workday.

VEh = human ambient default minute volume (20 m³ breathed during the entire day) (U.S. EPA, 1994). This value is consistent with the average of the daily average inhalation rates for males, in age groups from 21–60 years, of 20.2 m³/day, from U.S. EPA (2009c), as summarized in Table 6-14 of U.S. EPA (2011).

2.1.3. Derivation of the Reference Concentration

Consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered when deriving the RfC. A **composite UF of 10** was applied to the selected duration-adjusted POD of 4.9 mg/m³ to derive the RfC of 0.5 mg/m³. An explanation of the five possible areas of uncertainty and variability follows:

- An intraspecies uncertainty factor, UF_H, of 10 was applied to account for potentially susceptible individuals in the absence of data evaluating variability of response to inhaled ammonia in the human population;

- An interspecies uncertainty factor, UF_A, of 1 was applied to account for uncertainty in extrapolating from laboratory animals to humans because the POD was based on human data from an occupational study;

- A subchronic to chronic uncertainty factor, UF_S, of 1 was applied because the occupational exposure period in the principal study (Holness et al., 1989), defined as the mean number of years at the present job for exposed workers, of approximately 12 years was considered to be of chronic duration;

- An uncertainty factor for extrapolation from a LOAEL to a NOAEL, UF_L, of 1 was applied because a NOAEL was used as the POD; and

- A database uncertainty factor, UF_D, of 1 was applied to account for deficiencies in the database. As discussed in Section 1.2, available epidemiological studies include studies of workers exposed in industrial settings, in agriculture, or through use of cleaning products.

There are also controlled human exposure studies involving short-duration exposure to ammonia vapors, and many case reports of acute exposures to high concentrations. Available animal studies include subchronic studies that investigated respiratory and systemic effects in rats, guinea pigs, and pigs. There are also several immunotoxicity studies, and one limited reproductive toxicity study in young female pigs. The database lacks developmental and multigenerational reproductive toxicity studies. The EPA's review of RfD and RfC processes (U.S. EPA, 2002) states,

> "If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD . . ."

Although the database lacks developmental and multigenerational reproductive toxicity studies, there are no data or suspicions of developmental toxicity at levels below the POD. The available studies identify the respiratory system as the principal target of toxicity for inhaled ammonia and do not suggest a likelihood of developmental or reproductive effects at lower levels (see Sections 1.2.3 and 1.3.1).

The RfC for ammonia was calculated as follows:

RfC = $NOAEL_{ADJ}$ ÷ UF
= 4.9 mg/m^3 ÷ 10
= 0.49 mg/m^3 or **0.5 mg/m^3 (rounded to one significant figure)**

2.1.4. Uncertainties in the Derivation of the Reference Concentration

As presented earlier in this section and in the Preamble, EPA standard practices and RfC guidance (U.S. EPA, 2002, 1995, 1994) were followed in applying an UF approach to a POD (from a NOAEL) to derive the RfC. Specific uncertainties were accounted for by the application of UFs (i.e., in the case of the ammonia RfC, a factor to address the absence of data to evaluate the variability in response to inhaled ammonia in the human population). The following discussion identifies additional uncertainties associated with the quantification of the RfC for ammonia.

Use of a NOAEL as a POD

Data sets that support benchmark dose modeling are generally preferred for reference value derivation because the shape of the dose-response curve can be taken into account in establishing the POD. For the ammonia RfC, no decreases in lung function or increases in the prevalence of respiratory symptoms were observed in the worker population studied by Holness et al. (1989), i.e., the principal study used to derive the RfC, and as such, the data from this study did not support dose-response modeling. Rather, a NOAEL from the Holness et al. (1989) study was used to estimate the POD. The availability of dose-response data from a study of ammonia, especially in humans, would increase the confidence in the estimation of the POD.

Comparison of Exhaled Ammonia to the RfC

Ammonia is generated endogenously in multiple organs, including the liver, kidneys, intestines, brain, and skeletal muscle, as a product of amino acid catabolism. Ammonia plays central roles in nitrogen balance and acid-base homeostasis (Weiner et al., 2014; Weiner and Verlander, 2013). Given its important metabolic role, free ammonia is homeostatically regulated to remain at low concentrations in blood (Souba, 1987). Elimination of ammonia occurs primarily in urine and exhaled breath. (See Appendix C, Section C.1.3 for additional information on production and regulation of endogenous ammonia.)

Further consideration was given to the presence of ammonia in exhaled air because the range of ammonia concentrations in exhaled breath overlaps the ammonia RfC. Specifically, ammonia has been measured in exhaled breath at concentrations ranging from 0.009–2 mg/m^3 (see Appendix C, Table C-1), a range that exceeds the RfC of 0.5 mg/m^3. This section reviews information related to the exhalation of ammonia that provides context for this comparison.

In general, the higher and more variable ammonia concentrations are reported in human breath exhaled from the mouth or oral cavity. Investigators reported concentrations ranging from 0.03 to 2 mg/m^3, with the majority of concentrations ≥0.2 mg/m^3 (Schmidt et al., 2013; Smith et al., 2008; Španěl et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977). Ammonia concentrations measured in breath derived from oral breathing largely reflect the production of ammonia via bacterial degradation of food protein in the oral cavity or gastrointestinal tract (Turner et al., 2006; Smith et al., 1999; Vollmuth and Schlesinger, 1984). Ammonia concentrations from exhaled breath can be influenced by factors such as diet, oral hygiene, and age (Solga et al., 2013; Španěl et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Norwood et al., 1992). Schmidt et al. (2013) reported that ammonia concentrations in breath from the mouth strongly depended on saliva pH.

Concentrations of ammonia in breath exhaled from the nose and trachea of humans (0.0092–0.1 mg/m^3) are lower than those in air exhaled from the mouth (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977). Whereas the upper end of the range of ammonia concentrations in mouth breath exceeds the RfC of 0.5 mg/m^3, concentrations from the nose and trachea are generally lower than the ammonia RfC by a factor of five or more. Ammonia concentrations in breath exhaled from the nose appear to better represent levels at the alveolar interface of the lung and are thought to be more relevant to understanding systemic levels of ammonia than breath exhaled from the mouth (Schmidt et al., 2013; Smith et al., 2008). Nevertheless, the relationship between nose ammonia concentrations and systemic levels is complicated by the possibility that nose ammonia concentrations are still influenced by the oral cavity (e.g., in individuals with the soft palate incompletely closed), and tracheobronchial fluids that, like saliva, can influence the airway concentration of ammonia. Further, measurements of exhaled ammonia reported in the literature were generally not conducted in ammonia-free environments, and thus the ammonia in inhaled air may account for some of the ammonia measured in exhaled air (e.g., see Španěl et al. (2013)).

Thus, ammonia concentrations in exhaled breath, and particularly those exhaled through the mouth, are not correlated with blood ammonia; factors identified as influencing exhaled

ammonia concentrations include bacterial populations in the oral cavity, salivary pH, diet, oral hygiene, and age (see Appendix C, Section C.1.4). Concentration in breath cannot be used to predict blood ammonia concentration or previous exposure to environmental (ambient) concentrations of ammonia.

Regardless, the level of ammonia in breath, even at concentrations that exceed the RfC, does not necessarily raise questions about the appropriateness of the RfC. The exhalation of ammonia is a clearance mechanism for a product of metabolism that is otherwise toxic in the body at sufficiently high concentrations. Ammonia concentrations in exhaled breath may be higher than inhaled concentrations, particularly when compared to exhaled air from the mouth or oral cavity. However, the fact that humans may exhale ammonia at concentrations higher than 0.5 mg/m^3 (i.e., the RfC) is not considered an uncertainty in the RfC.

Consideration of Tolerance and the Healthy Worker Effect on Selection of the POD

As discussed in Section 1.2.1, two controlled-exposure studies provide some evidence of habituation to eye, nose, and throat irritation in volunteers after repeated ammonia exposure. Following exposure to ammonia at concentrations ranging from 7 to 35 mg/m^3 for 4 hours/day on five consecutive days, Ihrig et al. (2006) reported higher mean intensities for irritative, olfactory, and respiratory symptoms in male volunteers unfamiliar with ammonia when compared to male chemical company workers exposed to ammonia vapor for several years in a urea department; differences were statistically significant only for olfactory symptoms. In a more limited study with only four male volunteers each exposed to 18, 35, or 71 mg/m^3 ammonia (exposure to each concentration was for one week, 2–6 hours/day, 5 days/week; individuals were exposed to each concentration twice), fewer occurrences of irritation were reported during week 2 than during week 1 at the same exposure concentration Ferguson et al. (1977). However, in the same Ferguson et al. (1977) study, the occurrences of irritation in two individuals exposed to 50 ppm for 6 hours/day, 5 days/week for 6 weeks was variable from week to week and did not show any clear trend. The study by Ihrig et al. (2006), and to a lesser extent the study by Ferguson et al. (1977), provide some evidence of decreased irritation following repeated exposure; the results of Ihrig et al. (2006) may also be influenced by attrition out of the workforce of those most affected by the irritation symptoms. These studies raise the possibility that repeated exposure could lead to the development of tolerance to ammonia (i.e., to decreased sensory responsiveness). It is possible, therefore, that industrially-exposed populations considered in deriving the RfC for ammonia (i.e., Holness et al. (1989), Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001)) may have developed some degree of tolerance to ammonia, and may underpredict responses to ammonia that would be observed in the general population. The magnitude of tolerance, if any, cannot be estimated from the available studies.

In addition, as discussed in the Literature Search Strategy | Study Selection and Evaluation section, the workers in the cross-sectional occupational studies used to derive the RfC were healthy enough to remain in the plant for a considerable time; mean employment duration ranged from 52 months to 18 years. In general, studies in these populations may result in a "healthy worker

survivor" bias and in an underestimate of the risk of health effects of ammonia exposure, as a healthy worker population may not exhibit health effects (such as decreased lung function or increased prevalence of respiratory symptoms) to the same degree that would be seen in the general population under the same conditions.

Therefore, there is potential for tolerance development in populations exposed occupationally to ammonia and "healthy worker" bias, both of which may result in underestimation of the general population response. However, the evidence is limited and not conclusive, and thus does not warrant increasing the intraspecies uncertainty factor.

2.1.5. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Confidence in the principal study (Holness et al., 1989) is medium. The design, conduct, and reporting of this occupational exposure study were adequate, but the study was limited by a small sample size and by the fact that workplace ammonia concentrations to which the study population was exposed were below those associated with ammonia-related effects (i.e., only a NOAEL was identified). However, the results from the principal study are supported by the results from other cross-sectional studies of workers in industrial settings, studies of ammonia exposure in a cleaning setting, studies in agricultural settings, multiple studies of acute ammonia exposure in volunteers, and the available inhalation data from animals.

Confidence in the database is medium. The inhalation ammonia database includes one limited study of reproductive and developmental toxicity in pigs that did not examine a complete set of reproductive or developmental endpoints. Normally, confidence in a database lacking these types of studies is considered to be lower due to the uncertainty surrounding the use of any one or several studies to adequately address all potential endpoints following chemical exposure at various critical lifestages. Unless a comprehensive array of endpoints is addressed by the database, there is uncertainty as to whether the critical effect chosen for RfC derivation is the most sensitive or appropriate. However, the likelihood of reproductive, developmental, and other systemic effects at the RfC is considered small because it is well documented that ammonia is endogenously produced in humans and animals, and any changes in blood ammonia levels at the POD would be small relative to normal blood ammonia levels. Further, EPA is not aware of any mechanisms by which effects at the point of contact (i.e., respiratory system) could directly or indirectly impact tissues or organs distal to the point of contact. Thus, confidence in the database, in the absence of these types of studies, is medium.

Reflecting medium confidence in the principal study and medium confidence in the database, the overall confidence in the RfC is medium.

2.1.6. Previous IRIS Assessment

The previous IRIS assessment for ammonia (posted to the database in 1991) presented an RfC of 0.1 mg/m^3 based on co-principal studies—the occupational exposure study of workers in a soda ash plant by Holness et al. (1989) and the subchronic study by Broderson et al. (1976) that examined the effects of ammonia exposure in F344 rats inoculated on day 7 of the study with the bacterium *M. pulmonis*. The NOAEL of 6.4 mg/m^3 (estimated as the mean concentration of the entire exposed group) from the Holness et al. (1989) study (duration adjusted: $NOAEL_{ADJ}$ = 2.3 mg/m^3) was used as the POD.[12]

The previous RfC was derived by dividing the exposure-adjusted POD of 2.3 mg/m^3 (from a NOAEL of 6.4 mg/m^3) by a composite UF of 30: 10 to account for the protection of sensitive individuals and 3 for database deficiencies to account for the lack of chronic data, the proximity of the LOAEL from the subchronic inhalation study in the rat (Broderson et al., 1976) to the NOAEL, and the lack of reproductive and developmental toxicity studies. A UF_D of 3 (rather than 10) was applied because studies in rats (Schaerdel et al., 1983b) showed no increase in blood ammonia levels at an inhalation exposure up to 32 ppm (22.6 mg/m^3) and only minimal increases at 300–1,000 ppm (212–707 mg/m^3), suggesting that no significant distribution is likely to occur at the human equivalent concentration.

[12]In this document, the lower confidence bound of the estimated mean exposure concentration in the high-exposure group from the Holness et al. (1989) study (13.6 mg/m^3, adjusted for continuous exposure to 4.9 mg/m^3) was identified as the POD because workers in this high-exposure group, as well as those in the two lower-exposure groups, showed no statistically significant increase in the prevalence of respiratory symptoms or decreases in lung function.

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