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Toxicological Review of Ammonia

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

August 2013

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ABBREVIATIONS

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BCG	bacillus Calmette-Guérin
BMCL	95% lower bound on the benchmark concentration
BMDL	95% lower bound on the benchmark dose
CAC	cumulative ammonia concentration
CCRIS	Chemical Carcinogenesis Research Information System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFU	colony forming unit
CI	confidence interval
DAP	diammonium phosphate
EPA	Environmental Protection Agency
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
HERO	Health and Environmental Research Online
HSDB	Hazardous Substances Data Bank
IgE	immunoglobulin E
IgG	immunoglobulin G
IRIS	Integrated Risk Information System
LD_{50}	50% lethal dose
LOAEL	lowest-observed-adverse-effect level
MAO	monoamine oxidase
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
MRM	murine respiratory mycoplasmosis
NCEA	National Center for Environmental Assessment
NH_3	ammonia
NH_4^+	ammonium ion
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
ORD	EPA's Office of Research and Development
PEFR	peak expiratory flow rate
pO_2	oxygen partial pressure
POD	point of departure
PPD	purified protein derivative
RfC	reference concentration
RfD	reference dose
RTECS	Registry of Toxic Effects of Chemical Substances
TSCATS	Toxic Substance Control Act Test Submission Database
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database deficiencies uncertainty factor
VEh	human occupational default minute volume
VEho	human ambient default minute volume

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This assessment was provided for review to scientists in EPA's program and regional Offices. Comments were submitted by:

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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
Council on Environmental Quality, Executive Office of the President
Food Safety and Inspection Service, U.S. Department of Agriculture

This assessment was released for public comment on June 8, 2012 and comments were due on August 7, 2012. A summary and EPA's disposition of the comments received from the public is included in Appendix G of the Supplemental Information to the Toxicological Review. Comments were received from the following entities:

The American Chemistry Council	Washington, DC
The Fertilizer Institute	Washington, DC

PREFACE

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

Ammonia and ammonium hydroxide are listed as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) 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 (RfC) 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, and summaries of toxicity studies and other information are provided as Supplemental Information to this assessment (see Appendices A to E).

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.

Implementation of the 2011 National Research Council Recommendations

On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law (U.S. Congress, 2011). 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 (NRC, 2011). The report language included the following:

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

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

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

Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. The IRIS assessment for ammonia is the first assessment in Phase 2 of implementation, which addresses all of the short-term NRC recommendations (see Appendix F, Table F-1). The IRIS Program is implementing all of these recommendations but recognizes that achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and external peer review committees. Chemical assessments in Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC (see Appendix F, Table F-2), including the development of a standardized approach to describe the strength of the evidence for noncancer effects. On May 16, 2012, EPA announced (U.S. EPA, 2012c) that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

Assessments by Other National and International Health Agencies

Toxicity information on ammonia has been evaluated by ATSDR, the National Research Council (NRC), 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 A of the Supplemental Information. It is important to recognize that these assessments may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

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 B.

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

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

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

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

1. Scope of the IRIS Program

Soon after the EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that the 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, the 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 exposure to chemicals and to characterize exposure-response relationships. In terms set forth by the National Research Council (NRC, 1983), IRIS assessments cover the hazard identification and dose-response assessment steps of risk assessment, not the exposure assessment or risk characterization steps that are conducted by the EPA's program and regional offices and by other federal, state, and local health agencies that evaluate risk in specific populations and exposure scenarios. IRIS assessments are distinct from and do not address political, economic, and technical considerations that influence the design and selection of risk management alternatives.

An IRIS assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. These agents may be found in air, water, soil, or sediment. 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).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state health agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. Agents may be considered for reassessment as significant new studies are published. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. Other agents may also be assessed in response to an urgent public health need.

2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009 and enhanced in July 2013) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. The EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2009).

Before beginning an assessment, the IRIS Program discusses the scope with other EPA programs and regions to ensure that the assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the studies and analytical approaches that might contribute to their resolution.

Step 1. Development of a draft Toxicological Review. The draft assessment considers all pertinent publicly available studies and applies

consistent criteria to evaluate study quality, identify health effects, identify mechanistic events and pathways, integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

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

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

Step 4. Public review and comment, followed by external peer review. The EPA releases the draft assessment for public review and comment. A public meeting provides an opportunity to discuss the assessment prior to peer review. Then the EPA releases a draft for external peer review. The peer review meeting is open to the public and includes time for oral 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, written public comments, and peer review report become part of the public record.

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary. 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 The draft assessment and summary are revised to address the 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. 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, 2000, 1998, 1996, 1991, 1986a, b) and other methods (U.S. EPA, 2012a, b, 2011, 2006a, b, 2002, 1994b). 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, the 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, Web of Science, and other databases listed in the EPA's HERO system (Health and Environmental Research Online, http://

hero.epa.gov/). Searches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.

Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. The EPA posts the results of the literature search on the IRIS web site and requests information from the public on additional studies and ongoing research.

The EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide 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, the EPA will have it peer-reviewed.

The 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, 2000, §2.1, 1986b, §2.2):

- 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, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological 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 ecological studies and case reports but reports details only if they suggest effects not identified by other studies.

3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental animal studies or human clinical studies.

- 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 animal 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 for identifying effects from lifetime human exposure. Such studies may be indicative of effects from less-than-lifetime human exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information.

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

After the subsets of pertinent epidemiologic and experimental studies have been selected from the literature searches, the assessment evaluates the quality of each individual study. This evaluation considers the design, methods, conduct, and documentation of each study, but not whether the results are positive, negative, or null. The objective is to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

4.1. Evaluating the quality of epidemiologic studies

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

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error (can lead to misclassification of exposure, health outcomes, and other factors) and other types of information bias.
- Potential confounding and other sources of bias 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 related to both exposure and outcome and is sufficiently prevalent to result in bias.

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 methodological aspects that can increase or decrease the weight given to each

experimental animal study, in-vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991). Research involving human subjects is considered only if conducted according to ethical principles.

- Documentation of study design, animals or study population, methods, basic data, and results.
- Nature of the assay and validity for its intended purpose.
- 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, §2.2.2.1.3).

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, §3.1.1.4, 1991, §3.1.2.4.5.4).

4.3. Reporting study results

The assessment uses evidence tables to present 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 quality characteristics in this section to identify the strongest studies or types of study. The tables present 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 information 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, the EPA asks peer reviewers to identify studies that were not adequately considered.

5. Evaluating the overall evidence of each effect

5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the

agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

Causal inference involves scientific judgment, and the considerations are nuanced and complex. Several health agencies have developed frameworks for causal inference, among them the U.S. Surgeon General (CDC, 2004; HEW, 1964), the International Agency for Research on Cancer (2006) , the Institute of Medicine (2008), and the EPA (U.S. EPA, 2010, §1.6, 2005a, §2.5). Although developed for different purposes, the frameworks are similar in nature and provide an established structure and language for causal inference. Each considers aspects of an association that suggest causation, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998) (U.S. EPA, 2005a, §2.2.1.7, 1994b, app. C).

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 causation 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 causation. 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 causation, 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 causation. A monotonic increase is not the only pattern consistent with causation. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

Biologic plausibility: An inference of causation is strengthened by data demonstrating plausible biologic mechanisms, if available. Plausibility may reflect subjective prior beliefs if there is insufficient understanding of the biologic process involved.

Coherence: An inference of causation 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, as it tests the hypothesis of causation. An example would be 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 causation.

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).

5.2. Evaluating evidence in humans

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole. The objective is to determine whether a credible association has been observed and, if so, whether that association is consistent with causation. In doing this, the assessment explores alternative explanations (such as chance, bias, and confounding) and draws a conclusion about whether these alternatives can satisfactorily explain any observed association.

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may select a standard descriptor to characterize the epidemiologic evidence of association between exposure to the agent and occurrence of a health effect.

Sufficient epidemiologic evidence of an association consistent with causation: The evidence establishes a causal association for which alternative explanations such as chance, bias, and confounding can be ruled out with reasonable confidence.

Suggestive epidemiologic evidence of an association consistent with causation: The evidence suggests a causal association but chance, bias, or confounding cannot be ruled out as explaining the association.

Inadequate epidemiologic evidence to infer a causal association: The available studies do not permit a conclusion regarding the presence or absence of an association.

Epidemiologic evidence consistent with no causal association: Several adequate studies covering the full range of human exposures and considering susceptible populations, and for which alternative explanations such as bias and confounding can be ruled out, are mutually consistent in not finding an association.

5.3. Evaluating evidence in animals

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, §2.2.1.7, 1994, app. C).

In weighing evidence from multiple experiments, U.S. EPA (2005a, §2.5) 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. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological differences (for example, relative sensitivity of the tests, differences in

dose levels, insufficient sample size, or timing of dosing or data collection).

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

In evaluating evidence of genetic toxicity:

- 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, The 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, §2.3).

5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

- The biologic plausibility of a causal interpretation of human studies.
- The generalizability of animal studies to humans.
- The susceptibility of particular populations or lifestages.

The focus of the analysis is to describe, if possible, mechanistic pathways that lead to a health effect. These pathways encompass:

- *Toxicokinetic processes* of absorption, distribution, metabolism, and elimination that lead to the formation of an active agent and its presence at the site of initial biologic interaction.
- *Toxicodynamic processes* that lead to a health effect at this or another site (also known as a *mode 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 the agent is causally related to an effect (U.S. EPA, 2005a, §2.5).

The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a, §2.4.3.4).

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 causation 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. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

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, §2.4.3.3). 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., 2008b).

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, §2.3.5).

5.5. Characterizing the overall weight of the evidence

After evaluating the human, animal, and mechanistic evidence pertinent to an effect, the assessment answers the question: Does the agent cause the adverse effect? (NRC, 2009, 1983). In doing this, the assessment develops a narrative that integrates the evidence pertinent to causation. To provide clarity and consistency, the narrative includes a standard hazard descriptor. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a, §2.5).

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, §2.5).

Another example of standard descriptors comes from the EPA's Integrated Science Assessments, which evaluate causation for the effects of the criteria pollutants in ambient air (U.S. EPA, 2010, §1.6).

Causal relationship: Sufficient evidence to conclude that there is a causal relationship. Observational studies cannot be explained by plausible alternatives, or they are supported by other lines of evidence, for example, animal studies or mechanistic information.

Likely to be a causal relationship: Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but co-exposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

Suggestive of a causal relationship: At least one high-quality epidemiologic study shows an association but other studies are inconsistent.

Inadequate to infer a causal relationship: The studies do not permit a conclusion regarding the presence or absence of an association.

Not likely to be a causal relationship: Several adequate studies, covering the full range of human exposure and considering susceptible populations, are mutually consistent in not showing an effect at any level of exposure.

The EPA is investigating and may on a trial basis use these or other 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 hazard descriptor.

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal:

- 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, the EPA asks peer reviewers to identify studies that were not adequately considered.

7. Deriving toxicity values

7.1. General framework for dose-response analysis

The EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a, §3).

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). If response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors to the point of departure in order to account for sources of uncertainty and variability (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, §3.2.6, 2000, §4.4).

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

7.2. Modeling dose to sites of biologic effects

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, §3.1, 1994b, §4.3).

Because toxicokinetic modeling can require many parameters and more data than are typically available, the 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, §3.1.1, 1991, §3.2).

- 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, 2011, 2005a, §3.1.3).
  - 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, 2012a, 1994b, §3).

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, §3.1.4).

In the absence of study-specific data on, for example, intake rates or body weight, the 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, §3.2.2).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, the 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. The EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2012b). Additional judgment or alternative analyses are used if 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, §3.2.3).

Modeling is used to derive a point of departure (U.S. EPA, 2012b, 2005a, §3.2.4). (See section 7.6 for alternatives if a point of departure cannot be derived by modeling.)

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).
- For nonlinear approaches, both statistical and biologic considerations are taken into account.
  - For dichotomous data, a response level of 10% extra risk is generally used for minimally adverse effects, 5% or lower for more severe effects.
  - For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for

minimally adverse effects, one-half standard deviation for more severe effects.

The point of departure is the 95% lower bound on the dose associated with the selected response level.

7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data, considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1, §3.3.2).

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 when data are insufficient to establish mode of action and when scientifically plausible.

   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 models are used for extrapolation 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. Nonlinear approaches generally should not be used in cases where mode of action has not ascertained. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

4) Both linear and nonlinear approaches may be used if there a multiple modes of action. For example, modeling to a low response level can be useful for estimating the response at doses where a high-dose mode of action would be less important.

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 or selects 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, §3.5).

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, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential 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, the EPA recommends (U.S. EPA, 2005b, §5):

   - 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, §4.2). 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 adverse 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. The assessment accounts 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. A factor of 10 is generally used to account for this variation. This factor is reduced only if the point of departure is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, §4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4).

Animal-to-human extrapolation. If animal results are used to make inferences about humans, the assessment adjusts for cross-species differences. These may arise from differences in toxicokinetics or toxicodynamics. Accordingly, if the point of departure is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. In most other cases, a factor of 10 is applied (U.S. EPA, 2011, 2002, §4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4).

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, §4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4).

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, §4.4.5, 1998, §4.2, 1994b, §4.3.9.1).

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§§4.4.5, 1998, §4.2, 1996, §4, 1994b, §4.3.9.1, 1991, §3.4). The size of the factor depends on the nature of the database deficiency. For example, the 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, §4.4.5).

In this way, the assessment derives candidate 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, 1994b, §4.3.9).

The assessment derives or selects an *organ- or system-specific reference value* for each organ or system affected by the agent. The assessment explains the rationale for each organ/system-specific reference value (based on, for example, the highest quality studies, the most sensitive outcome, or a clustering of values). By providing these organ/system-specific reference values, IRIS assessments facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (NRC, 2009)..

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. This is generally the most sensitive organ/system-specific reference value, though consideration of study quality and confidence in each value may lead to a different selection.

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, 1994b, §4.3.9.2).

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. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). 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 in humans 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 in humans. 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 from biting into capsules of ammonia smelling salts; the relevance of these acute findings to chronic, low-level ammonia exposure is unclear. 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, 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 in industrial settings provide evidence of an increased prevalence of respiratory symptoms (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Bhat and Ramaswamy, 1993). Other occupational studies of exposure to ammonia when used as a disinfectant or cleaning product, for example in health care workers and cleaning workers, provide additional evidence of effects on asthma, asthma symptoms, and pulmonary function, using a variety of study designs (Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005). Additional evidence of respiratory effects of ammonia is seen in studies of pulmonary function in livestock workers, specifically in the studies that accounted for effects of co-exposures to other agents such as endotoxin and dust (Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Heederik et al., 1990). Controlled volunteer studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia provide 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. Little evidence exists for these effects relative to the evidence 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 respiratory symptoms Occupational epidemiology studies Holness et al. (1989), supported by Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001)	$NOAEL_{ADJ}$: 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), with support from three studies in urea fertilizer plants by Rahman et al. (2007), Ballal et al. (1998), and Ali et al. (2001), was identified as the principal study for RfC derivation. Respiratory effects, characterized as increased respiratory symptoms (including cough, wheezing, and other asthma-related symptoms) and decreased lung function in workers exposed to ammonia, were selected as the critical effect. Holness et al. (1989) found no differences in the prevalence of respiratory symptoms or lung function between workers (mean exposure 6.5 mg/m^3) and the control group, and no differences when stratified by exposure level (highest exposure group, >8.8 mg/m^3). Rahman et al. (2007) observed an increased prevalence of respiratory symptoms and decreased lung function in workers exposed in a plant with a mean ammonia concentration of 18.5 mg/m^3, but not in workers in a second plant exposed to a mean concentration of 4.9 mg/m^3. Ballal et al. (1998) observed an increased prevalence of respiratory symptoms among workers in one factory with exposures ranging from 2 to 27.1 mg/m^3,[1] but no increase in another factory with exposures ranging from 0.02–7 mg/m^3. A companion study by Ali et al. (2001) also observed decreased lung function among workers in the higher exposure factory.

Considerations in selecting the principal study for RfC derivation include the higher confidence placed in the measures of ammonia exposure in Holness et al. (1989), evaluation of both respiratory symptoms and lung function parameters in this study, and the fact that the estimate of the no-observed-adverse-effect level (NOAEL) for respiratory effects of 8.8 mg/m^3 from Holness et al. (1989) was the highest of the studies with adequate exposure-response information. Because a high level of control of exposures in the plant studied by Holness et al. (1989) resulted in relatively

1This concentration range does not include exposures in the urea store (number of employees = 6; range of ammonia concentrations = 90–130.4 mg/m^3) because employees in this area were required to wear full protective clothing, thus minimizing potential exposure.

low ammonia levels in this facility, the Holness et al. (1989) study does not demonstrate a relationship between ammonia exposure and respiratory effects. Therefore, the Holness et al. (1989) study is identified as the principal study only as part of a collection of epidemiology studies of industrial settings that includes studies with higher workplace ammonia concentrations in which respiratory effects were observed.

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

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

Consistent with 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., 1992b). 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 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 (0.0092–0.1 mg/m^3) (Appendix E, Section E.1 (Elimination) and Table E-1). 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 appear to better represent levels at the alveolar interface of the lung or in the tracheo-bronchial region and are thought to be more relevant to understanding systemic levels of ammonia than ammonia in breath exhaled from the mouth.

The studies of ammonia in exhaled breath were conducted in environments with measureable levels of ambient (exogenous) ammonia and not in ammonia-free environments. Because concentrations of trace compounds in exhaled breath may be correlated with their ambient concentrations (e.g., Spanel et al. (2013) found that approximately 70% of inhaled ammonia is retained in exhaled breath), it is likely that ammonia concentrations in breath exhaled from the nose would be lower if the inspired air were free of ammonia Therefore, levels of ammonia in exhaled breath reported in the literature would need to be adjusted if they were to be used as a measure of systemic ammonia.

Ammonia concentrations measured in breath exhaled from the nose and trachea, considered to be more representative of systemic ammonia levels than breath exhaled from the mouth, are lower than the ammonia RfC of 0.3 mg/m^3 by a factor of threefold or more. Although the RfC falls within the range of concentrations measured in the mouth or oral cavity, ammonia exhaled by an individual is rapidly diluted in the larger volume of ambient air and would not

contribute significantly to ammonia exposure. Further, such endogenous exposures existed in the occupational epidemiology studies that served as the basis for the ammonia RfC.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

The primary, peer-reviewed literature pertaining to ammonia was identified through a keyword search of the databases listed in Table LS-1. The detailed search string used for searching these databases is provided in Appendix D, Table D-1. The original literature search was conducted through March 2012; an updated literature search was conducted using the same strategy from March 2012 through March 2013. References from health assessments developed by other national and international health agencies were also examined. References were also identified by reviewing the list of references cited in key health effects studies of ammonia ("backwards searching"), and a "forward search" of studies citing the development of an asthma-specific job exposure matrix (Kennedy et al., 2000); see Appendix D for additional search strategy details. Other peer-reviewed information, including review articles and literature necessary for the interpretation of ammonia-induced health effects, were retrieved and included in the assessment where appropriate. EPA requested the public submit additional data on December 21, 2007 and November 2, 2009 (U.S. EPA, 2009b, 2007); no submissions were received.

Figure LS-1 depicts the literature search and study selection strategy and the number of references obtained at each stage of literature screening. Approximately 23,000 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 1,032 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, and a review of references within identified papers, pared this to 40 epidemiological studies (i.e., studies of workers exposed to ammonia in industrial settings or through the use of ammonia in cleaning products, livestock farmers, or short-term exposure in volunteers as well background epidemiology method papers), 44 case reports, 61 unique oral or inhalation animal studies and 105 other studies (e.g., studies that provided supporting information on physical and chemical properties, mode of action, 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 employed for ammonia

Database	Keywords[a]
Pubmed Toxcenter Toxline Current Contents (2008 and 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 (see Appendix D for specific keywords used)</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 of 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 was 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 C, Table C-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.

CASRN = Chemical Abstracts Service Registry Number; CCRIS = Chemical Carcinogenesis Research Information System; HSDB = Hazardous Substances Data Bank; RTECS = Registry of Toxic Effects of Chemical Substances; TSCATS = Toxic Substance Control Act Test Submission Database

1

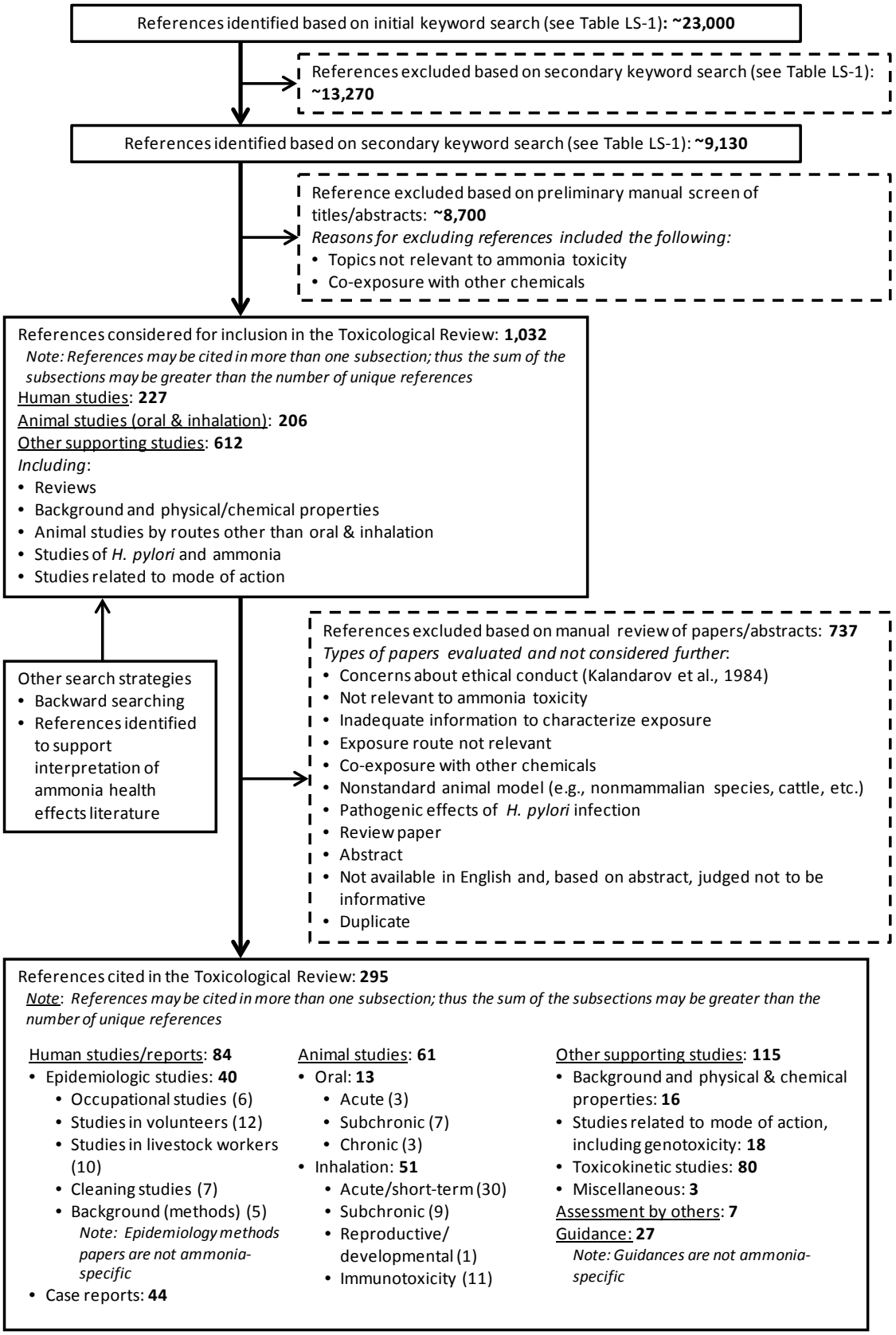

Figure LS-1. Study selection strategy.

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

Considerations for evaluation of epidemiology studies

Case reports are often anecdotal and describe unusual or extreme exposure situations, providing little information that would be useful for characterizing chronic health hazards. Ammonia case studies were only briefly reviewed; representative citations from the collection of case reports are provided as supplemental information in Appendix E, Section E.2.

Epidemiology studies of chronic exposure to ammonia have primarily focused on industrial worker populations, workers exposed to ammonia as a cleaning or disinfectant product, and livestock farmers. The observational epidemiology studies identified in Figure LS-1 (i.e., the studies considered most informative for evaluating ammonia toxicity from chronic exposure) are summarized in evidence tables (i.e., Tables 1-1, 1-2, and 1-7). Evaluation of the studies summarized in the evidence tables is provided in Appendix D (Tables D-2, D-3, and D-4 corresponding to Tables 1-1, 1-2, and 1-7, respectively). This evaluation process addressed aspects relating to the selection of study participants, exposure parameters, outcome measurement, confounding, and statistical analysis, as discussed below for each set of studies.

Studies of Industrial Settings

Selection of study participants

All of the studies were cross-sectional analyses in occupational settings. The workers were healthy enough to remain in the work area for a considerable time; with one exception, mean duration ranged from 52 months to 18 years. One study (Bhat and Ramaswamy, 1993) grouped workers into those exposed for up to 10 years and those with more than 10 years of exposure; a minimum exposure duration was not provided. In general, these designs may result in a "healthy worker" bias. In addition, the workers in these studies are not representative of the general population, as they do not include children or women. These aspects of the study design may result in an underestimate of the risk of health effects of ammonia exposure, as the worker population may not exhibit health effects (such as decreased lung function or increased prevalence of respiratory symptoms) to the same degree that would be seen in the general population under the same conditions.

Exposure parameters

Exposure methods differ across these occupational studies, which makes comparison of ammonia measurements among the studies difficult. Spectrophotometric absorption measures of areas samples (Ali et al., 2001; Ballal et al., 1998) are not directly comparable to direct-reading diffusion methods used to analysis personal samples (Rahman et al., 2007) or to the NIOSH-

recommended protocol for personal sampling and analysis of airborne contaminants (Holness et al., 1989). In the study by Rahman et al. (2007), exposure concentrations were determined by both the Dräger tube and Dräger PAC III methods. The Dräger tube method yielded concentrations of ammonia in the two plants studied that were approximately fourfold higher than the concentrations obtained by the Dräger PAC III method; a strong correlation between measurements by the two methods was reported. Rahman et al. (2007) stated that their measurements indicated only relative differences in exposures between workers and production areas, and did not identify one analytical measure as the more valid of the two. Based on communication with technical support at Dräger Safety Inc. (Bacom and Yanosky, 2010), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Ammonia concentrations based on the PAC III method were also in line with concentrations reported in other studies. Therefore, exposure levels based on PAC III air measurements of ammonia were used in the current health assessment to characterize the exposure-response relationship in the Rahman et al. (2007) study.

In the Hamid and El-Gazzar (1996) study, no direct measurement of ammonia exposure was made; blood urea was used as a surrogate measure of ammonia exposure. The correlation of blood urea with ammonia is not reported by the authors. EPA considered this a major limitation of this study, based on other data indicating no correlation between ammonia levels in air and serum urea levels in a study of six groups of workers with varying types of exposure (Giroux and Ferrières, 1998). No exposure measurements of ammonia were used in the study by Bhat and Ramaswamy (1993); EPA considers the lack of exposure measure in this study to be a major limitation.

Outcome measurement

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

Confounding

Co-exposures to other ambient chemicals in urea fertilizer factories included inorganic gases (nitrogen dioxide and sulfur dioxide) and dust. In one of these studies (Rahman et al., 2007), nitrogen dioxide was measured concurrently with ammonia and found to be below detection limits for all areas (urea plant, ammonia plant, and administration area). The other urea fertilizer studies (Ali et al., 2001; Ballal et al., 1998; Hamid and El-Gazzar, 1996) did not describe potential co-exposures. [It appears from the exposure measurements that the plant in Ali et al. (2001) is "Factory A" in Ballal et al. (1998)]. In the fertilizer plant in Bhat and Ramaswamy (1993), co-exposures are not discussed, but the workers are grouped based on different parts of the plant (ammonia, urea, and diammonium phosphate); effects observed with respect to lung function tests were similar in magnitude, albeit slightly stronger, in the ammonia plant workers compared with the urea plant workers. One study was conducted in a soda ash production plant (Holness et al., 1989). No measurements of co-exposures were described in this study, but the authors note the high level of control of exposures (resulting in low ammonia levels) in this facility. Because of the lack of demonstration of co-exposures correlated with ammonia levels in these studies, and lack of demonstration of stronger associations between potential co-exposures and respiratory outcomes, EPA concluded that confounding by other workplace exposures, although a potential concern, was unlikely to be a major limitation.

The analyses of respiratory symptoms and lung function may also be confounded by smoking. In these five studies, analyses accounted for smoking as follows: the analysis included either an adjustment for smoking (Rahman et al., 2007; Holness et al., 1989), the exclusion of smokers (Bhat and Ramaswamy, 1993), or stratification of the results by smoking status (Ali et al., 2001; Ballal et al., 1998). EPA did not consider potential confounding by smoking to be a major limitation of these studies. In reviewing the study of liver function by Hamid and El-Gazzar (1996), however, EPA noted the lack of information on smoking habits or use of alcohol (another exposure potentially affecting liver function tests) to be a major limitation.

Statistical analysis

EPA considered the statistical analysis in the epidemiological studies (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Hamid and El-Gazzar, 1996; Bhat and Ramaswamy, 1993; Holness et al., 1989) to be adequate and appropriate. Although the type of statistical testing was not specified in Hamid and El-Gazzar (1996), the results were presented in sufficient detail to allow interpretation of the data and analysis. Sample size, an important consideration with respect to statistical power, was also considered. EPA noted the small number of exposed workers and low levels of exposure in the study by Holness et al. (1989) as limitations that could result in "false negative" results (i.e., a test result indicating a lack of association, whereas, in fact, a positive association between exposure and a health effect exists).

Studies of Health Care and Cleaning Settings

EPA also evaluated the studies that examined exposure to ammonia when used as a cleaning or disinfectant product. EPA noted the potential for the "healthy worker" bias arising from movement out of jobs by affected individuals in most of these studies (Le Moual et al., 2008). This issue was less of a concern in the study by Zock et al. (2007), which was conducted in a general (non-occupational) population sample, focusing on cleaning activities in the home.

None of these studies used a direct measure of ammonia exposure in the analysis, precluding interpretation of the results in relation to an absolute level of exposure. The limited data available concerning exposure levels in cleaning scenarios found median exposures of 0.6 to 5.4 ppm (0.4 to 3.8 mg/m^3), with peaks exceeding 50 ppm (35 mg/m^3), in a small study (n = 9) using personal samples during a domestic cleaning session (Medina-Ramón et al., 2005). Although an absolute level of exposure is not available, the relative ranking of exposure used in these studies does allow examination of relative risk in relation to relative levels of exposure. Key considerations regarding the validity of the exposure measures are the specificity of the classification and the extent to which classification could be influenced by knowledge of the disease or symptoms under study. Methodological research has reported underestimation of self-reported exposure to specific products by health care workers, and differential reporting by disease status (i.e., asthma) for self-reported use of cleaning products in patient care, but not in instrument cleaning or building materials (Donnay et al., 2011; Delclos et al., 2009; Kennedy et al., 2000). Two of these studies used an exposure assessment protocol that incorporated an independent, expert review, blinded to disease status (Dumas et al., 2012; Lemiere et al., 2012), and one study collected exposure information using a 2-week daily diary (Medina-Ramón et al., 2006). EPA considered these to be the strongest of the exposure protocols used within this set of studies.

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

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

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

Studies of Livestock Farmers

EPA also evaluated a set of studies conducted among livestock farmers. As with the other occupational studies discussed above, the selection of sensitive individuals out of the workforce would be a potential bias in cross-sectional studies in this type of population.

Among the studies examining pulmonary function, two studies used area-based exposure sampling in animal confinement buildings (Monsó et al., 2004; Zejda et al., 1994), one study used area samples taken in conjunction with specific tasks and calculated a personal exposure measure taking into account duration spent in specific locations and tasks (Heederik et al., 1990), and four studies collected personal samples over a workshift (Donham et al., 2000; Reynolds et al., 1996; Preller et al., 1995), or an unspecified time period (Donham et al., 1995). EPA considered the use of the area-based samples without consideration of duration to be limitations of the studies by Zejda et al. (1994) and Monsó et al. (2004).

All of the studies reported using a standard spirometric technique; five studies compared two measures per individual (i.e., pre- and post-shift) (Monsó et al., 2004; Donham et al., 2000; Reynolds et al., 1996; Heederik et al., 1990) and two studies used a single pulmonary function measure, adjusted for height, age, and smoking variables (Preller et al., 1995; Zejda et al., 1994). EPA did not consider any of these outcome measures to be limitations in these studies.

Five of these studies controlled for co-exposures (e.g., endotoxin, dust, disinfectants) (Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995), noted only weak correlations (i.e., Spearman $r < 0.20$) between ammonia and dust or endotoxin (Donham et al., 2000), or observed associations with ammonia but not with endotoxin or dust measures (Heederik et al., 1990). The two studies that did not address confounding were those that also used the more limited exposure measure (Monsó et al., 2004; Zejda et al., 1994).

Based on these considerations, EPA considered the studies by Reynolds et al. (1996), Preller et al. (1995), Donham et al. (2000), Donham et al. (1995), and Heederik et al. (1990) to be the methodologically strongest studies of this set. Because of the variety of exposures in the type of environment examined in these studies (including dust, endotoxin, mold, and disinfectant products) and the availability of sets of studies in settings with a lesser degree of co-exposures, this set of studies is considered to be supporting material.

Considerations for evaluation of animal studies

Relatively few repeat-dose toxicity studies of ammonia in experimental animals are available. Many of the available animal studies come from the older toxicological literature and are limited in terms of study design (e.g., small group sizes) and reporting of results. These studies were evaluated consistent with EPA principles and practices for evaluating study quality (U.S. EPA, 2005a, 1998b, 1996, 1994, 1991); however, detailed documentation of the methodological features of the available animal studies was not necessary to convey the limitations of this body of ammonia

literature. The animal studies are summarized in detail in Appendix E, Section E.3. Essentially all the animal toxicology studies were included in this assessment. Any studies excluded from the hazard identification as uninformative are identified in Section 1.1, along with the basis for exclusion.

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

[3]HERO (Health and Environmental Research On-line) 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. Five cross-sectional occupational epidemiology studies in industrial settings (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998; Bhat and Ramaswamy, 1993; Holness et al., 1989) examined the association between inhaled ammonia and prevalence of respiratory symptoms or changes in lung function (Table 1-1). Another set of studies examined pulmonary function or asthma symptoms in relation to ammonia exposure in health care workers and domestic cleaners (Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005) (Table 1-2). The association between ammonia exposure and respiratory effects indicated by these studies is also informed by studies of pulmonary function in livestock farmers, volunteer studies involving acute exposures to inhaled ammonia, and human case reports (see Supplemental Material, Appendix E, Section E.2), and in subchronic inhalation toxicity studies in various experimental animal species (Table 1-3). The evidence of respiratory effects in humans and experimental animals exposed to ammonia is summarized in an exposure-response array in Figure 1-1 at the end of this section.

Respiratory Symptoms

Respiratory symptoms (including cough, wheezing, and other asthma-related symptoms) were reported in two cross-sectional studies of industrial worker populations exposed to ammonia at levels greater than or equal to approximately 18 mg/m^3 (Rahman et al., 2007; Ballal et al., 1998) (Table 1-1). One of these studies also examined frequency of respiratory symptoms by cumulative ammonia concentration (CAC, mg/m^3-years) and observed significantly higher relative risks (2.5–5.3) with higher CAC (>50 mg/m^3-years) compared to those with a lower CAC (≤50 mg/m^3-years) (Ballal et al., 1998). In three studies examining lower exposures settings (Rahman et al., 2007; Ballal et al., 1998; Holness et al., 1989) (Table 1-1), no differences were observed in the prevalence of respiratory symptoms between ammonia-exposed workers and controls. Ammonia concentrations reported in these lower exposure settings included a mean ammonia concentration of 6.5 mg/m^3 and a high-exposure group defined as >8.8 mg/m^3 in Holness et al. (1989), an exposure range of 0.2—7 mg/m^3 in "Factory B" of Ballal et al. (1998), and a mean concentration of 4.9 mg/m^3 in Rahman et al. (2007). The primary limitation noted in all of these studies was the potential under-ascertainment of effects inherent in the study of a long-term worker population (i.e., "healthy worker" effect) (see Literature Search Strategy | Study Selection and Evaluation section and Table D-2 in the Supplemental Information). Confounding by other workplace

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

Studies of health care workers or hospital workers (Arif and Delclos, 2012; Dumas et al., 2012) (Table 1-2) provide evidence that exposure to ammonia as a cleaning or disinfectant product is associated with increased risk of asthma or asthma symptoms. Use of ammonia as a cleaning product in other settings has also been associated with asthma and respiratory symptoms (Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2005) (Table 1-2). Occupational exposure to ammonia was associated with work-exacerbated asthma (compared to non-work related asthma) in a study at two occupational asthma specialty clinics by Lemiere et al. (2012) (Table 1-2). Each of six studies, from Europe, Canada, and the United States, observed elevated odds ratios, generally between 1.5 and 2.0, with varying degrees of precision. These studies were conducted using a variety of designs, including a prospective study (Zock et al., 2007) and a nested case-control study (Medina-Ramón et al., 2005). Criteria used to define current asthma or asthma symptoms were generally well defined and based on validated methods. A major limitation of this collection of studies is the lack of direct measures of ammonia exposure. Two of the studies included expert assessment of exposure (blinded to case status); expert assessment, improves reliance on self-reported exposure (Dumas et al., 2012; Lemiere et al., 2012). Confounding by other cleaning products is an unlikely explanation for these results, as two of the studies noted only weak correlations between ammonia and other product use (Zock et al., 2007; Medina-Ramón et al., 2005), and another study observed stronger associations with ammonia than with bleach (Dumas et al., 2012). All of the studies addressed smoking as a potential confounder.

Studies in swine and dairy farmers analyzing prevalence of respiratory symptoms (including cough, phlegm, wheezing, chest tightness, and eye, nasal, and throat irritation) in relation to ammonia exposure provided generally negative results (Melbostad and Eduard, 2001; Preller et al., 1995; Zejda et al., 1994) (Appendix E, Table E-7). Two other studies that measured ammonia, but did not present an analysis in relation to variability in ammonia levels, reported an increased prevalence of respiratory symptoms in pig farmers exposed to ammonia from animal waste (Choudat et al., 1994; Crook et al., 1991) (Appendix E, Table E-8). In addition to ammonia, these studies also documented exposures to other compounds, such as airborne dust, endotoxin, mold, and disinfectants.

Reports of irritation and hyperventilation in volunteers acutely exposed to ammonia at concentrations ranging from 11 to 354 mg/m^3 ammonia for durations up to 4 hours under controlled exposure conditions (Petrova et al., 2008; Smeets et al., 2007; Ihrig et al., 2006; Verberk, 1977; Silverman et al., 1949) provide support for ammonia as a respiratory irritant (Appendix E, Section E.2 and Table E-9). 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, persistent, productive cough, bilateral diffuse rales and rhonchi, mucous production, pulmonary edema, marked hypoxemia, and necrosis of the lung) (Appendix E, Section E.2).

Experimental studies in laboratory animals also provide consistent evidence that repeated exposure to ammonia can affect the respiratory system (Table 1-3 and Appendix E, Section E.3). The majority of available animal studies did not look at measures of respiratory irritation, in contrast to the majority of human studies, but rather examined histopathological changes of respiratory tract tissues. Histopathological changes in the nasal passages were observed in Sherman rats after 75 days of exposure to 106 mg/m^3 ammonia and in F344 rats after 35 days of exposure to 177 mg/m^3 ammonia, with respiratory and nasal epithelium thicknesses increased 3–4 times that of normal (Broderson et al., 1976). Thickening of nasal and tracheal epithelium (50–100%) was also observed in pigs exposed to 71 mg/m^3 ammonia continuously for 1–6 weeks (Doig and Willoughby, 1971). Nonspecific inflammatory changes (not further described) were reported in the lungs of Sprague-Dawley and Long-Evans rats 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 did not identify respiratory effects in laboratory animals exposed to ammonia. No increase in the incidence of respiratory or other diseases common to young pigs was observed after continuous exposure to ammonia and inhalable dust at concentrations representative of those found in commercial pig farms (≤26 mg/m^3 ammonia) for 5 weeks (Done et al., 2005). No gross or histopathological changes in the turbinates, trachea, and lungs of pigs were observed after continuous exposure to 35 or 53 mg/m^3 ammonia for up to 109 days (Curtis et al., 1975). No signs of toxicity in rats or dogs were observed after continuous exposure to 40 mg/m^3 ammonia for 114 days or after intermittent exposure (8 hours/day) to 155 mg/m^3 ammonia for 6 weeks (Coon et al., 1970). Only one study reported respiratory effects at concentrations <50 mg/m^3 (i.e., lung congestion, edema, and hemorrhage in guinea pigs and mice exposed to 14 mg/m^3 ammonia for up to 42 days; Anderson et al. (1964)), but confidence in the findings from this study is limited by inadequate reporting and small numbers of animals tested.

Lung Function

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

One of the studies of domestic cleaning workers described in Table 1-2 included a measure of pulmonary function (Medina-Ramón et al., 2006). Ammonia use was associated with a decrease in peak expiratory flow (PEF) (-9.4 [95% CI, -17, -2.3]). A limitation of this study was the use of lung function measurements conducted by the participant; the reliability of this procedure has not been established.

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

Changes in lung function following acute exposure to ammonia have been observed in some, but not all, controlled exposure studies conducted in volunteers (Appendix E, Section E.2 and Table E-9). Cole et al. (1977) reported reduced lung function as measured by reduced expiratory minute volume and changes in exercise tidal volume in volunteers exposed for a half-day in a chamber at ammonia concentrations ≥106 mg/m^3, but not at 71 mg/m^3. Bronchoconstriction was reported in volunteers exposed to ammonia through a mouthpiece for 10 inhaled breaths of ammonia gas at a concentration of 60 mg/m^3 (Douglas and Coe, 1987); however, there were no bronchial symptoms

reported in volunteers exposed to ammonia in an exposure chamber at concentrations of up to 35 mg/m^3 for 10 minutes (MacEwen et al., 1970). Similarly, no changes in bronchial responsiveness or lung function (as measured by FVC and FEV_1) were reported in healthy volunteers exposed to ammonia at concentrations up to 18 mg/m^3 for 1.5 hours during exercise (Sundblad et al., 2004). There were no changes in lung function as measured by FEV_1 in 25 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers exposed to ammonia concentrations up to 354 mg/m^3 ammonia for up to 2.5 hours (Petrova et al., 2008), or in 6 healthy volunteers and 8 mildly asthmatic volunteers exposed to 11–18 mg/m^3 ammonia for 30-minute sessions (Sigurdarson et al., 2004).

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

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

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

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

Study design and reference	Results
Holness et al. (1989) (Canada) Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant)[c]. Average exposure: 12.2 years **Exposure:** Personal samples, one work-shift per person, mean 8.4 hours Low: <6.25 ppm (<4.4 mg/m^3); n = 34 Medium: 6.25–12.5 ppm (4.4–8.8 mg/m^3); n = 12 High: >12.5 ppm (>8.8 mg/m^3); n = 12 All exposed workers (mean): 6.5 mg/m^3 **Outcome**: Respiratory symptoms based on American Thoracic Society questionnaire	Percentage of workers reporting symptoms (%): Cough: Control (n = 31) 10; Exposed (n = 58) 16; *p*-value 0.53 Sputum: 16; 22; 0.98 Bronchitis: 19; 22; 0.69 Wheeze: 10; 10; 0.91 Chest tightness: 6; 3; 0.62 Dyspnea (shortness of breath): 13; 7; 0.05 Chest pain: 6; 2; 0.16 Rhinitis (nasal complaints): 19; 10; 0.12 Throat irritation: 3; 7; 0.53 No increased risk seen in analyses stratified by exposure group.
Lung function	
Rahman et al. (2007) (Bangladesh) Urea fertilizer factory worker (all men); 24 ammonia plant workers, 64 urea plant workers, and 25 controls (staff from administration building). Mean employment duration: 16 years **Exposure:** Personal samples (2 methods[a]; correlation = 0.80) Low-exposure group (ammonia plant), mean: 6.9 ppm (4.9 mg/m^3); range: 2.8–11.1 ppm (2–8 mg/m^3) High-exposure group (urea plant), mean: 26.1 ppm (18.5 mg/m^3); range: 13.4–43.5 ppm (9–31 mg/m^3) **Outcome**: Lung function (standard spirometry)	Pre-shift / Post-shift / *p*-value Ammonia plant (low-exposure group, 4.9 mg/m^3); n = 24 ammonia plant workers FVC: 3.308; 3.332; 0.67 FEV_1: 2.627; 2.705; 0.24 PEFR: 8.081; 8.313; 0.22 Urea plant (high-exposure group, 18.5 mg/m^3); n = 64 urea plant workers FVC: 3.362; 3.258; 0.01 FEV_1: 2.701; 2.646; 0.05 PEFR: 7.805; 7.810; 0.97 *p*-value reflects the comparison of pre- and post-shift values.
Ali et al. (2001) (Saudi Arabia) Urea fertilizer factory workers (all men)—(additional study of "Factory A" in Ballal et al. (1998)); 73 exposed workers and 348 unexposed controls. Mean employment duration: not reported **Exposure:** 4-hour measurements. Cumulative exposure calculated based on exposure and duration; dichotomized to high and low at 50 mg/m^3-years **Outcome**: Lung function (standard spirometry; morning measurement)	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 (n = 45) / >50 mg/m^3-y (n = 28) / *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)

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

Study design and reference	Results
Bhat and Ramaswamy (1993) (India) Fertilizer chemical plant workers; 30 diammonium phospate (DAP) plant workers, 30 urea plant workers, 31 ammonia plant workers, and 68 controls (people with comparable body surface area chosen from the same socio-economic status and sex as exposed workers) **Exposure:** Measurements not reported; duration dichotomized as ≤10 and >10 years **Outcome**: Lung function (standard spirometry)	Controls (n = 68) / DAP plant (n = 30) / Urea plant (n = 30) / Ammonia plant (n = 31) FVC 3.4 ± 0.21 / 2.5 ± 0.06* / 3.3 ± 0.11 / 3.2 ± 0.07 FEV_1 2.8 ± 0.10 / 2.1 ± 0.08* / 2.7 ± 0.10 / 2.5 ± 0.1* PEFR 383 ± 7.6 / 228 ± 18* / 307 ± 19* / 314 ± 20* *$p < 0.05$
Holness et al. (1989) (Canada) Soda ash plant workers (all men); 58 exposed workers and 31 controls (from stores and office areas of plant)[c]. Average exposure: 12.2 years **Exposure:** Personal samples, one work-shift per person, mean 8.4 hours Low: <6.25 ppm (<4.4 mg/m^3); n = 34 Medium: 6.25–12.5 ppm (4.4–8.8 mg/m^3); n = 12 High: >12.5 ppm (>8.8 mg/m^3); n = 12 All exposed workers (mean): 6.5 mg/m^3 **Outcome**: Lung function (standard spirometry; beginning and end of shift, at least two test days per worker)	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

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

[a]Exposure concentrations were determined by both the Dräger tube and Dräger PAC III methods. Using the Dräger tube method, concentrations of ammonia in the ammonia and urea plants were 17.7 and 88.1 mg/m^3, respectively; using the Dräger PAC III method, ammonia concentrations were 4.9 and 18.5 mg/m^3, respectively (Rahman et al. (2007). The study authors observed that their measurements indicated only relative differences in exposures between workers and production areas, and that the validity of the exposure measures could not be evaluated based on their results. Based on communication with technical support at Dräger Safety Inc (telephone conversations and e-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, SRC, Inc., contractor to NCEA, ORD, U.S. EPA), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, higher confidence is attributed to the PAC III air measurements of ammonia for the Rahman et al. (2007) study.

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

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

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Table 1-2. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

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

Table 1-2. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

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

Table 1-2. Evidence pertaining to respiratory effect in humans following inhalation exposure in cleaning settings

Study design and reference	Results
Medina-Ramón et al. (2005) (Spain) Nested case-control, cleaning workers; case (n = 40; 74% participation rate) based on asthma and/or bronchitis at both assessments. Controls (n = 155, 69% participation rate)—no history of respiratory symptoms in preceding year and no asthma at either assessment. **Exposure:** Structured interview; frequency of use of 22 products; ammonia prevalence 16% undiluted, 56% diluted **Outcome:** Asthma: asthma attack or being woken by attack or shortness of breath in past 12 months; Chronic bronchitis: regular cough or regular bringing up phlegm for at least 3 months each year	Odds ratio (95% CI) (unadjusted), ≥12 compared with <12 times per year Undiluted 3.1 (1.2, 8.0) Diluted 0.8 (0.4, 1.7)
Pulmonary function and respiratory symptoms	
Medina-Ramón et al. (2006) (Spain) Panel study, sample selected from participants in nested case-control study by Medina-Ramón et al. (2005). Current asthma symptoms or chronic bronchitis in 2000–2001 survey; n = 51 of 80 (64%); 8 excluded for possible recording errors, outliers, learning effects **Exposure:** Daily diary of use of products **Outcome:** Respiratory symptoms based on 2-week daily diary (7 symptoms, 5 point intensity scale); summed score for upper respiratory symptoms (blocked nose, throat irritation, watery eyes) and lower respiratory symptoms (chest tightness, wheezing, shortness of breath, and cough); PEF measured with mini-Wright peak flow meter (with training and written instructions); measured morning, lunchtime, night (3 measurements each; highest recorded)	Diluted and undiluted / Diluted only OR (95% CI) Upper respiratory symptoms: 1.8 (0.7, 4.9) / 1.3 (0.3, 5.0) Lower respiratory symptoms: 1.6 (0.6, 4.4) / 3.0 (1.0, 9.1) Beta (95% CI) PEF at night: -9.4 (-17, -2.3) / -10.3 (-18, -2.7) PEF, following morning: -1.2 (-8.5, 6.2) / -2.9 (-11, 6.2) Adjusted for respiratory infection, use of maintenance medication, and age; daily number of cigarettes smoked, years of employment in domestic cleaning, and/or weekly working hours in domestic cleaning also assessed as potential confounders

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Table 1-3. Evidence pertaining to respiratory effects in animals

Study design and reference	Results
Effects on the lungs	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*Saimiri sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	Gross necropsies were normal; focal pneumonitis in one of three monkeys at 155 mg/m^3. Nonspecific lung inflammation observed in guinea pigs and rats, but not in other species, at 770 mg/m^3.[a]
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	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]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d or 455 mg/m^3 for 65 d	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]
Anderson et al. (1964) Swiss albino mouse; male and female; 4/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d	Lung congestion, edema, and hemorrhage observed at 14 mg/m^3 after 42 d.[a]
Anderson et al. (1964) 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	Lung congestion, edema, and hemorrhage observed at 14 and 35 mg/m^3 after 42 d.[a]
Done et al. (2005) Pig (several breeds); sex not specified; 24/group 0, 0.6, 10, 18.8, or 37 ppm (0, 0.4, 7, 13.3, or 26 mg/m^3) and 1.2, 2.7, 5.1, or 9.9 mg/m^3 inhalable dust for 5 wks (Exposure to ammonia and inhalable dust at concentrations commonly found at pig farms)	No increase in the incidence of respiratory or other diseases.
Curtis et al. (1975) Pig (crossbred); sex not specified; 4–8/group 0, 50, or 75 ppm (0, 35, or 53 mg/m^3 for 109 d)	Turbinates, trachea, and lungs of all pigs were classified as normal.
Effects on the upper respiratory tract	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	Dyspnea in rats and dogs exposed to 770 mg/m^3 during wk 1 only; no indication of irritation after wk 1; nasal tissues not examined for gross or histopathologic changes.
Broderson et al. (1976)[c] Sherman rat; 5/sex/group 10 or 150 ppm (7 or 106 mg/m^3) from bedding for 75 d	↑ thickness of the nasal epithelium (3–4 times) and nasal lesions at 106 mg/m^3.[a]

Table 1-3. Evidence pertaining to respiratory effects in animals

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

[a]Incidence data not provided.
[b]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.

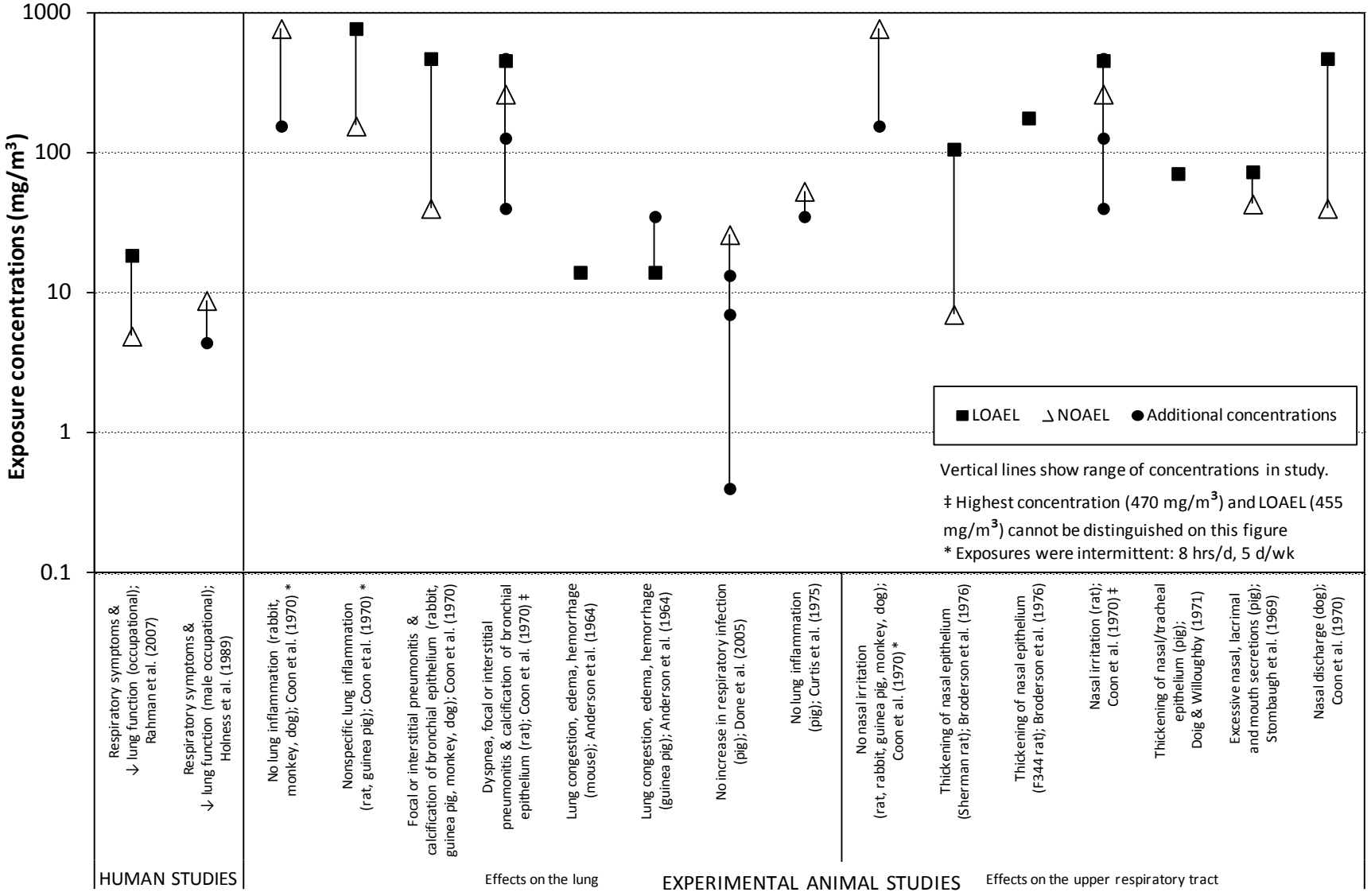

Figure 1-1. Exposure-response array of respiratory effects following inhalation exposure to ammonia.

Mode-of-Action Analysis—Respiratory Effects

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

Summary of Respiratory Effects

Evidence for respiratory toxicity associated with exposure to ammonia comes from studies in humans and animals. Multiple occupational studies involving chronic exposure to ammonia in industrial settings provide evidence of an increased prevalence of respiratory symptoms (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali et al., 2001; Bhat and Ramaswamy, 1993) (Table 1-1 and Appendix E, Section E.2). An increase in respiratory effects was reported both with higher workplace ammonia concentrations (Rahman et al., 2007; Ballal et al., 1998) and with greater cumulative ammonia concentration (expressed in mg/m^3-years) (Ali et al., 2001; Ballal et al., 1998). Additional evidence is provided by studies of asthma, asthma symptoms, and pulmonary function in health care and cleaning workers, in a variety of study designs and populations (Arif and Delclos, 2012; Dumas et al., 2012; Lemiere et al., 2012; Vizcaya et al., 2011; Zock et al., 2007; Medina-Ramón et al., 2006; Medina-Ramón et al., 2005) (Table 1-2) and in studies of pulmonary function in livestock workers, specifically in the studies that accounted for effects of co-exposures such as endotoxin and dust (Donham et al., 2000; Reynolds et al., 1996; Donham et al., 1995; Preller et al., 1995; Heederik et al., 1990) (Appendix E, Table E-7). The livestock farmer studies, however, do not provide evidence of associations between ammonia and respiratory symptoms. Controlled volunteer studies of ammonia inhalation and case reports of injury in humans with inhalation exposure to ammonia provide additional support for the respiratory system as a target of ammonia toxicity when inhaled (Appendix E, Section E.2).

Evidence from animal studies supports an association between inhaled ammonia and respiratory effects. Short-term and subchronic animal studies show histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens (Gaafar et al., 1992; Broderson et al., 1976; Doig and Willoughby, 1971; Coon et al., 1970; Anderson et al., 1964) (Table 1-3 and Appendix E, Section E.3). In general, responses in respiratory tissues increased with increasing ammonia exposure concentration.

Based on evidence of respiratory effects in multiple human and animal studies (including epidemiological studies in different settings and populations), respiratory system effects are identified as a hazard associated with inhalation exposure to ammonia.

1.1.2. 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) (Appendix E, Section E.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. These effects appear to reflect the corrosive properties of ammonia, and their relevance to effects associated with chronic low-level exposure to ammonia is unclear.

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 (Appendix E, Section E.3). Statistically significant decreases of 40–60% in the thickness of the antral gastric mucosa were reported in Sprague-Dawley rats administered ammonia in drinking water at concentrations ≥0.01% for durations of 2–8 weeks (Tsujii et al., 1993; Kawano et al., 1991); estimated doses in two studies by the same group of investigators 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.[4] 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

[4]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.

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 that 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 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 1-4 and as an exposure-response array in Figure 1-2.

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

Study design and references	Results[a]
Histopathologic changes of the gastric mucosa	
Kawano et al. (1991) 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	*% 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*%
Tsujii et al. (1993) 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	*% 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)
Hata et al. (1994) 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 d and 1, 4, 8, 12, or 24 wks	*% 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]Percent 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/day (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/day (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 ($p < 0.05$).

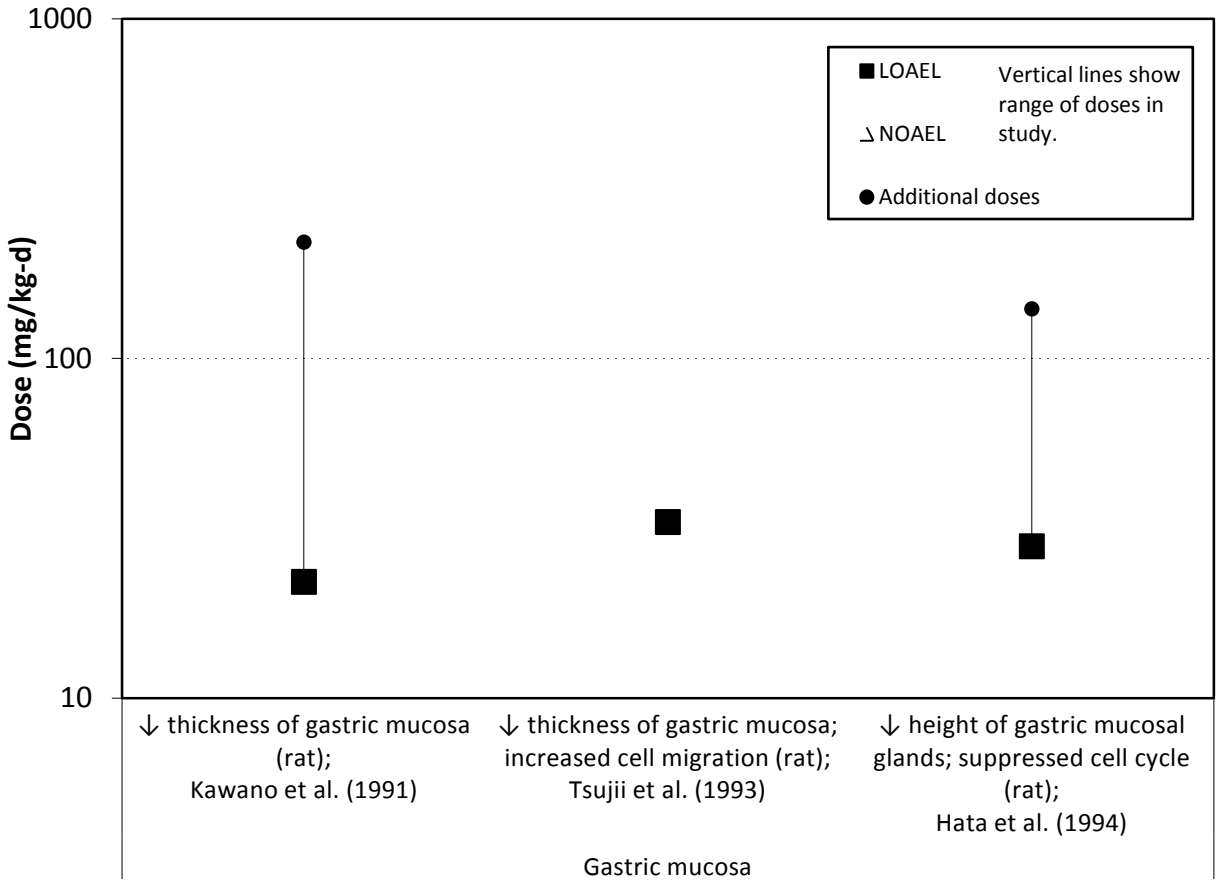

Figure 1-2. Exposure-response array of gastrointestinal effects following oral exposure to ammonia.

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., 1992a). Rather, the available evidence suggests that the ability of ammonia to damage the gastric mucosa is related to its ionization state. Ammonia (NH_3) (in its non-ionized state) can easily penetrate cell membranes, whereas the ionized form (NH_4^+) is less permeable to cell membranes (Tsujii et al., 1992a). 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 NH_4^+. In contrast, in the antral mucosa (a nonacid-secreting area), the pH was higher, the ratio of ammonia to NH_4^+ 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., 1992a). 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. Tsujii et al. (1992b) suggested that ammonia may accelerate mucosal cell desquamation and stimulate cell proliferation by a compensatory mechanism. Overall, although hypotheses have been proposed, a specific mechanism(s) by which ammonia may induce cellular toxicity has not been established,

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) (Appendix C, Table C-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. In summary, the evidence primarily from human case reports as supported by mechanistic studies in experimental animals suggests that gastric effects are a potential hazard associated with oral exposure to ammonia.

1.1.3. Immune System Effects

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

Animal studies that examined ammonia immunotoxicity were conducted using short-term inhalation exposures and were measured by three general types of immune assays: host resistance, T cell proliferation, and delayed-type hypersensitivity. Immunotoxicity studies of ammonia using measures of host resistance provide the most relevant data for assessing immune function since they directly measure ability of the immune system to control microorganism growth. Other available studies of ammonia employed assays that evaluated immune function. Changes in immune cell populations without corresponding functional data are considered to be the least predictive, and studies that looked only at these endpoints (Gustin et al., 1994; Neumann et al., 1987) were 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. The available studies also do not correlate increased bacterial colonization with reduced immune function. Lung lesions, both gross and microscopic, were positively correlated with ammonia concentration in F344 rats continuously exposed to ammonia in an inhalation chamber for 7 days prior to inoculation with 10^8 colony forming units [CFU] of *Mycoplasma pulmonis* followed by up to 42 days of ammonia exposure post inoculation (Broderson et al., 1976). (Inoculation with the respiratory pathogen *M. pulmonis* causes murine respiratory mycoplasmosis [MRM] characterized by lung lesions.) The incidence of lung lesions was significantly increased at ammonia concentrations ≥35 mg/m^3, suggesting that ammonia exposure decreased bacterial clearance resulting in the development of *M. pulmonis*-induced MRM. However, increasing ammonia concentration was not associated with increased CFU of *M. pulmonis* isolated from the respiratory tract. The high number of inoculating

CFU could have overwhelmed the innate immune response and elicited a maximal response that could not be further increased in immunocompromised animals.

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

OF1 mice exposed to 354 mg/m^3 ammonia for 7 days prior to inoculation with a 50% lethal dose (LD_{50}) of *Pasteurella multocida* exhibited significantly increased mortality compared to controls (86 versus 50%, respectively); however, an 8-hour exposure was insufficient to affect mortality (Richard et al., 1978a). 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 lung lesions. Additional data obtained from pigs suggest that ammonia exposure eliminates the commensal flora of the nasal cavities, which allows for increased colonization of *P. multocida*; however, this effect abates following cessation of ammonia exposure (Hamilton et al., 1999; Hamilton et al., 1998).

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

Study design and reference	Results
Host resistance	
Broderson et al. (1976) F344 rat; male and female; 11–12/sex/ group ≤5 (control), 25, 50, 100, or 250 ppm (≤3.5 [control], 18, 35, 71, or 177 mg/m^3), 7 d (continuous exposure) pre-inoculation/28–42 d post-inoculation with *M. pulmonis*	*% of animals with gross lung lesions:* 16, 46, 66*, 33, and 83% No effect on CFU.
Schoeb et al. (1982) F344 rat; 5-15/group (sex unknown) <2 or 100 ppm (<1.4 [control] or 71 mg/m^3), 7 d (continuous exposure) pre-inoculation/ 28 d post-inoculation with *M. pulmonis*	↑ bacterial colonization (as a result of reduced bacterial clearance).
Richard et al. (1978a) OF1 mouse; male; 99/group 0 or 500 ppm (0 or 354 mg/m^3), 8 hrs or 7 d (continuous exposure), prior to infection with *P. multocida*	*% Mortality:* 50 and 86%*
Andreasen et al. (2000) Landrace X large white pigs; 10/group (sex unknown) <5 (control), 50, or 100 ppm (3.5, 35, or 71 mg/m^3), 63 d (continuous exposure) inoculated with *M. hyopneumoniae* on day 9 and *P. multocida* on d 28, 42, and 56	*% of animals with positive day 49 nasal swab:* 24, 100*, and 90%*
Hamilton et al. (1998) Large white pigs; 4–7/group (sex unknown) 0 or 20 ppm (0 or 14 mg/m^3), 14 d (continuous exposure), inoculated with *P. multocida* on d 0	↑ bacterial colonization
Hamilton et al. (1999) Large white pigs; 5/group (sex unknown) 0 or 50 ppm (0 or 35 mg/m^3), 1 wk pre-inoculation with *P. multocida*, 3 wks post-inoculation	↑ bacterial colonization *Bacteria isolated from nasal cavities*: 3.18 and 4.30* CFU
T cell proliferation	
Targowski et al. (1984) Hartley guinea pig; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m^3), 3 wks (continuous exposure)	↓ proliferation in blood and bronchial T cells.
Delayed-type hypersensitivity	
Targowski et al. (1984) Hartley guinea pig, BCG immunized; 8/group (sex unknown) <15, 50, or 90 ppm (<11 [control], 35, or 64 mg/m^3), 3 wks (continuous exposure) followed by PPD challenge	*Mean diameter of dermal lesion (mm):* 12, 12.6, and 8.7*

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

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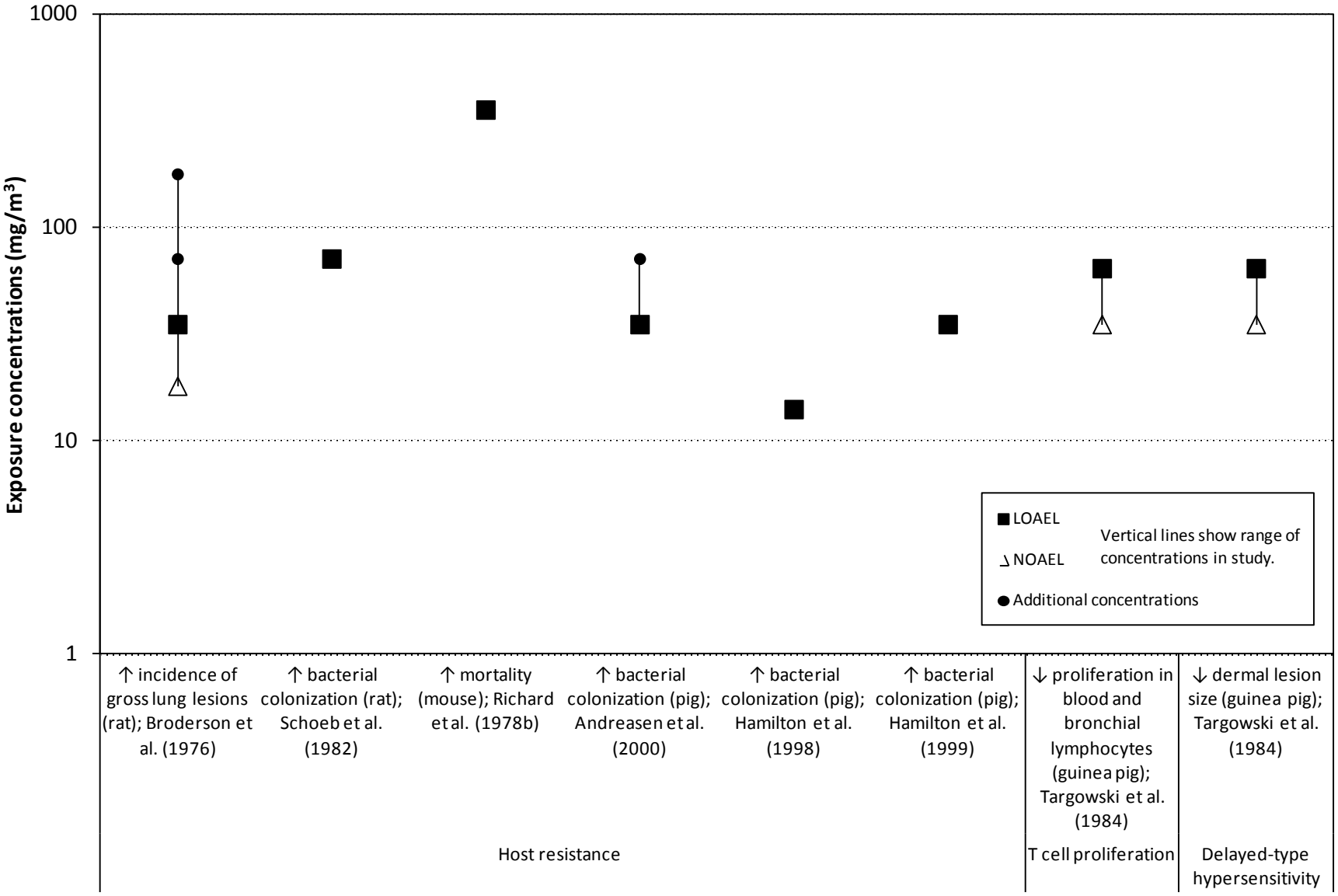

Figure 1-3. Exposure-response array of immune system effects following inhalation exposure to ammonia.

Summary of Immune System Effects

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

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

Overall, the evidence in humans and animals indicates that ammonia exposure may be associated with immunotoxicity, but it is unclear if elevated bacterial colonization is the result of damage to the protective mucosal epithelium of the respiratory tract or the result of suppressed immunity. Therefore, the evidence does not support the immune system as a potential hazard of ammonia exposure.

1.1.4. 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 reported in workers in an Egyptian urea fertilizer production plant (Hamid and El-Gazzar, 1996); there were no direct measurements of workplace exposure to ammonia and information on control for potentially confounding exposures was not provided (Table 1-6).

Evidence of liver toxicity 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; Table 1-8), suggesting that animals can recover from intermittent exposure to elevated ammonia levels (Coon et al., 1970). In addition, no effects on nonrespiratory system organs 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) in rats exposed to 262 mg/m^3 ammonia for 90 days 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 exposed monkeys or 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; the study authors stated that differences in pH leveled off at 10 and 15 days (Manninen et al., 1988). In another study, blood pH in rats was not affected by exposure to ammonia at concentrations up to 818 mg/m^3 for up to 24 hours (Schaerdel et al., 1983).

Encephalopathy related to ammonia may occur in humans following disruption of the body's normal homeostatic regulation of the glutamine and urea cycles, e.g., due to severe liver or kidney disease resulting in elevated ammonia levels in blood (Minana et al., 1995; Souba, 1987). Acute inhalation exposure studies have identified alterations in amino acid levels and neurotransmitter metabolism (including glutamine concentrations) in the brain of rats and mice (Manninen and Savolainen, 1989; Manninen et al., 1988; Sadasivudu et al., 1979; Sadasivudu and

Radha Krishna Murthy, 1978). It has been suggested that glutamate and γ-amino butyric acid play a role in ammonia-induced neurotoxicity (Jones, 2002). There is no evidence, however, that ammonia is neurotoxic in humans or animals following chronic inhalation exposures.

In the only study of the reproductive and developmental toxicity of ammonia, no changes in reproductive or developmental endpoints were found between two groups of female pigs (crossbred gilts) exposed to ammonia via inhalation for 6 weeks at mean concentrations of 5 or 25 mg/m^3 and then mated (Diekman et al., 1993). A control group without ammonia exposure was not evaluated. Age at puberty did not differ significantly between the two groups. Gilts exposed to 25 mg/m^3 ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m^3; however, body weights of the two groups were similar at gestation day 30. Conception rates in the mated females were similar between the two groups (94.1 versus 100% in 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 systemic toxicity in humans and experimental animals exposed to ammonia is summarized in Tables 1-6 and 1-7 and as an exposure-response array in Figure 1-4.

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

Study design and reference	Results
Hamid and El-Gazzar (1996) (Egypt) Urea fertilizer plant workers (all men); 30 exposed and 30 control subjects (from administrative departments). Average employment duration: 12 yrs **Exposure:** No direct measurement of ammonia concentrations; blood urea used as surrogate measure **Outcome:** Blood sample measurements of AST, ALT, hemoglobin, and catalase and monoamine oxidase enzyme activities	↑ 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

Study design and reference	Results
Liver effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes observed.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Fatty liver changes in plate cells at 470 mg/m^3.[a]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d	Fatty liver changes in plate cells at 470 mg/m^3.[a,b]
Anderson et al. (1964) Swiss albino mouse; male and female; 4/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d	No visible signs of liver toxicity.
Weatherby (1952) Guinea pig (strain not specified); male; 6–12/group 0 or 170 ppm (0 or 120 mg/m^3) for 6 hrs/d, 5 d/wk for 6, 12 or 18 wks	Congestion of the liver at 18 wks, not observed at earlier times.[a]
Anderson et al. (1964) 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	Congestion of the liver at 35 mg/m^3 for 42 d.[a]
Adrenal gland effects	
Weatherby (1952) 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	"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 1-7. Evidence pertaining to other systemic effects in animals

Study design and reference	Results
Fazekas (1939) Rabbit (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 and 120–230 mg/kg-d, respectively[c]	*Mean adrenal weight compared to control:* 95% *Fat content of adrenal gland compared to control:* 4.5-fold ↑. Note: results by dose level were not provided.
Kidney and spleen effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes observed.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3.[a]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d	Calcification and proliferation of renal tubular epithelium at 470 mg/m^3.[a,b]
Anderson et al. (1964) Swiss albino mouse; male and female; 4/group 0 or 20 ppm (0 or 14 mg/m^3) for 7–42 d	No visible signs of toxicity.
Weatherby (1952) 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	Congestion of the spleen and kidneys.[a]
Anderson et al. (1964) 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	Enlarged and congested spleens at 35 mg/m^3.[a]
Myocardial effects	
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	No histopathologic changes observed.

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

Study design and reference	Results
Coon et al. (1970) New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Myocardial fibrosis at 470 mg/m^3.[a,b]
Coon et al. (1970) Sprague-Dawley or Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d	Myocardial fibrosis at 470 mg/m^3.[a]
Ocular effects	
Coon et al. (1970) Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	No ocular irritation observed.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group New Zealand albino rabbit; male; 3/group Princeton-derived guinea pig; male and female; 15/group Squirrel monkey (*S. sciureus*); male; 3/group Beagle dog; male; 2/group 0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks	No ocular irritation observed.
Coon et al. (1970) Sprague-Dawley and Long-Evans rat; male and female; 15–51/group 0 or 40 mg/m^3 for 114 d or 127, 262, or 470 mg/m^3 for 90 d	No ocular irritation observed.
Coon et al. (1970) New Zealand albino rabbit; male; 3/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Erythema, discharge, and ocular opacity over ¼–½ of cornea at 470 mg/m^3.[a]
Coon et al. (1970) Beagle dog; male; 2/group 0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d	Heavy lacrimation at 470 mg/m^3.[a]
Blood pH changes	
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25 or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5, 10 or 15 d	↓ blood pH at 5 days; pH differences "leveled off at later time points (data not shown)". *Blood pH (day 5)*: 7.43, 7.34*, 7.36*
Schaerdel et al. (1983) Crl:COBS CD(SD) rat; male; 8/group [blood pO_2 based on n = 5] 15, 32, 310, or 1,157 ppm (11, 23, 219, or 818 mg/m^3) for 0 (control), 8, 12, or 24 hrs	↑ blood pO_2 at 11 and 23 mg/m^3 at 8-, 12-, and 24-hr time points; no change at higher concentrations; no change in blood pH. *Percent change in pO_2 from time 0 (at 24 hours of exposure*[d]: 20*, 17*, 1, -2%

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

Study design and reference	Results
Amino acid levels and neurotransmitter metabolism in the brain	
Manninen and Savolainen (1989) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5 d	*% change compared to control*:[e] Brain glutamine: 42*, 40*%
Manninen et al. (1988) Wistar rat; female; 5/group 0, 25, or 300 ppm (0, 18, or 212 mg/m^3) 6 hrs/d for 5, 10, or 15 d	*% change compared to control at 212 mg/m^3*:[e] Blood glutamine (5, 10, 15 d): 44*, 13, 14% Brain glutamine (5, 10, 15 d): 40*, 4, 2%
Reproductive and developmental effects	
Diekman et al. (1993) Crossbred gilt (female pig); 4.5 mo old; 40/group 7 ppm (5 mg/m^3), range 4–12 ppm (3–8.5 mg/m^3) or 35 ppm (25 mg/m^3), range 26–45 (18–32 mg/m^3) for 6 wks[f]	No change in any of the reproductive or developmental parameters measured (age at puberty, conception rates, body weight of pregnant gilts, number of corpora lutea, number of live fetuses, and weight or length of fetuses).

[a]Incidence data not provided.
[b]Exposure to 470 mg/m^3 ammonia increased mortality in rats.
[c]Ammonia doses estimated using assumed average default body weight of 3.5–4.1 kg for adult rabbits (U.S. EPA, 1988).
[d]Measurements at time zero were used as a control; the study did not include an unexposed control group.
[e]Percent change compared to control calculated as: (treated value – control value)/control value x 100.
[f]A control group was not included. Prior to exposure to ammonia, pigs were also exposed naturally in conventional grower units to *Mycoplasma hypopneumoniae* and *Pasteurella multocida*, which cause pneumonia and atrophic rhinitis, respectively.

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

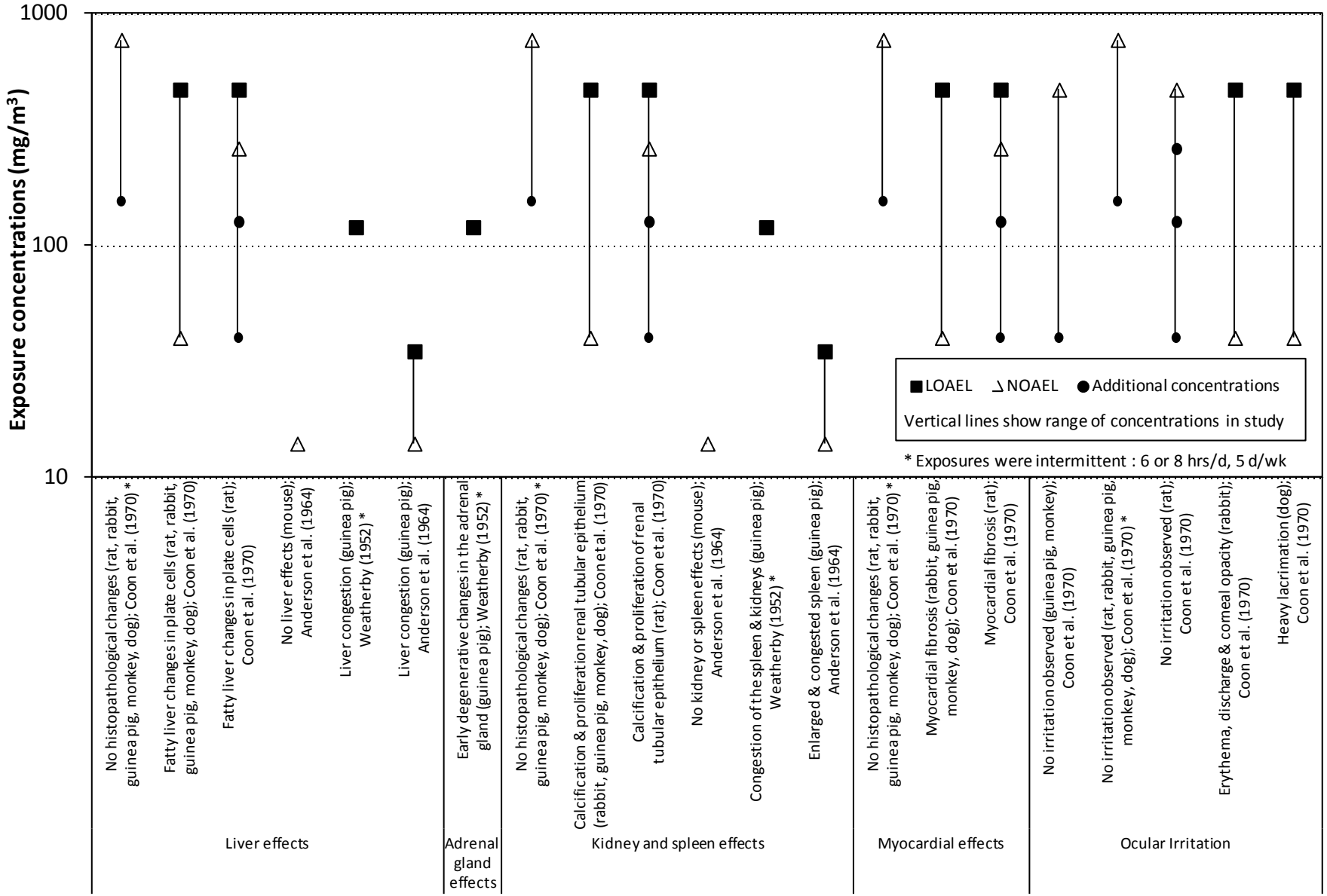

Figure 1-4. Exposure-response array of systemic effects following inhalation exposure to ammonia.

Summary of Other Systemic Effects

Effects of ammonia exposure on organs distal from the portal of entry are based largely on evidence in animals and, to a more limited extent, in humans. 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. Evidence of ocular irritation in experimental animals was inconsistently observed, and then only at high ammonia concentrations (470 mg/m^3).

Studies of ammonia toxicity that examined other systemic effects were all published in the older toxicological literature. 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.

Ammonia is produced endogenously in all human and animal tissues during fetal and adult life, and concentrations of free ammonia in 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. 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 Appendix E, Section E.1, Metabolism, for a more detailed summary of the available literature that describes the relationship between environmental ammonia concentrations and changes in ammonia homeostasis.

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.

1.1.5. 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., 1992b). 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., 1992b).

The evidence of carcinogenicity in experimental animals exposed to ammonia is summarized in Table 1-8.

Table 1-8. Evidence pertaining to cancer in animals

Study design and reference	Results
Carcinogenesis studies	
Toth (1972) 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]	Tumor incidence was not increased in ammonia-exposed mice; however, concurrent control tumor incidence data were not reported.
Toth (1972) C3H mouse; 40/sex/group 0.1% ammonium hydroxide in drinking water for their lifetime [191 (males) and 214 mg/kg-d (females)][b]	Tumor incidence was 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	
Tsujii et al. (1992b) 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	*Gastric tumor incidence:* 31, 70*% *# of gastric tumors/tumor-bearing rat:* 1.3, 2.1*
Tsujii et al. (1995) 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	*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 E, Section E.4, Tables E-14 and E-15). 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. Weight of Evidence for Effects Other than Cancer

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

Evidence for an association between inhaled ammonia exposure and effects on other organ systems distal from the portal of entry, 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 target of 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 of 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. Reproductive effects were not associated with inhaled ammonia in the only animal study that examined the reproductive effects of ammonia (i.e., a limited-design inhalation study in the pig). Further, ammonia is produced endogenously in human and animal tissues during fetal and adult life, and concentrations of free ammonia in physiological fluids are homeostatically regulated to remain at low levels (Souba, 1987). Thus, 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 hazard for systemic effects, including effects on the developing fetus or reproductive tissues.

1.2.2. Weight of Evidence for 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., 1992b). 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. Controlled human studies that examined both healthy volunteers and volunteers with asthma (Petrova et al., 2008; Sigurdarson et al., 2004) did not demonstrate greater respiratory sensitivity in asthmatics than healthy volunteers after acute exposure to ammonia. Under longer-term exposure conditions, however, as seen among livestock farmers, one study observed associations between ammonia exposure and decreased lung function among workers with chronic respiratory symptoms, but not among the asymptomatic workers (Preller et al., 1995). Additional research focusing on the question of variability in response to ammonia exposure is needed.

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% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to these points of departure (PODs) to reflect limitations of the data used.

The available human and animal 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 following intentional or accidental ingestion of household cleaning solutions containing ammonia or ammonia inhalant capsules. Case reports can indicate the nature of acute effects of ammonia exposure and thus inform hazard identification. Because of short exposure durations and incomplete or missing quantitative exposure information, data from case reports are inadequate for dose-response analysis and subsequent derivation of a chronic reference value.

The experimental animal database for ammonia lacks standard toxicity studies that systematically 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 the 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, 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 amount of toxicity testing that has been conducted on ingested ammonia and questions concerning the adversity of the observed gastric mucosal findings in rats, the available oral database for ammonia was considered insufficient to adequately characterize toxicity outcomes and dose-response relationships. Accordingly, **an RfD for ammonia was not derived**.

Previous IRIS Assessment

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

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

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

Respiratory effects, characterized as increased prevalence of respiratory symptoms or decreased lung function, have been observed in worker populations exposed to ammonia concentrations ≥18.5 mg/m^3 (Rahman et al., 2007; Ali et al., 2001; Ballal et al., 1998). Decrements in lung function parameters and increased prevalence of respiratory symptoms such as wheezing, chest tightness, and cough/phlegm, have been identified as adverse respiratory health effects by the American Thoracic Society (ATS, 2000) and are similarly noted as adverse in the EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Respiratory effects have also been observed in animals, but at ammonia concentrations higher than those associated with respiratory effects in humans and in studies involving exposure durations (up to 114 days) shorter than those in occupational studies.

In general human data are preferred over animal data for deriving reference values because these data are more relevant for assessing human health effects than animal studies and avoid the uncertainty associated with interspecies extrapolation when animal data serve as the basis for the RfC. In the case of ammonia, the available occupational studies provide adequate data for the quantitative analysis of health outcomes considered relevant to potential general population exposures. In addition, ammonia concentrations associated with respiratory effects in human studies were generally lower than effect levels identified in animal studies (Section 1.1.1). Therefore, data on respiratory effects in humans were used for the derivation of the RfC and respiratory effects in animals were not further considered.

Of the available human data, associations between ammonia exposure and respiratory effects have been examined in epidemiology studies of industrial worker populations (Table 1-1), workers using ammonia as a cleaning product (Table 1-2), and livestock farmers. Studies of

workers using ammonia as a cleaning product provide evidence of an association between ammonia exposure and increased risk of asthma; however, these studies did not measure ammonia concentrations in workplace air and thus are not useful for dose-response analysis. Studies in livestock farmers also support an association between ammonia exposure and decreased pulmonary function; however, because of co-exposures to other agents in these studies (including dust, endotoxin, mold, and disinfectant products) and the availability of studies with fewer co-exposures, studies of livestock farmers were considered to be supportive of the association between ammonia exposure and respiratory effects but were not carried forward for dose-response analysis.

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

In general, the four cross-sectional occupational studies provide a coherent set of estimated NOAELs (i.e., workplace exposures up to 8.8 mg/m^3) and effect levels, and are considered candidate principal studies for RfC derivation. Rahman et al. (2007) observed an increased prevalence of respiratory symptoms and decreased lung function in fertilizer plant workers exposed to a mean ammonia concentration of 18.5 mg/m^3, but not in workers in a second plant exposed to a mean ammonia concentration of 4.9 mg/m^3. Ballal et al. (1998) observed an increased prevalence of respiratory symptoms among workers in one factory (Factory A) with ammonia exposures ranging from 2–27.1 mg/m^3,[5] but no increase in symptoms in another factory (Factory B) with exposures ranging from 0.02–7 mg/m^3. A companion study by Ali et al. (2001) observed decreased lung function among workers in the factory with the higher ammonia exposures (Factory A); the factory with the lower ammonia exposures, also studied by Ballal et al. (1998), was not included in this companion study by Ali et al. (2001). Holness et al. (1989) found no differences in the prevalence of respiratory symptoms or lung function between workers (mean exposure 6.5 mg/m^3) and the control group, and also no differences in respiratory symptoms or lung function when workers were stratified by ammonia exposure level (lowest exposure group, <4.4 mg/m^3; middle exposure group, 4.4–8.8 mg/m^3; highest exposure group, >8.8 mg/m^3).

The NOAEL of 8.8 mg/m^3 from the Holness et al. (1989) study represents the low end of the high-exposure group (defined as those exposed to >8.8 mg/m^3) from this study. The authors state that 3 of the 12 workers in the high-exposure group were exposed to concentrations >17.7 mg/m^3; therefore, the majority of workers in the high-exposure group (9 of 12) would have been exposed to

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

ammonia concentrations in the range of 8.8–17.7 mg/m^3. In the absence of more detailed exposure information, the low-end of the range was considered a reasonable estimate of the NOAEL from the Holness et al. (1989) study.

Of the four candidate principal studies, higher confidence is associated with the exposure measures from Holness et al. (1989). Both Holness et al. (1989) and Rahman et al. (2007) collected personal air samples, but confidence in the analytical method used by Holness et al. (1989) is higher than that used by Rahman et al. (2007). Rahman et al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air (i.e., Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by four- to fivefold, indicating some uncertainty across the two measurement methods, although ammonia concentrations measured by the two methods were strongly correlated (correlation coefficient of 0.8). In contrast, the Holness et al. (1989) study used an established analytical method for measuring exposure to ammonia recommended by the National Institute for Occupational Safety and Health (NIOSH) that involved the collection of air samples on acid-treated silica gel absorption tubes. Ballal et al. (1998) used area monitors rather than personal air sampling methods; the latter method provides a better estimate of an individual's exposure. Both Holness et al. (1989) and Rahman et al. (2007) examined both respiratory symptoms and lung function, which provides stronger evidence of respiratory effects than symptom data alone. Ballal et al. (1998) evaluated only respiratory symptoms. Ali et al. (2001), the companion study to Ballal et al. (1998), examined pulmonary function; however, because Ali et al. (2001) evaluated only workers in the higher exposure setting, the data cannot be used to estimate a NOAEL.

Considerations in selecting the principal study for RfC derivation include the higher confidence placed in the measures of ammonia exposure in Holness et al. (1989) as compared to the other candidate studies, evaluation of both respiratory symptoms and lung function parameters in the Holness et al. (1989) study, and the fact that the estimate of the NOAEL for respiratory effects of 8.8 mg/m^3 from Holness et al. (1989) was the highest of the NOAELs estimated from the candidate principal studies. The Holness et al. (1989) study does not demonstrate a relationship between ammonia exposure and respiratory effects probably because of the relatively low levels of ammonia in the workplace that reflect the controlled nature of the operations at the plant. The Holness et al. (1989) study is identified as the principal study for derivation of the RfC, but only with support from the collection of occupational epidemiology studies that includes studies with higher workplace ammonia concentrations.

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

2.2.2. Methods of Analysis

A **NOAEL of 8.8 mg/m^3, identified from the Holness et al. (1989) study, was used as the point of departure (POD) for RfC derivation.**

Because the RfC assumes continuous human exposure over a lifetime, the POD was adjusted to account for the noncontinuous exposure associated with occupational exposure (i.e., 8-hour workday and 5-day workweek). 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, 2011a)

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

2.2.3. Derivation of the Reference Concentration

Consistent with EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5), also described in the Preamble, five possible areas of uncertainty and variability were considered when deriving the RfC. A **composite UF of 10** was applied to the selected duration-adjusted POD of 3.1 mg/m^3 to derive the RfC of 0.3 mg/m^3. An explanation of the five possible areas of uncertainty and variability follows:

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

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

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

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

- A database uncertainty factor, UF_D, of 1 was applied to account for deficiencies in the database. The ammonia inhalation database consists of epidemiological studies and experimental animal studies. The epidemiological studies include industrial worker populations, populations exposed to ammonia through the use of cleaning products, 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. 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 (Jóźwik et al., 2005; Jóźwik et al., 1999; 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). 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 (i.e., 1.0–1.4 µg/mL in umbilical arterial and venous blood compared to 0.5 µg/mL in the mothers' venous 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 E, Section E.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, exposure to 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:

RfC = $NOAEL_{ADJ}$ ÷ UF
= 3.1 mg/m^3 ÷ 10
= 0.31 mg/m^3 or **0.3 mg/m^3 (rounded to one significant figure)**

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.,

[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.

in the case of the ammonia RfC, a factor to address the absence of data to evaluate the variability in response to inhaled ammonia in the human population). The following discussion identifies additional uncertainties associated with the quantification of the RfC for ammonia.

Use of a NOAEL as a POD

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

Endogenous Ammonia

Ammonia, which is produced endogenously, has been detected in breath exhaled from the nose and trachea of humans (range: 0.0092–0.1 mg/m^3) (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977). Higher and more variable ammonia concentrations are reported in human 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 (Schmidt et al., 2013; 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: 0.0092–0.1 mg/m^3) (Schmidt et al., 2013; Smith et al., 2008; Larson et al., 1977) and appear to better represent levels at the alveolar interface of the lung or in the tracheo-bronchial region and are thought to be more relevant to understanding systemic levels of ammonia than ammonia in breath exhaled from the mouth (Schmidt et al., 2013; Smith et al., 2008) (Appendix E, Section E.1 and Table E-1).

It is important to recognize that ammonia in ambient air is the source of some of the ammonia in exhaled breath. Studies of ammonia in exhaled breath (Appendix E, Table E-1) were conducted in environments with measureable levels of ambient (exogenous) ammonia rather than in ammonia-free environments, and it has been established that concentrations of certain trace compounds in exhaled breath are correlated with their ambient concentrations (Spanel et al., 2013). Spanel et al. (2013) found that 70% (± 13%) of inhaled ammonia is retained in exhaled breath. It is likely that ammonia concentrations in breath exhaled from the nose would be lower if the inspired air were free of ammonia. Therefore, levels of ammonia in exhaled breath reported in the literature would need to be adjusted if they are to be used as a measure of systemic ammonia.

Ammonia concentrations measured in breath exhaled from the nose and trachea, considered to be more representative of systemic levels of ammonia than breath exhaled from the mouth, are lower than the ammonia RfC of 0.3 mg/m^3 by a factor of threefold or more. The range of ammonia breath concentrations measured in samples collected from the mouth (0.085 to 2.1 mg/m^3), i.e., concentrations that are largely influenced by such factors as ammonia production via bacterial degradation of food protein, includes the value of the ammonia RfC. Ammonia exhaled by an individual, whether through the nose or mouth, is rapidly diluted in the larger volume of ambient air and would not contribute significantly to overall ammonia exposure. Further, such endogenous exposures existed in the occupational epidemiology studies that served as the basis for the ammonia RfC.

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, the results from the principal study are supported by the results from other cross-sectional studies of workers in industrial settings, studies of workers using ammonia as a cleaning product, studies of livestock farmers, multiple studies of acute ammonia exposure in volunteers, and the available inhalation data from animals.

Confidence in the database is medium. The inhalation ammonia database includes one limited study of reproductive and developmental toxicity in pigs that did not examine a complete set of reproductive or developmental endpoints. Normally, confidence in a database lacking these types of studies is considered to be lower due to the uncertainty surrounding the use of any one or several studies to adequately address all potential endpoints following chemical exposure at various critical lifestages. Unless a comprehensive array of endpoints is addressed by the database, there is uncertainty as to whether the critical effect chosen for 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

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]

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 up to 32 ppm (22.6 mg/m^3) and only minimal increases at 300–1,000 ppm (212–707 mg/m^3), suggesting that no significant distribution is likely to occur at the human equivalent concentration. 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 of ammonia also did not include a carcinogenicity assessment.

[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 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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