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Toxicological Review of Ammonia

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

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CONTENTS

TABLES AND FIGURES

ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BCG	bacillus Calmette-Guérin
BMC	benchmark concentration
BMD	benchmark dose
CAC	cumulative ammonia concentration
CCRIS	Chemical Carcinogenesis Research Information System
CFU	colony forming unit
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
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
LC_{50}	50% lethal concentration
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
PBPK	physiologically based pharmacokinetic
PEF	peak expiratory flow
PEFR	peak expiratory flow rate
POD	point of departure
PPD	purified protein derivative
RD_{50}	50% response dose
RfC	reference concentration
RfD	reference dose
RTECS	Registry of Toxic Effects of Chemical Substances
TLV	threshold limit value
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:

Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention, Department of Health & Human Services
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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 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) and ammonia is found at about 8% of hazardous waste sites on the National Priorities List (ATSDR, 2004). 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 for effects other than cancer. New information has become available, and this assessment reviews information on all health effects by all exposure routes.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. The findings of this assessment and related documents produced during its development are available on the IRIS website (http://www.epa.gov/iris/). Appendices for chemical and physical properties, the toxicity of ammonium salts, toxicokinetic information, summaries of toxicity studies and other information are provided as *Supplemental Information* to this assessment (see Appendices A to D).

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.

On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law[1]. The report language included direction to EPA for the IRIS Program related to recommendations provided by the National Research Council (NRC) in their review of EPA's draft IRIS assessment of formaldehyde. The NRC's recommendations, provided in Chapter 7 of their review report, offered suggestions to EPA for improving the development of IRIS assessments. The report language included the following:

[1]Pub. L. No. 112-74, Consolidated Appropriations Act, 2012.

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

Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in Appendix E. This documentation also includes an explanation for why certain recommendations were not incorporated.

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.

Chemical Properties and Uses

Ammonia is a corrosive gas with a pungent odor. It is highly soluble in water (up to 482 g/L) and is a weak base (Lide, 2008; O'Neil et al., 2006; Eggeman, 2001; Dean, 1985). Additional information on the chemical and physical properties of ammonia is presented in Appendix A.

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, 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).

Ammonia is a component of the global nitrogen cycle and is essential to many biologic 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). This assessment compares endogenous levels of ammonia in humans to the toxicity values that it derives.

Consideration of Ammonium Salts for Inclusion in This Assessment

EPA considered whether to include ammonium salts (e.g., ammonium acetate, chloride, and sulfate) in this assessment. These salts readily dissolve in water through dissociation into an ammonium cation (NH_4^+) and an anion. Oral toxicity studies on ammonium chloride and ammonium sulfate suggest that these salts may differ in toxicity (see Appendix B for a summary of subchronic/chronic toxicity information for selected ammonium salts), but it is not clear whether this reflects differences between the salts or in the effects that were studied. If the toxicity of the salts is affected by the anion, then it would not be correct to attribute toxic effects to the ammonium cation. ATSDR considered this question and concluded, ". . . that it would be inappropriate to extrapolate findings obtained with ammonium chloride (or any ammonium salt) to equivalent amounts of ammonium, but derived from a different salt" (ATSDR, 2004). Similarly, the World Health Organization considered ammonium chloride-induced kidney hypertrophy and observed that the extent to which it results from ammonium chloride-induced acidosis or from a direct effect of the ammonium ion is not clear (IPCS, 1986). Thus, in light of the uncertain influence of the anion on toxicity, ammonium salts were not used in the identification of effects or in the derivation of reference values for ammonia and ammonium hydroxide.

Assessments by Other National and International Health Agencies

Toxicity information on ammonia has been evaluated by ATSDR, the National Research Council, the American Conference of Governmental Industrial Hygienists, the National Institute for Occupational Safety and Health, and the Food and Drug Administration. The results of these assessments are presented in Appendix C. It is important to recognize that these earlier assessments were prepared for different purposes using different methods and could consider only the studies that were available at the time.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that EPA provide "an ample margin of safety to protect public health"; the Safe Drinking Water Act, that "no adverse effects on the health of persons may reasonably be anticipated to occur, allowing an adequate margin of safety." Accordingly, EPA uses information on the adverse effects of chemicals and on exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects from long-term exposure to chemicals and to characterize exposure-response relationships. An assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. Exceptions are chemicals currently used exclusively as pesticides, ionizing and non-ionizing radiation, and criteria air pollutants listed under section 108 of the Clean Air Act (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides; EPA's Integrated Science Assessments evaluate the effects from these pollutants in ambient air).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state government agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. These agents may be found in air, water, soil, or sediment. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. IRIS may assess other agents as an urgent public health need arises. IRIS also reassesses agents as significant new studies are published.

2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2009).

Step 1. Development of a draft Toxicological Review (usually about 11-1/2 months duration). The draft assessment considers all pertinent publicly available studies and applies consistent criteria to evaluate the studies, identify health effects, weigh the evidence of causation for each effect, identify mechanistic events and pathways, and derive toxicity values.

Step 2. Internal review by scientists in EPA programs and regions (2 months). The draft assessment is revised to address comments from within EPA.

Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President (1-1/2 months). The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and EPA's response to major comments become part of the public record.

Step 4. External peer review, after public review and comment (3-1/2 months or more, depending on the review process). EPA releases the draft assessment for public review and comment, followed by external peer review. The peer review meeting is open to the public and includes time for oral public comments. The peer reviewers also receive the written public comments. The peer reviewers assess whether the evidence has been assembled and evaluated according to guidelines and whether the conclusions are justified by the evidence. The peer review draft, peer review report, and written public comments become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary (2 months). The draft assessment is revised to reflect the peer review comments, public comments, and newly published studies that are critical to the conclusions of the assessment. The disposition of peer review

comments and public comments becomes part of the public record.

Step 6. Final EPA review and interagency science discussion with other federal agencies and the Executive Offices of the President (1-1/2 months). The draft assessment and summary are revised to address EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

Step 7. Completion and posting (1 month). The Toxicological Review and IRIS summary are posted on the IRIS website (http://www.epa.gov/iris/).

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (U.S. EPA, 2005a, b, 2000b, 1998, 1996, 1991, 1986a, b) and other methods (U.S. EPA, 2011b, 2006a, b, 2002, 2000a, 1994). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

3. Identifying and selecting pertinent studies

3.1. Identifying studies

Before beginning an assessment, EPA conducts a comprehensive search of the primary scientific literature. The literature search follows standard practices and includes the PubMed and ToxNet databases of the National Library of Medicine and other databases listed in EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. EPA posts the results of the literature search on the IRIS website and requests information from the public on additional studies and ongoing research.

EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act. Material submitted as Confidential Business Information is considered only if it includes health and safety data that can be publicly released. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, EPA will have it peer-reviewed.

EPA also examines the toxicokinetics of the agent to identify other chemicals (for example, major metabolites of the agent) to include in the assessment if adequate information is available, in order to more fully explain the toxicity of the agent and to suggest dose metrics for subsequent modeling.

In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components. The literature search seeks, in decreasing order of preference (U.S. EPA, 2000b, 1986b):

- Studies of the mixture being assessed.
- Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.
- Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

3.2. Selecting pertinent epidemiologic studies

Study design is the key consideration for selecting pertinent epidemiologic studies from the results of the literature search.

- Cohort studies and case-control studies provide the strongest epidemiologic evidence, as they collect information about individual exposures and effects.
- Ecologic studies (geographic correlation studies) relate exposures and effects by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.
- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare effect or about the relevance of analogous results in animals.

The assessment briefly reviews ecologic studies and case reports but reports details only if they suggest effects not identified by other epidemiologic studies.

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental studies from the results of the literature search.

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier

and are considered most pertinent to human environmental exposure.

- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

Exposure duration is also a key design consideration for selecting pertinent experimental studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from less-than-chronic exposure are pertinent but less preferred than studies of chronic exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information. Research involving human subjects is considered only if conducted according to ethical principles.

For developmental toxicity and reproductive toxicity, irreversible effects may result from a brief exposure during a critical period of development. Accordingly, specialized study designs are used for these effects (U.S. EPA, 2006b, 1998, 1996, 1991).

4. Evaluating the quality of individual studies

4.1. Evaluating the quality of epidemiologic studies

The assessment evaluates design and methodologic aspects that can increase or decrease the weight given to each epidemiologic study in the overall evaluation (U.S. EPA, 2005a, 1998, 1996, 1994, 1991):

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study and comparison populations.
- Ascertainment of exposure and the potential for misclassification.
- Ascertainment of disease or effect and the potential for misclassification.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects, including latent effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and the resulting potential for selection bias.
- Potential confounding and other sources of bias are identified and addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is prevalent in the population and is related to both exposure and outcome.

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating epidemiologic studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991).

4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodologic aspects that can increase or decrease the weight given to each experimental study in the overall evaluation (U.S. EPA, 2005a, 1998, 1996, 1994, 1991):

- Documentation of study design, animals or study population, methods, basic data, and results.
- Relevance to humans of the animal model and experimental methods.
- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.
- Sample sizes and statistical power to detect dose-related differences or trends.
- Ascertainment of survival, vital signs, disease or effects, and cause of death.
- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may

show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (U.S. EPA, 2005a).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991). In multi-generation studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (U.S. EPA, 1998, 1991).

4.3. Reporting study results

The assessment uses evidence tables to summarize details of the design and key results of pertinent studies. There may be separate tables for each site of toxicity or type of study.

If a large number of studies observe the same effect, the assessment considers the study characteristics in this section to identify the strongest studies or types of study. The tables report details from these studies, and the assessment explains the reasons for not reporting details of other studies or groups of studies that do not add new information. Supplemental material provides references to all studies considered, including those not summarized in the tables.

The assessment discusses strengths and limitations that affect the interpretation of each study. If the interpretation of a study in the assessment differs from that of the study authors, the assessment discusses the basis for the difference.

As a check on the selection and evaluation of pertinent studies, EPA asks peer reviewers to identify studies that were not adequately considered.

5. Weighing the overall evidence of each effect

5.1. Weighing epidemiologic evidence

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole to determine the extent to which any observed associations may be causal. Positive, negative, and null results are given weight according to study quality. This evaluation considers aspects of an association that suggest causality, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998) (U.S. EPA, 2005a; CDC, 2004; U.S. EPA, 2002, 1994).

Strength of association: The finding of a large relative risk with narrow confidence intervals strongly suggests that an association is not due to chance, bias, or other factors. Modest relative risks, however, may reflect a small range of exposures, an agent of low potency, an increase in an effect that is common, exposure misclassification, or other sources of bias.

Consistency of association: An inference of causality is strengthened if elevated risks are observed in independent studies of different populations and exposure scenarios. Reproducibility of findings constitutes one of the strongest arguments for causality. Discordant results sometimes reflect differences in study design, exposure, or confounding factors.

Specificity of association: As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less informative aspect of causality, unless the effect is rare or unlikely to have multiple causes.

Temporal relationship: A causal interpretation requires that exposure precede development of the effect.

Biologic gradient (exposure-response relationship): Exposure-response relationships strongly suggest causality. A monotonic increase is not the only pattern consistent with causality. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causality is strengthened by data demonstrating plausible biologic mechanisms, if available.

Coherence: An inference of causality is strengthened by supportive results from animal experiments, toxicokinetic studies, and short-term tests. Coherence may also be found in other lines of evidence, such as changing disease patterns in the population.

"Natural experiments": A change in exposure that brings about a change in disease frequency provides strong evidence of causality, for example, an intervention to reduce exposure in the workplace or environment that is followed by a reduction of an adverse effect.

Analogy: Information on structural analogues or on chemicals that induce similar mechanistic events can provide insight into causality.

These considerations are consistent with guidelines for systematic reviews that evaluate the quality and weight of evidence. Confidence is increased if the magnitude of effect is large, if there is evidence of an exposure-response relationship, or if an association was observed and the plausible biases would tend to decrease the magnitude of the reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al., 2008a; Guyatt et al., 2008b).

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may choose a descriptor such as *sufficient evidence*, *suggestive evidence*, *inadequate evidence*, or *evidence suggestive of no causal relationship* to characterize the epidemiologic evidence of each effect (CDC, 2004).

5.2. Weighing experimental animal evidence

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Several concepts discussed by Hill (1965) are pertinent to the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 2005a, 2002, 1994).

In weighing evidence from multiple experiments, (U.S. EPA, 2005a) distinguishes

Conflicting evidence (that is, mixed positive and negative results in the same sex and strain using a similar study protocol) from

Differing results (that is, positive results and negative results are in different sexes or strains or use different study protocols).

Negative or null results do not invalidate positive results in a different experimental system. EPA regards all as valid observations and looks to methodological differences or, if available, mechanistic information to reconcile differing results.

It is well established that there are critical periods for some developmental and reproductive effects. Accordingly, the assessment determines whether critical periods have been adequately investigated (U.S. EPA, 2006b, 2005a, b, 1998, 1996, 1991). Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

In evaluating evidence of genotoxicity:

- Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (*in vivo*) provides the strongest evidence.
- This is followed by positive results in lower organisms or in cultured cells (*in vitro*) or for other genetic events.
- Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC, 2006).

For germ-cell mutagenicity, EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (U.S. EPA, 1986a).

5.3. Characterizing modes of action

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. Information on mode of action is not required for a conclusion that an effect is causally related to an agent (U.S. EPA, 2005a).

The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a).

(1) **Is the hypothesized mode of action sufficiently supported in test animals?** Strong support for a key event being necessary to a mode of action can come from experimental challenge to the hypothesized mode of action, in which studies that suppress a key event observe suppression of the effect. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, much more so than by replicate experiments in the same model. The assessment may consider various aspects of causality in addressing this question.

(2) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed

between animals and humans, though it may hold for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis but is not used to determine relevance. Similarly, anticipated levels of human exposure are not used to determine relevance.

(3) **Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?** The assessment reviews the key events to identify populations and lifestages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or lifestages.

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (U.S. EPA, 2005a). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008a).

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity relationships to mutagenic carcinogens (U.S. EPA, 2005a).

5.4. Characterizing the overall weight of the evidence

After weighing the epidemiologic and experimental studies pertinent to each effect, the assessment may select a standard descriptor to characterize the overall weight of the evidence. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a).

Carcinogenic to humans: There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive animal evidence, identification of key precursor events in animals, and strong evidence that they are anticipated to occur in humans.

Likely to be carcinogenic to humans: The evidence demonstrates a potential hazard to humans but does not meet the criteria for *carcinogenic*. There may be a plausible association in humans, multiple positive results in animals, or a combination of human, animal, or other experimental evidence.

Suggestive evidence of carcinogenic potential: The evidence raises concern for effects in humans but is not sufficient for a stronger conclusion. This descriptor covers a range of evidence, from a positive result in the only available study to a single positive result in an extensive database that includes negative results in other species.

Inadequate information to assess carcinogenic potential: No other descriptors apply. *Conflicting evidence* can be classified as *inadequate information* if all positive results are opposed by negative studies of equal quality in the same sex and strain. *Differing results*, however, can be classified as *suggestive evidence* or as *likely to be carcinogenic*.

Not likely to be carcinogenic to humans: There is robust evidence for concluding that there is no basis for concern. There may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

Multiple descriptors may be used if there is evidence that carcinogenic effects differ by dose range or exposure route (U.S. EPA, 2005a).

EPA is investigating and may on a trial basis propose standard descriptors to characterize the overall weight of the evidence for effects other than cancer.

6. Selecting studies for derivation of toxicity values

For each effect where there is credible evidence of an association with the agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The decision to derive toxicity values may be linked to the weight-of-evidence descriptor. For example, EPA typically derives toxicity values for agents classified as *carcinogenic to humans* or as *likely to be carcinogenic* (U.S. EPA, 2005a).

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal (U.S. EPA, 2005a, 1994):

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.
- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model can be used to extrapolate across exposure routes.
- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.
- Studies with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with non-monotonic exposure-response relationships are not necessarily excluded from the analysis. A diminished effect at higher exposure levels may be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, exposure misclassification, or selection bias.

If a large number of studies are suitable for dose-response analysis, the assessment considers the study characteristics in this section to focus on the most informative data. The assessment explains the reasons for not analyzing other groups of studies. As a check on the selection of studies for dose-response analysis, EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a).

Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis. The modeling yields a *point of departure* (an exposure level near the lower end of the observed range, without significant extrapolation to lower doses) (sections 7.2-7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (sections 7.4-7.5). When response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors that account for sources of uncertainty and variability to the point of departure (section 7.6).

For a group of agents that induce an effect through a common mode of action, the dose-response analysis may derive a *relative potency factor* for each agent. A full dose-response analysis is conducted for one well-studied *index chemical* in the group, then the potencies of other members are expressed in relative terms based on relative toxic effects, relative absorption or metabolic rates, quantitative structure-activity relationships, or receptor binding characteristics (U.S. EPA, 2005a, 2000b).

Increasingly, EPA is basing toxicity values on combined analyses of multiple data sets or multiple responses. EPA also considers multiple dose-response approaches when they can be supported by robust data.

7.2. Modeling dose

The preferred approach for analysis of dose is toxicokinetic modeling because of its ability to incorporate a wide range of data. The preferred dose metric would refer to the active agent at the site of its biologic effect or to a close, reliable surrogate measure. The active agent may be the administered chemical or a metabolite. Confidence in the use of a toxicokinetic model depends on the robustness of its validation process and on the results of sensitivity analyses (U.S. EPA, 2006a, 2005a, 1994).

Because toxicokinetic modeling can require many parameters and more data than are typically available, EPA has developed standard approaches that can be applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration (U.S. EPA, 2005a, 1998, 1996, 1991).

- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.
  - Oral doses are scaled allometrically using $mg/kg^{3/4}$-d as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011b, 2005a).
  - Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 1994).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a).

In the absence of study-specific data on, for example, intake rates or body weight, EPA has developed recommended values for use in dose-response analysis (U.S. EPA, 1988).

7.3. Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to an effect. Such models require sufficient data to ascertain a mode of action and to quantitatively support model parameters associated with its key events. Because different models may provide equivalent fits to the observed data but diverge substantially at lower doses, critical biologic parameters should be measured from laboratory studies, not by model fitting. Confidence in the use of a toxicodynamic model depends on the robustness of its validation process and on the results of sensitivity analyses. Peer review of the scientific basis and performance of a model is essential (U.S. EPA, 2005a).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, EPA has developed a standard set of empirical ("curve-fitting") models (http://www.epa.gov/ncea/bmds/) that can be applied to typical data sets, including those that are nonlinear. EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2000a). Additional judgment or alternative analyses are used when the procedure fails to yield reliable results, for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses (U.S. EPA, 2005a).

Modeling is used to derive a point of departure (U.S. EPA, 2005a, 2000a). (See section 7.6 for alternatives if a point of departure cannot be derived by modeling.)

- For dichotomous responses, the point of departure is often the 95% lower bound on the dose associated with a 10% response, but a lower response that falls within the observed range may be used instead. For example, reproductive or developmental studies often have power to detect a 5% response; epidemiologic studies, 1% or lower.
- For continuous responses, the point of departure is ideally the dose where the effect becomes biologically significant. In the absence of such definition, both statistical and biologic factors are considered.

7.4. Extrapolating to lower doses

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation is typically used for known and likely carcinogens. Low-dose extrapolation considers what is known about modes of action (U.S. EPA, 2005a).

(1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model below the observed range if significant model uncertainty can be ruled out with reasonable confidence.

(2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:
  - Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.
  - Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

  Linear extrapolation is also used if the evidence is insufficient to establish a mode of action.

  The result of linear extrapolation is described by an *oral slope factor* or an *inhalation unit risk*, which is the slope of the dose-response curve at lower doses or concentrations, respectively.

(3) Nonlinear extrapolation is used if there are sufficient data to ascertain the mode of action

and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

If linear extrapolation is used, the assessment develops a candidate slope factor or unit risk for each suitable data set. These results are arrayed, using common dose metrics, to show the distribution of relative potency across various effects and experimental systems. The assessment then derives an overall slope factor and an overall unit risk for the agent, considering the various dose-response analyses, the study preferences discussed in section 6, and the possibility of basing a more robust result on multiple data sets.

7.5. Considering susceptible populations and lifestages

The assessment analyzes the available information on populations and lifestages that may be particularly susceptible to each effect. A tiered approach is used (U.S. EPA, 2005a).

(1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or lifestage, these data are analyzed to derive separate toxicity values for susceptible individuals.

(2) If data on risk-related parameters allow comparison of the general population and susceptible individuals, these data are used to adjust the general-population toxicity values for application to susceptible individuals.

(3) In the absence of chemical-specific data, EPA has developed *age-dependent adjustment factors* for early-life exposure to suspected carcinogens that have a mutagenic mode of action. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, EPA recommends (U.S. EPA, 2005b):

- 10-fold adjustment for exposures before age 2 years.
- 3-fold adjustment for exposures between ages 2 and 16 years.

7.6. Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a necessary key event does not occur below a specific dose. Reference values provide no information about risks at higher exposure levels.

The assessment characterizes effects that form the basis for reference values as adverse, considered to be adverse, or a precursor to an adverse effect. For developmental toxicity, reproductive toxicity, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1998, 1996, 1991).

To account for uncertainty and variability in the derivation of a lifetime human exposure where effects are not anticipated to occur, reference values are calculated by applying a series of *uncertainty factors* to the point of departure. If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level or a lowest-observed-adverse-effect level is used instead. The assessment discusses scientific considerations involving several areas of variability or uncertainty.

Human variation. A factor of 10 is applied to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. This factor is reduced only if the point of departure is derived specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, 1998, 1996, 1994, 1991).

Animal-to-human extrapolation. A factor of 10 is applied if animal results are used to make inferences about humans. This factor is often regarded as comprising toxicokinetics and toxicodynamics in equal parts. Accordingly, if the point of departure is based on toxicokinetic modeling, dosimetry modeling, or allometric scaling across species, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicodynamic differences. An animal-to-human factor is not applied if a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species (U.S. EPA, 2011b, 2002, 1998, 1996, 1994, 1991).

Adverse-effect level to no-observed-adverse-effect level. If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such

effects are not expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, 1998, 1996, 1994, 1991).

Subchronic-to-chronic exposure. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure. This factor may also be applied for developmental or reproductive effects if exposure covered less than the full critical period. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response (U.S. EPA, 2002, 1998, 1994).

Incomplete database. If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor (U.S. EPA, 2002, 1998, 1996, 1994, 1991). The size of the factor depends on the nature of the database deficiency. For example, EPA typically follows the suggestion that a factor of 10 be applied if both a prenatal toxicity study and a two-generation reproduction study are missing and a factor of $10^{1/2}$ if either is missing (U.S. EPA, 2002).

In this way, the assessment derives candidate reference values for each suitable data set and effect that is credibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (U.S. EPA, 1994). The assessment then selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur.

The assessment may also report reference values for each effect. This would facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (U.S. EPA, 2002).

7.7. Confidence and uncertainty in the reference values

The assessment selects a standard descriptor to characterize the level of confidence in each reference value, based on the likelihood that the value would change with further testing. Confidence in reference values is based on quality of the studies used and completeness of the database, with more weight given to the latter. The level of confidence is increased for reference values based on human data supported by animal data (U.S. EPA, 1994).

High confidence: The reference value is not likely to change with further testing, except for mechanistic studies that might affect the interpretation of prior test results.

Medium confidence: This is a matter of judgment, between high and low confidence.

Low confidence: The reference value is especially vulnerable to change with further testing.

These criteria are consistent with guidelines for systematic reviews that evaluate the quality of evidence. These also focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al., 2008a).

All assessments discuss the significant uncertainties encountered in the analysis. EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

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EXECUTIVE SUMMARY

Occurrence and Health Effects

Ammonia occurs naturally in air, soil and water and is produced by humans and other animals as part of normal biological processes. Ammonia is also used as an agricultural fertilizer. Exposure to ammonia occurs primarily through breathing air containing ammonia gas, and may also occur via diet or direct skin contact.

Health effects observed at levels exceeding naturally-occurring concentrations are generally limited to the site of direct contact with ammonia (skin, eyes, respiratory tract, and digestive tract). Short-term exposure to high levels of ammonia can cause irritation and serious burns on the skin and 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. Studies in experimental animals similarly suggest 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 or water may adversely affect other organs, such as the stomach, liver, adrenal gland, kidney, and spleen. There is inadequate information to evaluate the carcinogenicity of ammonia.

Effects Other Than Cancer Observed Following Oral Exposure

There are few oral toxicity studies for ammonia. Gastric toxicity may be a hazard for ammonia based on evidence from case reports in humans and mechanistic studies in experimental animals. Evidence in humans is limited to case reports of individuals suffering from gastrointestinal effects from ingesting household cleaning solutions containing ammonia or biting into capsules of ammonia smelling salts. The experimental animal toxicity database for ammonia lacks standard toxicity studies that evaluate a range of tissues/organs and endpoints. In rats, gastrointestinal effects, characterized as increased epithelial cell migration in the mucosa of the stomach leading to decreased thickness of the gastric mucosa, were reported following short-term and subchronic exposures to ammonia via ingestion (Hata et al., 1994; Tsujii et al., 1993; Kawano et al., 1991). While these studies provide consistent evidence of changes in the gastric mucosa associated with exposure to ammonia in drinking water, the investigators reported no evidence of microscopic lesions of the stomach, gastritis, or ulceration in the stomachs of these rats.

Given the limited scope of toxicity testing of ingested ammonia and questions concerning the adversity of the gastric mucosal findings in rats, the available oral database for ammonia was

considered insufficient to characterize toxicity outcomes and dose-response relationships, and **an oral reference dose (RfD) for ammonia was not derived**.

Effects Other Than Cancer Observed Following Inhalation Exposure

Respiratory effects have been identified as a hazard following inhalation exposure to ammonia. Evidence for respiratory toxicity associated with inhaled ammonia comes from studies in humans and animals. Cross-sectional occupational studies involving chronic exposure to ammonia have consistently demonstrated an increased prevalence of symptoms consistent with respiratory irritation and decreased lung function (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Holness et al., 1989). Cross-sectional studies of livestock farmers exposed to ammonia, controlled volunteer studies of ammonia inhalation, and case reports of injury in humans with inhalation exposure to ammonia provide additional, consistent support for the respiratory system as a target of ammonia toxicity. Additionally, respiratory effects were observed in several animal species following short-term and subchronic inhalation exposures to ammonia.

The experimental toxicology literature for ammonia also provides evidence that inhaled ammonia may be associated with toxicity to target organs other than the respiratory system, including the liver, adrenal gland, kidney, spleen, heart, and immune system, at concentrations higher than those associated with respiratory system effects. Less evidence exists for these effects than for respiratory effects.

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 increased respiratory symptoms Occupational epidemiology study Holness et al. (1989)	$NOAEL_{ADJ}$: 3.1 mg/m^3	10	0.3 mg/m^3

[a]Because the study involved workplace exposure conditions, the NOAEL of 8.8 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) was identified as the principal study for RfC derivation. Respiratory effects, characterized as increased respiratory symptoms (including cough, phlegm. chronic bronchitis, wheeze, chest tightness, and dyspnea) and decreased lung function, observed 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 in any of the three exposure categories, including the high-exposure category (>8.8 mg/m^3), and the control group. The Holness et al. (1989) study in conjunction with a second occupational study by Rahman et al. (2007) collectively provide information useful for examining the relationship between chronic ammonia exposure and increased prevalence of respiratory symptoms and decreased lung function. Both studies reported either the presence or absence of respiratory effects in workers exposed to ammonia over a range of concentrations (approximately 4–18 mg/m^3), with the no-observed-adverse-effect level (NOAEL) of 8.8 mg/m^3 from the Holness et al. (1989) study falling between the NOAEL and lowest-observed-adverse-effect level (LOAEL) (4.9 and 18.5 mg/m^3, respectively) from the Rahman et al. (2007) study. The NOAEL of 8.8 mg/m^3 ($NOAEL_{ADJ}$ = 3.1 mg/m^3, i.e., adjusted to continuous exposure) from the Holness et al. (1989) study was used as the point of departure (POD) for RfC derivation.

An RfC of 0.3 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

Under 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 and volunteer 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, reproductive, developmental, and other systemic effects are not expected at the RfC because it is well documented that ammonia is endogenously produced in humans and animals, ammonia concentrations in blood are homeostatically regulated to remain at low levels, and ammonia concentrations in air at the POD are not expected to alter homeostasis.

Evidence of Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "**inadequate information to assess carcinogenic potential**" for ammonia, based on the absence of ammonia carcinogenicity studies in humans and a single lifetime drinking water study of ammonia in mice (Toth, 1972) that showed no evidence of carcinogenic potential. There is limited evidence that ammonia may act as a cancer promoter (Tsujii et al., 1995; Tsujii et al., 1992a). The

available genotoxicity studies are inadequate to characterize the genotoxic potential of ammonia. **A quantitative cancer assessment for ammonia was not conducted.**

Susceptible Populations and Lifestages

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.

Studies of the toxicity of ammonia in children or young animals compared to other lifestages that would support an evaluation of childhood susceptibility have not been conducted.

Key Issues Addressed in the Assessment

Endogenous Ammonia

Ammonia, which is produced endogenously, has been detected in the expired air of healthy volunteers. Ammonia concentrations in breath exhaled from the mouth or oral cavity (0.085–2.1 mg/m^3) are higher and more variable than concentrations measured in breath exhaled from the nose and trachea (0.013–0.078 mg/m^3). See Appendix D, Section D.3 (Elimination) and Table D-1 for further discussion of studies that examined ammonia in exhaled breath. Concentrations exhaled from the mouth and oral cavity are largely attributed to the production of ammonia via bacterial degradation of food protein in the oral cavity or gastrointestinal tract, and can be influenced by factors such as diet, oral hygiene, and age. In contrast, the lower ammonia concentrations measured in breath exhaled from the nose and trachea more likely reflect levels of ammonia circulating in the blood. These levels are lower than the ammonia RfC of 0.3 mg/m^3 by a factor of at least fourfold. Although the RfC falls within the range of concentrations measured in the mouth or oral cavity, exhaled ammonia is rapidly diluted in the larger volume of ambient air and would not contribute significantly to ammonia exposure. Further, occupational epidemiology studies served as the basis for the ammonia RfC; the worker populations in these studies would have been exposed to ammonia that also included endogenously produced ammonia, and as such the RfC accounts for ammonia exposures from endogenous sources.

LITERATURE SEARCH STRATEGY | STUDY SELECTION

The primary, peer-reviewed literature pertaining to ammonia was identified through a keyword search of the databases listed in Table LS-1. References from health assessments developed by other national and international health agencies and review articles were also examined. EPA requested the public submit additional data on December 21, 2007 (U.S. EPA, 2007); no submissions were received. The last search was conducted in March 2012.

Figure LS-1 depicts the literature search and study selection strategy and the number of references obtained at each stage of literature screening. Approximately 22,400 references were identified with the initial keyword search. Based on a secondary keyword search followed by a preliminary manual screen of titles or abstracts by a toxicologist, approximately 1,022 references were identified that provided information potentially relevant to characterizing the health effects or physical and chemical properties of ammonia. A more detailed review of titles, abstracts, and/or papers pared this to 32 epidemiological studies (i.e., studies of occupational or livestock worker populations or short-term exposure studies in volunteers), 43 case reports, 62 oral or inhalation animal studies, 104 other studies (e.g., studies that provided supporting information on physical and chemical properties, mechanisms, and toxicokinetics). The majority of the toxicokinetics studies came from the ATSDR (2004) *Toxicological Profile of Ammonia*[2] or were identified based on a focused keyword search (e.g., for studies on ammonia in exhaled breath or ammonia in fetal circulation).

[2]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 (ATSDR, 2004) 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 to share scientific information.

Table LS-1. Details of the literature search strategy

Database	Keywords[a]
Pubmed Toxcenter Toxline Current Contents (2008 & 2010 only)	**Chemical names (CASRN):** ammonia (7664-41-7); ammonium hydroxide (1336-21-6)[b] Synonyms: spirit of hartshorn; aquammonia **Initial keyword search** <u>Standard toxicology search</u> Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors <u>Chemical-specific keywords</u> Respiration; metabolism; breath tests; inhalation; air; breath; exhalation; biological markers; analysis **Secondary keyword search**[c] reproductive; developmental; teratogen; gastrointestinal; stomach; gastric AND mucosa, cancer OR tumor; genotoxicity; kidney OR spleen AND toxicity; exhaled breath; respiratory irritation, symptom OR disease, including dyspnea, bronchitis, pneumonitis, asthma; lung; pulmonary function; chest tightness; inflammation; congestion; edema; hemorrhage; discharge; epithelium; immune; immunosuppression; hypersensitivity; skin lesion; erythema; host resistance; bacterial colonization; T-cell; liver function OR toxicity; fatty liver; clinical chemistry; adrenal; heart AND toxicity; myocardium; lacrimation; ocular symptoms; blood pH; brain AND amino acid; neurotransmitter. The following terms were used to filter out reference not relevant to the evaluation of the health effects of ammonia: hyperammonemia; ammonemia; hepatic coma; liver failure; Reye syndrome; hepatic encephalopathy; cirrhosis; fish; daphnia; crustaceans; amphibians.
TSCATS ChemID Chemfinder CCRIS HSDB GENETOX RTECS	Searched by chemical names (including synonyms) and CASRNs[b]

[a]The use of certain keywords in a given database was contingent on number and type of results. The large number of search results required restriction search terms to filter out references not relevant to evaluation of ammonia health effects and limiting metabolism results to studies in animals and humans.

[b]As discussed in the Preface, literature on ammonium salts were not included in this review because of the uncertainty as to whether the anion of the salt can influence the toxicity of the ammonium compound (see also Appendix B, Table B-1).

[c]Secondary keywords were selected from an understanding of the targets of ammonia toxicity gained from review of papers identified in literature searches conducted at the start of document development and relevant review documents.

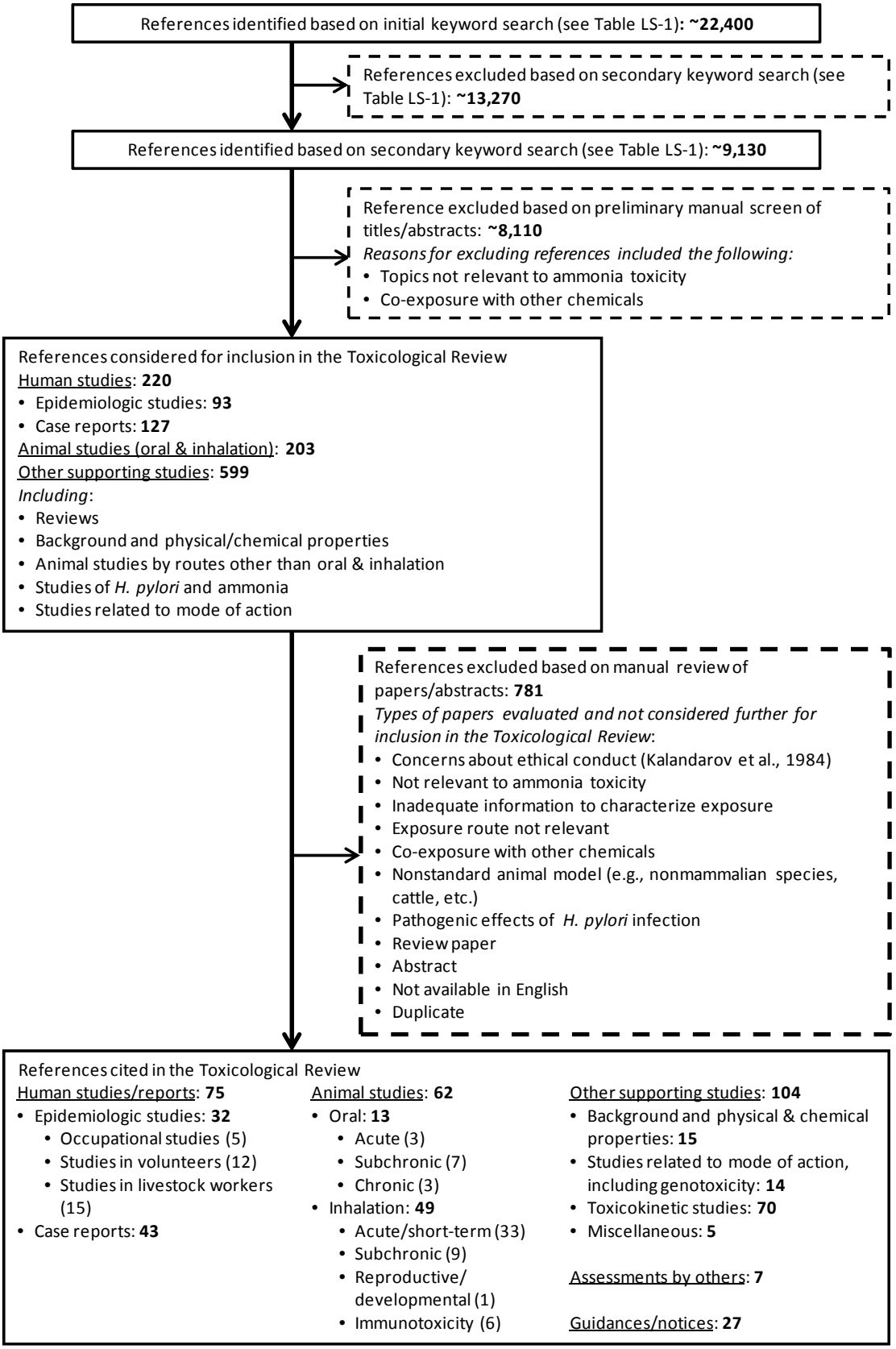

Figure LS-1. Literature search and study selection strategy for ammonia.

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 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 majority of the human studies consisted of case reports involving acute ammonia exposure; because case reports are generally anecdotal and thereby provide little information that would be useful for characterizing chronic health hazards. These studies were only briefly reviewed, and representative citations from this collection of literature are provided as supplemental information in Appendix D, Section D.2.

The references considered for inclusion, as well as those cited in this document, including bibliographic information and abstracts, can be found on the Health and Environmental Research On-line (HERO) website[3] (http://hero.epa.gov/ammonia).

[3]HERO is a database of scientific studies and other references used to develop EPA's risk 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 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

1. HAZARD IDENTIFICATION

1.1. Synthesis of Evidence

1.1.1. Respiratory Effects

The respiratory system is the primary target of toxicity of inhaled ammonia in humans and experimental animals. Four cross-sectional occupational epidemiology studies (Rahman et al., 2007; Ali et al., 2001; Holness et al., 1989) examined the association between inhaled ammonia and prevalence of respiratory symptoms and changes in lung function. The association between ammonia exposure and respiratory effects suggested by these cross-sectional studies is also informed by studies of livestock farmers, volunteer studies involving acute exposures to inhaled ammonia, human case reports, and subchronic inhalation toxicity studies in various experimental animal species. The evidence of respiratory effects in humans and experimental animals exposed to ammonia is summarized in Tables 1-1 and 1-2, respectively, and as an exposure-response array in Figure 1-1 at the end of this section.

Respiratory Symptoms

Ammonia is an upper respiratory tract irritant in humans. Respiratory symptoms (including cough, chest tightness, stuffy/runny nose, sneezing, phlegm, wheezing, dyspnea, chronic bronchitis, and asthma) were reported in two cross-sectional studies of industrial worker populations exposed to ammonia (Rahman et al., 2007; Ballal et al., 1998) (see Table 1-1 at the end of this section). Rahman et al. (2007)[4] found up to a 4.1-fold higher prevalence of respiratory symptoms (cough, chest tightness, stuffy nose, runny nose, and sneezing) in workers exposed to a mean ammonia concentration of 18.5 mg/m^3 (high-exposure group) for about 16 years compared to a control group (administration building workers); the prevalence of cough and chest tightness were statistically significantly elevated in the high-exposure group compared to the control group. The prevalences of respiratory symptoms in the low-exposure group exposed to a mean ammonia concentration of 4.9 mg/m^3 were up to threefold higher than those in the control group, but none were statistically significantly different from control.

Significantly higher relative risks (ranging from 1.6- to 4.7-fold) for cough, phlegm, wheezing, dyspnea, chronic bronchitis, and asthma were also observed in workers from another cross-sectional study (Ballal et al., 1998) with ammonia exposure concentrations higher than the

[4]Rahman et al. (2007) examined respiratory effects in workers from two plants in a urea fertilizer factory. Workers in the urea plant were exposed to higher concentrations of ammonia (arithmetic mean = 18.5 mg/m^3) than workers in the ammonia plant (arithmetic mean = 4.9 mg/m^3). Therefore, the urea plant workers represented the high-exposure group, and the ammonia plant workers represented the low-exposure group. Exposure to dusts and other contaminants, except for nitrogen dioxide, were not measured; however, based on information about the production process and previous literature, the authors considered ammonia to be the major exposure agent in this work environment.

American Conference of Governmental Industrial Hygienists [ACGIH] threshold limit value [TLV] of 18 mg/m^3 compared with workers exposed to levels below the TLV. Distribution of respiratory symptoms by cumulative ammonia concentration (CAC, mg/m^3-years) also showed significantly higher relative risks for respiratory symptoms among workers with higher CAC (>50 mg/m^3-years) compared to those with a lower CAC (<50 mg/m^3-years) (Ballal et al., 1998). Only Ballal et al. (1998) evaluated respiratory symptoms in terms of cumulative ammonia exposure.

In a third cross-sectional study of ammonia-exposed male workers, no differences were observed in the prevalence of respiratory symptoms, eye irritation, or odor detection threshold between the ammonia-exposed workers (concentrations were relatively lower than those in Rahman et al. [2007] and Ballal et al. [1998]) and the control group (Holness et al., 1989), when evaluating all ammonia-exposed workers as one group or when stratifying them into three exposure categories: high = >8.8 mg/m^3, medium = 4.4–8.8 mg/m^3, or low = <4.4 mg/m^3. Although respiratory irritation prevalence was similar across groups, the exposed workers reported that exposure in the plant aggravated some of their reported respiratory symptoms (cough, sputum, chronic bronchitis, wheeze, chest tightness, dyspnea, chest pain, rhinitis); however, no further information was provided as to how the authors evaluated aggravation of symptoms. Co-exposures to dust and inorganic gases such as nitrogen dioxide and sulfur dioxide were possible in these cross-sectional studies; however, except for the low levels of nitrogen dioxide identified in the Rahman et al. (2007) study, these workplace exposures were not measured or reported.

Overall, the cross-sectional occupational epidemiology studies that evaluated the prevalence of respiratory symptoms provide consistent estimates of the effect level associated with exposure to ammonia. Rahman et al. (2007) observed that exposure to 18.5 mg/m^3 ammonia increased the prevalence of respiratory symptoms (up to 4.1-fold). This is consistent with the observation by Ballal et al. (1998) that workers in a factory with ammonia concentrations exceeding the TLV of 18 mg/m^3 had significantly higher relative risks (up to 4.7-fold) for respiratory symptoms. The prevalence of respiratory symptoms was not increased following occupational exposures at lower workplace concentrations; i.e., >8.8 mg/m^3 (Holness et al., 1989) and 4.9 mg/m^3 ammonia (Rahman et al., 2007).

Elevated prevalence of respiratory symptoms, including cough, phlegm, wheezing, chest tightness, and eye, nasal, and throat irritation, have been reported in livestock farmers and stable workers compared to controls (Melbostad and Eduard, 2001; Preller et al., 1995; Choudat et al., 1994; Zejda et al., 1994; Crook et al., 1991; Heederik et al., 1990); (Monsó et al., 2004) (see Appendix D, Section D.2 and Table D-7 for more detailed information). Additionally, bronchial hyperreactivity to methacholine or histamine challenge (tests used to assist in the diagnosis of asthma by provoking bronchoconstriction) was increased in farmers exposed to ammonia compared to control workers (Vogelzang et al., 2000; Vogelzang et al., 1997; Choudat et al., 1994), indicating that exposure to ammonia and other air contaminants in farm settings may contribute to chronic airway inflammation. In addition to ammonia, these studies also documented exposures to airborne dust, bacteria, fungal spores, endotoxin, and mold—agents that could also induce respiratory symptoms and airway effects. The release of other volatiles on livestock farms is likely,

but measurements for other volatile chemicals were not conducted. Therefore, while several studies have reported associations between ammonia exposure in livestock farmers or stable workers and an increase in respiratory symptoms, these findings are of limited use because of exposures to other constituents in air that likely confound this association.

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; Altmann et al., 2006; Ihrig et al., 2006; Verberk, 1977; Silverman et al., 1949) provide support for ammonia as a respiratory irritant (see Appendix D, Section D.2 and Table D-8 for more detailed information, including documentation of human subjects research ethics procedures). Two controlled-exposure studies report habituation to eye, nose, and throat irritation in volunteers after several weeks of ammonia exposure (Ihrig et al., 2006; 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, dyspnea, persistent, productive cough, bilateral diffuse rales and rhonchi, mucous production, pulmonary edema, marked hypoxemia, and necrosis of the lung) (see Appendix D, Section D.2, for more detailed information and references).

Experimental studies in laboratory animals also provide consistent evidence that repeated exposure to ammonia can affect the respiratory system (see Appendix D, Section D.3 for more detailed information). 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 or 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 continuously exposed to 127 mg/m^3 ammonia for 90 days and 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 show that ammonia does not produce respiratory effects in laboratory animals. Lung congestion, edema, and hemorrhage were observed in guinea pigs and mice exposed to 14 mg/m^3 ammonia for 42 days (Anderson et al. (1964). However, no increase in the incidence of respiratory or other diseases common to young pigs were 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).

Lung Function

Decreased lung function in ammonia-exposed workers has been reported in two cross-sectional studies of industrial worker populations (Rahman et al., 2007; Ali et al., 2001) that measured lung function (Rahman et al., 2007; Ali et al., 2001; Holness et al., 1989). Ammonia exposure was correlated with a significant decline in lung function over the course of a work shift (cross-shift) as measured by forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1 % predicted) in the high-exposure worker group (mean ammonia concentration of 18.5 mg/m^3) in a fertilizer factory (Rahman et al., 2007). In a second study (Ali et al., 2001), the FVC% predicted was higher in fertilizer factory workers exposed to ammonia than in controls (4.6% increase, $p \leq 0.002$); FEV_1 % predicted was higher (1.5%) in the exposed workers but the difference was not statistically significant. When Ali et al. (2001) based their analysis on measures of cumulative exposure, workers with cumulative exposure >50 mg/m^3-years had significantly lower FVC% predicted (5.4% decrease, $p \leq 0.030$) and FEV_1% predicted (7.4% decrease, $p < 0.006$) than workers with cumulative ammonia exposure ≤50 mg/m^3-years, but had similar FEV_1/FVC%. The authors did not explain the inconsistent findings across the analyses of noncumulative and cumulative exposures.

Lung function did not appear to be affected in worker populations chronically exposed to ammonia at concentrations below approximately 18 mg/m^3. Baseline lung function, based on spirometry (test measuring lung function volume and flow) conducted at the beginning and end of the work shift, differed very slightly relative to control in workers exposed to ammonia concentrations ranging from <4.4 to >8.8 mg/m^3 in a cross-sectional study of male workers in a soda ash plant (Holness et al., 1989), but was not statistically significant. Additionally, no changes in lung function were observed over either work shift (days 1 or 2) or over the work week in the exposed group compared with controls. Similarly, measures of lung function (FVC, FEV_1, and PEFR [peak expiratory flow rate]) in workers exposed to a mean concentration of 4.9 mg/m^3 ammonia (low-exposure group) in a urea [$CO(NH_2)_2$] fertilizer factory showed no significant cross-shift changes.

Decreased lung function (e.g., measured as decreased FEV_1, FVC) was reported in farmers with ammonia exposure from animal waste (Monsó et al., 2004; Cormier et al., 2000; Donham et al., 2000; Vogelzang et al., 1998; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Crook et al., 1991; Heederik et al., 1990) (see Appendix D, Section D.2 and Table D-7). These findings are of limited use because of the failure of these studies to account and control for exposures to other constituents in air (including respirable dust, bacteria, fungal spores, endotoxin, and mold) that can affect lung function, and likely confound the association between exposure to ammonia and decreased lung function observed in these study populations.

Changes in lung function following acute exposure to ammonia have been observed in some, but not all, controlled exposure studies conducted in volunteers (see Appendix D, Section D.2 and Table D-8). 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 at concentrations of up to 35 mg/m^3 for 10 minutes in an exposure chamber (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.

1

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

Study design and reference	Results
Respiratory symptoms	
Cross-sectional occupational study of soda ash plant workers in Canada; 58 exposed workers and 31 controls (from stores and office areas of plant)[a] 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 Average exposure: 12 y Holness et al. (1989)	Percentage of workers reporting symptoms (%): Flu: Control (n = 31) 3; Exposed (n = 58) 7; *p*-value 0.63 Cough: 10; 16; 0.53 Sputum: 16; 22; 0.98 Bronchitis: 19; 22; 0.69 Wheeze: 10; 10; 0.91 Chest tightness: 6; 3; 0.62 Dyspnea: 13; 7; 0.05 Chest pain: 6; 2; 0.16 Rhinitis: 19; 10; 0.12 Throat: 3; 7; 0.53
Cross-sectional occupational study of urea fertilizer factory in Bangladesh; 63 ammonia plant workers, 77 urea plant workers, and 25 controls (administration building staff) Low-exposure group (ammonia plant)[b]: 6.9 ppm (4.9 mg/m^3) High-exposure group (urea plant)[b]: 26.1 ppm (18.5 mg/m^3) Mean employment duration: 16 y Rahman et al. (2007)	Percentage of workers reporting symptoms (%): Cough: Control (n = 25) 8; Low exposed (*p*-value)[1] (n = 63) 17 (0.42); High exposed (*p*-value)[2] (n = 77) 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 and for urea plant compared to ammonia plant
Cross-sectional occupational study of two urea fertilizer factories in Saudi Arabia; 161 exposed workers and 355 unexposed controls[c] Exposures were stratified > or < the ACGIH TLV of 18 mg/m^3 Mean employment duration: 51.8 mo (exposed workers) and 73.1 mo (controls) Ballal et al. (1998)	Relative risks for those exposed to ammonia at concentrations >TLV (>18 mg/m^3) as compared to those exposed at levels ≤TLV (≤18 mg/m^3): Cough: 4-fold Phlegm: 4.7-fold Wheezing: 2.2-fold Dyspnea: 4-fold Chronic bronchitis: 1.6-fold Asthma: 3.7-fold

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

Study design and reference	Results
Lung function	
Cross-sectional occupational study of soda ash plant workers in Canada; 58 exposed workers and 31 controls (from stores and office areas of plant)[a] 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 Average exposure: 12 y Holness et al. (1989)	Control (n = 31) / Exposed (n = 58) / *p*-value Lung function (% predicted values): FVC 98.6 / 96.8 / 0.094 FEV_1 95.1 / 94.1 / 0.35 FEV_1/FVC 96.5 / 97.1 / 0.48 Change in lung function over work shift: FVC day1 -0.9 / -0.8 / 0.99 day 2 +0.1 / -0.0 / 0.84 FEV_1 day 1 -0.2 / -0.2 / 0.94 day 2 +0.5 / +0.7 / 0.86
Cross-sectional occupational study of urea fertilizer factory in Bangladesh; 63 ammonia plant workers, 77 urea plant workers, and 25 controls (staff from administration building) Low-exposure group (ammonia plant)[b]: 6.9 ppm (4.9 mg/m^3) High-exposure group (urea plant)[b] : 26.1 ppm (18.5 mg/m^3) Mean employment duration: 16 y Rahman et al. (2007)	Pre-shift / Post-shift / *p*-value Ammonia plant (low-exposure group); n = 24 of 63 ammonia plant workers[d] 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); n = 64 of 77 urea plant workers[d] 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.
Cross-sectional occupational study of a urea fertilizer factory in Saudi Arabia—follow-up of Ballal et al. (1998); 73 exposed workers and 348 unexposed controls Exposures were stratified < or > the ACGIH TLV of 18 mg/m^3 Mean employment duration: not reported Ali et al. (2001)	Control (n = 348) / Exposed (n = 73) / *p*-value FEV_1% predicted 96.6 / 98.1 / NS FVC% predicted 101.0 / 105.6 / 0.002 FEV_1/FVC% 83.0 / 84.2 / NS ≤50 mg/m^3-y / >50 mg/m^3-y / *p*-value FVC_1% predicted 100.7 / 93.4 / 0.006 FVC% predicted 105.6 / 100.2 / 0.03 FEV_1/FVC% 84.7 / 83.4 / NS NS = not significant (*p*-values not provided by study authors)

[a]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.

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

Study design and reference	Results

[b]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, more confidence is attributed to the PAC III air measurements of ammonia for the Rahman et al. (2007) study.

[c]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.

[d]Lung function testing was not performed on all workers; only the morning shift was chosen for data collection for practical reasons and workers who planned to have less than a 4-hr working day were excluded.

1

Table 1-2. Evidence pertaining to respiratory effects in animals following inhalation exposure

Study design and reference	Results
Effects on the lungs	
Squirrel monkey (*Saimiri sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Sprague-Dawley & Long-Evans rat; male and female; 15–51/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks Coon et al. (1970)	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]
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	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]
Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d or 455 mg/m^3 for 65 d Coon et al. (1970)	Dyspnea (mild) at 455 mg/m^3. Focal or diffuse interstitial pneumonitis in all animals, and calcification of bronchial epithelium observed in several animals at 470 mg/m^3.[a,b]
Guinea pig (strain not specified); male and female; 2/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d or 50 ppm (35 mg/m^3) for 42 d Anderson et al. (1964)	Lung congestion, edema and hemorrhage observed at 14 and 35 mg/m^3 after 42 d.[a]
Swiss albino mouse; male and female; 4/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d Anderson et al. (1964)	Lung congestion, edema, and hemorrhage observed at 14 mg/m^3 after 42 d.[a]
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) Done et al. (2005)	No increase in the incidence of respiratory or other diseases.
Pig (crossbred); sex not specified; 4–8/group 0, 50, or 75 ppm (0, 35, or 53 mg/m^3 for 109 d) Curtis et al. (1975)	Turbinates, trachea, and lungs of all pigs were classified as normal.
Effects on the upper respiratory tract	
Sherman rat; 5/sex/group 10 or 150 ppm (7 or 106 mg/m^3) from bedding for 75 d Broderson et al. (1976)[c]	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 106 mg/m^3.[a]

Table 1-2. Evidence pertaining to respiratory effects in animals following inhalation exposure

Study design and reference	Results
F344 rat; 6/sex/group 0 or 250 ppm (0 or 177 mg/m^3) in an inhalation chamber for 35 d Broderson et al. (1976)[c]	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 177 mg/m^3.[a]
Yorkshire-Landrace pig; sex not specified; 6/group 0 or 100 ppm (0 or 71 mg/m^3) for 6 wks Doig and Willoughby (1971)	↑ thickness of nasal and tracheal epithelium (50–100% increase).[a]
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Sprague-Dawley & Long-Evans rat; male and female; 15–51/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 weeks Coon et al. (1970)	Dyspnea in rats and dogs exposed to 770 mg/m^3 during week 1 only; no indication of irritation after week 1; nasal tissues not examined for gross or histopathologic changes.
Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	Nasal discharge at 470 mg/m^3.[a]
Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d or 455 mg/m^3 for 65 d Coon et al. (1970)	Nasal irritation in all animals at 455 mg/m^3.[a,b]
White albino mouse; male; 50 Ammonia vapor of 0 or 12% ammonia solution for 15 min/d, 6 d/wk, for 8 wks Gaafar et al. (1992)	Histological changes in the nasal mucosa.[a]
Duroc pig; both sexes; 9/group 12, 61, 103, 145 ppm (8, 43, 73, or 103 mg/m^3) for 5 wks Stombaugh et al. (1969)	Excessive nasal, lacrimal, and mouth secretions and ↑ frequency of cough at 73 and 103 mg/m^3.[a]

[a]Incidence data not provided.

[b]Exposure to 455 and 470 mg/m^3 ammonia increased mortality in rats.

[c]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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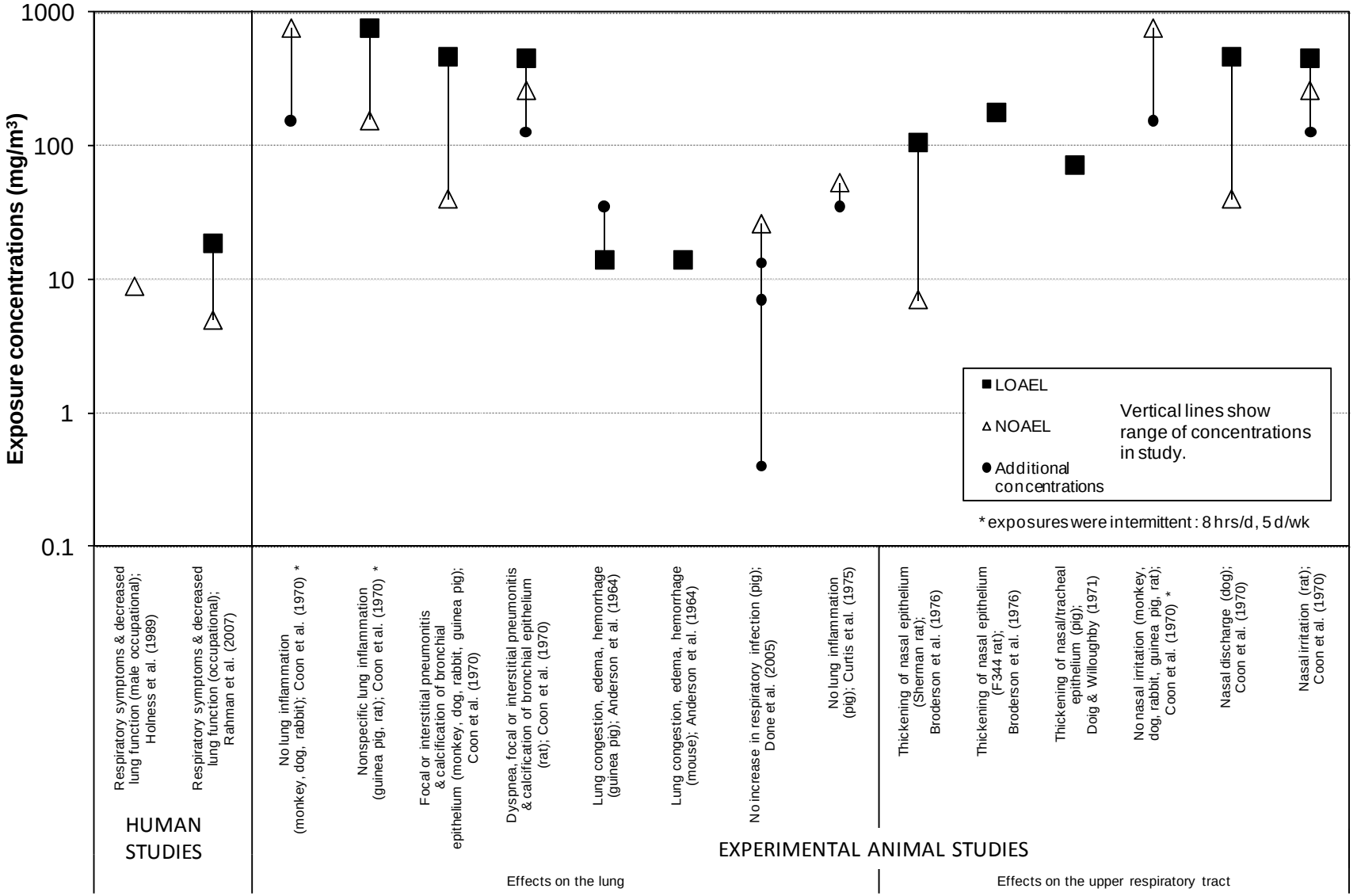

Figure 1-1. Exposure-response array of respiratory effects following inhalation exposure.

Mode-of-Action Analysis—Respiratory Effects

Data regarding 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. Cross-sectional occupational studies involving chronic exposure to ammonia have consistently demonstrated an increased prevalence of symptoms consistent with respiratory irritation (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali et al., 2001) (see Appendix D, Section D.2 and Tables D-3 to D-6). Cross-sectional studies of livestock farmers exposed to ammonia, controlled volunteer studies of ammonia inhalation, and case reports of injury in humans with inhalation exposure to ammonia provide additional and consistent support for the respiratory system as a target of ammonia toxicity when inhaled (see Appendix D, Section D.2 and Tables D-7 and D-8).

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 dose regimens (Gaafar et al., 1992; Broderson et al., 1976; Doig and Willoughby, 1971; Coon et al., 1970; Anderson et al., 1964) (see Appendix D, Section D.3). In general, responses in respiratory tissues increased with increasing ammonia exposure concentration. The evidence of observed respiratory effects seen across multiple human and animal studies identifies respiratory system effects as a hazard from ammonia exposure via inhalation.

1.1.2. Gastrointestinal Effects

Reports of gastrointestinal effects of ammonia in humans are limited to case reports involving intentional or accidental ingestion of household cleaning solutions or ammonia inhalant capsules (Dworkin et al., 2004; Rosenbaum et al., 1998; Christesen, 1995; Wason et al., 1990; Lopez et al., 1988; Klein et al., 1985; Klendshoj and Rejent, 1966) (see Appendix D, Section D.2). Clinical signs of gastrointestinal effects reported in these case studies include stomachache, nausea, diarrhea, drooling, erythematous and edematous lips, reddened and blistered tongues, dysphagia,

vomiting, oropharyngeal burns, laryngeal and epiglottal edema, erythmatous esophagus with severe corrosive injury, and hemorrhagic esophago-gastro-duodeno-enteritis.

The experimental animal toxicity database for ammonia lacks standard toxicity studies that evaluate a range of tissues/organs and endpoints. Exposure to ammonia in drinking water has, however, been associated with effects on the gastric mucosa. Evidence for this association comes from animal studies (Hata et al., 1994) designed to investigate the mechanisms by which the bacterium *Helicobacter pylori*, which produces a potent urease that increases ammonia production, may have a significant role in the etiology of chronic atrophic gastritis (see Appendix D, Section D.3). Statistically significant decreases of 40–60% in the thickness of the antral gastric mucosa were reported in Sprague-Dawley rats administered 0.01% ammonia in drinking water for durations of 2–8 weeks (Tsujii et al., 1993; Kawano et al., 1991); estimated doses were 22 mg/kg-day (Kawano et al., 1991) and 33 mg/kg-day (Tsujii et al., 1993). The magnitude of the decrease in gastric mucosal thickness increased with dose and duration of ammonia exposure (Tsujii et al., 1993; Kawano et al., 1991). Further, the effect was more prominent in the mucosa of the antrum region of the stomach than in the body region of the stomach.[5] Antral gastric mucosal thickness decreased significantly (by 56–59% of the tap water control) at 4 and 8 weeks of exposure to 0.01% ammonia in drinking water, but there was no significant effect on the thickness of the body gastric mucosa. Similarly, the height of fundic and pyloric glands in the gastric mucosa was decreased by approximately 30% in Donryu rats exposed to ammonia in drinking water for up to 24 weeks at concentrations of 0.02 and 0.1% (estimated doses of 28 and 140 mg/kg-day, respectively) (Hata et al., 1994).

Mucosal cell proliferation and migration (as measured by 5-bromo-2'-deoxyuridine labeling) were also significantly increased in rats exposed to ammonia (Tsujii et al., 1993). The authors observed that it was not clear whether mucosal cell proliferation was primarily stimulated directly by ammonia or indirectly by increased cell loss followed by compensatory cell proliferation. Cell proliferation in the gastric mucosa was also affected in the 24-week drinking water study in Donryu rats (Hata et al., 1994), although the pattern differed from that reported by Tsujii et al. (1993). The labeling index in gastric mucosal glands was increased at earlier time points (up to week 1 for fundic glands and up to week 4 for pyloric glands), suggesting enhanced cell cycling subsequent to repeated erosion and repair. At later time points (up to 24 weeks of exposure), however, the labeling index was decreased, a finding the authors' attributed to reduced capability of the generative cell zone of the mucosal region.

The gastric changes observed by Kawano et al. (1991), Tsujii et al. (1993), and Hata et al. (1994) were characterized by the study authors as consistent with changes observed in human atrophic gastritis; however, Kawano et al. (1991) and Tsujii et al. (1993) observed that no mucosal lesions were found macroscopically or microscopically in the stomachs of rats after exposure to ammonia in drinking water for 4–8 weeks, and Hata et al. (1994) reported that there was no

[5]The body is the main, central region of the stomach. The antrum is the distal part of the stomach near the pyloric sphincter and adjacent to the body.

evidence of ammonia-induced gastritis or ulceration in rats following 24-weeks of exposure to 0.1% ammonia in drinking water.

A relationship between ammonia ingestion and gastrointestinal effects is supported by findings from three acute oral studies in rats following gavage administration of ammonium hydroxide (Nagy et al., 1996; Takeuchi et al., 1995; Murakami et al., 1990). Takeuchi et al. (1995) reported hemorrhagic necrosis of the gastric mucosa in male Sprague-Dawley rats that received a single gavage dose of ammonium hydroxide (concentration ≥1%). Nagy et al. (1996) observed severe hemorrhagic mucosal lesions in female Sprague-Dawley rats 15 minutes after exposure to an estimated dose of 48 mg/kg ammonium hydroxide via gavage. Lesions of the gastric mucosa, including necrosis, were observed in male Sprague-Dawley rats 15 minutes after being given 1 mL of ammonia by intubation at concentrations of 0.5–1%, but not at concentrations of 0.025–0.1% (Murakami et al., 1990).

The evidence of gastrointestinal effects in experimental animals following oral exposure to ammonia is summarized in Table 1-3 and as an exposure-response array in Figure 1-2.

Table 1-3. Evidence pertaining to gastrointestinal effects in animals following oral exposure

Study design and references	Results[a]
Histopathologic changes of the gastric mucosa	
Sprague-Dawley rat; male; 6/group 0, 0.01 or 0.1% in drinking water (0, 22, or 220 mg/kg-d)[b] for 2 or 4 wks Kawano et al. (1991)	*% change in thickness of mucosa compared to control*: Antrum: Wk 2: 0, -5, -20*%; Wk 4: 0, -38*, -61*% Body: Wk 2: 0, -1, 3%; Wk 4: 0, -22, -30*%
Sprague-Dawley rat; male; 36/group 0 or 0.01% in drinking water (0 or 33 mg/kg-d)[c] for 3 d or 1, 2, 4, or 8 wks; tap water provided for the balance of the 8-wk study Tsujii et al. (1993)	*% change in thickness of mucosa compared to control (at d 3, wks 1, 2, 4, and 8)*: Antrum: D 3: 0, 8%; Wk 1: 0, -4%; Wk 2: 0, 6%; Wk 4: 0, -44%*; Wk 8: 0, -41%* Body: D 3: 0, 5%; Wk 1: 0, 1%; Wk 2: 0, 4%; Wk 4: 0, -1%; Wk 8: 0, -5% (extracted from Figure 3 of Tsujii et al., 1993)
Donryu rat; male; 6/group and time point 0, 0.02, or 0.1% in drinking water (0, 28, or 140 mg/kg-d)[c] for 1, 3, or 5 days and 1, 4, 8, 12, or 24 weeks Hata et al. (1994)	*% change in gland height compared to control (week 24):* Fundic region: 0, -18*, -34*% Pyloric region: 0, -17*, -26*% (estimated from Figure 3 of Hata et al., 1994) *% change in labeling index compared to control (week 24):* Fundic region: 0, -35*, -27*% Pyloric region: 0, -17*, -11*%

[a]% change compared to control calculated as: (treated value – control value)/control value x 100.

[b]Doses were estimated based on a body weight of 230 g for male rats and an estimated drinking water intake of 50 mL/d (as reported by study authors).

[c]Doses were estimated based on an initial body weight of 150 g and an estimated drinking water intake of 50 mL/d (as reported by study authors).

[d]Body weights and drinking water intakes were not provided by the authors. Doses were estimated assuming a body weight of 267 g (subchronic value for a male Sprague-Dawley rat, Table 1-2, (U.S. EPA, 1988)) and a drinking water intake of 37 mL/d (subchronic value for a male Sprague-Dawley rat, Table 1-5, (U.S. EPA, 1988)).

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

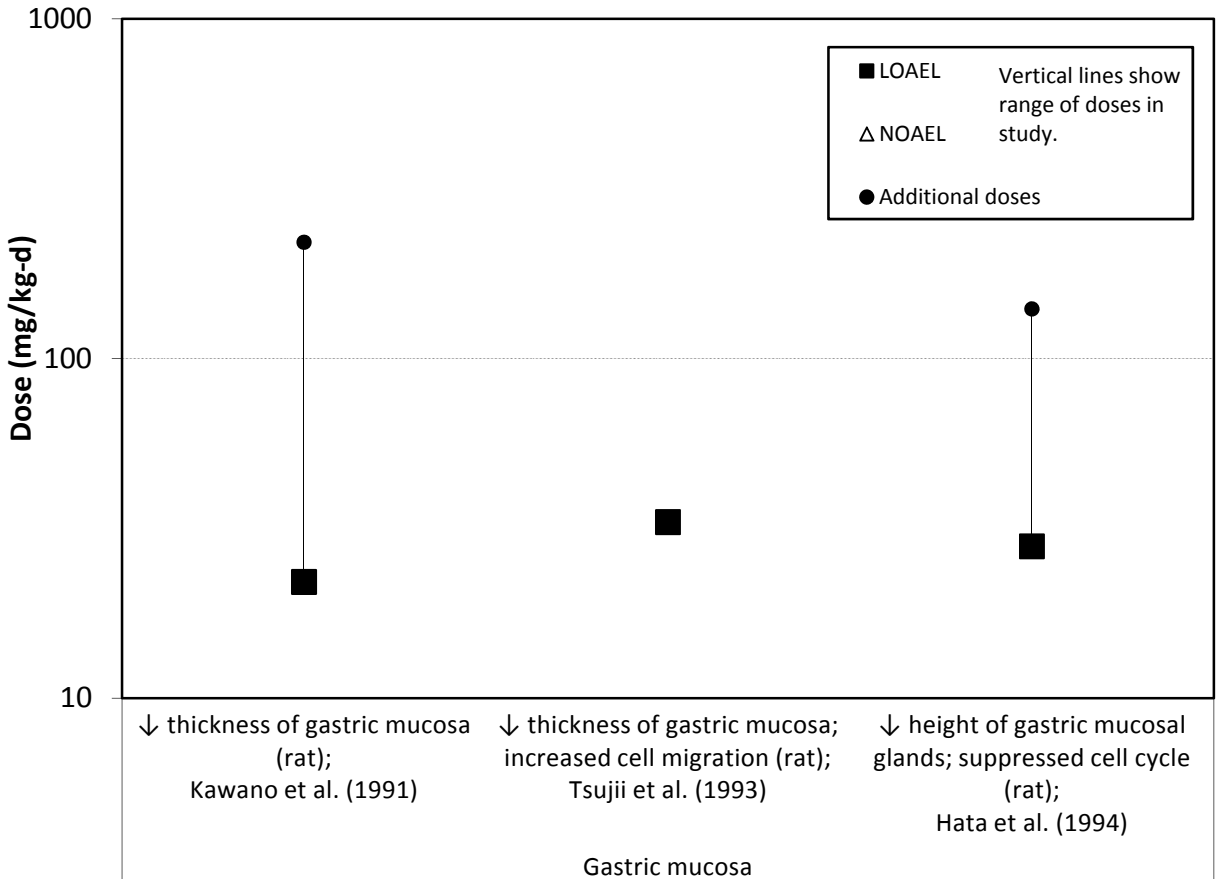

Figure 1-2. Exposure-response array of gastrointestinal effects following oral exposure.

Mode-of-Action Analysis—Gastrointestinal Effects

The alkalinity of the ammonia solution does not seem to play a direct role in the gastric effects associated with ammonia. An ammonia solution (pH 10.3) produced dose-related acute macroscopic mucosal lesions, whereas a glycine-sodium hydroxide buffer (pH 10.3) or ammonium chloride (pH 4.5) did not (Tsujii et al., 1992b). Rather, the ability of ammonia to damage the gastric mucosa may be related to its ionization state. Ammonia (NH_3) can easily penetrate cell membranes, subsequently reacting to form NH_4^+ and OH^- in the interior of the membrane (Tsujii et al., 1992b). The finding that antral and body regions of the rat stomach mucosa responded differently following administration of 33 mg/kg-day ammonia in drinking water for 8 weeks (Tsujii et al., 1993) is consistent with the influence of ionization. The hydrogen chloride secreted by the mucosa in the body of the stomach resulted in a lower pH in the body mucosa and a corresponding decrease in the ratio of ammonia to ammonium ion. In contrast, in the antral mucosa (a nonacid-secreting area), the pH was higher, the ratio of ammonia to ammonium ion was increased, and measures of gastric mucosal changes were increased compared to those observed in the stomach body where there was relatively higher exposure to NH_4^+.

Several specific events that may contribute to the induction of gastric mucosal changes by ammonia have been proposed. Increased cell vacuolation and decreased viability of cells were associated with increasing ammonia concentration in an in vitro system (Mégraud et al., 1992); the effect was not linked to pH change because of the high buffering properties of the medium. Using an in situ rat stomach model, hemorrhagic mucosal lesions induced by ammonia were associated with the rapid release and activation of cathepsins—which are mammalian cysteine proteases that are released from lysosomes or activated in the cytosol and can be damaging to cells, tissues, or organs (Nagy et al., 1996). Ammonia also appears to inhibit cellular and mitochondrial respiration, possibly by elevating intracellular or intraorganelle pH or by impairing adenosine triphosphate synthesis (Tsujii et al., 1992b). Mori et al. (1998) proposed a role for increased release of endothelin-1 and thyrotropin-releasing hormone from the gastric mucosa in ammonia-induced gastric mucosal injury based on findings in rats given ammonia intragastrically. Although a specific mechanism(s) by which ammonia may induce cellular toxicity has not been established, the available evidence suggests that ammonia-related acceleration of mucosal cell desquamation and stimulation of cell proliferation occurs via a compensatory mechanism (Tsujii et al., 1992a).

Summary of Gastrointestinal Effects

Evidence that oral exposure to ammonia causes gastrointestinal effects is based on human case reports and studies in rats that focused on mechanistic understandings of effects of ammonia on the gastric mucosa. Acute gastric toxicity observed in case reports involving intentional or accidental ingestion of cleaning solutions or ammonia inhalant capsules appears to reflect the corrosive properties of ammonia. Whether these acute effects are relevant to toxicity following chronic low-level ammonia exposure is not known. Indirect evidence for the biological plausibility of gastric tissue as a target of ammonia toxicity is provided by the association between the bacterium *H. pylori*, which produces urease that catalyzes urea into ammonia, and human diseases of the upper gastrointestinal tract (including chronic gastritis, gastric ulcers, and stomach cancer).

Three mechanistic studies in male rats (Hata et al., 1994; Tsujii et al., 1993; Kawano et al., 1991) provide consistent evidence of changes in the gastric mucosa associated with exposure to ammonia in drinking water, including decreased thickness or gland height. These gastric changes did not correlate, however, with other lesions in the stomach; no evidence of other microscopic lesions, gastritis, or ulceration was found in the stomachs of these rats. It is also interesting to note that chronic toxicity studies of other ammonia compounds have not identified the gastrointestinal tract as a target of ammonia toxicity. For example, no treatment-related changes in the stomach or other parts of the gastrointestinal tract were observed in Wistar rats exposed to ammonium chloride in the diet for 130 weeks at doses up to 1,200 mg/kg-day (Lina and Kuijpers, 2004) or in F344 rats exposed to ammonium sulfate for 104 weeks at a dose up to 1,371 mg/kg-day (Ota et al., 2006) (see Appendix B, Table B-1). Therefore, while drinking water studies with a mechanistic focus provide evidence for ammonia-related changes in rat gastric mucosa, adverse changes of the gastrointestinal tract were not identified in standard toxicity bioassays of ammonia compounds.

Mechanistic studies in rodent models support the biological plausibility that ammonia exposure may be associated with gastric effects in humans. Conditions that favor the un-ionized form of ammonia (pH>9.25) facilitate penetration of the cell membrane and are associated with greater gastric cytotoxicity. Given the evidence primarily from human case reports as supported by mechanistic studies in experimental animals, gastric effects may be a hazard from ammonia exposure.

1.1.3. Reproductive and Developmental Effects

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) in the only study of the reproductive and developmental toxicity of ammonia. 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 low- versus high-exposure 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 and possible confounding by exposures to bacterial and mycoplasm pathogens. The evidence of reproductive and developmental effects in experimental animals exposed to ammonia is provided in Table 1-4.

Table 1-4. Evidence pertaining to reproductive and developmental effects in animals following inhalation exposure

Study Design and Reference	Results
Crossbred gilts (female pigs); 4.5 months 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[a] Diekman et al. (1993)	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]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.

Summary of Reproductive/Developmental Effects

No studies of the potential reproductive or developmental toxicity of ammonia in humans are available, and only one animal study that examined the reproductive effects of ammonia in the pig has been conducted. This study did not use a conventional test species and did not include a control group with no ammonia exposure. Further, animals were exposed naturally to bacterial and mycoplasm pathogens.

Although the reproductive and developmental toxicity database for ammonia is limited, information on the endogenous formation of ammonia can inform the potential for ammonia to present a reproductive and developmental hazard. Ammonia is endogenously produced in humans and animals during fetal and adult life, and concentrations in blood are homeostatically regulated to remain at low levels. Studies in humans and animals demonstrate that ammonia is present in fetal circulation. In vivo studies in several animal species and in vitro studies of human placenta demonstrate that ammonia is produced within the uteroplacenta and released into the fetal and maternal circulations (Bell et al., 1989; Johnson et al., 1986; Hauguel et al., 1983; Meschia et al., 1980; Remesar et al., 1980; Holzman et al., 1979; Holzman et al., 1977; Rubaltelli and Formentin, 1968; Luschinsky, 1951). Jóźwik et al. (2005) reported that ammonia levels in human fetal blood (specifically, umbilical arterial and venous blood) at birth were 1.0–1.4 µg/mL, compared to 0.5 µg/mL in the mothers' venous blood. Ammonia was also present in human umbilical arterial and venous blood collected at delivery (range of 25–43 weeks of gestation), with umbilical arterial ammonia concentrations significantly higher than venous concentrations (DeSanto et al. (1993); there was no correlation between umbilical ammonia level and gestational age. In sheep, uteroplacental tissues are a site of ammonia production, with outputs of ammonia into both the uterine and umbilical circulations (Jóźwik et al., 1999). In late-gestation pregnant sheep that were catheterized to allow measurement of ammonia exposure to the fetus, concentrations of ammonia in umbilical arterial and venous blood and uterine arterial and venous blood ranged from approximately 0.39 to 0.60 µg/mL (Jóźwik et al., 2005; Jóźwik et al., 1999). Thus, the developing fetus and reproductive tissues are normally exposed to ammonia in blood, and external concentrations that do not alter homeostasis would not be expected to pose a developmental or reproductive hazard. Experimental animal data suggest that ammonia exposures below 18 mg/m^3 will not increase blood ammonia levels (Manninen et al., 1988; Schaerdel et al., 1983; see also Appendix D.1, Metabolism); however, information is not available to identify air concentrations of ammonia that could alter homeostasis.

1.1.4. 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 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, namely 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 immune system's ability 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 excluded from the hazard identification for 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. Further, the available studies 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 *Mycoplasma pulmonis* (10^8 colony forming units [CFU]) 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 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., 1978b). 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. (2000) 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 pulmonary 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 (DTH) 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 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 1-3.

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

Study design and reference	Results
Host resistance	
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* Broderson et al. (1976)	*% of animals with gross lesions:* 16 , 46, 66*, 33, and 83% No effect on CFU.

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

Study design and reference	Results
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* Schoeb et al. (1982)	↑ bacterial colonization (as a result of reduced bacterial clearance).
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* Richard et al. (1978b)	*% Mortality:* 50 and 86%*
Landrace X large white pigs; 10/group (sex unknown) <5 (control), 50, 100 ppm (3.5, 35, 71 mg/m^3), 63 d (continuous exposure) inoculated with *M. hyopneumoniae* on day 9 and *P. multocida* on d 28, 42, 56 Andreasen et al. (2000)	*% of animals with positive day 49 nasal swab:* 24, 100*, 90%*
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 Hamilton et al. (1998)	↑ bacterial colonization
Large white pigs; 5/group (sex unknown) 0 or 50 ppm (0 or 35 mg/m^3), 1 week pre-inoculation with *P. multocida*, 3 weeks post-inoculation Hamilton et al. (1999)	↑ bacterial colonization *Bacteria isolated from nasal cavities*: 3.18 and 4.30* CFU
T cell proliferation	
Hartley guinea pig; 8/group (sex unknown) <15, 50 or 90 ppm (<11 (control), 35 or 64 mg/m^3), 3 wks (continuous exposure) Targowski et al. (1984)	↓ proliferation in blood and bronchial T cells.
Delayed-type hypersensitivity	
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 Targowski et al. (1984)	*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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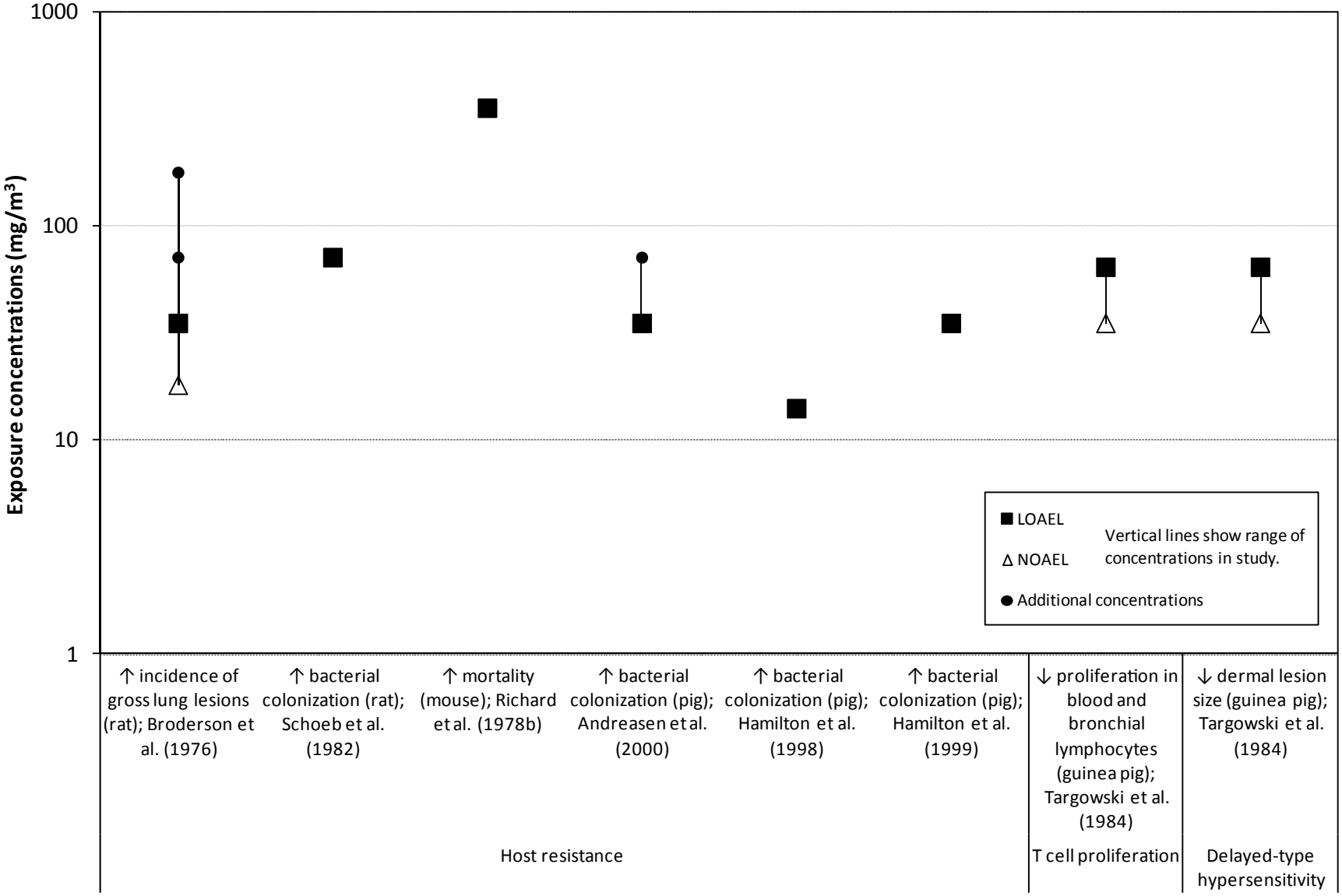

Figure 1-3. Exposure-response array of immune system effects following inhalation exposure.

Summary of Immune System Effects

The evidence for ammonia immunotoxicity is based on two epidemiological studies and seven 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 provide little support for ammonia immunotoxicity.

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., 1978b) 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. One study suggested that T cells are inhibited by ammonia (Targowski et al., 1984), but the data were not dose responsive.

Mechanistic data are not available that would support a biologically plausible mechanism for immunosuppression. Because ammonia damages the protective mucosal epithelium of the respiratory tract, it is unclear if elevated bacterial colonization is the result of damage to this barrier or the result of suppressed immunity. Overall, the evidence in humans and animals indicates that ammonia exposure may be associated with these effects, but does not support the immune system as a sensitive target of ammonia toxicity.

1.1.5. Other Systemic Effects

Although the majority of information suggests that ammonia induces effects in and around the portal of entry, there is limited evidence that ammonia can produce effects on organs distal from the portal of entry, including the liver, adrenal gland, kidney, spleen, and heart. Alterations in liver function, based on elevated mean levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea, decreased hemoglobin, and inhibition of catalase and monoamine oxidase (MAO) activities, were observed in workers exposed to ammonia over an average exposure duration of 12 years at an Egyptian urea production plant; measurements of workplace exposure concentrations were not provided (Hamid and El-Gazzar, 1996).

Evidence of hepatotoxicity in animals comes from observations of histopathological alterations in the liver. Fatty changes in liver plate cells were consistently reported at exposure concentrations ≥470 mg/m^3 ammonia in rats, guinea pigs, rabbits, dogs, and monkeys following identical subchronic inhalation exposure regimens (Coon et al., 1970). Congestion of the liver was observed in guinea pigs following subchronic and short-term inhalation exposure to 35 and 120 mg/m^3 (Anderson et al., 1964; Weatherby, 1952); no liver effects were observed in similarly exposed mice at 14 mg/m^3 (Anderson et al., 1964; Weatherby, 1952).

No histopathological or hematological effects 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; see Table 1-8 for

additional information); suggesting that animals can recover from intermittent exposure to elevated ammonia levels (Coon et al., 1970). In addition, no effects on organs distal from the respiratory system were observed in mice exposed to 14 mg/m^3 for up to 6 weeks (Anderson et al., 1964).

Adrenal effects were observed in animals following subchronic and short-term exposure to ammonia. Increased mean adrenal weights and fat content of the adrenal gland, as well as histological changes in the adrenal gland (enlarged cells of the zona fasiculata of the adrenal cortex that were rich in lipid), were observed in rabbits exposed via gavage to ammonium hydroxide for durations ranging from 5.5 days to 17 months (Fazekas, 1939). The strength of these findings is limited by inadequate reporting and study design. A separate study identified early degenerative changes in the adrenal glands of guinea pigs exposed to 120 mg/m^3 ammonia by inhalation for 18 weeks (Weatherby, 1952), providing additional limited evidence for effects on the adrenal gland.

Evidence that inhaled ammonia can affect the kidney and spleen is limited to studies in experimental animals. Nonspecific degenerative changes in the kidneys (not further described) of rats exposed 262 mg/m^3 ammonia were reported (Coon et al., 1970). Histopathological evaluation of other animal species in the same study exposed to 470 mg/m^3, an ammonia concentration that induced a high rate of mortality in rats, consistently showed alterations in the kidneys (calcification and proliferation of tubular epithelium; incidence not reported). Exposure of guinea pigs to inhaled ammonia at a concentration of 120 mg/m^3 for 18 weeks (but not 6 or 12 weeks) resulted in histopathological alterations (congestion) of the kidneys and spleen, although incidence was not reported (Weatherby, 1952). Enlarged and congested spleens were reported in guinea pigs exposed to 35 mg/m^3 ammonia for 6 weeks in a separate study (Anderson et al., 1964).

Myocardial fibrosis was observed in monkeys, dogs, rabbits, guinea pigs, and rats following subchronic inhalation exposure to 470 mg/m^3 ammonia; 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 dogs and rabbits, but was not observed in similarly treated monkeys and rats.

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; 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., 1983). Oxygen partial pressure (pO_2) in rats exposed to 11 and 23 mg/m^3 ammonia were statistically significantly increased, but remained within the normal range; exposure to 219 and 818 mg/m^3 over the same time period resulted in no change in pO_2 (Schaerdel et al., 1983). No explanation for a change in pO_2 only at the lower exposure concentrations was provided.

Encephalopathy related to ammonia may occur following disruption of the body's normal homeostatic regulation of the glutamine and urea cycles resulting in elevated ammonia levels in blood, e.g., as a result of severe liver or kidney disease (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 exposures.

The evidence of systemic toxicity in humans and experimental animals exposed to ammonia is summarized in Tables 1-6 to 1-8 and as an exposure-response array in Figure 1-4.

Table 1-6. Evidence pertaining to other systemic effects in humans following inhalation exposure

Study design and reference	Results
Occupational study workers in an Egyptian urea plant; 30 exposed and 30 control subjects No measurement of exposure concentrations Average employment time: 12 yrs Hamid and El-Gazzar (1996)	↑ AST, ALT, and blood urea in exposed workers; ↓ hemoglobin and inhibition of catalase and MAO.

Table 1-7. Evidence pertaining to other systemic effects in animals following oral exposure

Study design and reference	Results
Adrenal effects	
Rabbits (strain and sex not specified); 16–33/group 50–80 mL of a 0.5 or 1.0% ammonium hydroxide solution by gavage; initially every other day, later daily; duration ranged from 5.5 d to 17 mo; estimated dose: 61–110 mg/kg-d and 120–230 mg/kg-d, respectively[a] Fazekas (1939)	*Mean adrenal weight -- response relative to control:* 95% *Fat content of adrenal gland--response relative to control:* 4.5-fold ↑.

[a]Ammonia doses estimated using assumed average default body weight of 3.5–4.1 kilograms for adult rabbits (U.S. EPA, 1988).

Table 1-8. Evidence pertaining to other systemic effects in animals following inhalation exposure

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

Table 1-8. Evidence pertaining to other systemic effects in animals following inhalation exposure

Study design and reference	Results
Kidney and spleen effects	
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks Coon et al. (1970)	No histopathologic changes observed.
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3.[a]
Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d Coon et al. (1970)	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3.[a,b]
Guinea pig (strain not specified); male; 6–12/ group 0 or 170 ppm (0 or 120 mg/m^3) 6 hrs/d, 5 d/wk for 6, 12, or 18 wks Weatherby (1952)	Congestion of the spleen and kidneys.[a]
Guinea pig (strain not specified); male and female; 2/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d or 50 ppm (35 mg/m^3) for 42 d Anderson et al. (1964)	Enlarged and congested spleens at 35 mg/m^3.[a]
Swiss albino mouse; male and female; 4/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d Anderson et al. (1964)	No visible signs of toxicity.
Myocardial effects	
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 ds/wk for 6 wks Coon et al. (1970)	No histopathologic changes observed.

Table 1-8. Evidence pertaining to other systemic effects in animals following inhalation exposure

Study design and reference	Results
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	Myocardial fibrosis at 470 mg/m^3.[a]
Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d Coon et al. (1970)	Myocardial fibrosis at 470 mg/m^3.[a,b]
Ocular effects	
Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	Heavy lacrimation at 470 mg/m^3.[a]
New Zealand albino rabbit; male; 3/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	Erythema, discharge and ocular opacity over ¼ to ½ of cornea at 470 mg/m^3.[a]
Squirrel monkey (*S. sciureus*); male; 3/group Princeton-derived guinea pig; male and female; 15/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Coon et al. (1970)	No ocular irritation observed.
Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d Coon et al. (1970)	No ocular irritation observed.
Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks Coon et al. (1970)	No ocular irritation observed.
Blood pH changes	
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 Manninen et al. (1988)	↓ 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*

Table 1-8. Evidence pertaining to other systemic effects in animals following inhalation exposure

Study design and reference	Results
Crl:COBS CD(SD) rat; male; 32 and 70 15, 32, 310, 1157 ppm (11, 23, 219, 818 mg/m^3) for 0, 8, 12, 24 hrs, 3 and 7 d Schaerdel et al. (1983)	↑ pO_2 at 11 and 23 mg/m^3 for 8, 12 and 24 hrs; no change at higher concentrations; no change in blood pH. *Percent change in pO_2 from time 0 (at 24 hours of exposure)*[c]: 20*, 17*, 1, -2%
Amino acid levels and neurotransmitter metabolism in the brain	
Wistar rat; female; 5/group 0, 25 or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5 d Manninen and Savolainen (1989)	*% change compared to control*:[d] Brain glutamine: 42*, 40*%
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 Manninen et al. (1988)	*% change compared to control at 212 mg/m^3*:[d] Blood glutamine (5, 10, 15 d): 44*, 13, 14% Brain glutamine (5, 10, 15 d): 40*, 4, 2%

[a]Incidence data not provided.
[b]Exposure to 470 mg/m^3 ammonia increased mortality in rats.
[c]Measurements at time zero were used as a control; the study did not include an unexposed control group.
[d]% change compared to control calculated as: (treated value – control value)/control value x 100.

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

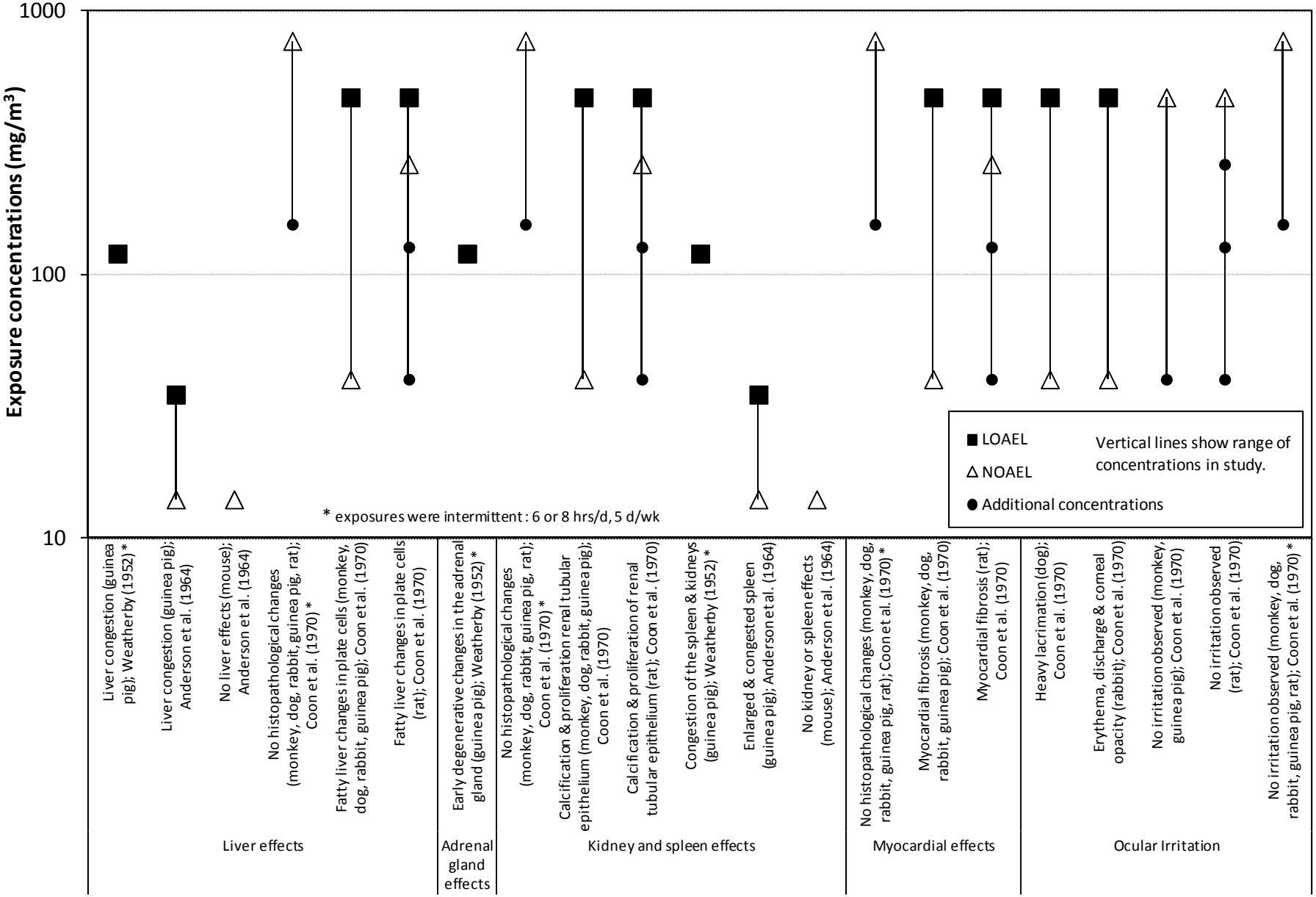

Figure 1-4. Exposure-response array of systemic effects following inhalation exposure.

Summary of Other Systemic Effects

Effects of ammonia exposure on organs distal from the portal of entry are based on evidence in animals and, to a more limited extent, in humans. One occupational epidemiology study of ammonia-exposed workers reported changes in serum enzymes indicative of altered liver function (Hamid and El-Gazzar, 1996). Because the study population was small and measurements of workplace ammonia concentrations were not provided, the evidence for liver effects in humans associated with ammonia exposure is weak.

Effects on various organs, including liver, adrenal gland, 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. For example, Coon et al. (1970) reported fatty liver and calcification and proliferation of renal tubular epithelium in monkeys, dogs, rabbits, and guinea pigs exposed continuously to ammonia for 90 days at a concentration of 470 mg/m^3, but no histopathological changes in these organs were observed in the same species following intermittent exposure (8 hours/day, 5 days/week for 6 weeks) to concentrations as high as 770 mg/m^3. It could be speculated that these differences in response reflect recovery from short-term (i.e., 8-hour) exposures, but the reason for the inconsistent findings is not known.

Studies of ammonia toxicity that examined other systemic effects were all published in the older toxicological literature. The only oral study of ammonium hydroxide was published in 1939 (Fazekas, 1939), and three subchronic inhalation studies were published between 1952 and 1970 (Coon et al., 1970; Anderson et al., 1964; Weatherby, 1952). In general, the information from these studies is limited by small group sizes, minimal characterization of some of the reported responses (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. (1964), 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.

As discussed in Section 1.1.3, ammonia is endogenously produced in all human and animal tissues, and concentrations in all physiological fluids are homeostatically regulated to remain at low levels (Souba, 1987). Thus, tissues are normally exposed to ammonia, and external concentrations that do not alter homeostasis would not be expected to pose a hazard for systemic effects. Overall, the evidence in humans and animals indicates that ammonia exposure may be associated with effects on organs distal from the portal of entry, but does not support the liver, adrenal gland, kidney, spleen, or heart as sensitive targets of ammonia toxicity.

1.1.6. Carcinogenicity

No information is available regarding the carcinogenic effects of ammonia in humans following oral or inhalation exposure. The carcinogenic potential of ammonia by the inhalation route has not been assessed in animals, and animal carcinogenicity data by the oral route of

exposure are limited. Toth (1972) concluded that tumor incidence was not increased in Swiss mice exposed for their lifetime (exact exposure duration not specified) to ammonium hydroxide in drinking water at concentrations up to 0.3% (equivalent to 410 and 520 mg/kg-day in female and male mice, respectively) or in C3H mice exposed to ammonium hydroxide in drinking water at a concentration of 0.1% (equivalent to 214 and 191 mg/kg-day in female and male mice, respectively). With the exception of mammary gland tumors in female C3H mice, concurrent control tumor incidence data were not reported and, therefore, comparison of tumor incidence in exposed and control mice could not be performed. The general lack of concurrent control data limits the ability to interpret the findings of this study.

The incidence of gastric cancer and the number of gastric tumors per tumor-bearing rat were statistically significantly higher in rats exposed to 0.01% ammonia solution in drinking water (equivalent to 10 mg/kg-day) for 24 weeks following pretreatment (for 24 weeks) with the initiator, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), compared with rats receiving only MNNG and tap water (Tsujii et al., 1992a). An ammonia-only exposure group was not included in this study. In another study with the same study design, Tsujii et al. (1995) reported similar increases in the incidence of gastric tumors in rats following exposure to MNNG and 10 mg/kg-day ammonia. Additionally, the size and penetration to deeper tissue layers of the MNNG-initiated gastric tumors were enhanced in the rats treated with ammonia (Tsujii et al., 1995). The investigators suggested that ammonia administered in drinking water may act as a cancer promoter (Tsujii et al., 1995; Tsujii et al., 1992a).

The evidence of carcinogenicity in experimental animals exposed to ammonia is summarized in Table 1-9.

Table 1-9. Evidence pertaining to cancer in animals following oral exposure

Study design and reference	Results
Carcinogenesis studies	
Swiss mouse; 50/sex/group 0.1, 0.2, and 0.3% ammonium hydroxide in drinking water for their lifetime [250, 440, and 520 mg/kg-d (males); 240, 370, and 410 mg/kg-d (females)][a] Toth (1972)	Tumor incidence not increased in ammonia-exposed mice; however, concurrent control tumor incidence data were not reported.
C3H mouse; 40/sex/group 0.1% ammonium hydroxide in drinking water for their lifetime [191 (males) and 214 mg/kg-d (females)][b] Toth (1972)	Tumor incidence not increased in ammonia-exposed mice; however, with the exception of mammary gland tumors in female mice, concurrent control tumor incidence data were not reported. *Mammary gland adenocarcinoma*: 76, 60%
Initiation-promotion studies	
Sprague Dawley rat; male; 40/group 0 or 0.01% ammonia in drinking water (0 or 10 mg/kg-d)[c] for 24 wks; both groups pretreated for 24 wks with the tumor initiator, MNNG; no ammonia-only group Tsujii et al. (1992a)	*Gastric tumor incidence:* 31, 70*% *# of gastric tumors/tumor-bearing rat:* 1.3, 2.1*
Sprague-Dawley rat; male; 43–44/group 0 or 0.01% ammonia in drinking water (0 or 10 mg/kg-d)[c] for 24 wks; both groups pretreated for 24 wks with the tumor initiator, MNNG; no ammonia-only group Tsujii et al. (1995)	*Gastric tumor incidence:* 30, 66*% *Penetrated muscle layer or deeper*: 12, 22*% *Size (mm):* 4.4, 5.3*

[a]Ammonium hydroxide doses estimated based on reported average daily drinking water intakes of 9.2, 8.2, and 6.5 mL/day for males and 8.3, 6.5, and 4.8 mL/day for females in the 0.1, 0.2, and 0.3% groups, respectively, and assumed average default body weights of 37.3 and 35.3 g for males and females, respectively (U.S. EPA, 1988).

[b]Ammonium hydroxide doses estimated based on reported average daily drinking water intakes of 7.9 and 8.4 mL/day for males and females, respectively, and assumed average default body weights of 37.3 and 35.3 g for males and females, respectively (U.S. EPA, 1988).

[c]Ammonia doses estimated based on reported drinking water intake of 50 mL/day and assumed average default body weight of 523 g for male Sprague-Dawley rats during chronic exposure (U.S. EPA, 1988).

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

A limited number of genotoxicity studies are available for ammonia vapor, including one study in exposed fertilizer factory workers in India that reported chromosomal aberrations and sister chromatid exchanges in lymphocytes (Yadav and Kaushik, 1997), two studies that found no evidence of DNA damage in rabbit gastric mucosal or epithelial cell lines (Suzuki et al., 1998; Suzuki et al., 1997), mutation assays in *Salmonella typhimurium* (not positive) and *Escherichia coli*

(positive) (Shimizu et al., 1985; Demerec et al., 1951), a micronucleus assay in mice (positive) (Yadav and Kaushik, 1997), one positive and one negative study in *Drosophila melanogaster* (Auerbach and Robson, 1947; Lobasov and Smirnov, 1934), and a positive chromosomal aberration test in chick fibroblast cells in vitro (Rosenfeld, 1932) (see Appendix D, Section D.4, Tables D-13 and D-14). The finding of chromosomal aberrations and sister chromatid exchanges in human lymphocytes (Yadav and Kaushik, 1997) was difficult to interpret because of the small number of samples and confounding in the worker population by smoking and alcohol consumption. In addition, the levels of ammonia in the plant were low compared to other fertilizer plant studies, raising questions about the study's exposure assessment. Positive findings in in vitro studies with nonhuman cell lines were difficult to interpret because of the presence of a high degree of toxicity (Demerec et al., 1951; Lobasov and Smirnov, 1934) or inadequate reporting (Rosenfeld, 1932). It is noteworthy that four of the eight available genotoxicity studies were published between 1932 and 1951. In two of the more recent studies, ammonia exposure did not induce DNA damage in rabbit gastric mucosal or epithelial cell lines in vitro (Suzuki et al., 1998; Suzuki et al., 1997). Overall, the available genotoxicity literature is inadequate to characterize the genotoxic potential of ammonia.

1.2. Summary and Evaluation

1.2.1. 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 that demonstrated an increased prevalence of respiratory symptoms consistent with irritation and changes in lung function. The findings of respiratory effects in cross-sectional studies of livestock farmers, controlled exposures in volunteers, and case reports of injury following acute exposure provide additional and consistent 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 seen 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, including the immune system, liver, adrenal gland, kidney, spleen, and heart, is less compelling than for the respiratory system. 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 sensitive target for ammonia toxicity**. Findings from animal studies indicate that **ammonia exposure may be associated with effects in the liver, adrenal gland,**

kidney, spleen, and heart; however, the weight of evidence indicates that these organs are not sensitive targets for ammonia toxicity.

A limited experimental toxicity database indicates that **oral exposure to ammonia may be associated with effects on the stomach mucosa**. Increased epithelial cell migration in the antral gastric mucosa leading to a statistically significant decrease in mucosal thickness was reported in male Sprague-Dawley rats exposed to ammonia in drinking water for durations up to 8 weeks (Tsujii et al., 1993; Kawano et al., 1991). Similarly, decreases in the height and labeling index of gastric mucosa glands were reported in Donryu rats exposed to ammonia in drinking water for up to 24 weeks (Hata et al., 1994). The gastric mucosal effects observed in rats were reported to resemble mucosal changes in human atrophic gastritis (Tsujii et al., 1993; Kawano et al., 1991); however, the investigators also reported an absence of microscopic lesions, gastritis, or ulceration in the stomach of these rats. Evidence that oral exposure to ammonia is associated with gastrointestinal effects in humans is limited to case reports of individuals suffering from gastrointestinal effects (e.g., stomach ache, nausea, diarrhea, distress, and burns along the digestive tract) from intentionally or accidentally ingesting household cleaning solutions containing ammonia or biting into capsules of ammonia smelling salts. Mechanistic studies in rodent models support the biological plausibility that ammonia exposure may be associated with gastric effects. **Given the weight of evidence from human, animal, and mechanistic studies, gastric effects may be a hazard from ammonia exposure**.

Studies of the potential reproductive or developmental toxicity of ammonia in humans are not available. No reproductive effects were 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). Toxicokinetic information provides support for the conclusion that **exposures to ammonia at levels that do not alter homeostasis (i.e., that do not alter normal blood or tissue ammonia levels) would not be expected to pose a developmental or reproductive hazard to the developing fetus and reproductive tissues**.

1.2.2. Carcinogenicity

The available information on carcinogenicity following exposure to ammonia is limited to oral animal studies. There was inadequate reporting in studies in Swiss or C3H mice administered ammonium hydroxide in drinking water for a lifetime (Toth, 1972). There is limited evidence that ammonia administered in drinking water may act as a cancer promoter (Tsujii et al., 1995; Tsujii et al., 1992a). The genotoxic potential cannot be characterized based on the available genotoxicity information. Thus, under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "**inadequate information to assess carcinogenic potential**" of ammonia.

1.2.3. Susceptible Populations and Lifestages

Studies of the toxicity of ammonia in children or young animals compared to other lifestages that would support an evaluation of childhood susceptibility have not been conducted.

Hyperammonemia is a condition of elevated levels of circulating ammonia that can occur in

individuals with severe diseases of the liver or kidney, organs that biotransform and excrete ammonia, or with hereditary urea cycle disorders (Córdoba et al., 1998; Schubiger et al., 1991; Gilbert, 1988; Jeffers et al., 1988; Souba, 1987). The elevated ammonia levels that accompany human diseases such as acute liver or renal failure 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 (Minana et al., 1995; Souba, 1987). 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 hypothesized susceptibility.

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; however, controlled human studies that examined both healthy volunteers and volunteers with asthma exposed to ammonia, as well as cross-sectional studies of livestock farmers exposed to ammonia (Petrova et al., 2008; Monsó et al., 2004; Sigurdarson et al., 2004; Vogelzang et al., 2000; Vogelzang et al., 1998; Vogelzang et al., 1997; Preller et al., 1995), generally did not demonstrate greater respiratory sensitivity after exposure to ammonia in populations with underlying respiratory disease.

2. DOSE-RESPONSE ANALYSIS

2.1. Oral Reference Dose for Effects Other than Cancer

The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral 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 no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95 percent lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

The available data are inadequate to derive an oral RfD for ammonia. Human data involving oral exposure to ammonia are limited to case reports of gastrointestinal effects involving intentional or accidental ingestion of household cleaning solutions or ammonia inhalant capsules. Human data were not considered for derivation of the RfD because, although case reports can indicate the nature of acute endpoints in humans and inform hazard identification, they are inadequate for dose-response analysis and for subsequent derivation of a chronic reference value due to short duration of exposure and incomplete or missing quantitative exposure information.

The experimental animal toxicity database for ammonia lacks standard toxicity studies that evaluate a range of tissues/organs and endpoints. Repeat-exposure animal studies of the noncancer effects of ingested ammonia are limited to three studies designed to investigate mechanisms by which ammonia can induce effects on rat gastric mucosa (Hata et al., 1994; Tsujii et al., 1993; Kawano et al., 1991). While these studies provide consistent evidence of changes in the gastric mucosa associated with exposure to ammonia in drinking water (see Section 1.1.2), the investigators reported no evidence of microscopic lesions of the stomach, gastritis, or ulceration in the stomachs of these rats. In addition, the gastrointestinal tract has not been identified as a target of ammonia toxicity in chronic toxicity studies of ammonium compounds, including ammonium chloride and sulfate (see Section 1.1.2).

Given the limited scope of toxicity testing of ingested ammonia and questions concerning the adversity of the gastric mucosal findings in rats, the available oral database for ammonia was considered insufficient to characterize toxicity outcomes and dose-response relationships. Accordingly, **an RfD for ammonia was not derived**.

Previous IRIS Assessment: Reference Dose

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

2.2. 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 percent lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

2.2.1. Identification of Candidate Principal Studies and Critical Effects

Figure 2-1 is an exposure-response array comparing effect levels for inhaled ammonia across a range of toxicological effects. As discussed in Section 1.2, the respiratory system is the primary and most sensitive target of inhaled ammonia toxicity 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 some evidence that inhaled ammonia may be associated with toxicity to target organs other than the respiratory system, including the liver, adrenal gland, kidney, spleen, heart, and immune system. The evidence for these associations is weak; therefore, they were not considered as the basis for RfC derivation.

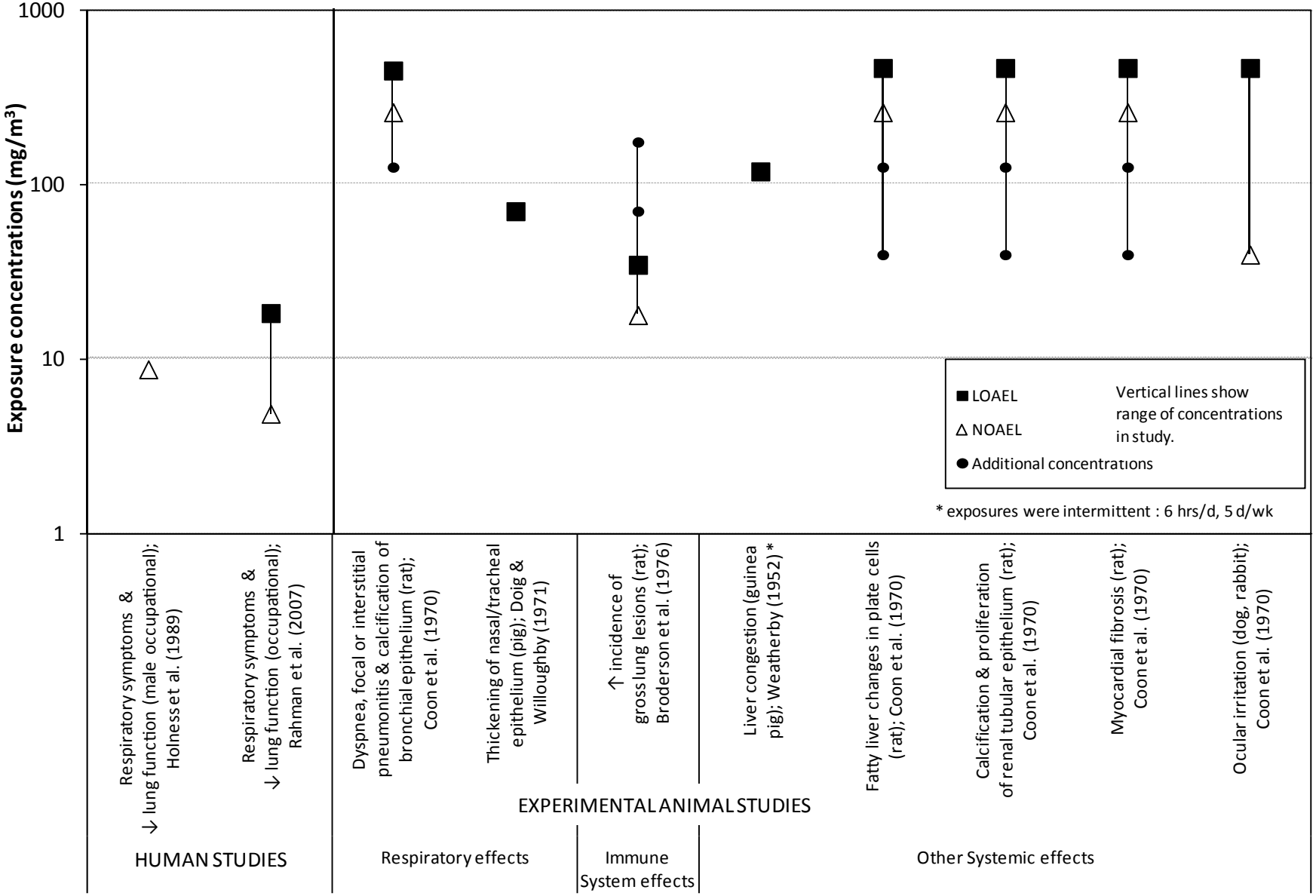

Figure 2-1. Exposure-response array of toxicological effects following inhalation exposure.

Respiratory effects, characterized as increased prevalence of respiratory symptoms and 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). Effects, including changes in lung function parameters and increased prevalence of 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). As shown in Figure 2-1, respiratory effects were also observed in animals, but at 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.

Human data are preferred over animal data for deriving reference values when possible because the use of human data is more relevant in the assessment of human health and avoids the uncertainty associated with interspecies extrapolation introduced when animal data serve as the basis for the RfC. In the case of ammonia, the available human occupational studies provide data adequate for quantitative analysis of health outcomes considered relevant to potential general population exposures. Further, human data provide a more sensitive measure of respiratory effects than do data from animal studies. Therefore, data on respiratory effects in humans were considered for derivation of the RfC and the respiratory effects in animals were not further considered.

Of the available human data, two occupational studies—Rahman et al. (2007) and Holness et al. (1989)—provide information useful for examining the relationship between chronic ammonia exposure and increased prevalence of respiratory symptoms and decreased lung function. Both studies reported either the presence or absence of respiratory effects in workers exposed to ammonia over a range of concentrations (approximately 4–18 mg/m^3). These studies are coherent, with the NOAEL of 8.8 mg/m^3 from the Holness et al. (1989) study falling between the NOAEL and LOAEL values (4.9 and 18.5 mg/m^3, respectively) from the Rahman et al. (2007) study. These studies are considered as candidate principal studies for RfC derivation. Other occupational epidemiology studies (Ali et al., 2001; Ballal et al., 1998) did not provide exposure information adequate for dose-response analysis and were thus not considered useful for RfC derivation.

Higher confidence is associated with the analytical methods used by Holness et al. (1989) than Rahman et al. (2007). Rahman et al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air (Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by four- to fivefold, indicating some uncertainty in these measurements, 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 (ATSG) absorption tubes.

In light of the greater confidence in the ammonia measurements in Holness et al. (1989) and considering the range of NOAELs and LOAELs reported in both studies [with a higher NOAEL being

reported by Holness et al. (1989)], 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 and respiratory effects as the critical effect**.

2.2.2. Methods of Analysis

The highest occupational exposure in the Holness et al. (1989) study, a **NOAEL of 8.8 mg/m^3, was used as the POD for RfC derivation**.

Because the RfC is a measure that 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). The duration-adjusted POD was calculated as follows:

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

Where:

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

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

2.2.3. Derivation of Reference Concentration

Under EPA's *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. A **composite UF of 10** was applied to the selected duration-adjusted POD of 3.1 mg/m^3 to derive an RfC. 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), i.e., mean number of years at 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. The ammonia inhalation database consists of epidemiological studies and experimental animal studies. The epidemiological studies include industrial worker populations, cross sectional studies in livestock farmers exposed to inhaled ammonia and other airborne agents, controlled exposure studies involving volunteers exposed to ammonia vapors for short periods of time, and a large number of case reports of acute exposure to high ammonia concentrations (e.g., accidental spills/releases) that examined irritation effects, respiratory symptoms, and effects on lung function. Studies of the toxicity of inhaled ammonia in experimental animals include subchronic studies in a number of species, including rats, guinea pigs, and pigs, that examined respiratory and other systemic effects of ammonia, several immunotoxicity studies, and one limited, reproductive toxicity study in young female pigs. (See Chapter 1 for more details regarding available studies.) The database lacks developmental and multigeneration reproductive toxicity studies.

  As noted in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), "the size of the database factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical and, consequently, the POD." While the database lacks multigeneration reproductive and developmental toxicity studies, these studies would not be expected to impact the determination of ammonia toxicity at the POD. Therefore, a database UF to account for the lack of these studies is not considered necessary. This determination was based on the observation that ammonia is endogenously produced and homeostatically regulated in humans and animals during fetal and adult life. Uteroplacental tissues produce ammonia, and ammonia concentrations in human umbilical vein and artery blood (at term) of healthy individuals have been shown to be higher than concentrations in maternal blood (Jóźwik et al., 2005). 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). This evidence provides some assurance that endogenous ammonia concentrations in the fetus are similar to other lifestages, and that baseline ammonia concentrations would not be associated with developmental toxicity. Additionally, evidence in animals (Manninen et al., 1988; Schaerdel et al., 1983) suggests that exposure to ammonia at concentrations up to 18 mg/m^3 does not alter blood ammonia levels (see Appendix D, Section D.1, for a more detailed discussion of ammonia distribution and elimination). Accordingly, exposure at the duration-adjusted POD (3.1 mg/m^3) would not be expected to alter ammonia homeostasis nor result in measureable increases in blood ammonia concentrations. Thus, the concentration of ammonia at the POD for the RfC would not be expected to result in systemic toxicity, including reproductive or developmental toxicity.

The RfC for ammonia[6] was calculated as follows:

$$\begin{aligned} \text{RfC} &= NOAEL_{ADJ} \div UF \\ &= 3.1\ mg/m^3 \div 10 \\ &= 0.31\ mg/m^3 \text{ or } \mathbf{0.3\ mg/m^3\ (rounded\ to\ one\ significant\ figure)} \end{aligned}$$

[6] Due to uncertainty concerning the possible influence of anions on the toxicity of ammonium, information on ammonium salts was not used to characterize the effects for ammonia and ammonium hydroxide. Therefore, the RfC derived in this assessment is applicable to ammonia and ammonium hydroxide, but not ammonium salts.

2.2.4. 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 BMD 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.

Endogenous Ammonia

Ammonia, which is produced endogenously, has been detected in breath exhaled from the nose and trachea (range: 0.013–0.078 mg/m^3) (Smith et al., 2008; Larson et al., 1977). Higher and more variable ammonia concentrations are reported in breath exhaled from the mouth or oral cavity, with the majority of ammonia concentrations from these sources ranging from 0.085 to 2.1 mg/m^3 (Smith et al., 2008; Spanel et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977). Ammonia in exhaled breath from the mouth or oral cavity is largely attributed to 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), and can be influenced by factors such as diet, oral hygiene, and age. In contrast, ammonia concentrations measured in breath exhaled from the nose and trachea are lower (range: 0.013–0.078 mg/m^3) (Smith et al., 2008; Larson et al., 1977) and more likely reflect systemic levels of ammonia (i.e., circulating levels in the blood) (Smith et al., 2008).

Ammonia concentrations measured in breath exhaled from the nose and trachea (i.e., concentrations expected to more closely correlate with circulating levels of ammonia in blood) are lower than the ammonia RfC of 0.3 mg/m^3 by a factor of fourfold or more; however, the RfC does fall within the more variable range of breath concentrations collected from the mouth or oral cavity. Although the RfC falls within the range of breath concentrations collected from the mouth or oral cavity, ammonia in exhaled breath is expected to be rapidly diluted in the much larger volume of ambient air and not contribute significantly to overall ammonia exposure. Further, occupational epidemiology studies served as the basis for the ammonia RfC; the worker populations in these

studies would have been exposed to any endogenously produced ammonia, and as such the RfC accounts for ammonia exposures from endogenous sources.

2.2.5. 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, this study is supported in the context of the entire database, which includes the NOAEL and LOAEL values identified in the Rahman et al. (2007) occupational exposure study, other occupational epidemiology studies, 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 study of reproductive toxicity and no studies of developmental toxicity. 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 the RfC derivation is the most sensitive or appropriate. However, reproductive, developmental, and other systemic effects are not expected at the RfC because it is well documented that ammonia is endogenously produced in humans and animals, ammonia concentrations in blood are homeostatically regulated to remain at low levels, and ammonia concentrations in air at the POD are not expected to alter homeostasis. 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**.

2.2.6. Previous IRIS Assessment: Reference Concentration

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.[7]

[7]In this document, the lower bound of the high exposure category from the Holness et al. (1989) study (8.8 mg/m^3, adjusted for continuous exposure to 3.1 mg/m^3) was identified as the POD because workers in

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., 1983) showed no increase in blood ammonia levels at an inhalation exposure 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. In this document, a UF_D of one was selected because a more thorough investigation of the literature on ammonia homeostasis and literature published since 1991 on fetoplacental ammonia levels provides further support that exposure to ammonia at the POD would not result in a measureable increase in blood ammonia, including fetal blood levels.

2.3. Cancer Risk Estimates

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, and unless otherwise stated, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per µg/m^3 air breathed.

As discussed in Section 1.2, there is **"inadequate information to assess carcinogenic potential"** of ammonia. Therefore, **a quantitative cancer assessment was not conducted and cancer risk estimates were not derived for ammonia**.

The previous IRIS assessment also did not include a carcinogenicity assessment.

this high exposure category, as well as those in the two lower exposure categories, showed no statistically significant increase in the prevalence of respiratory symptoms or decreases in lung function.

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