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Toxicological Review of

Ammonia

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Washington, DC

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5 ACGIH American Conference of Governmental
6 Industrial Hygienists
7 ALP alkaline phosphatase
8 ALT alanine aminotransferase
9 AST aspartate aminotransferase
10 ATSDR Agency for Toxic Substances and Disease
11 Registry
12 ATSG acid-treated silica gel
13 BAL bronchioalveolar lavage
14 BMD benchmark dose
15 BMI body mass index
16 BrDU 5-bromo-2-deoxyuridine
17 BUN blood urea nitrogen
18 CAC cumulative ammonia concentration
19 CASRN Chemical Abstracts Service Registry
20 Number
21 CI confidence interval
22 EPA Environmental Protection Agency
23 EU endotoxin unit
24 FEF forced expiratory flow
25 FEV_1 forced expiratory volume in 1 second
26 FVC forced vital capacity
27 GABA γ-amino butyric acid
28 IgE immunoglobin E
29 IgG immunoglobin G
30 IRIS Integrated Risk Information System
31 LOAEL lowest-observed-adverse-effect level
32 MAO monoamine oxidase
33 MMEF mean midexpiratory flow
34 MNNG N-methyl-N'-nitro-N-nitrosoguanidine
35 MRM murine respiratory mycoplasmosis
36 NH_3 ammonia
37 NH_4^+ ammonium ion
38 NIOSH National Institute for Occupational Safety
39 and Health
40 NOAEL no-observed-adverse-effect level
41 NOx nitrogen oxide
42 NRC National Research Council
43 OR odds ratio
44 PBPK physiologically based pharmacokinetic
45 PEF peak expiratory flow
46 PEFR peak expiratory flow rate
47 PHA phytohemagglutin
48 POD point of departure
49 PPD purified protein derivative
50 RfC inhalation reference concentration
51 RfD oral reference dose
52 RNA ribonucleic acid
53 SD standard deviation
54 SIFT-MS selected ion flow tube mass spectrometry
55 TLV threshold limit value
56 TWA time-weighted average
57 UF uncertainty factor

58 UF_A interspecies uncertainty factor
59 UF_H intraspecies uncertainty factor
60 UF_L LOAEL to NOAEL uncertainty factor
61 UF_S subchronic-to-chronic uncertainty factor
62 UF_D database deficiencies uncertainty factor
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PREAMBLE

1. Scope of the IRIS Program

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

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

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

2. Process for developing and peer-reviewing IRIS assessments

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

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

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

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

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

Step 5. Revision of draft Toxicological Review and development of draft IRIS summary (2 months). The draft assessment is revised to reflect the peer review comments, public comments, and newly available studies. 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 White House offices (1-1/2 months). The draft assessment and summary are revised to address

EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

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

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, 1986a, 1986b, 1991, 1996, 1998, 2000a, 2005a, 2005b, 2006a) and other descriptions of "best practices" (U.S. EPA, 1994, 2000b, 2002, 2006b, 2011a). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

3. Identifying and selecting pertinent studies

3.1 Identifying studies

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

Each assessment also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted to EPA under the Toxic Substances Control Act. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, EPA will have it peer-reviewed.

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

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

– Studies of the mixture being assessed.

– Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.

– Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

3.2 Selecting pertinent epidemiologic studies

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

– Cohort studies and case-control studies provide the strongest epidemiologic evidence, as they collect information about individual exposures and disease.

– Cross-sectional studies provide useful evidence if they relate exposures and disease at the individual level and it is clear that exposure preceded the onset of disease.

– Ecologic studies (geographic correlation studies) relate exposures and disease 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 disease or about the relevance of analogous results in animals.

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

3.3 Selecting pertinent experimental studies

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

– Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered most pertinent to human environmental exposure.

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

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

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.

- Studies of effects from subchronic exposure are pertinent but less preferred than studies of chronic exposure.

- Short-term and acute studies are less pertinent but are useful for obtaining toxicokinetic or mechanistic information. The assessment reviews short-term and acute studies if they suggest distribution or effects at a site not identified by longer-term studies.

- 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, 1991, 1996, 1998).

4. Evaluating the quality of individual studies

4.1 Evaluating the quality of epidemiologic studies

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

- Documentation of study design, methods, population characteristics, and results.

- Definition and selection of the study and comparison populations.

- Ascertainment of exposure and the potential for misclassification.

- Ascertainment of disease or effect and the potential for misclassification.

- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects, including latent effects.

- Characterization of exposure during critical periods for the development of effects.

- Sample size and statistical power to detect anticipated effects.

- Participation rates and the resulting potential for selection bias.

- Potential confounding and other sources of bias are identified and addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is prevalent in the population and is related to both exposure and outcome.

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

4.2 Evaluating the quality of experimental studies

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

- Documentation of study design, animals or study population, methods, basic data, and results.

- Relevance of the animal model or study population and the experimental methods.

- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.

- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.

- Sample sizes and statistical power to detect dose-related differences or trends.

- Ascertainment of survival, vital signs, disease or effects, and cause of death.

- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (U.S. EPA, 2005a).

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

4.3 Reporting study results

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

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

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

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

5. Weighing the overall evidence of each effect

5.1 Weighing epidemiologic evidence

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

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 a disease that is common, exposure misclassification, or other sources of bias.

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

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

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

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

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

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

"Natural experiments": A change in exposure that brings about a change in disease frequency provides strong evidence of causality.

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

These considerations are consistent with contemporary guidelines 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,b).

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

5.2 Weighing experimental evidence

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Although causality is not at issue in controlled experiments, several concepts discussed by Hill (1965) affect the weight of

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experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 1994, 2002, 2005a).

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

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

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

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

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

5.3 Characterizing modes of action

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a).

(a) **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, where suppressing a key event suppresses the disease. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, but not by replicate experiments in the same model. The assessment may consider various aspects of causality in addressing this question.

(b) **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 modes of action. Information suggesting quantitative differences is considered in dose-response analyses but is not used to determine relevance. Similarly, anticipated levels of human exposure are not used to determine relevance.

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

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

Studies of genetic toxicity are often available, and the assessment evaluates the evidence of a mutagenic mode of action.

– Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (*in vivo*) provides the strongest evidence.

– This is followed by positive results in lower organisms or in cultured cells (*in vitro*) or for other genetic events.

– Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC, 2006).

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

5.4 Characterizing the overall weight of the evidence

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

Carcinogenic to humans: There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive

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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 data.

Suggestive evidence of carcinogenic potential: The data raise concern for effects in humans but are 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 are robust data 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.

6. Selecting studies for derivation of toxicity values

For each effect associated with an agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The derivation of toxicity values may be linked to the weight-of-evidence descriptor. For example, EPA typically derives toxicity values for agents classified as *carcinogenic to humans* or *likely to be carcinogenic*, but not for agents with *inadequate information* or that are *not likely to be carcinogenic* (U.S. EPA, 2005a).

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

– 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 that show an exposure-response gradient are preferred, as long as lack of a monotonic relationship at higher exposure levels can be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, misclassification bias, or selection bias.

– Among studies that show an exposure-response gradient, those with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

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

7. Deriving toxicity values

7.1 General framework for dose-response analysis

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

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

Extrapolation to lower doses considers what is known about the modes of action for each effect (sections 7.4-7.5). An alternative to low-dose extrapolation is derivation of reference values, which are calculated by adjusting the point of departure by factors that account for several sources of uncertainty and variability (section 7.6).

Increasingly, EPA is making use of multiple data sets or combining multiple responses in deriving toxicity values. EPA also considers multiple dose-response approaches when they can be supported by robust data.

7.2 Modeling dose

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

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

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

– 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. As allometric scaling is typically based on adult body weight, it is not used for early-life exposure or for developmental effects (U.S. EPA, 2005a, 2011a).

   – Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 1994).

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

7.3 Modeling response in the range of observation

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

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

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

– For dichotomous responses, the point of departure is the 95% lower bound on the dose associated with a small increase of a biologically significant effect.

   – If linear extrapolation to lower doses will be used, a standard value near the low end of the observable range is used (10% response for animal data, 1% for epidemiologic data, depending on the observed response rates).

   – If nonlinear extrapolation will be used, both statistical and biologic factors are considered (10% response for minimally adverse effects, 5% or lower for more severe effects or for developmental toxicity data on individual offspring).

– For continuous responses, the point of departure is ideally a level where the effect is considered minimally adverse. In the absence of such definition, both statistical and biologic factors are considered in selecting a response level.

7.4 Extrapolating to lower doses

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

(1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model beyond the observed range if significant model uncertainty can be ruled out with reasonable confidence. Below the range where confidence bounds on the predictions are reasonably precise, extrapolation may continue using a linear model.

(2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:

– Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.

– Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

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

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

(3) Nonlinear extrapolation is used if there are sufficient data to ascertain the mode of action and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. If nonlinear extrapolation is appropriate but no model is developed, a default is to calculate reference values.

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

7.5 Considering susceptible populations and life-stages

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

(1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or life-stage, 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, application of *age-dependent adjustment factors* is recommended for early-life exposure to suspected carcinogens. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, EPA recommends:

– 10-fold adjustment for exposures before age 2 years.

– 3-fold adjustment for exposures between ages 2 and 16 years.

These adjustments are generally applied only for a mutagenic mode of action, though early-life susceptibility has been observed for several carcinogens that are not mutagenic (U.S. EPA, 2005b).

7.6 Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a threshold can be based on prevention of an early key event. Reference values provide no information about risks at exposures above the reference value.

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, reproductive, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1991, 1996, 1998).

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

Human variation. A factor of 10 is applied to account for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who

are most susceptible to the effect. This factor is reduced only if the point of departure is derived specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 1991, 1994, 1996, 1998, 2002).

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

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, 1991, 1994, 1996, 1998, 2002).

Subchronic-to-chronic exposure. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure would have effects at lower levels. 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, 1994, 1998, 2002).

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

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

The assessment may also report reference values for each effect. This would facilitate subsequent cumulative risk assessments, where it may be important to consider the combined effect of chemicals acting at a common site or operating through common mechanisms (U.S. EPA, 2002).

7.7 Confidence and uncertainty in the reference values

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

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

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

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

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

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

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

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This assessment was provided for review to other federal agencies and White House offices. The federal agencies and White House offices that commented are listed below. Comments submitted by the agencies listed below are available on the IRIS website.

Agency for Toxic Substances and Disease Registry, Public Health Service, Department of Health & Human Services

Office of Public Health Service, Food Safety Inspection Service, U.S. Department of Agriculture

A public listening session was held by EPA on [month] [date], [year]. Attendees external to the EPA are listed below.

NAME	Affiliation
NAME	Affiliation
NAME	Affiliation
NAME	Affiliation

This assessment was released for public comment on [month] [date], 2012; the public comment period ended on [month] [date], [year]. Comments were received from the following entities.

NAME	Affiliation, Location
NAME	Affiliation, Location
NAME	Affiliation, Location
NAME	Affiliation, Location

This assessment was peer-reviewed by independent expert scientists external to EPA (specify SAB or NAS panel) and a peer review meeting was held on [month] [date], [year]. The external peer review comments are available on the IRIS website. EPA's response to the external peer review and public comments is included in Appendix C and is also available on the IRIS website.

NAME	Affiliation, Location
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NAME	Affiliation, Location

PREFACE

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to exposure to ammonia. This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of ammonia. The appendices to this document include information addressing chemical and physical properties, ammonium salts, toxicokinetics, toxicity study summaries, and external peer review, and are included in a separate volume: the *Supplemental Information for the Toxicological Review of Ammonia*.

The Toxicological Review of Ammonia is an update of a previous IRIS assessment for ammonia posted to the IRIS database in 1991. The previous assessment included an inhalation RfC only. A reassessment of ammonia was conducted because of concerns related to ammonia emissions generated from its use in selective catalytic reduction-based diesel engine aftertreatment technology to reduce nitrogen oxide (NOx) to N2 gas and the presence of ammonia at hazardous waste National Priorities List (NPL) sites. Ammonia is found in over 8% of the hazardous waste NPL sites (ATSDR, 2004).

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) 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.

Background

Ammonia is a corrosive gas with a very pungent odor (O'Neil et al., 2006). It is highly soluble in water (4.82×10^5 mg/L) and is a weak base (Lide, 2008; Eggeman, 2001; Dean, 1985). When ammonia (NH_3) is present in water at environmental pH, a pKa of 9.25 indicates that the equilibrium will favor the formation of the conjugate acid, the ammonium ion (NH_4^+) (Lide, 2008). A solution of ammonia in water is sometimes referred to as ammonium hydroxide because the ammonia and water both ionize to form ammonium cations and hydroxide anions (Eggeman, 2001). Ammonium salts are easily dissolved in water and disassociate into the ammonium ion and the anion. At physiological pH (7.4), the equilibrium between NH_3 and

1 NH_4^+ favors the formation of NH_4^+. Additional chemical and physical properties information for

2 ammonia is provided in Appendix A, Section A.1.

3 Low levels of ammonia occur naturally in the environment in air, soil, and water.

4 Ammonia is a major component of the geochemical nitrogen cycle and is essential for many

5 biological processes (Rosswall, 1981). Nitrogen-fixing bacteria convert atmospheric nitrogen

6 into ammonia available for plant uptake (Socolow, 1999; Rosswall, 1981). Organic nitrogen

7 released from biota is converted into ammonia through nitrogen mineralization (Rosswall, 1981).

8 Ammonia in water and soil is naturally converted into nitrite and nitrate through the process of

9 nitrification (Rosswall, 1981). Ammonia is endogenously produced in humans and animals, is

10 an essential mammalian metabolite used in nucleic acid and protein synthesis, is necessary for

11 maintaining acid-base balance, and is an integral part of nitrogen homeostasis (Nelson and Cox,

12 2008).

13 With regard to exogenous exposure, the largest and most significant use of ammonia is

14 the agricultural application of fertilizers, which represents about 80-85% of commercially-

15 produced ammonia in the form of urea, ammonium nitrate, ammonium sulfate, ammonium

16 phosphate, and other nitrogen compounds (Eggeman, 2001). Ammonia is also used as a

17 corrosion inhibitor, in the purification of water supplies, as a component of household cleaners,

18 as a refrigerant, as a chemical intermediate in pharmaceuticals, explosives and other chemicals,

19 as a stabilizer in the rubber industry, and as a hydrogen source for the hydrogenation of fats and

20 oils. Ammonia (generated from urea injected into the exhaust stream) is also used in the

21 reduction of NOx emissions from the exhaust of diesel vehicles and stationary combustion

22 sources such as industrial and municipal boilers and power generators (Eggeman, 2001; HSDB,

23 2009; Johnson et al., 2009).

24

25 **Scope of the Assessment**

26 This assessment presents a review of hazard and dose-response information for ammonia,

27 including gaseous ammonia (NH_3) and ammonia dissolved in water (ammonium hydroxide,

28 NH_4OH). Because ammonium salts (e.g., ammonium acetate, chloride, and sulfate) readily

29 dissolve in water through disassociation into the ammonium ion (NH_4^+) and the anion, EPA

30 considered whether or not the literature on ammonium salts could inform the toxicity of

31 ammonia. The toxicology literature for ammonium salts includes several oral toxicity studies of

32 ammonium chloride and ammonium sulfate. No inhalation toxicity studies of ammonium salts

33 are available. The toxicity data for ammonium chloride and ammonium sulfate demonstrate that

34 these two salts present distinctly different toxicity profiles, suggesting that the anion can

35 influence the toxicity of the ammonium compound, and that the toxicity of the salts cannot

36 necessarily be attributed to the cation (i.e., NH_4^+) only (for detailed ammonium salts information

37 see Appendix A, Section A.2 and Table A-2). Accordingly, information on the toxicity of

ammonium salts was not used to characterize the toxicity of ammonia or ammonium hydroxide in this assessment.

Other Agency and International Assessments

Assessments and regulatory limits for ammonia developed by other health agencies, including the Agency for Toxic Substances and Disease Registry (ATSDR), the National Research Council (NRC), the American Conference of Governmental Industrial Hygienists (ACGIH), the National Institute of Occupational Safety and Health (NIOSH), and the Food and Drug Administration (FDA), are identified in Appendix B of the Supplemental Material.

EXECUTIVE SUMMARY

Effects other than cancer observed following oral exposure

 The oral toxicity database for ammonia is very limited. Gastric toxicity is identified as a hazard for ammonia based on evidence from case reports in humans, two animal studies, and mechanistic studies. Evidence in humans is limited to case reports of individuals suffering from gastrointestinal effects from ingesting household cleaning solutions containing ammonia or biting into capsules of ammonia smelling salts. In rats, gastrointestinal effects, characterized as increased epithelial cell migration in the mucosa of the stomach and decreased thickness of the gastric mucosa, were reported following subchronic and short-term exposure to ammonia. These gastric mucosal effects observed in rats resemble mucosal changes in human atrophic gastritis; indicating this effect is biological plausible and relevant to humans.

 Given the limited number of studies available and the small number of toxicological evaluations, there are uncertainties associated with the oral database for ammonia and **a 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 exposure to ammonia comes from studies in humans and animals. Cross-sectional occupational studies involving chronic exposure to ammonia have consistently demonstrated an increased prevalence of respiratory effects and decreased lung function. Cross-sectional studies of livestock farmers exposed to ammonia, controlled human volunteer studies of ammonia inhalation, and case reports of injury in humans with inhalation exposure to ammonia provide additional and consistent support for the respiratory system as a target of ammonia toxicity. Additionally, respiratory effects were observed in several animal species following subchronic and short-term 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. The weight of evidence for these effects is less robust than for respiratory effects.

Inhalation reference concentration (RfC) for effects other than cancer

Table ES-1. Reference Concentration

Critical Effect	Point of Departure*	UF	Chronic RfC
Lack of decreased lung function and increased respiratory irritation Occupational epidemiology study Holness et al., 1989	NOAEL$_{ADJ}$: 3.1 mg/m^3	10	0.3 mg/m^3

*Because the POD (NOAEL = 8.8 mg/m^3) involved workplace exposure conditions, the NOAEL was adjusted for continuous exposure based on the ratio of VE$_{ho}$ (human occupational default minute volume of 10 m^3 breathed during an 8-hour workday) to VE$_h$ (human ambient default minute volume of 20 m^3 breathed during the entire day) and an exposure of 5 days out of 7 days.

The occupational exposure study of ammonia exposure in workers in a soda ash plant by **Holness et al. (1989) was identified as the principal study for RfC derivation**. Respiratory effects, characterized as increased respiratory irritation and decreased lung function, observed in workers exposed to ammonia were selected as the critical effect. In the evaluation of the prevalence of increased respiratory irritation and decreased lung function in workers exposed to ammonia (Holness et al., 1989), a NOAEL$_{ADJ}$ of 3.1 mg/m^3 (adjusted for continuous exposure from 8.8 mg/m^3; see calculation below) was identified based on the absence of statistically significant increases in the prevalence of the respiratory effects. BMD modeling was not utilized because ammonia concentrations in the Holness et al. (1989) study were not associated with changes in respiratory effects in the study population (i.e., data from Holness et al. could not be subjected to dose-response modeling). Thus, the Holness et al. (1989) data were analyzed using a NOAEL approach and the **NOAEL$_{ADJ}$ of 3.1 mg/m^3 was used as the POD for RfC derivation**.

The RfC was calculated by dividing the POD (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

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. Although there are no studies of developmental toxicity and studies of reproductive and other systemic endpoints are limited, 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 human carcinogenicity

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "**inadequate information to assess the carcinogenic potential**" of ammonia based on the absence of ammonia carcinogenicity studies in humans and a single lifetime drinking water study of ammonia in mice that showed no evidence of carcinogenic potential. There is limited evidence that ammonia may act as a cancer promoter based on the findings of *H. pylori*-induced gastric cancer. The available studies of ammonia genotoxicity are inadequate to characterize the genotoxic potential of this compound. **A quantitative cancer assessment for ammonia was not conducted.**

Susceptible Populations and Life Stages

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 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 life stages 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. Higher and more variable ammonia concentrations are reported in breath exhaled from the mouth or oral cavity (0.09 to 2.1 mg/m^3). These levels 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, age, and living

1   conditions (i.e., urban vs. rural setting). In contrast, ammonia concentrations measured in breath

2   exhaled from the nose and trachea are lower (0.013–0.078 mg/m^3) and more likely reflect levels

3   of ammonia circulating in the blood. These levels are lower than the ammonia RfC of 0.3 mg/m^3

4   by a factor of approximately fourfold or more. Although the RfC falls within the range of breath

5   concentrations collected from the mouth or oral cavity, ammonia in exhaled breath is expected to

6   be rapidly diluted in the much larger volume of ambient air.

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LITERATURE SEARCH STRATEGY & STUDY EVALUATION FOR HAZARD IDENTIFICATION

Literature Search Strategy and Study Selection

The literature search strategy employed for ammonia was conducted with the keywords listed in Table LS-1. Primary, peer-reviewed literature was identified through a literature search using the databases listed in Table LS-1. The literature search was last conducted on November 11, 2011. A data call-in was announced by EPA on December 21, 2007 (U.S. EPA, 2007); no submissions in response to the data call-in were received. Other peer-reviewed information, including health assessments developed by other health agencies, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate.

Table LS-1. Details of the search strategy employed for ammonia

Databases	Limits	Keywords
Pubmed Toxcenter Toxline Current Contents (2008 & 2010 only)	Search constraints: 2003-current[b] Pre-2003—ATSDR (2004) was used as the source of references published before 2003 Last search: November 11, 2011	**Chemical name and synonyms[a]:** ammonia (7664-41-7); ammonium hydroxide (1336-21-6); ammonium; spirit of hartshorn; aquammonia **Other keywords:** toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors; respiration; metabolism; breath tests; inhalation; air; breath; exhalation; biological markers; analysis
TSCATS	2011	NA
ChemID	2011	NA
Chemfinder	2011	NA
CCRIS	2011	NA
HSDB	2011	NA
GENETOX	2008	NA
RTECS	2011	NA

[a]The initial search conducted in 2008 included ammonia salts (i.e., ammonium nitrate [6484-52-2], ammonium fluoride [12125-01-8], ammonium sulfate [7783-20-2], ammonium persulfate [7727-54-0], and ammonium chloride [12125-02-9]) as keywords. Once the determination was made not to include data on ammonium salts in the assessment, updated searches focused on ammonia and ammonium hydroxide only.
[b]The search using search terms related to concentrations of ammonia in exhaled breath was conducted for the period 1/1/2002–11/11/2011.

Approximately 4,900 references were identified in the literature search for ammonia using the literature search strategy identified in Table LS-1; the references captured in this search can be found on the EPA's HERO website.[1] From this list, approximately 250 references were identified that provided information relevant to the human health effects of ammonia or information on the physical and chemical properties of ammonia.

The references cited in this document, as well as those that were considered but not included in the Toxicological Review of Ammonia, can be found on the HERO website (http://hero.epa.gov/{chemical}). This site contains HERO links to lists of references, including bibliographic information and abstracts, which were considered for inclusion in the Toxicological Review of Ammonia.

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[1]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.

Study Evaluation for Hazard Identification

This document is not intended to be a comprehensive treatise on the chemical or toxicological nature of ammonia. In general, the quality and relevance of health effects studies were evaluated as outlined in the Preamble to this assessment. In addition, *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 Inhalation Dosimetry* (U.S. EPA, 1994) were consulted for guidance in evaluating the scientific quality of the available studies.

The health effects literature for ammonia is not extensive; therefore, essentially all of the available epidemiology and toxicity studies of ammonia and ammonium hydroxide were considered in the characterization of the potential health hazards associated with ammonia exposure. As discussed in the preface, literature on ammonium salts were not included in this review because the available data suggest that the anion of the salt can influence the toxicity of the ammonium compound. Approximately 100 case reports involving acute ammonia exposure were identified; because case reports generally provide little information that would be useful for characterizing chronic health hazard, these studies were only briefly reviewed and citations to this literature are provided as supplemental materials in Appendix A. Human studies that provided unreliable measures of exposure (e.g., self-reporting) or intentional dosing studies that raised concerns of ethical conduct were excluded from consideration; two human studies fell into this category.

The hazard identification analysis for each health endpoint in Chapter 1 includes a synthesis of the relevant health effects literature and an analysis of the weight of the evidence for an association between ammonia exposure and the health effects. The available studies examining health effects of ammonia exposure in humans (four cross-sectional occupational exposure studies, studies in livestock farmers and stable workers, and acute controlled-exposure studies in volunteers) are discussed and evaluated, with specific limitations of individual studies and of the collection of studies noted. The evaluation of the effects seen in experimental animal studies focuses on the available subchronic toxicity studies and a single reproductive toxicity study. Chronic toxicity studies were limited to oral exposure studies that did not adequately evaluate the noncancer effects of ammonia.

1

1. HAZARD IDENTIFICATION

1.1. Synthesis of Major Toxicological Effects

1.1.1. Respiratory Effects

Respiratory Irritation

The respiratory system is the primary target of toxicity of inhaled ammonia in humans and experimental animals. Symptoms consistent with respiratory irritation were reported in two cross-sectional studies of industrial worker populations exposed to ammonia (Rahman et al., 2007; Ballal et al., 1998) (see Table 1-1). Rahman et al. (2007)[2] found a higher prevalence, by up to 20%, of respiratory irritation (cough, chest tightness, runny nose, stuffy nose, and sneezing) in urea fertilizer factory workers exposed to a mean ammonia concentration of 18.5 mg/m^3 (high-exposure group) for about 16 years compared to a control group (staff workers); the prevalences of cough and chest tightness were statistically significantly elevated in the high-exposure ammonia group compared to the control group. Respiratory irritation prevalence between the low-exposure group exposed to a mean ammonia concentration of 4.9 mg/m^3 was not statistically significantly different from the control group. Significantly higher relative risks for cough, phlegm, wheezing, dyspnea, and bronchial asthma were also observed in workers from another cross-sectional study (Ballal et al., 1998) with ammonia exposure concentrations higher than the American Conference of Governmental Industrial Hygienists [ACGIH] threshold limit value [TLV] of 18 mg/m^3 [25 ppm]) compared with workers exposed to levels below the TLV. Distribution of respiratory irritation effects by cumulative ammonia concentration (CAC, mg/m^3-years) also showed significantly higher relative risk for these respiratory irritation effect among workers with higher CAC (>50 mg/m^3-years) compared to those with a lower CAC (< 50 mg/m^3-years) (Ballal et al., 1998). Only Ballal et al. (1998) evaluated respiratory endpoints in terms of cumulative exposure.

In a third cross-sectional study of male ammonia-exposed workers, no differences were observed in the prevalence of respiratory irritation, eye irritation, or odor detection threshold between any of the ammonia-exposed workers and the control group (Holness et al., 1989), either as one group or when stratified into three exposure categories: high = >8.8 mg/m^3, medium = 4.4–8.8 mg/m^3, or low = <4.4 mg/m^3. Although respiratory irritation prevalence was similar across groups, the exposed workers reported that exposure in the plant aggravated some

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[2] Rahman et al. (2007) examined respiratory effects in workers from two plants in a urea fertilizer factory. Workers in the urea plant were exposed to higher concentrations of ammonia (arithmetic mean ammonia concentration of 18.5 mg/m^3) than workers in the ammonia plant (arithmetic mean ammonia concentration of 4.9 mg/m^3). Therefore, the urea plant workers represented the high-exposure group, and the ammonia plant workers represented the low-exposure group.

1 of their reported respiratory symptoms (cough, sputum, chronic bronchitis, wheeze, chest

2 tightness, dyspnea, chest pain, rhinitis) (no further information provided). Co-exposures to dust

3 and inorganic gases such as nitrogen dioxide and sulfur dioxide were possible in these cross-

4 sectional studies; however, except for the low levels of nitrogen dioxide identified in the Rahman

5 et al. (2007) study, these workplace exposures were not measured or reported.

6   Overall, these cross-sectional occupational epidemiology studies provide consistent

7 estimates of the effect level for respiratory irritation by ammonia. Rahman et al. (2007)

8 observed that exposure to 18.5 mg/m^3 ammonia increased the prevalence of respiratory effects.

9 This is consistent with the observation by Ballal et al. (1998) that workers in a factory with

10 ammonia concentrations exceeding the TLV of 18 mg/m^3 had significantly higher relative risks

11 for respiratory irritation effects. The prevalence of respiratory effects was not increased

12 following occupational exposures at lower workplace concentrations (i.e., >8.8 mg/m^3 ammonia

13 [Holness et al., 1989] and 4.9 mg/m^3 [Rahman et al., 2007]).

14   Respiratory irritation, indicated by elevated prevalences of respiratory symptoms,

15 including cough, phlegm, wheezing, chest tightness, and eye, nasal and throat irritation, has been

16 reported in livestock farmers and stable workers compared to controls (Melbostad and Eduard,

17 2001; Preller et al., 1995; Choudat et al., 1994; Zejda et al., 1994; Crook et al., 1991; Heederik et

18 al., 1990). Additionally, bronchial hyperreactivity to methacholine or histamine challenge was

19 increased in farmers exposed to ammonia compared to control workers (Vogelzang et al., 2000,

20 1997; Choudat et al., 1994), indicating that exposure to ammonia and other air contaminants in

21 farm settings may contribute to chronic airway inflammation. In addition to ammonia, these

22 studies also documented exposures to airborne dust, bacteria, fungal spores, endotoxin, and

23 mold—agents that could also induce respiratory effects. The release of other volatiles on

24 livestock farms is likely, but measurements for other volatile chemicals were not conducted.

25 Therefore, while several studies have reported associations between ammonia exposure in

26 livestock farmers or stable workers and respiratory irritation, these findings are limited by

27 exposures to other constituents in air that likely confound the association between ammonia

28 exposure and the respiratory effects observed in the study populations.

29   Support for ammonia as a respiratory irritant is also provided by reports of irritation and

30 hyperventilation in volunteers acutely exposed to ammonia at concentrations ranging from

31 11−354 mg/m^3 ammonia for durations up to 4 hours under controlled exposure conditions

32 (Petrova et al., 2008; Smeets et al., 2007; Altmann et al., 2006; Ihrig et al., 2006; Verberk, 1977;

33 Silverman et al., 1949) (see Appendix A, Section A.4). Two controlled-exposure studies

34 reported habituation to eye, nose, and throat irritation in volunteers after several weeks of

35 ammonia exposure (Ihrig et al., 2006; Ferguson et al., 1977). Numerous case reports document

36 the acute respiratory effects of inhaled ammonia, ranging from mild symptoms (including nasal

37 and throat irritation and perceived tightness in the throat) to moderate effects (including

38 pharyngitis, tachycardia, dyspnea, rapid and shallow breathing, cyanosis, transient

1 bronchospasm, and rhonchi in the lungs) to severe effects (including burns of the nasal passages,

2 soft palate, posterior pharyngeal wall, and larynx, upper airway obstruction, bronchospasm,

3 dyspnea, persistent, productive cough, bilateral diffuse rales and rhonchi, mucous production,

4 pulmonary edema, marked hypoxemia, and necrosis of the lung) (see Appendix A, Section A.4,

5 for more detailed information and references).

6 Experimental studies in laboratory animals also provide consistent evidence that

7 ammonia exposure for 35 days or more can produce respiratory irritation. Histopathological

8 changes in the nasal passages were observed in Sherman rats after 75 days of exposure to 106

9 mg/m^3 ammonia or 35 days of exposure to 177 mg/m^3 ammonia, with respiratory and olfactory

10 epithelium thickness increased three- to four times that of normal thickness (Broderson et al.,

11 1976). Thickening of nasal and tracheal epithelium (50 to 100%) was observed in pigs exposed

12 to 71 mg/m^3 ammonia continuously for 1–6 weeks (Doig and Willoughby, 1971). Nonspecific

13 inflammatory changes (not further described) were reported in the lungs of Sprague-Dawley and

14 Longs-Evans rats continuously exposed to 127 mg/m^3 ammonia for 90 days and rats and guinea

15 pigs intermittently exposed to 770 mg/m^3 ammonia (or 183 mg/m^3, adjusted to continuous

16 exposure[3]) (Coon et al., 1970). Focal or diffuse interstitial pneumonitis was observed in all

17 Princeton-derived guinea pigs, New Zealand white rabbits, beagle dogs, and squirrel monkeys

18 exposed to 470 mg/m^3 ammonia that were examined (Coon et al., 1970). Additionally, under

19 these exposure conditions, dogs exhibited nasal discharge and other signs of irritation (marked

20 eye irritation, heavy lacrimation). Nasal discharge was observed in 25% of rats exposed to

21 262 mg/m^3 ammonia for 90 days (Coon et al., 1970).

22 At lower concentrations, approximately 50 mg/m^3 and below, the majority of studies of

23 inhaled ammonia show that ammonia does not produce respiratory irritation effects in laboratory

24 animals. No increase in the incidence of respiratory or other diseases common to young pigs

25 were observed after continuous exposure to ammonia and inhalable dust at concentrations

26 representative of those found in commercial pig farms (26 mg/m^3 ammonia) for 5 weeks (Done

27 et al., 2005). No gross or histopathological changes in the turbinates, trachea, and lungs of pigs

28 were observed after continuous exposure to 53 mg/m^3 ammonia for up to 109 days (Curtis et al.,

29 1975). No signs of toxicity in rats were observed after continuous exposure to 40 mg/m^3

30 ammonia for 114 days or after intermittent exposure to 155 mg/m^3 ammonia (or 36.9 mg/m^3,

31 adjusted to continuous exposure) for 6 weeks (Coon et al., 1970).

32

33 ***Lung Function***

34 Decreased lung function in ammonia-exposed workers has been reported in two cross-

35 sectional studies of industrial worker populations (Rahman et al., 2007; Ali et al., 2001) of three

36 such studies that measured lung function (Rahman et al., 2007; Ali et al., 2001; Holness et al.,

37 (1989). Ammonia exposure was correlated with a significant decline in lung function over the

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[3]$C_{adjusted} = C \times 8$ hours/24 hours $\times$ 5 days/7 days, where C is the exposure concentration.

1   course of a work shift (cross-shift) as measured by forced vital capacity (FVC) and forced

2   expiratory volume in one second (FEV_1) in the high-exposure worker group (mean ammonia

3   concentration of 18.5 mg/m^3) in a fertilizer factory (Rahman et al., 2007). In a second study (Ali

4   et al., 2001), the FVC% predicted was higher in fertilizer factory workers exposed to ammonia

5   than in controls (4.6% increase, $p \leq 0.002$); FEV_1 was higher (1.5%) in the exposed workers but

6   the difference was not statistically significant. When Ali et al. (2001) based their analysis on

7   measures of cumulative exposure, workers with cumulative exposure >50 mg/m^3-years had

8   significantly lower FVC% predicted (5.4% decrease, $p \leq 0.030$) and FEV_1% predicted (7.4%

9   decrease, $p < 0.006$) than workers with cumulative exposure ≤50 mg/m^3-years, but similar

10   FEV_1/FVC%. The authors did not explain the inconsistent findings across the analyses of

11   noncumulative and cumulative exposures.

12   Lung function did not appear to be affected in worker populations chronically exposed to

13   ammonia at concentrations below approximately 18 mg/m^3. Baseline lung function, based on

14   spirometry conducted at the beginning and end of the work shift, differed very slightly relative to

15   control in workers exposed to ammonia concentrations ranging from <4.4 mg/m^3 to >8.8 mg/m^3

16   in a cross-sectional study of male workers in a soda ash plant (Holness et al., 1989), but was not

17   statistically significant. Additionally, no changes in lung function were observed over either

18   work shift (days 1 or 2) or over the work week in the exposed group compared with controls.

19   Similarly, measures of lung function (FVC, FEV_1, and PEFR [peak expiratory flow rate]) in

20   workers exposed to a mean concentration of 4.9 mg/m^3 ammonia in a urea fertilizer factory

21   showed no significant cross-shift changes (Rahman et al., 2007).

22   Decreased lung function (e.g., measured as decreased FEV_1, FVC) was reported in

23   farmers with ammonia exposure (Cormier et al., 2000; Donham et al., 2000, 1995; Vogelzang et

24   al., 1998; Reynolds et al., 1996; Preller et al., 1995; Crook et al., 1991; Heederik et al., 1990).

25   These findings are limited by exposures to other constituents in air (including respirable dust,

26   bacteria, fungal spores, endotoxin, and mold) that can affect lung function, and likely confound

27   the association between exposure to ammonia and decreased lung function observed in the study

28   populations.

29   Changes in lung function following acute exposure to ammonia have been observed in

30   some but not all controlled exposure studies conducted in volunteers. Cole et al. (1977) reported

31   reduced lung function as measured by reduced expiratory minute volume and changes in exercise

32   tidal volume in volunteers exposed for a half-day in a chamber at ammonia concentrations

33   ≥106 mg/m^3 but not at 71 mg/m^3. Bronchioconstriction was reported in volunteers exposed to

34   ammonia through a mouthpiece for 10 inhaled breaths of ammonia gas at a concentration of

35   60 mg/m^3 (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in

36   volunteers exposed to ammonia at concentrations of up to 35 mg/m^3 for 10 minutes in an

37   exposure chamber (MacEwen et al., 1970). Similarly, no changes in bronchial responsiveness or

38   lung function (as measured by forced vital capacity and FEV_1) were reported in healthy

1 volunteers exposed to ammonia at concentrations up to 18 mg/m^3 for 1.5 hours during exercise

2 (Sundblad et al., 2004). There were no changes in lung function as measured by FEV$_1$ in 25

3 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers exposed to ammonia

4 concentrations up to 354 mg/m^3 ammonia for up to 2.5 hours (Petrova et al., 2008), or in six

5 healthy volunteers and eight mildly asthmatic volunteers exposed to 11–18 mg/m^3 ammonia for

6 30-minute sessions (Sigurdarson et al., 2004).

7 　　　Lung function effects following ammonia exposure were not evaluated in the available

8 animal studies.

9 　　　The evidence of respiratory effects in humans and experimental animals exposed to

10 ammonia is provided in Tables 1-1 and 1-2, respectively, and presented visually as an exposure-

11 response array in Figure 1-1.

12

13

Table 1-1. Respiratory effects in humans following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m³)
Respiratory irritation	Cross-sectional occupational study of soda ash plant workers in Canada; 58 exposed workers and 31 controls (from stores and office areas of plant)[b] Low (<4.4 mg/m³), medium (4.4–8.8 mg/m³), high (>8.8 mg/m³); adjusted[c] concentration ranges <1.6 mg/m³, 1.6–3.1 mg/m³ and >3.1 mg/m³ Average exposure: 12 y Holness et al., 1989	No statistically significant differences in subjective symptomology relative to the control. Control Exposed p-value Flu 3 7 0.6299 Cough 10 16 0.5289 Sputum 16 22 0.9770 Bronchitis 19 22 0.6938 Wheeze 10 10 0.9068 Chest tightness 6 3 0.6221 Dyspnea 13 7 0.0470 Chest pain 6 2 0.1563 Rhinitis 19 10 0.1185 Throat 3 7 0.5296	NOAEL: 3.1 LOAEL: not identified

	Cross-sectional occupational study of urea fertilizer factory in Bangladesh; 63 ammonia plant workers, 77 urea plant workers, and 25 controls (from administration building)
	
	Ammonia plant: 4.9 mg/m³ [d] (1.8 mg/m³ adjusted[c])
	Urea plant: 18.5 mg/m³ [d] (6.6 mg/m³ adjusted[c])
	Mean employment duration: 16 y
	
	Rahman et al., 2007
		Exposure-related increase in respiratory symptoms.
		
		Respiratory symptom prevalence (%):
		        Control Ammonia Urea
		        (admin) plant plant
		Cough 8 17 (0.42)[a] 28 (0.05, 0.41)[b]
		Chest tightness 8 17 (0.42)[a] 33 (0.02, 0.19)[b]
		Stuffy nose 4 12 (0.35)[a] 16 (0.17, 1.0)[b]
		Runny nose 4 4 (1.0)[a] 16 (0.17, 0.28)[b]
		Sneeze 8 0 (0.49)[a] 22 (0.22, 0.01)[b]
		
		[a] p-value for ammonia plant compared to control
		[b] p-value for urea plant compared to control and urea plant compared to ammonia plant
			NOAEL: 1.8
			LOAEL: 6.6

	Cross-sectional study of two urea fertilizer factories in Saudi Arabia; 161 exposed workers and 355 unexposed controls[e] Exposures were stratified > or < the ACGIH TLV of 18 mg/m³ Mean of employment duration: 51.8 mo (exposed workers) and 73.1 mo (controls) Ballal et al., 1998	Higher relative risks for those exposed to ammonia at concentrations >TLV as compared to those exposed at levels ≤TLV: Cough: 4-fold Phlegm: 4.7-fold Wheezing: 2.2-fold Dyspnea: 4-fold Chronic bronchitis:1.6-fold Asthma: 3.7-fold	NOAEL and LOAEL values were not identified because exposures were not adequately characterized

Table 1-1. Respiratory effects in humans following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m^3)
Lung function	Cross-sectional occupational study of soda ash plant workers in Canada; 58 exposed workers and 31 controls (from stores and office areas of plant)[b] Low (<4.4 mg/m^3), medium (4.4–8.8 mg/m^3), high (>8.8 mg/m^3) adjusted[c] concentration ranges <1.6 mg/m^3, 1.6–3.1 mg/m^3 and >3.1 mg/m^3 Average exposure: 12 y Holness et al., 1989	No statistically significant differences in lung function relative to the control. <table><tr><td></td><td>Exposed</td><td>Control</td><td>p value</td></tr><tr><td colspan=4>Lung function (% predicted values):</td></tr><tr><td>FVC</td><td>96.8</td><td>98.6</td><td>0.0944</td></tr><tr><td>FEV$_1$</td><td>94.1</td><td>95.1</td><td>0.3520</td></tr><tr><td>FEV$_1$/FVC</td><td>97.1</td><td>96.5</td><td>0.4801</td></tr><tr><td colspan=4>Change in lung function over work shift:</td></tr><tr><td>FVC day1</td><td>-0.8</td><td>-0.9</td><td>0.9940</td></tr><tr><td>day 2</td><td>-0.0</td><td>+0.1</td><td>0.8378</td></tr><tr><td>FEV$_1$ day 1</td><td>-0.2</td><td>-0.2</td><td>0.9363</td></tr><tr><td>day 2</td><td>+0.7</td><td>+0.5</td><td>0.8561</td></tr></table>	NOAEL: 3.1 LOAEL: not identified
	Cross-sectional occupational study of urea fertilizer factory in Bangladesh; 63 ammonia plant workers, 77 urea plant workers, and 25 controls (from administration building) Ammonia plant: 4.9 mg/m^3 [d] (1.8 mg/m^3 adjusted[c]) Urea plant: 18.5 mg/m^3 [d] (6.6 mg/m^3 adjusted[c]) Mean employment duration: 16 y Rahman et al., 2007	Dose-related decrease in lung function parameters. <table><tr><td></td><td>Pre-shift</td><td>Post-shift</td><td>p-value</td></tr><tr><td colspan=4>Ammonia plant</td></tr><tr><td>FVC</td><td>3.308</td><td>3.332</td><td>0.67</td></tr><tr><td>FEV$_1$</td><td>2.627</td><td>2.705</td><td>0.24</td></tr><tr><td>PEFR</td><td>8.081</td><td>8.313</td><td>0.22</td></tr><tr><td colspan=4>Urea plant</td></tr><tr><td>FVC</td><td>3.362</td><td>3.258</td><td>0.01</td></tr><tr><td>FEV$_1$</td><td>2.701</td><td>2.646</td><td>0.05</td></tr><tr><td>PEFR</td><td>7.805</td><td>7.810</td><td>0.97</td></tr></table>	NOAEL: 1.8 LOAEL: 6.6
	Cross-sectional study of a urea fertilizer factory in Saudi Arabia—follow-up of Ballal et al. (1998); 73 exposed workers and 343 unexposed controls Exposures were stratified < or > the ACGIH TLV of 18 mg/m^3 Mean of employment duration: not reported Ali et al., 2001	Lung function results based on exposure concentration and cumulative exposure: <table><tr><td></td><td>Control</td><td>Exposed</td><td>p-value</td></tr><tr><td>FVC$_1$% predicted</td><td>96.6</td><td>98.1</td><td>NS</td></tr><tr><td>FVC% predicted</td><td>101.0</td><td>105.6</td><td>0.002</td></tr><tr><td>FEV$_1$/FVC%</td><td>83.0</td><td>84.2</td><td>NS</td></tr><tr><td></td><td>≤50 mg/m^3-y</td><td>>50 mg/m^3-y</td><td>p-value</td></tr><tr><td>FVC$_1$% predicted</td><td>100.7</td><td>93.4</td><td>0.006</td></tr><tr><td>FVC% predicted</td><td>105.6</td><td>100.2</td><td>0.03</td></tr><tr><td>FEV$_1$/FVC%</td><td>84.7</td><td>83.4</td><td>NS</td></tr></table>NS = not significant	NOAEL and LOAEL values were not identified because exposures were not adequately characterized

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]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.

[c]Adjusted to 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 (i.e., measured concentration × 10/20 × 5/7).

[d]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 (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 (telephone conversations and e-mails dated June 22, 2010, from Michael Yanosky, Dräger Safety Inc., Technical Support Detection Products to Amber Bacom, SRC, Inc., contractor to NCEA, ORD, U.S. EPA), EPA considered the PAC III instrument to be a more sensitive monitoring technology than the Dräger tubes. Therefore, more confidence is attributed to the PAC III air measurements of ammonia for the Rahman et al. (2007) study.

[e]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.

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Table 1-2. Respiratory effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m^3)
Pulmonary inflammation and congestion	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No visible signs of toxicity, gross necropsies were normal, focal pneumonitis in 1 of 3 monkeys at 36.9 mg/m^3. Nonspecific lung inflammation observed in guinea pigs and rats but not in other species at 183 mg/m^3	NOAEL: 36.9 LOAEL: 183
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group Coon et al., 1970	Focal or interstitial pneumonitis in all animals, calcification of bronchial epithelium was observed in several animals. Hemorrhagic lung lesion in 1 of 2 dogs. Moderate lung congestion in 2 of 3 rabbits.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d of 455 mg/m^3 for 65 d Sprague-Dawley or Long-Evans rat; male and female; 15-51/group Coon et al., 1970	Dyspnea (mild) at 455 mg/m^3. Focal or interstitial pneumonitis in all animals, calcification of bronchial epithelium observed in several animals at 470 mg/m^3. (Exposure to 455 and 470 mg/m^3 ammonia increased mortality in rats.)	NOAEL: 262 LOAEL: 455
	0 or 14 for 7-42 days or 35 mg/m^3 for 42 days Guinea pig (strain not specified); male and female; 2/group Anderson et al., 1964	Pulmonary congestion, edema and hemorrhage were observed at 14 and 35 mg/m^3 after 42 d.	NOAEL: NA LOAEL:14
	0 or 14 mg/m^3 for 7-42 days Swiss albino mouse; male and female; 4/group Anderson et al., 1964	Pulmonary congestion, edema and hemorrhage were observed at 14 mg/m^3 after 42 d.	NOAEL: not identified LOAEL: 14

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Table 1-2. Respiratory effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m^3)
	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 Pig (several breeds); sex not specified; 24/group Done et al., 2005	No increase in the incidence of respiratory or other disease.	NOAEL: 26 LOAEL: not identified
	0, 35, or 53 mg/m^3 for 109 d Pig (crossbred); sex not specified; 4–8/group Curtis et al., 1975	Turbinates, trachea, and lungs of all pigs were classified as normal.	NOAEL: 53 LOAEL: not identified
Thickening of airway epithelium	7 or 106 mg/m^3 from bedding for 75 d Sherman rat; 5/sex/group Broderson et al., 1976[c]	Thickening of the nasal epithelium (3-4 times) and nasal lesions.	NOAEL: 7 LOAEL:106
	0 or 177 mg/m^3 in an inhalation chamber for 35 d F344 rat; 6/sex/group Broderson et al., 1976	Thickening of the nasal epithelium (3-4 times) and nasal lesions.	NOAEL: not identified LOAEL:177
	0 or 71 mg/m^3 for 6 wks Yorkshire-Landrace pig; sex not specified; 6/group Doig and Willoughby, 1971	Thickening of nasal and tracheal epithelium (50-100% increase).	NOAEL: not identified LOAEL:71

Table 1-2. Respiratory effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m^3)
Nasal inflammation and lesions	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No nasal irritation observed.	NOAEL: 183 LOAEL: not identified
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Beagle dog; male; 2/group Coon et al., 1970	Nasal discharge.	NOAEL: 40 LOAEL: 470
	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 Sprague-Dawley or Long-Evans rat; male and female; 15-51/group Coon et al., 1970	Nasal irritation in all animals at 455 mg/m^3. (Exposure to 455 and 470 mg/m^3 ammonia increased mortality in rats.)	NOAEL: 262 LOAEL: 455
	7 or 106 mg/m^3 from bedding for 75 d Sherman rat; 5/sex/group Broderson et al., 1976	Nasal lesions at 106 mg/m^3.	NOAEL: 7 LOAEL:106
	0 or 177 mg/m^3 in an inhalation chamber for 35 d F344 rat; 6/sex/group Broderson et al., 1976	Nasal lesions at 177 mg/m^3.	NOAEL: not identified LOAEL:177
	Ammonia vapor of 0 or 12% ammonia solution for 15 min/d, 6 d/wk, for 8 wks White albino mouse; male; 50 Gaafar et al., 1992	Histological changes in the nasal mucosa.	NOAEL and LOAEL values were not identified because of inadequate reporting of exposure concentrations.

Table 1-2. Respiratory effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL[a] (mg/m^3)
	8, 43, 73, or 103 mg/m^3 for 5 wks Duroc pig; both sexes; 9/group Stombaugh et al., 1969	Excessive nasal, lacrimal, and mouth secretions and increased frequency of cough at 73 and 103 mg/m^3.	NOAEL and LOAEL values were not identified because of the absence of a control group.

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]Adjusted to continuous exposure based on the ratio of hours exposed per day and days exposed per week (i.e., measured concentration × 8/24 × 5/7).

[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 *M. 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.
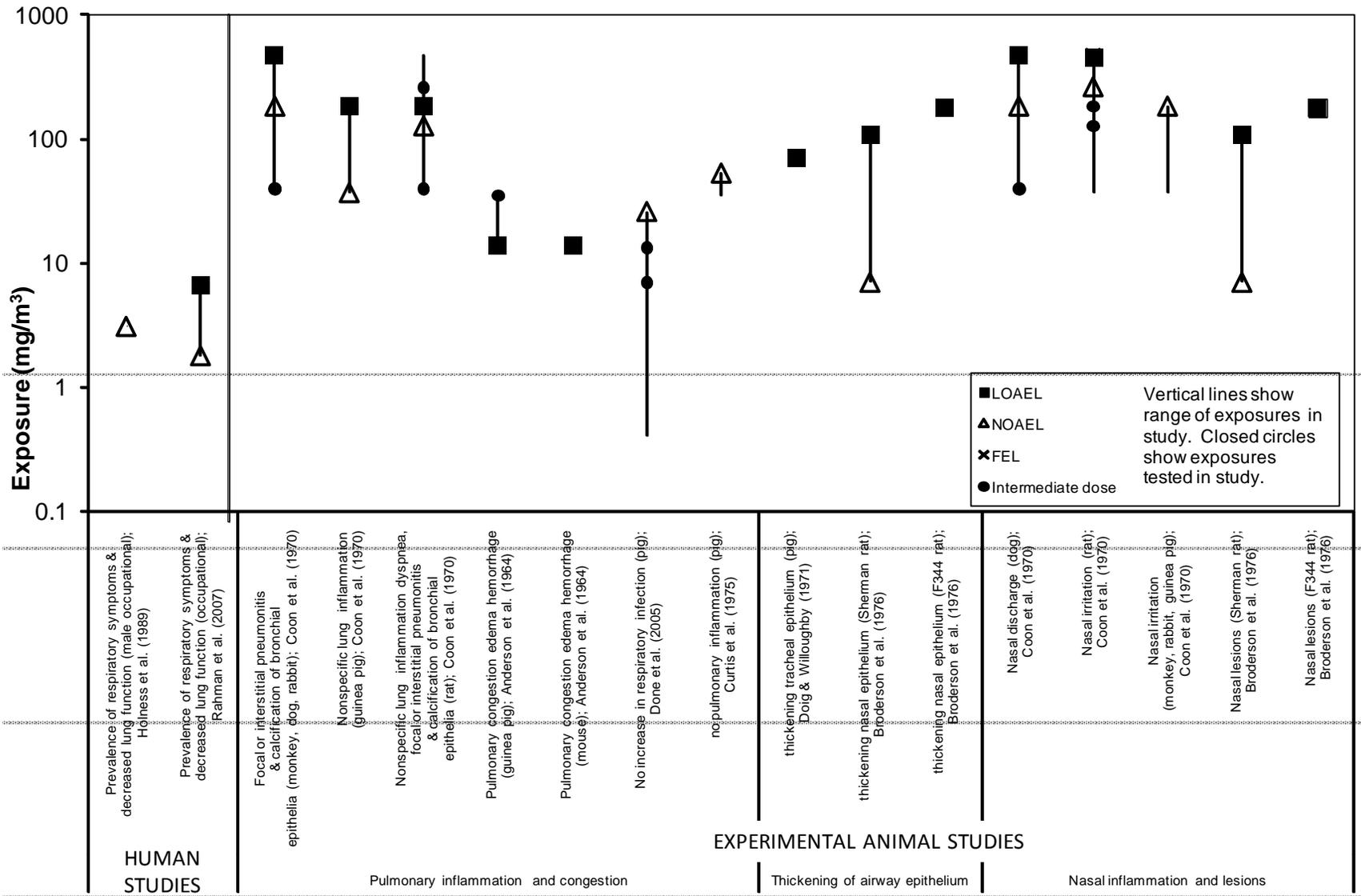
1

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

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1   ➢ **Mode of Action Analysis – Respiratory Effects**

2   Data regarding the potential mode of action for respiratory effects associated with chronic

3 exposure to ammonia are limited. However, it is well established that injury to respiratory

4 tissues resulting from acute exposure to inhaled ammonia is primarily due to its alkaline

5 properties and its solubility. Given its high solubility, ammonia readily dissolves in the moisture

6 on the mucous membranes, forming ammonium hydroxide, which causes liquefaction necrosis of

7 the tissues. Ammonia directly denatures tissue proteins due to the production of alkaline

8 proteinates. Specifically, ammonium hydroxide causes saponification of cell membrane lipids

9 that leads to cell disruption and death (necrosis). As cell proteins break down, water is extracted,

10 resulting in an inflammatory response, which further damages the surrounding tissues (Amshel et

11 al, 2000; Mellea, 1989; Jarudi and Golden, 1973).

12

13 ## 1.1.2. Gastrointestinal Effects

14   Reports of gastrointestinal effects of ammonia in humans are limited to case reports

15 involving intentional or accidental ingestion of household cleaning solutions or ammonia

16 inhalant capsules (Dworkin et al., 2004; Rosenbaum et al., 1998; Christesen, 1995; Wason et al.,

17 1990; Lopez et al., 1988; Klein et al., 1985; Klendshoj and Rejent, 1966). Clinical signs reported

18 in these case studies include stomachache, nausea, dizziness, diarrhea, drooling, erythematous

19 and edematous lips, reddened and blistered tongues, dysphagia, vomiting, oropharyngeal burns,

20 laryngeal and epiglottal edema, erythmatous esophagus with severe corrosive injury, and

21 hemorrhagic esophago-gastro-duodeno-enteritis.

22   In animals following oral exposure, statistically significant decreases of 40−60% in the

23 thickness of the gastric mucosa were reported in Sprague-Dawley rats administered 0.01%

24 ammonia in drinking water for durations of 2–8 weeks (Tsujii et al., 1993; Kawano et al., 1991);

25 estimated doses were 22 mg/kg-day (Kawano et al., 1991) and 33 mg/kg-day (Tsujii et al., 1993).

26 These studies were designed to investigate the hypothesis that the bacterium *Helicobacter pylori*,

27 which produces a potent urease that increases ammonia production, plays a significant role in the

28 etiology of chronic atrophic gastritis. Kawano et al. (1991) reported that the magnitude of the

29 decrease in gastric mucosal thickness increased with dose and duration of exposure and that the

30 effect was more prominent in the mucosa of the antrum region of the stomach than in the body

31 region of the stomach.[4] As discussed further under Mode of Action – Gastrointestinal Effects

32 (see below), the difference in response to ammonia in drinking water in the two regions of the rat

33 stomach may be a function of differences in pH in these regions and resulting differences in the

34 extent of ionization of ammonia to NH_4^+. Parietal cell number per oxyntic gland also decreased

35 in a statistically significant dose- and time-dependent fashion, up to approximately 35% at 0.01%

---

[4]The body is the main, central region of the stomach. The antrum is located in the distal part of the stomach
adjacent to the body.

1 ammonia in drinking water after 4 weeks. In a follow-up study (Tsujii et al., 1993), antral

2 mucosal thickness decreased significantly (by 56–59% of the tap water control) at 4 and 8 weeks

3 of exposure to 0.01% ammonia in drinking water, but there was no significant effect on the

4 thickness of the body mucosa. Increased mucosal cell proliferation and migration (as measured

5 by 5-bromo-2'-deoxyuridine [BrDU] labeling) were significantly increased. The authors

6 observed that it was not clear whether mucosal cell proliferation was primarily stimulated

7 directly by ammonia or indirectly by increased cell loss followed by compensatory cell

8 proliferation. They further observed that the ammonia-related changes in rat stomach resembled

9 mucosal changes in human atrophic gastritis (Tsujii et al., 1993; Kawano et al., 1991).

10 A relationship between ammonia ingestion and gastrointestinal effects is supported by

11 findings from two acute oral studies in rats following gavage administration of ammonium

12 hydroxide (Takeuchi et al., 1995; Nagy et al., 1996). Takeuchi et al. (1995) reported

13 hemorrhagic necrosis of the gastric mucosa in male Sprague-Dawley rats that received a single

14 gavage dose of ammonium hydroxide (concentration $\geq$1%). Nagy et al. (1996) observed severe

15 hemorrhagic mucosal lesions in female Sprague-Dawley rats 15 minutes after exposure to an

16 estimated dose of 48 mg/kg ammonium hydroxide via gavage.

17 The evidence of gastrointestinal effects in experimental animals following oral exposure

18 to ammonia is provided in Table 1-3.

19

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Table 1-3. Gastrointestinal effects in animals following oral exposures

Health Effect	Study Design and References	Results	NOAEL/LOAEL[a] (mg/kg-day)

Histopathologic changes of the gastric mucosa	0, 22, or 220 mg/kg-day in drinking water for 2 or 4 weeks[b]
	
	Sprague-Dawley rat; male; 6/group
	
	Kawano et al., 1991
		Statistically significant decrease in the thickness of the gastric mucosa that was dose and duration related; effect was more prominent in the mucosa of the antrum region of the stomach than the body region.
		
		Thickness of mucosa relative to control:
		Antrum
		  Week 2: 96, 80[d]%
		  Week 4: 62[d], 39[d]%
		Body
		  Week 2: 99, 103%
		  Week 4: 78, 71[d]%
			NOAEL: not identified
			LOAEL: 22

	0 or 33 mg/kg-day in drinking water for 3 days or 1, 2, 4, or 8 weeks; tap water provided for the balance of the 8-week study[c] Sprague-Dawley rat; male; 36/group Tsujii et al., 1993	Antral mucosal thickness decreased significantly at 4 and 8 weeks of exposure; there was no significant effect on the thickness of the body mucosa. Cell migration was significantly increased. *Thickness of mucosa relative to control (d 3, wk 1, 2, 4, 8)*: Antrum: 108, 96, 106, 56[d], 59[d]% Body: 105, 101, 104, 99, 95% (extracted from Figure 3 of Tsujii et al., 1993)	NOAEL: not identified LOAEL: 33

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]Ammonia was provided in drinking water at concentrations of 0, 0.01 or 0.1%. Doses were estimated based on a body weight of 230 g for male rats and estimated daily water intake of 50 mL/day.

[c]Ammonia was provided in drinking water at concentrations of 0 or 0.01%. Doses were estimated based on an initial body weight of 150 g and estimated daily water intake of 50 mL.

[d]Statistically significant from controls.

1

2 ➢ **Mode of Action Analysis – Gastrointestinal Effects**

3 The mode of action for the gastric effects of ammonia has not been established; however,

4 relevant mechanistic information that informs ammonia mode of action comes largely from

5 investigation of the action of the bacterium *Helicobacter pylori* on the stomach. *H. pylori*

6 produces urease, which breaks down urea that is normally present in the stomach into ammonia

7 (Mégraud et al. 1992; Tsujii et al. 1992a), and has been linked to chronic gastritis, gastric ulcers,

8 and stomach cancer in humans.

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

Several specific events have been identified that may contribute to the induction of gastric lesions by ammonia. Increased cell vacuolation and decreased viability of cells in vitro 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, mammalian cysteine proteases that are released from lysosomes or activated in the cytosol and that 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 (ATP) synthesis (Tsujii et al., 1992b). Mori et al. (1998) proposed a role for increased release of endothelin-1 and thyrotropin releasing hormone from the gastric mucosa in ammonia-induced gastric mucosal injury based on findings in rats given ammonia intragastrically. Regardless of the specific mechanism(s) by which ammonia induces cellular toxicity, gastric injury appears to accelerate mucosal cell desquamation and stimulate cell proliferation via a compensatory mechanism (Tsujii et al., 1992a).

1.1.3. Reproductive and Developmental Effects

No statistically significant differences in reproductive or developmental endpoints were found between two groups of female pigs (crossbred gilts) exposed to ammonia for 6 weeks at mean concentrations of 5 or 25 mg/m^3 and then mated (Diekman et al., 1993) in the only study of the reproductive and developmental toxicity potential of ammonia (see Table 1-4). Age at puberty did not differ significantly between the two groups. Gilts exposed to 25 mg/m^3 ammonia weighed 7% less ($p < 0.05$) at puberty than those exposed to 5 mg/m^3; however, body weights of the two groups were similar at gestation day 30. Conception rates in the mated females were

similar between the two groups (94.1 versus 100% in low- versus high-exposure groups). At

sacrifice on day 30 of gestation, there were no significant differences between the two exposed

groups in body weights of the pregnant gilts, number of corpora lutea, number of live fetuses, or

weight and length of the fetuses. The strength of the findings from this study are limited by the

absence of a control group and possible confounding by exposures to bacterial and mycoplasm

pathogens. The evidence of reproductive and developmental effects in experimental animals

exposed to ammonia is provided in Table 1-4.

Table 1-4. Reproductive and developmental effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Reproductive and developmental parameters	5 mg/m^3 (range, 3–8.5 mg/m^3) or 25 mg/m^3 (range, 18–32 mg/m^3) for 6 weeks[b] Crossbred gilts (female pigs), 4.5 months old, 40/group Diekman et al., 1993	No effect on 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).	NOAEL: 5 LOAEL: not identified

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]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.

1.1.4. Immune System Effects

A limited number of studies have evaluated the immunotoxicity of ammonia in human

populations and in experimental animal models. Immunological function was evaluated in two

independent investigations of livestock farmers exposed to ammonia; immunoglobulin G (IgG)

and IgE-specific antibodies for pig skin and urine (Crook et al., 1991), elevated neutrophils from

nasal washes, and increased white blood cell counts (Cormier et al., 2000) were reported. These

data are suggestive of immunostimulatory effects; however, the test subjects were also exposed

to a number of other respirable agents in addition to ammonia such as endotoxin, bacteria, fungi,

and mold that are known to stimulate immune responses. Data in humans following exposure to

ammonia only are not available.

Animal studies that examined ammonia immunotoxicity were conducted using short-term

inhalation exposures and three general types of immune assays. Immunotoxicity studies of

ammonia using measures of host resistance provide the most relevant data for assessing immune

function since they directly measure the immune system's ability to control microorganism

growth. Other available studies of ammonia employed assays that evaluated immune function.

Changes in immune cell populations without corresponding functional data are considered to be the least predictive and were excluded from the hazard identification for ammonia (Neumann et al, 1987; Gustin et al, 1994).

Evidence of immunosuppression was observed in several host resistance studies utilizing lung pathogens to measure reduced bacterial clearance following ammonia exposure. Inoculation with the respiratory pathogen *Mycoplasma pulmonis* causes murine respiratory mycoplasmosis (MRM) characterized by lung lesions. 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 *M. pulmonis* (10^8 colony forming units [CFU]) followed by up to 42 days of ammonia exposure post inoculation (Broderson et al., 1976). The incidence of lesions was significantly increased at ammonia concentrations ≥ 35 mg/m^3, and suggests that ammonia exposure decreased bacterial clearance resulting in the development of *M. pulmonis*-induced MRM. However, the 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 immune response and elicited a maximal response that could not be further magnified in immunocompromised animals. Conversely, significantly increased CFU of *M. pulmonis* bacteria isolated in the trachea, nasal passages, lungs, and larynx was 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* 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.

Suppressed cell-mediated immunity and decreased T cell proliferation was also observed following ammonia exposure. Using a delayed-type hypersensitivity (DTH) test to evaluate cell-mediated immunity, Hartley guinea pigs were vaccinated with *Mycobacterium bovis* BCG and exposed to ammonia followed by intradermal challenge with purified protein derivative (PPD). Dermal lesion size was reduced in animals exposed to 64 mg/m^3 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

1 reduced viability, indicating nonspecific effects of ammonia-induced immunosuppression

2 (Targowski et al., 1984). These data suggest that T cells may be the target of ammonia since

3 specific macrophage effects were not observed.

4   The evidence of immune system effects in experimental animals exposed to ammonia is

5 provided in Table 1-5, and presented visually in an exposure-response array in Figure 1-2.

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Table 1-5. Immune system effects in animals following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Host resistance	≤3.5 (control), 18, 35, 71, 177 mg/m^3, 7 days (continuous exposure) pre inoculation/28-42 days post inoculation with *M. pulmonis* F344 rat; male and female; 11-12/sex/ group Broderson et al., 1976	Increased incidence of gross lung lesions; no effect on CFU. *% of animals with gross lesions:* 16 (control), 46, 66[b], 33, 83%	NOAEL: 18 LOAEL: 35[c]
	<1.4 (control) or 71 mg/m^3, 7 days (continuous exposure) pre inoculation/ 28 days post inoculation with *M. pulmonis* F344 rat; 5-15/group (sex unknown) Schoeb et al., 1982	Increased bacterial colonization (as a result of reduced bacterial clearance). No quantitative data available.	NOAEL: not identified LOAEL: 71
	0 or 354 mg/m^3, 8 hours or 7 days (continuous exposure), prior to infection with *P. multocida* OF1 mouse; male; 99/group Richard et al., 1978	Increased mortality. *Mouse mortality:* 50% (control) and 86%[b]	NOAEL: not identified LOAEL: 354
T cell proliferation	<11 (control), 35 or 64 mg/m^3, 3 weeks (continuous exposure) Hartley guinea pig; 8/group (sex unknown) Targowski et al., 1984	Reduced proliferation in blood and bronchial T cells. No quantitative data available.	NOAEL: 35 LOAEL: 64
Delayed-type hypersensitivity	<11 (control), 35 or 64 mg/m^3, 3 weeks (continuous exposure) followed by PPD challenge in BCG immunized Hartley guinea pig; 8/group (sex unknown) Targowski et al., 1984	Reduced dermal lesion size. *Mean diameter (mm):*12 (control), 12.6 and 8.7[b]	NOAEL: 35 LOAEL: 64

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]Statistically significant from controls.

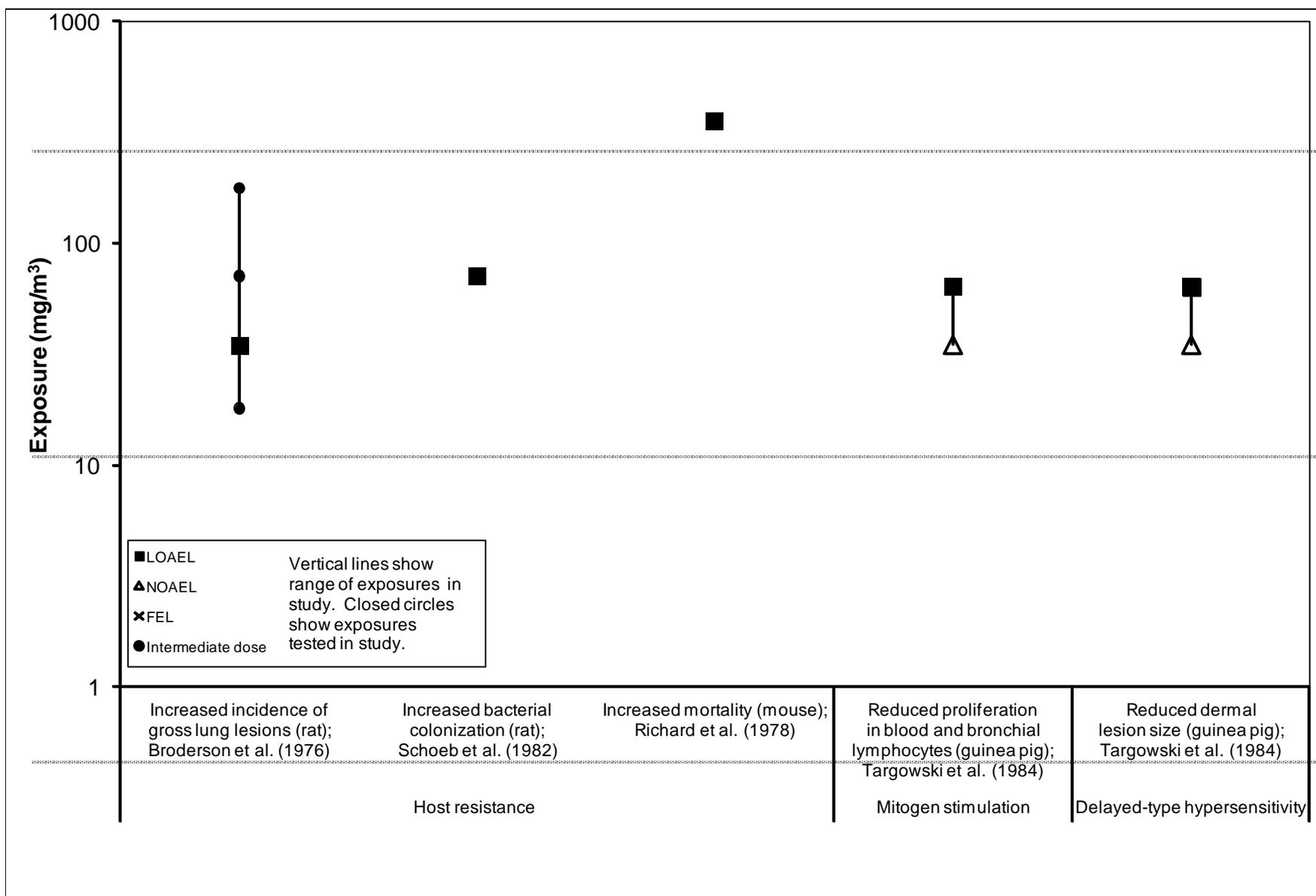[c]Study did not find statistical significance despite a large increase in the response at the lowest dose measured.

Figure 1-2. Exposure-response array for immune system effects following inhalation exposure.

1.1.5. Other Systemic Effects

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

Evidence of hepatotoxicity in animals comes from observations of histopathological alterations in the liver. Fatty changes in the liver were consistently reported at 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), suggesting that mammals can recover from short-term exposure to elevated ammonia levels (Coon et al., 1970). In addition, no effects 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; data in humans were not found. 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 orally via gavage to ammonium hydroxide for durations ranging from 5.5 days to 17 months (Fazekas, 1939). While the strength of these findings is limited by inadequate reporting and study design, a separate study identified early degenerative changes in the adrenal glands of guinea pigs exposed to 120 mg/m^3 ammonia by inhalation for 18 weeks (Weatherby, 1952), providing additional limited evidence for effects on the adrenal gland.

Evidence that inhaled ammonia can affect the kidney and spleen is limited to studies in experimental animals. Nonspecific degenerative changes in the kidneys (not further described) of rats exposed to 262 mg/m^3 were reported (Coon et al., 1970). Histopathological evaluation of other animal species in the same study exposed to 470 mg/m^3, a 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 not observed in similarly treated monkeys and rats. Additionally, there is limited evidence of biochemical or metabolic effects of acute or short-term ammonia exposure. Acidosis, as evidenced by a decrease in blood pH and an increase in arterial blood carbon dioxide partial pressure (pCO_2), occurred in rats exposed to 212 mg/m^3 ammonia for 5–15 days (Manninen et al., 1988). Blood pH and pCO_2 did not change in rats exposed to ≤818 mg/m^3 for up to 24 hours, although statistically significant increases in oxygen partial pressure (pO_2) were reported in rats exposed to 10.6 and 22.6 mg/m^3 ammonia, but not at 219 and 818 mg/m^3 over the same time period (Schaerdel et al., 1983).

Encephalopathy related to ammonia may occur following disruption of the body's normal homeostatic regulation of the glutamine and urea cycles resulting in elevated ammonia levels in blood, e.g., as a result of severe liver or kidney disease (Miñana 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 Murthy, 1978). It has been suggested that glutamate and γ-amino butyric acid (GABA) play a role in ammonia-induced neurotoxicity (Jones, 2002). There is no evidence, however, that ammonia is neurotoxic in humans or animals following chronic exposures.

The evidence of systemic toxicity in humans and experimental animals exposed to ammonia is provided in Tables 1-6 to 1-8, and presented visually in an exposure-response array in Figure 1-3.

Table 1-6. Systemic effects in humans following inhalation exposure

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL
Serum clinical chemistry; liver function	Occupational study workers in an Egyptian urea plant; 30 exposed and 30 control subjects No measurement of exposure concentrations Average employment time: 12 y Hamid and El-Gazzar, 1996	Elevated AST, ALT and blood urea in exposed workers; lower hemoglobin and inhibition of catalase and MAO.	Not identified because the study did not report measurements of exposure.

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Table 1-7. Systemic effects in animals following oral exposure

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL
Adrenal effects	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 days to 17 months; estimated dose: 61–110 mg/kg-day and 120–230 mg/kg-day, respectively[a] Rabbits (strain and sex not specified); 16-33/group Fazekas, 1939	Increased mean adrenal weights and fat content of the adrenal gland. *Response relative to control (adrenal weight):* 95% increase *Response relative to control (fat):* 4.5-fold increase	Not identified.

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

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Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Liver toxicity	0 or 120 mg/m^3 6 h/day, 5 days/week for 6, 12 or 18 weeks; (24.1 mg/m^3 adjusted[b]), Guinea pig (strain not specified); male; 6–12/group Weatherby, 1952	Congestion of the liver at 18 weeks, not observed at earlier times.	NOAEL: not identified LOAEL: 24.1
	0 or 14 for 7-42 days or 35 mg/m^3 for 42 days Guinea pig (strain not specified); male and female; 2/group Anderson et al., 1964	Congestion of the liver at 35 mg/m^3 for 42 days.	NOAEL: 14 LOAEL: 35

Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
	0 or 14 mg/m^3 for 7-42 days Swiss albino mouse; male and female; 4/group Anderson et al., 1964	No visible signs of liver toxicity.	NOAEL: 14 LOAEL: not identified
	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No histopathologic changes observed.	NOAEL: 183 LOAEL: not identified
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group Coon et al., 1970	Fatty liver changes in plate cells.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d; Sprague-Dawley or Long-Evans rat; male and female; 15-51/group Coon et al., 1970	Fatty liver changes in plate cells.	NOAEL: 262 LOAEL: 470
Adrenal gland toxicity	0 and 120 mg/m^3 6 h/day, 5 days/week for 6, 12 or 18 weeks; (24.1 mg/m^3 adjusted[b]) Guinea pig (strain not specified); male; 6–12/group Weatherby, 1952	"Early" degenerative changes in the adrenal gland (swelling of cells, degeneration of the cytoplasm with loss of normal granular structure) at 18 weeks, not observed at earlier times.	NOAEL: not identified LOAEL: 24.1

Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Kidney and spleen toxicity	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No histopathologic changes observed.	NOAEL: 183 LOAEL: not identified
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group Coon et al., 1970	Calcification and proliferation of renal tubular epithelium.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d; Sprague-Dawley or Long-Evans rat; male and female; 15-51/group Coon et al., 1970	Calcification and proliferation of renal tubular epithelium.	NOAEL: 262 LOAEL: 470
	0 or 120 mg/m^3 6 h/day, 5 days/week for 6, 12 or 18 weeks; (24.1 mg/m^3 adjusted[b]) Guinea pig (strain not specified); male; 6–12/group Weatherby, 1952	Congestion of the spleen and kidneys.	NOAEL: not identified LOAEL: 24.1
	0 or 14 for 7-42 days or 35 mg/m^3 for 42 days Guinea pig (strain not specified); male and female; 2/group Anderson et al., 1964	Enlarged and congested spleens.	NOAEL: 14 LOAEL: 35
	0 or 14 mg/m^3 for 7-42 days Swiss albino mouse; male and female; 4/group Anderson et al., 1964	No visible signs of toxicity.	NOAEL: 14 LOAEL: not identified

Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Myocardial toxicity	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No histopathologic changes observed.	NOAEL: 183 LOAEL: not identified
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group Coon et al., 1970	Myocardial fibrosis.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d; Sprague-Dawley or Long-Evans rat; male and female; 15-51/group Coon et al., 1970	Myocardial fibrosis.	NOAEL: 262 LOAEL: 470
Ocular Irritation	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Beagle dog; male; 2/group Coon et al., 1970	Heavy lacrimation.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d New Zealand albino rabbit; male; 3/group Coon et al., 1970	Erythema, discharge and ocular opacity over ¼ to ½ of cornea.	NOAEL: 40 LOAEL: 470
	0 or 40 mg/m^3 for 114 d or 470 mg/m^3 for 90 d Squirrel monkey (*Saimiri sciureus*); male; 3/group and 3/group and Princeton-derived guinea pig; male and female; 15/group Coon et al., 1970	No ocular irritation observed.	NOAEL: 470 LOAEL: not identified

Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
	0 or 40 mg/m^3 for 114 d or 127, 262 or 470 mg/m^3 for 90 d; Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No ocular irritation observed.	NOAEL: 470 LOAEL: not identified
	0, 155, or 770 mg/m^3 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m^3 adjusted[b]) Squirrel monkey (*Saimiri sciureus*); male; 3/group and beagle dog; male; 2/group and New Zealand albino rabbit; male; 3/group and Princeton-derived guinea pig; male and female; 15/group and Sprague-Dawley and Long-Evans rat; male and female; 15-51/group Coon et al., 1970	No ocular irritation observed.	NOAEL: 183 LOAEL: not identified
Blood pH changes	0, 18, or 212 mg/m^3 6 h/day for 5, 10 or 15 days; (4.5, 53 mg/m^3 adjusted[b]) Wistar rat; female; 5/group Manninen et al., 1988	Statistically significant decrease in blood pH at 5 days. pH differences "leveled off at later time points (data not shown)". *Response difference from control*: 0.09[c] and 0.07[c]	NOAEL: 53 LOAEL: not identified
	10.6–818 mg/m^3 for 0, 8, 12, 24 hours, 3 and 7 days Crl:COBS CD(SD) rat; male; 32 and 70 Schaerdel et al., 1983	Statistically significant increase in pO$_2$ at 10.6 and 22.6 mg/m^3 exposure at 8, 12 and 24 hours ($p<0.05$). No change at higher exposures. No change in blood pH or pCO$_2$. *Response relative to control[d]*: 16, 6, 20% at 10.6 mg/m^3 and at 8, 12, 24 hrs; 18, 26, 17% at 22.6 mg/m^3 and at 8, 12, 24 hrs	NOAEL: 818 LOAEL: not identified

Table 1-8. Systemic effects in animals following inhalation exposures

Health Effect	Study Design and Reference	Results	NOAEL/LOAEL[a] (mg/m^3)
Amino acid levels and neurotrans-mitter metabolism in the brain	0, 18, or 212 mg/m^3 6 h/day for 5 days; (4.5, 53 mg/m^3 adjusted[b]) Wistar rat; female; 5/group Manninen and Savolainen, 1989	Statistically significant increase in brain glutamine (p< 0.05). *Response relative to control*: 42[c], 40[c]% for 18 and 212 mg/m^3, respectively	NOAEL: not identified LOAEL: 4.5[e]
	0, 18, or 212 mg/m^3 6 h/day for 5, 10 or 15 days; (4.5, 53 mg/m^3 adjusted[b]) Wistar rat; female; 5/group Manninen et al., 1988	Brain and blood glutamine statistically significantly increased (p< 0.05 and 0.01, respectively) at 212 mg/m^3 at 5 days, no statistically significant difference from control at 10 and 15 days. *Response relative to control at 212 mg/m^3*: 44[c], 13 and 14% increase in blood glutamine at 5, 10, 15 days; 40[c], 4 and 2% increase in brain glutamine at 5, 10, 15 days	NOAEL: 53 LOAEL: not identified

[a]The NOAEL and LOAEL values presented were identified by EPA.

[b]Adjusted to continuous exposure based on the ratio of hours exposed per day and days exposed per week (i.e., measured concentration × 8/24 × 5/7).

[c]Statistically significant difference from controls.

[d]Measurements at time zero were used as a control. The study did not include an unexposed, control group.
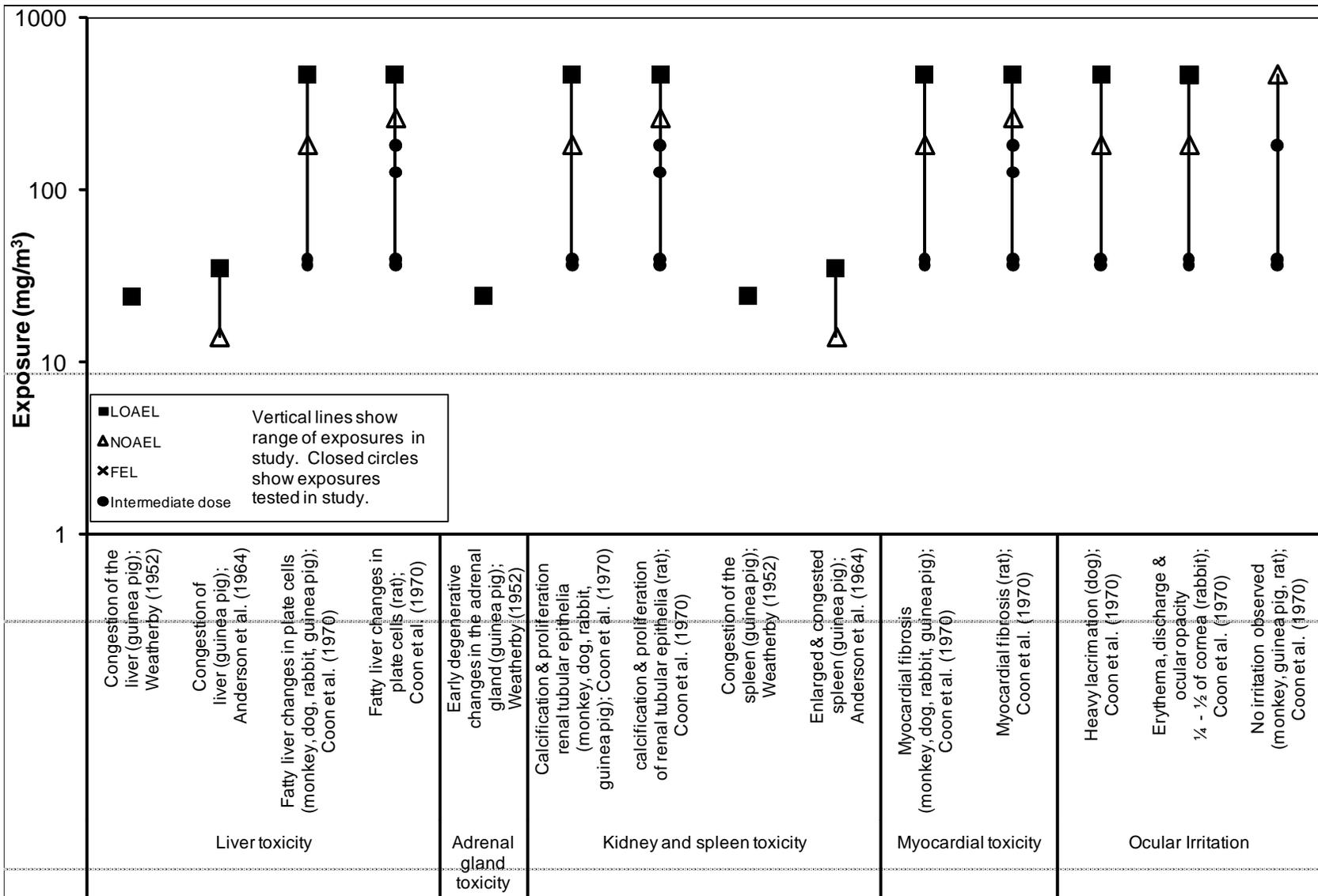
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Figure 1-3. Exposure-response array for systemic effects following inhalation exposure.

1.1.6. Cancer

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 (not further 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 C_3H 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 C_3H mice (a tumor with a high background incidence), concurrent control tumor incidence data were not reported and comparison of tumor incidence in exposed and control mice could not be performed. The general lack of concurrent control data limits the ability to interpret the findings of this study.

The incidence of gastric cancer and the number of gastric tumors per tumor-bearing rat were statistically significantly higher in rats exposed to 0.01% ammonia solution in drinking water (equivalent to 10 mg/kg-day) for 24 weeks following pretreatment (for 24 weeks) with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) compared with rats receiving only MNNG and tap water (Tsujii et al., 1992a). In an almost identically designed study, reported by Tsujii et al. (1995), similar increases in the incidence of gastric tumors were observed 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, 1992a); however, in the absence of an ammonia-only exposure group in these studies, it is not possible to distinguish between possible promotion and initiator activity.

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

Table 1-9. Cancer bioassays following oral exposure

Health Effect	Study Design and Reference	Results
Tumor incidence	250, 440, and 520 mg/kg-day (males); 240, 370, and 410 mg/kg-day (females) [0.1, 0.2, and 0.3% ammonium hydroxide in drinking water[a]] for their lifetime (not further specified) Swiss mouse, 50/sex/group Toth, 1972	The authors reported that tumor incidence was not increased in ammonia-exposed mice; however, concurrent control tumor incidence data were not reported and comparison of tumor incidence in exposed and control mice could not be performed.
	191 (males) and 214 mg/kg-day (females) [0.1% ammonium hydroxide in drinking water[b]] for their lifetime (not further specified) C_3H mouse, 40/sex/group Toth, 1972	The authors reported that 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 and comparison of tumor incidence in exposed and control mice could not be performed. *Mammary gland adenocarcinoma*: 76, 60%
	0 or 10 mg/kg-day [0 or 0.01% ammonia in drinking water[c]] for 24 weeks; both groups pretreated for 24 weeks with the tumor initiator MNNG Sprague Dawley rat, male; 40/group Tsujii et al.,1992a	Statistically significantly increased incidence of gastric cancers and number of gastric tumors per tumor-bearing rat in ammonia + MNNG group compared to MNNG only group *Gastric tumor incidence:* 31, 70[d]% *# of gastric tumors/tumor-bearing rat:* 1.3, 2.1[d]
	0 or 10 mg/kg-day [0 or 0.01% ammonia in drinking water[c]] for 24 weeks; both groups pretreated for 24 weeks with the tumor initiator MNNG Sprague Dawley rat; male; 43-44/group Tsujii et al., 1995	Statistically significantly increased incidence of gastric cancers, size, and penetration to deeper tissue layers in ammonia + MNNG group compared to MNNG only group *Gastric tumor incidence:* 30, 66[d]% *Penetrated muscle layer or deeper*: 12, 22[d]% *Size (mm):* 4.4, 5.3[d]

[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).
[d]Statistically significantly different from control.

1

2   A limited number of genotoxicity studies are available for ammonia vapor, including one

3   study in exposed fertilizer factory workers in India that reported chromosomal aberrations and

4   sister chromatid exchanges in lymphocytes (Yadav and Kaushik, 1997), mutation assays in *S.*

1 *typhimurium* and *E. coli* (Shimizu et al., 1985; Demerec et al., 1951), a micronucleus assay in

2 mice (Yadav and Kaushik, 1997), studies in *D. melanogaster* (Auerbach and Robson, 1947;

3 Lobasov and Smirnov, 1934), and a chromosomal aberration test in chick fibroblast cells in vitro

4 (Rosenfeld, 1932) (see Appendix A, Section A.5). Four of the six available studies were

5 published between 1932 and 1951, and the available genotoxicity database in general is

6 inadequate to characterize the genotoxic potential of ammonia.

7

1.1.7. Susceptible Populations and Life Stages

9 Studies of the toxicity of ammonia in children or young animals compared to other life

10 stages that would support an evaluation of childhood susceptibility have not been conducted.

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

12 in individuals with severe diseases of the liver or kidney, organs that biotransform and excrete

13 ammonia, or with hereditary urea cycle disorders (Córdoba et al., 1998; Schubiger et al., 1991;

14 Gilbert, 1988; Jeffers et al., 1988; Souba, 1987). The elevated ammonia levels that accompany

15 human diseases such as acute liver or renal failure can predispose an individual to

16 encephalopathy due to the ability of ammonia to cross the blood-brain barrier; these effects are

17 especially marked in newborn infants (Miñana et al., 1995; Souba, 1987). Thus, individuals with

18 disease conditions that lead to hyperammonemia may be more susceptible to the effects of

19 ammonia from external sources, but there are no studies that specifically support this

20 susceptibility.

21 Because the respiratory system is a target of ammonia toxicity, individuals with

22 respiratory disease (e.g., asthmatics) might be expected to be a susceptible population; however,

23 controlled human studies that examined both healthy volunteers and volunteers with asthma

24 exposed to ammonia as well as cross-sectional studies of livestock farmers exposed to ammonia

25 (Petrova et al., 2008; Sigurdarson et al., 2004; Vogelzang et al., 2000, 1998, 1997; Preller et al.,

26 1995) generally did not observe a greater sensitivity to respiratory effects in populations with

27 underlying respiratory disease.

28

1.2. Weight of Evidence Evaluation for Toxicological Effects

30 The available evidence for ammonia toxicity indicates that respiratory effects are

31 associated with inhalation exposure and gastrointestinal effects are associated with oral exposure

32 to ammonia. Ammonia exposure may not be associated with reproductive or developmental

33 toxicity, at least at levels in which respiratory and gastrointestinal effects are observed. Immune

34 system and other systemic effects (i.e., effects on the liver, kidney, heart, spleen, and adrenal

35 gland) may be associated with exposure to ammonia but are not sensitive targets of ammonia

36 toxicity. The evidence for these health effects are presented in more detail below. Figure 1-4 is

1   an exposure-response array comparing effect levels for inhaled ammonia across a range of

2   toxicological effect categories.

3

4   *Respiratory Effects*

5   Evidence for respiratory toxicity associated with exposure to ammonia comes from

6   studies in humans and animals. Cross-sectional occupational studies involving chronic exposure

7   to ammonia have consistently demonstrated an increased prevalence of respiratory effects

8   (Rahman et al., 2007; Ballal et al., 1998) and decreased lung function (Rahman et al., 2007; Ali.,

9   2001). Cross-sectional studies of livestock farmers exposed to ammonia, controlled human

10   volunteer studies of ammonia inhalation, and case reports of injury in humans with inhalation

11   exposure to ammonia provide additional and consistent support for the respiratory system as a

12   target of ammonia toxicity.

13   Short-term and subchronic animals studies show respiratory effects in several animal

14   species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys,

15   dogs, rabbits and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in

16   rats and pigs; nasal inflammation or lesions in rats and mice) across different dose regimens and

17   show respiratory effects across ranges of concentrations suggesting a dose-response (Coon et al.,

18   1970; Anderson et al., 1964; Broderson et al., 1976; Doig and Willoughby, 1971; Gaafar et al.,

19   1992). EPA considers the respiratory effects associated with ammonia exposure to be

20   biologically plausible and adverse. The evidence of observed respiratory effects seen across

21   multiple human and animal studies identifies the respiratory system as a hazard for ammonia.

22

23   *Gastrointestinal Effects*

24   Effects on gastric mucosa associated with oral exposure to ammonia are based on

25   evidence in animals and, to a more limited extent, in humans. Acute gastric toxicity observed in

26   case reports involving intentional or accidental ingestion of cleaning solutions or ammonia

27   inhalant capsules appears to reflect the corrosive properties of ammonia. Whether these acute

28   effects are relevant to toxicity following chronic low-level ammonia exposure is not known.

29   Indirect evidence is provided by the association between the stomach bacterium *H. pylori*, which

30   produces urease that catalyzes urea into ammonia, and human diseases of the upper

31   gastrointestinal tract (including chronic gastritis, gastric ulcers, and stomach cancer).

32   In vivo experimental evidence that ammonia is associated with gastric effects is provided

33   by two short-term studies in male Sprague-Dawley rats (Tsujii et al., 1993; Kawano et al., 1991).

34   These studies provide consistent findings of decreased gastric mucosal thickness that increased

35   with ammonia dose (Kawano et al., 1991) and duration of exposure (Tsujii et al., 1993; Kawano

36   et al., 1991); Tsujii et al. (1993) employed only one ammonia drinking water concentration and

37   therefore did not provide information on dose-response. Evidence for ammonia-related gastric

38   toxicity is limited to male rats of one strain and to investigations conducted by one research

group (Kawano et al. and Tsujii et al. were both affiliated with Osaka University Medical School).

Mechanistic studies in rodent models support the biological plausibility that ammonia exposure may be associated with gastric effects. Conditions that favor the unionized form of ammonia facilitate the penetration of the cell membrane and induce greater gastric toxicity. Multiple specific mechanistic events have been proposed that may contribute to the induction of gastric lesions, including ammonia-induced release of proteases, inhibition of mitochondrial respiration, and increased release of endothelin-1 and thyrotropin-releasing hormone. EPA considers the gastric effects associated with ammonia exposure to be biologically plausible and adverse, and relevant to humans. Given the evidence from human, animal, and mechanistic studies, gastric effects are identified as a hazard for ammonia.

Reproductive/Developmental Effects

No studies of the potential reproductive or developmental toxicity of ammonia in humans are available, and only one animal study that examined the reproductive effects of ammonia in the pig has been conducted. This study did not use a conventional test species and did not include a control group with no ammonia exposure. Further, animals were exposed naturally to bacterial and mycoplasm pathogens. Although the reproductive and developmental toxicity database for ammonia is limited, evidence on the endogenous formation of ammonia can inform the potential for ammonia to present a reproductive and developmental hazard.

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

tissues are normally exposed to ammonia in blood, and external concentrations that do not alter homeostasis would not be expected to pose a developmental or reproductive hazard.

*** Immune System Effects***

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

Animal studies consistently provide 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 (% 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, but the data were not dose responsive (Targowski et al, 1984).

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

*** Systemic Effects***

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

Effects on various organs, including liver, adrenal gland, kidney, spleen, and heart, were observed in several studies that examined responses to ammonia exposure in a number of laboratory species. While effects on many of these organs were observed in multiple species, including monkey, dog, rabbit, guinea pig, and rat, effects were not consistent across exposure protocols. For example, Coon et al. (1970) reported fatty liver and calcification and proliferation of renal tubular epithelium in monkeys, dogs, rabbits, and guinea pigs exposed continuously to

1   ammonia for 90 days at a concentration of 470 mg/m^3, but no histopathological changes in these

2   organs were observed in the same species following intermittent exposure (8 hours/day, 5

3   days/week for 6 weeks) to concentrations as high as 770 mg/m^3. It could be speculated that these

4   differences in response reflect recovery from short-term (i.e., 8-hour exposures), but the reason

5   for the inconsistent findings is not known.

6   Studies of ammonia toxicity that examined systemic effects were all published in the

7   older toxicological literature. The only oral study of ammonium hydroxide was published in

8   1939 (Fazekas, 1939), and three subchronic inhalation studies were published between 1952 and

9   1970 (Coon et al., 1970; Anderson et al., 1964; Weatherby, 1952). In general, the information

10 from these studies is limited by small group sizes, minimal characterization of some of the

11 reported responses (e.g., "congestion," "enlarged," "fatty liver"), insufficiently detailed reporting

12 of study results, and incomplete if any incidence data. In addition, Weatherby (1952), Anderson

13 et al. (1964), and some of the experiments reported by Coon et al. (1970) used only one ammonia

14 concentration in addition to the control, so no dose-response information is available the majority

15 of experimental studies to inform the evidence for systemic effects of ammonia.

16   As discussed above, ammonia is endogenously produced in all human and animal tissues,

17 and concentrations in all physiological fluids are homeostatically regulated to remain at low

18 levels (Souba, 1987). Thus, tissues are normally exposed to ammonia, and external

19 concentrations that do not alter homeostasis would not be expected to pose a hazard for systemic

20 effects. Overall, the evidence in humans and animals indicates that ammonia exposure may be

21 associated with these effects but does not support the liver, adrenal gland, kidney, spleen, or

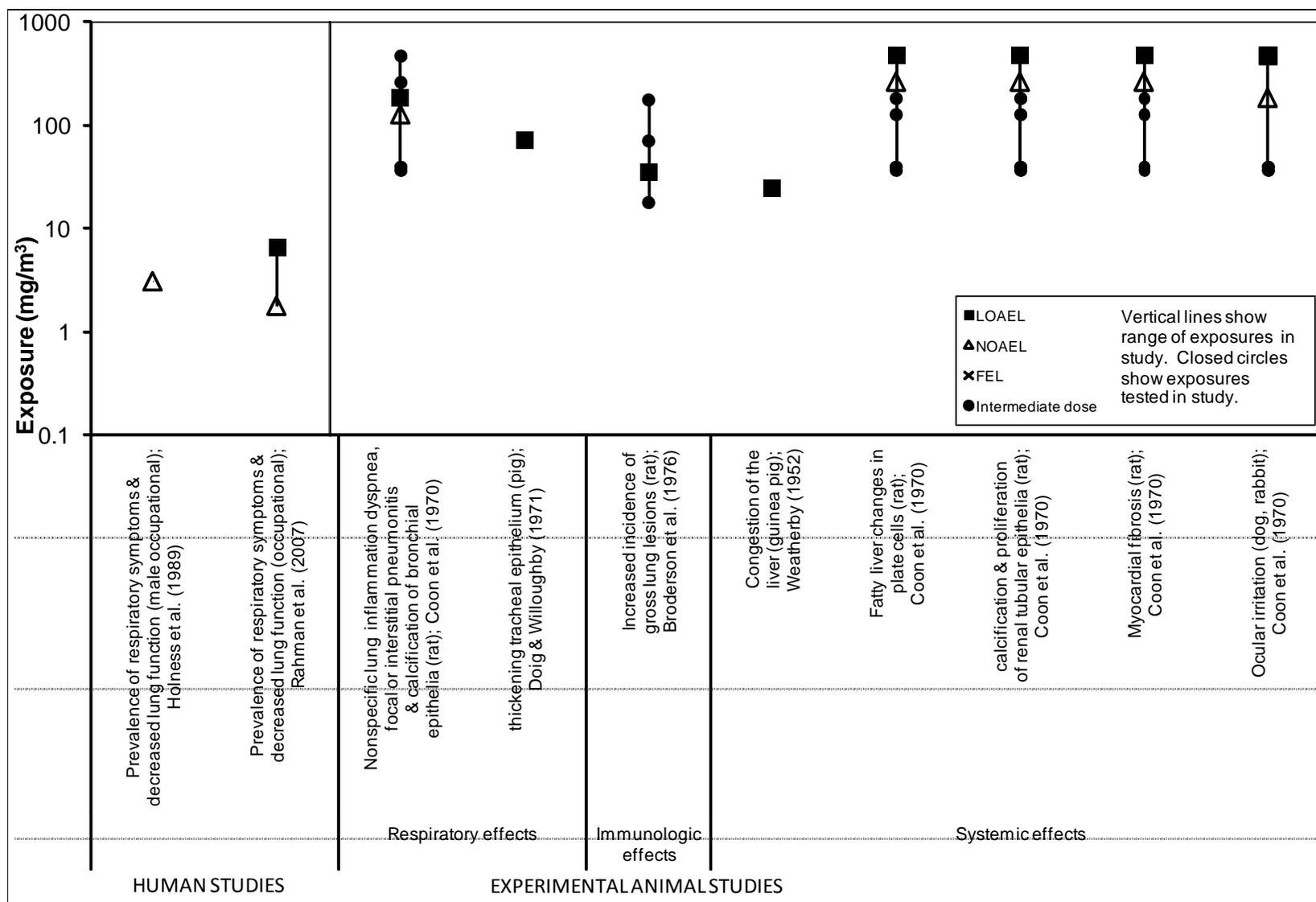22 heart as sensitive targets for ammonia toxicity.

23

Figure 1-4. Exposure-response array for toxicological effects following inhalation exposure.

Cancer

The available information on carcinogenicity following exposure to ammonia is limited to oral animal studies. There was no evidence of carcinogenicity in Swiss or C_3H 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 based on the findings of studies designed to examine *H. pylori*-induced gastric cancer (Tsujii et al., 1995, 1992a). Additionally, 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 the carcinogenic potential" of ammonia.

1

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 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 benchmark dose (BMD), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

The oral toxicity database for ammonia is very limited, although as noted in Section 1.2, gastric toxicity is identified as a hazard for ammonia based on evidence from case reports in humans, two animal studies, and mechanistic studies. Evidence in humans is limited to case reports of individuals suffering from gastrointestinal (e.g., stomach ache, nausea, diarrhea, distress, and burns along the digestive tract) effects from ingesting household cleaning solutions containing ammonia or biting into capsules of ammonia smelling salts. The data in humans were not considered for derivation of the RfD because although case reports can suggest the nature of acute endpoints in humans they are inadequate for dose-response analysis and derivation of a chronic reference value due to short duration of exposure and incomplete or missing quantitative exposure information.

Two studies reported gastrointestinal effects, characterized as increased epithelial cell migration in the mucosa of the stomach (in particular the antrum) leading to a statistically significant decrease in the thickness of the antral mucosa, in rats following subchronic (Tsujii et al., 1993) and short-term (Kawano et al., 1991) oral exposure to ammonia. These studies are repeated dose studies that analyzed gastrointestinal effects of ammonia and did not evaluate a comprehensive array of endpoints. Additionally, although both studies included a control group, Tsujii et al. (1993) employed one dose group and Kawano et al. (1991) included two dose groups. However, the decreased gastric antral mucosal thickness was consistently observed across these two studies. Prevalence of this effect was observed to generally increase with dose and duration, and the magnitude of decreases in thickness was 40-60%. Tsujii et al. (1993) and Kawano et al. (1991) reported that the gastric mucosal effects observed in rats resemble mucosal changes in human atrophic gastritis; indicating this effect is biological plausible and relevant to humans. Therefore, decreased gastric antral mucosal thickness is an effect considered by EPA to be adverse.

Given the limited number of studies available and the small number of toxicological evaluations, there are uncertainties associated with the oral database for ammonia. Although the oral database is limited, derivation of a RfD was considered due to the toxicological significance

1 of the reported gastrointestinal effects. However, uncertainties with extrapolations from the

2 available data (described below) were too high to support derivation of a chronic RfD; thus, in

3 consideration of the limited oral database and associated uncertainties **a RfD for ammonia was**

4 **not derived**.

5   In considering the derivation of a RfD, the subchronic study by Tsujii et al. (1993) was

6 considered as a potential principal study due to the relatively longer duration of exposure

7 compared with the short-term study by Kawano et al. (1991). Decreased gastric antral mucosal

8 thickness was considered as a potential critical effect. This effect was characterized as a portal-

9 of-entry effect based on the following. Tsujii et al. (1993) postulated that the difference in

10 response of the mucosa in the stomach body versus the mucosa of the antrum relates to

11 differences in pH in the two stomach regions. Most ammonia is transformed to ammonium ion

12 in solution at physiological pH; the ratio of ammonia to ammonium ion increases 10-fold with

13 each unit rise in pH. In the mucosa of the stomach body—an acid-secreting mucosa—ammonia

14 is protonated to the ammonium ion, which reduces the cytotoxicity associated with nonionized

15 ammonia. In the antral mucosa—a nonacid secreting area of the stomach—the pH is higher,

16 resulting in a relatively higher concentration of ammonia and thus enhanced cytotoxicity.

17   EPA identified a potential point of departure (POD) based on the LOAEL of 33 mg/kg-

18 day, for decreased gastric antral mucosal thickness in rats, from this study. BMD modeling was

19 not utilized because the Tsujii et al. (1993) employed only one dose level and a control, a data set

20 that is not amenable to dose-response analysis.

21   In U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation*

22 *of the Oral Reference Dose* (U.S. EPA, 2011a), the Agency endorses a hierarchy of approaches

23 to derive human equivalent oral exposures from data from laboratory animal species, with the

24 preferred approach being physiologically based pharmacokinetic modeling. Other approaches

25 may include using some chemical-specific information, without a complete physiologically

26 based pharmacokinetic model. In lieu of chemical-specific models or data to inform the

27 derivation of human equivalent oral exposures, EPA endorses body weight scaling to the ¾

28 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally

29 administered agents from laboratory animals to humans for the purpose of deriving a RfD. More

30 specifically, the use of $BW^{3/4}$ scaling for deriving a RfD is recommended when the observed

31 effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry

32 effects.

33   No PBPK model or chemical-specific information exists to inform the generation of

34 human equivalent oral exposures for ammonia. Furthermore, because ammonia oral toxicity

35 appears to be a function of the physical/chemical environment at the mucosal surface (i.e., a

36 portal-of-entry effect) and it is not clear if regions of the stomach scale allometrically across

37 species, a surface area adjustment would be the most relevant for interspecies extrapolation;

38 however, a dose scaling approach involving mass per unit surface area has not been developed

1 (U.S. EPA, 2011a). Therefore, because effects on the gastric antral mucosa are not expected to
2 scale allometrically, a $BW^{3/4}$ scaling approach (in combination with a reduced default UF for
3 interspecies extrapolation) was not applied.

4   The composite UF for ammonia that would be applied to the POD (LOAEL of 33 mg/kg-
5 day) from the Tsujii et al. (1993) study would be 10,000, consisting of four areas of uncertainty.
6 These areas of uncertainty, and the UFs that address each, are based on EPA's *A Review of the*
7 *Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and
8 include the following: uncertainties associated with intraspecies extrapolation (i.e., to account for
9 human variability in susceptibility to ammonia; $UF_H = 10$), uncertainties associated with
10 extrapolation of data from the rat to humans in the absence of information on species differences
11 in toxicokinetics and toxicodynamics (i.e., interspecies extrapolation; $UF_A = 10$), uncertainties
12 associated with extrapolation of data from a subchronic study (i.e., 8-week study) to a reference
13 value for chronic exposure scenarios ($UF_S = 10$), uncertainties associated with extrapolation
14 from a LOAEL to NOAEL ($UF_L = 10$), and database deficiencies ($UF_D = 1$; see Section 2.2.2 for
15 the justification for this UF).

16   In the report, *A Review of the Reference Dose and Reference Concentration*
17 *Processes* (U.S. EPA, 2002), the RfD/RfC technical panel concluded that, in cases where
18 maximum uncertainty exists in four or more areas of uncertainty, or when the total UF is
19 $\geq 10,000$, it is unlikely that the database is sufficient to derive a reference value. Therefore,
20 consistent with the recommendations in U.S. EPA (2002), the available oral data for ammonia
21 were considered insufficient to support reference value derivation and an RfD for ammonia was
22 not derived.

23   Route-to-route extrapolation from inhalation data was considered for deriving the oral
24 RfD; however, in the absence of a PBPK model and because the critical effect from the
25 inhalation literature is a portal-of-entry effect (respiratory irritation and decreased lung function),
26 route-to-route extrapolation is not supported (U.S. EPA, 1994).

27

28 ***Previous IRIS Assessment: Reference Dose***
29   No RfD was derived in the previous IRIS assessment for ammonia
30

31 **2.2. Inhalation Reference Concentration for Effects other than Cancer**
32   The RfC (expressed in units of mg/m^3) is defined as an estimate (with uncertainty
33 spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human
34 population (including sensitive subgroups) that is likely to be without an appreciable risk of
35 deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark
36 concentration (BMC), with UFs generally applied to reflect limitations of the data used.

As discussed in Section 1.2, respiratory effects have been identified as a hazard following inhalation exposure to ammonia. The studies in humans and animals examining inhalation exposure to ammonia provide evidence that inhaled ammonia is associated with toxicity to the respiratory system. 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. The weight of evidence for these effects is less robust than for respiratory effects. Therefore, the respiratory system is the primary and most sensitive target of inhaled ammonia toxicity in humans and experimental animals.

Human data are preferred over animal data for deriving reference values when possible because the use of human data is more relevant in the assessment of human health and avoids the uncertainty associated with interspecies extrapolation introduced when animal data serve as the basis for the RfC. Additionally, the respiratory effects in animals were observed at ammonia concentrations higher than those associated with respiratory effects in humans and represent much shorter durations (up to 114 days) of exposure, and thus were considered to carry less weight than the available human data. Therefore, data in humans were considered for derivation of the RfC and the respiratory effects in animals were not further considered.

Of the available human data, two occupational studies—Rahman et al. (2007) and Holness et al. (1989)—provide information useful for examining the relationship between chronic ammonia exposure and respiratory irritation and decreased lung function (quantitative dose-response analysis of ammonia respiratory tract toxicity data). Both studies reported the presence or absence of respiratory effects in workers exposed to ammonia over a range of concentrations (approximately 1 to 7 mg/m^3). Both studies provide consistent estimates of the effect level for ammonia, with the NOAEL$_{ADJ}$ of 3.1 mg/m^3 identified from the Holness et al. (1989) study falling between the NOAEL$_{ADJ}$ and LOAEL$_{ADJ}$ values (1.8 and 6.6 mg/m^3, respectively) from the Rahman et al. (2007) study. These studies are considered as candidate principal studies for RfC derivation. Other occupational epidemiology studies (Ali et al., 2001; Ballal et al., 1998) did not provide exposure information adequate for dose-response analysis and thus were not useful for RfC derivation.

Consideration of analytical methods suggests that higher confidence is associated with the exposure measures reported by Holness et al. (1989) than Rahman et al. (2007). Rahman et al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air (Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by four- to fivefold, indicating some uncertainty in these measurements, although ammonia concentrations measured by the two methods were strongly correlated. In contrast, the Holness et al. (1989) study used an established analytical method for measuring exposure to ammonia recommended by NIOSH that involved the collection of air samples on acid-treated silica gel (ATSG) absorption tubes.

1   Due to the greater confidence in the ammonia measurements in Holness et al. (1989) and

2   considering the range of NOAELs and LOAELs reported in both studies (in which a higher

3   NOAEL was reported by Holness et al. [1989]) the occupational exposure study of ammonia

4   exposure in workers in a soda ash plant by **Holness et al. (1989) was identified as the principal**

5   **study for RfC derivation**. Respiratory effects, characterized as increased respiratory irritation

6   and decreased lung function, observed in workers exposed to ammonia concentrations ≥6.6

7   mg/m^3 were selected as the critical effect. Respiratory effects, including changes in measures of

8   lung function and increased prevalence of wheezing, chest tightness, and cough/phlegm, have

9   been identified as adverse respiratory health effects by the American Thoracic Society (2000),

10 and are similarly noted as adverse in the EPA's *Methods for Derivation of Inhalation Reference*

11 *Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994).

12

2.2.1. Methods of Analysis

14   In the evaluation of the prevalence of increased respiratory irritation and decreased lung

15 function in workers exposed to ammonia (Holness et al., 1989), a NOAEL$_{ADJ}$ of 3.1 mg/m^3

16 (adjusted for continuous exposure from 8.8 mg/m^3; see calculation below) was identified based

17 on the absence of statistically significant increases in the prevalence of the respiratory effects.

18 BMD modeling was not utilized because ammonia concentrations in the Holness et al. (1989)

19 study were not associated with changes in respiratory effects in the study population (i.e., data

20 from Holness et al. could not be subjected to dose-response modeling). Thus, the Holness et al.

21 (1989) data were analyzed using a NOAEL approach and the **NOAEL$_{ADJ}$ of 3.1 mg/m^3 was**

22 **used as the POD for RfC derivation**.

23   Because the RfC is a measure that assumes continuous human exposure over a lifetime,

24 the POD derived from an occupational exposure was adjusted to account for the noncontinuous

25 exposure associated with occupational exposure (i.e., 8-hour workday and 5-day workweek).

26 The duration-adjusted POD was calculated as follows:

27

28      $\text{NOAEL}_{ADJ} = \text{NOAEL} \times \text{VEho}/\text{VEh} \times 5 \text{ days}/7 \text{ days}$

29              $= 8.8 \text{ mg/m}^3 \times 10 \text{ m}^3/20 \text{ m}^3 \times 5 \text{ days}/7 \text{ days}$

30              $= 3.1 \text{ mg/m}^3$

31

32   Where:

33   VEho = human occupational default minute volume (10 m^3 breathed during the 8-hour workday,

34   corresponding to a light to moderate activity level [U.S. EPA, 2011b])

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

36

2.2.2. Derivation of Reference Concentration

The UFs, selected based on EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002; Section 4.4.5) and described in the Preamble of this document, address five areas of uncertainty resulting in a **composite UF of 10**. This composite UF was applied to the selected POD (3.1 mg/m^3) to derive an RfC.

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

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

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

- A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied because a NOAEL value was used as the POD; and

- A database uncertainty factor, UF_D, of 1 was applied to account for deficiencies in the database. The ammonia inhalation database consists of studies of occupational exposure focused on effects of ammonia on respiratory irritation and lung function, studies in livestock farmers, controlled exposure studies involving volunteers exposed to ammonia vapors for short periods of time to evaluate irritation effects and changes in lung function, and a large number of case reports of acute exposure to high ammonia concentrations (e.g., accidental spills/releases). Studies of the toxicity of inhaled ammonia in experimental animals include subchronic studies in rats, guinea pigs, and pigs that examined respiratory and other systemic effects of ammonia and one limited, reproductive toxicity study in young female pigs. 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." Multigeneration reproductive and developmental toxicity studies would not be expected to impact the determination of ammonia toxicity at the POD, and therefore a database UF to account for the lack of these studies is not 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. Baseline blood levels in healthy individuals range from 0.1 to 1.0 µg/mL (Monsen, 1987; Conn, 1972; Brown et al., 1957). The fetoplacental unit produces ammonia, and concentrations in human umbilical vein and artery blood (at term) have been shown to be higher than concentrations in

maternal blood (Jóźwik et al., 2005), providing some assurance that developmental toxicity would not be associated with concentrations of ammonia at or below the POD. DeSanto et al. (1993) reported that human fetal umbilical blood levels of ammonia at birth were not influenced by gestational age based on deliveries ranging from gestation week 25–43. Finally, 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 A, Section A.3, for a more detailed discussion of ammonia distribution and elimination). Accordingly, exposure at the POD (3.1 mg/m^3) would not be expected to alter ammonia homeostasis or result in measureable increases in blood ammonia concentrations. Thus, the concentration of ammonia at the POD for the RfC would not be expected to result in systemic toxicity, including reproductive or developmental toxicity.

The RfC for ammonia was calculated as follows:

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

2.2.3. Uncertainties in the Derivation of the RfC

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

Use of a NOAEL as a POD

Data sets that support BMD modeling are generally preferred for reference value derivation because the shape of the dose-response curve can be taken into account in establishing the POD. For the ammonia RfC, no decreases in lung function or respiratory irritation 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 single study of ammonia would increase the confidence in the estimation of the POD.

Endogenous ammonia

Ammonia, which is produced endogenously, has been detected in the expired air of healthy volunteers at levels generally ranging from 0.013 to 2.1 mg/m^3 (Boshier et al., 2010; Smith et al., 2008; Spanel et al., 2007a, b; Turner et al., 2006; Diskin et al., 2003; Kearney et al.,

1 2002; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977). The higher and more

2 variable ammonia concentrations within this range are reported in breath exhaled from the mouth

3 or oral cavity, with the majority of ammonia concentrations from these sources ranging from

4 0.09 to 2.1 mg/m^3 (Smith et al., 2008; Spanel et al., 2007a, b; Turner et al., 2006; Diskin et al.,

5 2003; Smith et al., 1999; Norwood et al., 1992; Larson et al., 1977). Ammonia in exhaled breath

6 from the mouth or oral cavity is largely attributed to the production of ammonia via bacterial

7 degradation of food protein in the oral cavity or gastrointestinal tract (Turner et al., 2006; Smith

8 et al., 1999; Vollmuth and Schlesinger, 1984), and can be influenced by factors such as diet, oral

9 hygiene, age, and living conditions (i.e., urban vs. rural setting). In contrast, ammonia

10 concentrations measured in breath exhaled from the nose and trachea are lower (range: 0.013–

11 0.078 mg/m^3; Smith et al., 2008; Larson et al., 1977) and more likely reflect systemic levels of

12 ammonia (i.e., circulating levels in the blood) (Smith et al., 2008).

13   Ammonia concentrations measured in breath exhaled from the nose and trachea, i.e.,

14 concentrations expected to more closely correlate with circulating levels of ammonia in blood,

15 are lower than the ammonia RfC of 0.3 mg/m^3 by a factor of approximately fourfold or more;

16 however, the RfC does fall within the more variable range of breath concentrations collected

17 from the mouth or oral cavity. Although the contribution of ammonia generated endogenously

18 and expired through exhalation to ammonia present in ambient air is not known, this contribution

19 is expected to be minimal considering the ammonia in expired air should rapidly mix with and be

20 diluted in the much larger volume of ambient air.

21

2.2.4. Confidence Statement

23   A confidence level of high, medium, or low is assigned to the study used to derive the

24 RfC, the overall database, and the RfC itself, as described in Section 4.3.9.2 of EPA's *Methods*

25 *for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*

26 (U.S. EPA, 1994b). **Confidence in the principal study (Holness et al., 1989) is medium**. The

27 design, conduct, and reporting of this occupational exposure study were adequate, but the study

28 was limited by a small sample size and by the fact that workplace ammonia concentrations to

29 which the study population was exposed were below those associated with ammonia-related

30 effects (i.e., only a NOAEL was identified). However, this study is supported in the context of

31 the entire database, including the NOAEL and LOAEL values identified in the Rahman et al.

32 (2007) occupational exposure study, multiple studies of acute ammonia exposure in volunteers,

33 and the available inhalation data from animals. **Confidence in the database is medium**. The

34 inhalation ammonia database includes limited studies of reproductive toxicity and no studies of

35 developmental toxicity; however, reproductive, developmental, and other systemic effects are

36 not expected at the RfC because it is well documented that ammonia is endogenously produced

37 in humans and animals, ammonia concentrations in blood are homeostatically regulated to

38 remain at low levels, and ammonia concentrations in air at the POD are not expected to alter

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

2.2.5. Previous IRIS Assessment: Reference Concentration

The previous IRIS assessment for ammonia (posted to the database in 1991) presented an RfC of 0.1 mg/m^3 based on co-principal studies—the occupational exposure study of workers in a soda ash plant by Holness et al. (1989) and the subchronic study by Broderson et al. (1976) that examined the effects of ammonia exposure in F344 rats inoculated on day 7 of the study with the bacterium *M. pulmonis*. The NOAEL of 6.4 mg/m^3 (estimated as the mean concentration of the entire exposed group) from the Holness et al. (1989) study (duration adjusted: NOAEL$_{ADJ}$ = 2.3 mg/m^3) was used as the POD.[5]

The previous RfC was derived by dividing the POD 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 toxicology studies. A UF$_D$ of 3 (rather than 10) was applied because studies in rats (Schaerdel et al., 1983) showed no increase in blood ammonia levels at an inhalation exposure to 32 ppm (22.6 mg/m^3) and only minimal increases at 300–1,000 ppm (212–707 mg/m^3), suggesting that no significant distribution is likely to occur at the human equivalent concentration. In this document, a UF$_D$ of one was selected because a more thorough investigation of the literature on ammonia homeostasis and literature published since 1991 on fetoplacental ammonia levels provides further support that exposure to ammonia at the POD would not result in a measureable increase in blood ammonia, including fetal blood levels.

2.3. Cancer Risk Estimates

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

As discussed in Section 1.2, there is **"inadequate information to assess the 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 did not include a carcinogenicity assessment.

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[5]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 respiratory irritation or decreases in pulmonary function.

3. REFERENCES

Abramovicz, I. (1924) Ocular injury caused by liquid ammonia. Br J Ophthalmol 9(5):241–242.

AIChE (American Institute of Chemical Engineers). (1999) Ammonia H3N. Physical and thermodynamic properties of pure chemicals. Design Institute for Physical Property Data. Philadelphia, PA: Taylor and Francis.

Ali, BA; Ahmed, HO; Ballal, SG; et al. (2001) Pulmonary function of workers exposed to ammonia: a study in the eastern province of Saudi Arabia. Int J Occup Environ Health 7(1):19–22.

Altmann, L; Berresheim, H; Kruell, H; et al. (2006) Odor and sensory irritation of ammonia measured in humans. Naunyn-Schmiedebergs Arch Pharmakol 372(Suppl. 1):99.

American Thoracic Society. (2000) What constitutes an adverse health effect of air pollution? Official statement of the American Thoracic Society. Am J Respir Crit Care Med 161:665-673.

Amshel, CE; Fealk, MH; Phillips, BJ; et al. (2000) Anhydrous ammonia burns: case report and review of the literature. Burns 26(5):493–497.

Anderson, DP; Beard, CW; Hanson, RP. (1964) The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Dis 8:369–379.

Andersson, BO. (1991) Lithuanian ammonia accident, March 20th 1989. Institution of Chemical Engineers Symposium Series 124:15–17.

Appelman, LM; ten Berge, WF; Reuzel, PGJ. (1982) Acute inhalation toxicity of ammonia in rats with variable exposure periods. Am Ind Hyg Assoc J 43(9):662–665.

Arwood, R; Hammond, J; Gillon Ward, G. (1985) Ammonia inhalation. J Trauma 25(5):444–447.

Atkinson, SA; Anderson, GH: Bryan, MH. (1980) Human milk comparison of the nitrogen composition in milk from mothers of premature and full-term infants. Am J Clin Nutr 33:811–815.

ATSDR (Agency for Toxic Substances and Disease Registry). (2004) Toxicological profile for ammonia (update). Atlanta, GA. Available online at http://www.atsdr.cdc.gov/ (accessed August 20, 2009).

Auerbach, C; Robson, JM. (1947) XXXIII. Test of chemical substances for mutagenic action. Proc R Soc Edinb [Nat Environ] 62:284–291.

Ballal, SG; Ali, BA; Albar, AA; et al. (1998) Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia. Int J Tuberc Lung Dis 2(4):330–335.

Barrow, CS; Steinhagen, WH. (1980) NH_3 concentrations in the expired air of the rat: importance to inhalation toxicology. Toxicol Appl Pharmacol 53:116–121.

Barzel, US; Jowsey, J. (1969) The effects of chronic acid and alkali administration on bone turnover in adult rat. Clin Sci 36:517–524.

Beare, JD; Wilson, RS; Marsh, RJ. (1988) Ammonia burns of the eye: old weapon in new hands. Br Med J 296:590.

Bell, AW; Kennaugh, JM; Battaglia, FC; et al. (1989) Uptake of amino acids and ammonia at midgestation by the fetal lamb. Q J Exp Physiol 74:635–643.

Bernstein, IL; Bernstein, DL. (1989) Reactive airways disease syndrome (RADS) after exposure to toxic ammonia fumes. J Allergy Clin Immunol 83:173.

Betterton, EA. (1992) Henry's law constants of soluble and moderately soluble organic gases: effects on aqueous phase chemistry. Tucson, AZ: John Wiley & Sons, pp. 1–50.

Bishop, JM; Verlander, JW; Lee, HW; et al. (2010) Role of the Rhesus glycoprotein, Rh B glycoprotein, in renal ammonia excretion. Am J Physiol Renal Physiol 299(5):F1065–F1077.

Bloom, GR; Suhail, F; Hopkins-Price, P; et al. (2008) Acute anhydrous ammonia injury from accidents during illicit methamphetamine production. Burns 34(5):713–718.

Bodega, G; Suarez, I; Boyano, MC; et al. (1993) High ammonia diet: its effect on the glial fibrillary acidic protein (GFAP). Neurochem Res 18(9):971–975.

Boshier, PR; Marczin, N; Hanna, GB. (2010) Repeatability of the measurement of exhaled volatile metabolites using selected ion flow tube mass spectrometry. J Am Soc Mass Spectrom 21(6):1070–1074.

Boyd, EM; MacLachlan, ML; Perry, WF. (1944) Experimental ammonia gas poisoning in rabbits and cats. J Ind Hyg Toxicol 26(1):29–34.

Brautbar, N; Wu, MP; Richter, ED. (2003) Chronic ammonia inhalation and interstitial pulmonary fibrosis: a case report and review of the literature. Arch Environ Health 58(9):592–596.

Broderson, JR; Lindsey, JR; Crawford, JE. (1976) The role of environmental ammonia in respiratory mycoplasmosis of rats. Am J Pathol 85:115–130.

Brown, RH; Duda, GD; Korkes, S; et al. (1957) A colorimetric micromethod for determination of ammonia; the ammonia content of rat tissues and human plasma. Arch Biochem Biophys 66:301–309.

Buckley, LA; Jiang, XJ; James, RA; et al. (1984) Respiratory tract lesions induced by sensory irritants at the RD_{50} concentration. Toxicol Appl Pharmacol 74:417–429.

Burns, TR; Greenberg, SD; Mace, ML; et al. (1985) Ultrastructure of acute ammonia toxicity in the human lung. Am J Forensic Med Pathol 6:204–210.

Caplin, M. (1941) Ammonia-gas poisoning. Forty-seven cases in a London smelter. Lancet 2:95–96.

Castell, DO; Moore, EW. (1971) Ammonia absorption from the human colon. Gastroenterology 60:33–42.

Chaung, H; Hsia, L; Liu, S. (2008) The effects of vitamin A supplementation on the production of hypersensitive inflammatory mediators of ammonia-induced airways of pigs. Food Agric Immunol 19(4):283–291.

ChemIDplus. (2009) Ammonia. Bethesda, MD: U.S. National Library of Medicine. Available online at http://sis.nlm.nih.gov/chemical.html (accessed August 17, 2009).

Choudat, D; Goehen, M; Korobaeff, M; et al. (1994) Respiratory symptoms and bronchial reactivity among pig and dairy farmers. Scand J Work Environ Health 20(1):48–54.

Christesen, HB. (1995) Prediction of complications following caustic ingestion in adults. Clin Otolaryngol 20(3):272–278.

Close, LG; Catlin, FI; Cohn, AM. (1980) Acute and chronic effects of ammonia burns of the respiratory tract. Arch Otolaryngol 106:151–158.

Cole, TJ; Cotes, JE; Johnson, GR. (1977) Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to O-chlorobenzylidene malonitrile (CS) and ammonia gas in low concentrations. Q J Exp Physiol 62:341–351.

Conn, HO. (1972) Studies of the source and significance of blood ammonia. IV. Early ammonia peaks after ingestion of ammonia salts. Yale J Biol Med 45:543–549.

Coon, RA; Jones, RA; Jenkins, LJ; et al. (1970) Animal inhalation studies on ammonia, ethylene, glycol, formaldehyde, dimethylamine and ethanol. Toxicol Appl Pharmacol 16:646–655.

Córdoba, J. (1998) Chronic hyponatremia exacerbates ammonia-induced brain edema in rats after portacaval anastomosis. J Hepatol 29(4):589–594.

Cormier, Y; Israel-Assayag, E; Racine, G; et al. (2000) Farming practices and the respiratory health risks of swine confinement buildings. Eur Respir J 15(3):560–565.

Counturier, Y; Barbotin, M; Bobin, P; et al. (1971) Apropos of three cases of toxic lung caused by vapors of ammonia and hydrogen sulfide. Bull Soc Med d'Afrique Noire de Langue Francaise 16(2):250–252.

Crook, B; Robertson, JF; Travers Glass, SA; et al. (1991) Airborne dust, ammonia, microorganisms, and antigens in pig confinement houses and the respiratory health of exposed farm workers. Am Ind Hyg Assoc J 52(7):271–279.

Curtis, SE; Anderson, CR; Simon, J; et al. (1975) Effects of aerial ammonia, hydrogen sulfide and swine-house dust on rate of gain and respiratory-tract structure in swine. J Anim Sci 41(3):735–739.

da Fonseca-Wollheim, F. (1995) The influence of pH and various anions on the distribution of NH_4+ in human blood. Eur J Clin Chem Clin Biochem 33(5):289–294.

Dalhamn, T. (1963) Effect of ammonia alone and combined with carbon particles on ciliary activity in the rabbit trachea in vivo, with studies of the absorption capacity of the nasal cavity. Int J Air Water Pollut 7:531–539.

Davies, BMA; Yudkin, J. (1952) Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. Biochem J 52:407–412.

Davies, S; Španěl, P; Smith, D.l. (1997) Quantitative analysis of ammonia on the breath of patients in end-stage renal failure. Kidney Int 52:223–228.

Dean, JA (1985) Lange's handbook of chemistry. New York, NY: McGraw-Hill Book Co., pp. 10-3, 10-23.

de la Hoz, RE; Schlueter, DP; Rom, WN. (1996) Chronic lung disease secondary to ammonia inhalation injury: a report on three cases. Am J Ind Med 29(2):209–214.

Demerec, M; Bertau, G; Flint, J. (1951) A survey of chemicals for mutagenic action on E. coli. Am Nat 85:119–136.

DeSanto, JT; Nagomi, W; Liechty, EA; et al. (1993) Blood ammonia concentration in cord blood during pregnancy. Early Hum Dev 33(1):1–8.

DHHS (Department of Health and Human Services). (2004) The health consequences of smoking: a report of the Surgeon General. http://.cdc.gov//_statistics///.htm.

Diack, C; Bois, FY. (2005) Pharmacokinetic-pharmacodynamic models for categorical toxicity data. Regul Toxicol Pharmacol 41(1):55–65.

Diekman, MA; Scheidt, AB; Sutton, AL; et al. (1993) Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. Am J Vet Res 54(12):2128–2131.

Dilli, D; Bostanci, I; Tiras, U; et al. (2005) A non-accidental poisoning with ammonia in adolescence. Child Care Health Dev 31(6):737–739.

Diskin, A et al. (2003) Time variation of ammonia, acetone, isoprene and ethanol in breath: a quantitative SIFT-MS study over 30 days. Physiol Meas 24: 107-119.

Dodd, KT; Gross, DR. (1980) Ammonia inhalation toxicity in cats: a study of acute and chronic respiratory dysfunction. Arch Environ Health 35:6–14.

Doig, PA; Willoughby, RA. (1971) Response of swine to atmospheric ammonia and organic dust. J Am Vet Med Assoc 159:1353–1361.

Done, SH; Chennells, DJ; Gresham, AC; et al. (2005) Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. Vet Rec 157(3):71–80.

Donham, KJ; Reynolds, SJ; Whitten, P; et al. (1995) Respiratory dysfunction in swine production facility workers: dose-response relationships of environmental exposures and pulmonary functions. Am J Ind Med 27(3):405–418.

Donham, KJ; Cumro, D; Reynolds, SJ; et al. (2000) Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. J Occup Environ Med 42(3):260–269.

Douglas, RB; Coe, JE. (1987) The relative sensitivity of the human eye and lung to irritant gases. Annal Occup Hyg 31(2):265–267.

Drummond, JG; Curtis, SE; Simon, J; et al. (1980) Effects of aerial ammonia on growth and health of young pigs. J Anim Sci 50(6):1085–1091.

Dworkin, MS; Patel, A; Fennell, M; et al. (2004) An outbreak of ammonia poisoning from chicken tenders served in a school lunch. J Food Prot 67(6):1299–1302.

Eggeman, T. (2001) Ammonia. In: Kirk-Othmer encyclopedia of chemical technology. New York, NY: John Wiley & Sons, Inc., pp. 678–710.

Egle, JL. (1973) Retention of inhaled acetone and ammonia in the dog. Am Ind Hyg Assoc J 34:533–539.

Elfman, L; Riihimaki, M; Pringle, J; et al. (2009) Influence of horse stable environment on human airways. J Occup Med Toxicol 4:10.

Fazekas, IG. (1939) Experimental suprarenal hypertrophy induced by ammonia. Endokrinologie 21:315–337.

Ferguson, WS; Koch, WC; Webster, LB; et al. (1977) Human physiological response and adaption to ammonia. J Occup Med 19:319–326.

Ferris, BG. (1978) Epidemiology Standardization Project. Am Rev Respir Dis 118:11–31.

Flessner, MF; Wall, SM; Knepper, MA. (1992) Ammonium and bicarbonate transport in rat outer medullary collecting ducts. Am J Phyiol 262(1 Pt 2):F1–F7.

Flury, KE; Dines, DE; Rodarte, JR; et al. (1983) Airway obstruction due to inhalation of ammonia. Mayo Clin Proc 58:389–393.

FDA (Food and Drug Administration). (2011a) Direct food substances affirmed as generally recognized as safe. Substances added directly to human food affirmed as generally recognized as safe (GRAS). 21 CFR 184.1139. Available online at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1139. Accessed December 22, 2011.

FDA. (2011b) Substances generally recognized as safe. General purpose food additives. 21 CFR 582.1139. Available online at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=582.1139. Accessed December 22, 2011.

Gaafar, H; Girgis, R; Hussein, M; et al. (1992) The effect of ammonia on the respiratory nasal mucosa of mice. a histological and histochemical study. Acta Oto Laryngologica 112(2):339–342.

Gamble, MR; Clough, G. (1976) Ammonia build-up in animal boxes and its effect on rat tracheal epithelium. Lab Anim. 10:93–104.

Gay, WMB; Crane, CW; Stone, WD. (1969) The metabolism of ammonia in liver disease: a comparison of urinary data following oral and intravenous loading of [^{15}N] ammonium lactate. Clin Sci 37:815–823.

George, A; Bang, RL; Lari, AR; et al. (2000) Liquid ammonia injury. Burns 26(4):409–413.

Gilbert, GJ. (1988) Acute ammonia intoxication 37 years after ureterosigmoidostomy. South Med J 81(11):1443–1445.

Giroux, M; Bremont, F; Salles, JP; et al. (2002) Exhaled NH_3 and excreted NH_4+ in children in unpolluted or urban environments. Environ Int 28(3):197–202.

Green, AR; Wathes, CM; Demmers, TG; et al. (2008) Development and application of a novel environmental preference chamber for assessing responses of laboratory mice to atmospheric ammonia. J Am Assoc Lab Anim Sci 47(2):49–56.

Gustin, P; Urbain, B; Prouvost, JF; et al. (1994) Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: interaction with endotoxins. Toxicol Appl Pharmacol 125(1):17–26.

Guyatt, GH; Oxman, AD; Vist, GE; Kunz, R; Falck-Ytter, Y; Alonso-Coello, P; Schünemann, HJ. (2008a) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. British Medical Journal 336: 924-926, http://www.bmj.com/content/336/7650/924.full.

Guyatt, GH; Oxman, AD; Kunz, R; Vist, GE; Falck-Ytter, Y; Schünemann, HJ. (2008b) GRADE: what is "quality of evidence" and why is it important to clinicians? British Medical Journal 336: 995-998, http://www.bmj.com/content/336/7651/995.full.

Guyton, AC (1981) The body fluids and kidneys. In: Textbook of medical physiology. 6th edition. Philadelphia, PA: WB Saunders Company, pp. 456–458, 889.

Hamid, HA; El-Gazzar, RM. (1996) Effect of occupational exposure to ammonia on enzymatic activities of catalase and mono amine oxidase. J Egypt Public Health Assoc 71(5-6):465–475.

Han, KH; Croker, BP; Clapp, WL; et al. (2006) Expression of the ammonia transporter, Rh C glycoprotein, in normal and neoplastic human kidney. J Am Soc Nephrol 17(10):2670–2679.

Handlogten, ME; Hong, SP; Westhoff, CM; et al. (2005) Apical ammonia transport by the mouse inner medullary collecting duct cell (mIMCD-3). Am J Physiol Renal Physiol 289(2):F347–358.

Hatton, DV; Leach, CS; Beaudet, AL; et al. (1979) Collagen breakdown and ammonia inhalation. Arch Environ Health 34:83–86.

Hauguel, S; Challier, JC; et al. (1983) Metabolism of the human placenta perfused in vitro: glucose transfer and utilization, O2 consumption, lactate and ammonia production. Pediatr Res 17:729-732.

Heederik, D; van Zwieten, R; Brouwer, R. (1990) Across-shift lung function changes among pig farmers. Am J Ind Med 17(1):57–58.

Heifer, U. (1971) Casuistic contribution to acute lethal inhalation poisoning by ammonia. Lebensversicher Med 22:60–62. (German)

Helmers, S; Top, FH; Knapp, LW. (1971) Ammonia injuries in agriculture. J Iowa Med Soc 61(5):271–280.

Highman, VN. (1969) Early rise in intraocular pressure after ammonia burns. Br Med J. 1(5640):359–360.

Hilado, CJ; Casey, CJ; Furst, A. (1977) Effect of ammonia on Swiss albino mice. J Combust Toxicol 4:385–388.

Hill, AB. (1965) The environment and disease: association or causation? Proceedings of the Royal Society of Medicine 58:295-300.

Hoeffler, HB; Schweppe, HI; Greenberg, SD. (1982) Bronchiectasis following pulmonary ammonia burn. Arch Pathol Lab Med 106:686–687.

Holness, DL; Purdham, JT; Nethercott, JR. (1989) Acute and chronic respiratory effects of occupational exposure to ammonia. Am Ind Hyg Assoc J 50(12):646–650.

Holzman, IR; Lemons, JA; Meschia, G; et al. (1977) Ammonia production by the pregnant uterus. Proc Soc Exp Biol Med 156(1):27–30.

Holzman, IR; Phillips, AF; Battaglia, FC. (1979) Glucose metabolism, lactate, ammonia production by the human placenta in vitro. Pediatr Res 13:117–120.

HSDB (Hazardous Substances Data Bank). (2009) Ammonia. National Library of Medicine. Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (accessed August 17, 2009).

Huizenga, JR; Tangerman, A; Gips, CH. (1994) Determination of ammonia in biological fluids. Ann Clin Biochem 31(6):529–543.

Igarashi, M; Kitada, Y; Yoshiyama, H; et al. (2001) Ammonia as an accelerator of tumor necrosis factor alpha-induced apoptosis of gastric epithelial cells in *Helicobacter pylori* infection. Infect Immun 69(2):816–821.

Ihrig, A; Hoffman, J; Triebig, G. (2006) Examination of the influence of personal traits and habituation on the reporting of complaints at experimental exposure to ammonia. Int Arch Occup Environ Health 79(4):332–338.

IARC (International Agency for Research on Cancer). (2006) Preamble to the IARC Monographs. http://monographs.iarc.fr/.

Jarudi, MD; Golden, MD. (1973) Ammonia eye injuries. J Iowa Med Soc 63:260–263.

Jeffers, LJ. (1988) Hepatic encephalopathy and orotic aciduria associated with hepatocellular carcinoma in a noncirrhotic liver. Hepatology 8(1):78–81.

Johnson, RL; Gilbert, M; Block, SM; et al. (1986) Uterine metabolism of the pregnant rabbit under chronic steady-state conditions. Am J Obstet Gynecol 154:1146–1151.

Johnson, DR; Bedick, CR; Clark, NN; et al. (2009) Design and testing of an independently controlled urea SCR retrofit system for the reduction of NOx emissions from marine diesels. Environ Sci Technol 43(10):3959–3963.

Jóźwik, M; Teng, C; Meschia, G; et al. (1999) Contribution of branched-chain amino acids to uteroplacental ammonia production in sheep. Biol Repro 61:792-796.

Jóźwik, M; Pietrzycki, B; Chojnowski, M; et al. (2005) Maternal and fetal blood ammonia concentrations in normal term human pregnancies. Biol Neonate 87(1):38–43.

Jones, EA. (2002) Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. Metabolic Brain Disease 17:275-281.

Kalandarov, S; Bychkov, VP; Frenkel, ID; et al. (1984) Effect of an increased ammonia content in an enclosed atmosphere on the adrenocortical system in man. Kosm Bioloi Aviak Med 18:75–77.

Kane, LE; Barrow, CS; Alarie, Y. (1979) A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40:207–229.

Kapeghian, JC; Mincer, HH; Jones, AL; et al. (1982) Acute inhalation toxicity of ammonia in mice. Bull Environ Contam Toxicol 29:371–378.

Kapeghian, JC; Jones, AB; Waters, IW. (1985) Effects of ammonia on selected hepatic microsomal enzyme activity in mice. Bull Environ Contam Toxicol 35:15–22.

Kass, I; Zamel, N; Dobry, CA; et al. (1972) Bronchiectasis following ammonia burns of the respiratory tract. Chest 62(3):282–285.

Katayama, K. (2004) Ammonia metabolism and hepatic encephalopathy. Hepatol Res 30S:73–80.

Kawano, S; Tsujii, M; Fusamoto, H; et al. (1991) Chronic effect of intragastric ammonia on gastric mucosal structures in rats. Dig Dis Sci 36(1):33–38.

Kearney, DJ; Hubbard, T; Putnam, D. (2002) Breath ammonia measurement in *Helicobacter pylori* infection. Dig Dis Sci 47(11):2523–2530.

Keiding, S; Sorensen, M; Bender, D; et al. (2006) Brain metabolism of ^{13}N-ammonia during acute hepatic encephalopathy in cirrhosis measured by positron emission tomography. Hepatology 43(1):42–50.

Keiding, S; Sorensen, M; Munk, OL; et al. (2010) Human ^{13}N-ammonia PET studies: the importance of measuring ^{13}N-ammonia metabolites in blood. Metab Brain Dis 25(1):49–56.

Kerstein, MD; Schaffzin, DM; Hughes, WB; et al. (2001) Acute management of exposure to liquid ammonia. Mil Med 166(10):913–914.

Klein, J; Olson, KR; McKinney, HE. (1985) Caustic injury from household ammonia. Am J Emerg Med 3:320.

Klendshoj, NC; Rejent, TA. (1966) Tissue levels of some poisoning agents less frequently encountered. J Forensic Sci 11(1):75–80.

Lalic, H; Djindjic-Pavicic, M; Kukuljan, M. (2009) Ammonia intoxication on workplace-case report and a review of literature. Coll Antropol 33(3):945–949.

Landahl, HD; Hermann, RG. (1950) Retention of vapors and gases in the human nose and lung. Arch Ind Hyg Occup Med 1:36–45.

Larson, T. (1980) The chemical neutralization of inhaled sulfuric acid aerosol. Am J Ind Med 1:449–452.

Larson, TV; Covert, DS; Frank, R; et al. (1977) Ammonia in the human airways: neutralization of inspired acid sulfate aerosols. Science 197:161–163.

Latenser, BA; Lucktong, TA. (2000) Anhydrous ammonia burns: case presentation and literature review. J Burn Care Rehabil 21(1 PT 1):40–42.

Leduc, D; Gris, P; Lheureux, P; et al. (1992) Acute and long term respiratory damage following inhalation of ammonia. Thorax 47(9):755–757.

Lee, HS; Chan, CC; Tan, KT; et al. (1993) Burnisher's asthma: a case due to ammonia from silverware polishing. Singapore Med J 34(6):565–566.

Lee, HW; Verlander, JW; Bishop, JM; et al. (2009) Collecting duct-specific Rh C glycoprotein deletion alters basal and acidosis-stimulated renal ammonia excretion. Am J Physiol Renal Physiol 296(6):F1364–F1375.

Lee, HW; Verlander, JW; Bishop, JM; et al. (2010) Effect of intercalated cell-specific Rh C glycoprotein deletion on basal and metabolic acidosis-stimulated renal ammonia excretion. Am J Physiol Renal Physiol 299(2):F369–F379.

Levy, DM; Divertie, MB; Litzow, TJ; et al. (1964) Ammonia burns of the face and respiratory tract. J Am Med Assoc 190:873–876.

Li, WL; Pauluhn, J. (2010) Comparative assessment of the sensory irritation potency in mice and rats nose-only exposed to ammonia in dry and humidified atmospheres. Toxicology 276(2):135–142.

Lide, DR (2008) CRC handbook of chemistry and physics. 88th edition. Boca Raton, FL: Taylor & Francis, pp. 4-46 to 4-48, 8-40.

Lina, BA; Kuijpers, MH. (2004) Toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats; effects of feeding NH(4)Cl, KHCO(3) or KCl. Food Chem Toxicol 42(1):135–153.

Lobasov, M; Smirnov, F. (1934) On the nature of the action of chemical agents on mutational process in *Drosophila melanogaster*. II. The effect of ammonia on the occurrence of lethal transgressions. C R Seances Acad Sci Roum 3:177–178.

Lopez, G; Dean, BS; Krenzelok, EP. (1988) Oral exposure to ammonia inhalants: a report of 8 cases. Vet Hum Toxicol 30:350.

Luschinsky, HL. (1951) The activity of glutaminase in the human placenta. Arch Biochem Biophys 31(1):132–140.

MacEwen, JD; Theodore, J; Vernot, EH (1970) Human exposure to eel concentrations of monomethylhydrazine. In: Wright-Patterson Air Force Base, OH: Aerospace Medical Research Laboratory, pp. 355–363.

Manninen, ATA; Savolainen, H. (1989) Effect of short-term ammonia inhalation on selected amino acids in rat brain. Pharmacol Toxicol 64(3):244–246.

Manninen, A; Anttila, S; Savolainen, H. (1988) Rat metabolic adaptation to ammonia inhalation. Proc Soc Exp Biol Med 187(3):278–281.

Manolis, A. (1983) The diagnosis potential of breath analysis. Clin Chem 29:5–15.

Mayan, MH; Merilan, CP. (1972) Effects of ammonia inhalation on respiration rate of rabbits. J Anim Sci 34:448–452.

Mayan, MH; Merilan, CP. (1976) Effects of ammonia inhalation on young cattle. NZ Vet J 24:221–224.

McGuinness, R. (1969) Ammonia in the eye. Br Med J:575.

Mégraud, F; Neman-Simha, V; Brugmann, D. (1992) Further evidence of the toxic effect of ammonia produced by *Helicobacter pylori* urease on human epithelial cells. Infect Immun 60(5):1858–1863.

Melbostad, E; Eduard, W. (2001) Organic dust-related respiratory and eye irritation in Norwegian farmers. Am J Ind Med 39(2):209–217.

Meschia, G; Battaglia, FC; Hay, WW; et al. (1980) Utilization of substrates by the ovine placenta in vivo. Fed Proc 39:245–249.

Millea, TP; Kucan, JO; Smoot, EC. (1989) Anhydrous ammonia injuries. J Burn Care Rehabil 10(5):448–453.

Miñana, MD; Marcaida, G; Grisolia, S; et al. (1995) Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. J Neuropathol Exp Neurol 54(5):644–650.

Monsen, ER. (1987) The journal adopts SI units for clinical laboratory values. J Am Diet Assoc 87(3):356–378.

Montague, TJ; Macneil, AR. (1980) Mass ammonia inhalation. Chest 77:496–498.

Morgan, SE. (1997) Ammonia pipeline rupture: risk assessment to cattle. Vet Hum Toxicol 39(3):159–161.

Mori, S; Kaneko, H; Mitsuma, T; et al. (1998) Implications of gastric topical bioactive peptides in ammonia-induced acute gastric mucosal lesions in rats. Scand J Gastroenterol 33(4):386–393.

Morton, K. (2005) Fatal exposure to anhydrous ammonia in a food manufacturing plant. J Occup Environ Hyg 2(6):D4–D43.

Mossberg, SM; Ross, G. (1967) Ammonia, movement in the small intestine: preferential transport by the ileum. J Clin Invest 46(4):490–498.

Mulder, JS; Van der Zalm, HO. (1967) Fatal case of ammonia poisoning. Tijdschrift voor Sociale Geneeskunde 45:458–460.

Muntwyler, E; Iacobellis, M; Griffin, GE. (1956) Kidney glutaminase and carbonic anhydrase activities and renal electrolyte excretion in rats. Am J Physiol 184:83–90.

Murakami, M. (1995) Products of neutrophil metabolism increase ammonia-induced gastric mucosal damage. Dig Dis Sci 40(2):268–273.

Nagy, L; Kusstatscher, S; Hauschka, PV; et al. (1996) Role of cysteine proteases and protease inhibitors in gastric mucosal damage induced by ethanol or ammonia in the rat. J Clin Invest 98(4):1047–1054.

Nelson, DL; Cox, MM. (2008) Amino acid oxidation and the production of urea. In: Lehninger principles of biochemistry. 5th edition. WH Freeman & Co., pp. 680, 683.

Neumann, R; Mehlhorn, G; Buchholz, I; et al. (1987) Experimental studies of the effect of chronic exposure of suckling pigs to aerogenous toxic gas with ammonia of varying concentrations. Zentralbl Veterinaermed Reihe B 34:241–253.

NIOSH (National Institute for Occupational Safety and Health) (1979) NIOSH manual of analytical methods. Vol 5, 2nd ed. (DHEW/NISOSH Pub. No. 79-141). Washington, D.C.: Government Printing Office,. p. S347.

NIOSH (2011). NIOSH Pocket Guide to Chemical Hazards. Ammonia. Available online at: http://www.cdc.gov/niosh/npg/npgd0028.html. Accessed December 22, 2011.

Norwood, DM; Wainman, T; Lioy, PJ; et al. (1992) Breath ammonia depletion and its relevance to acidic aerosol exposure studies. Arch Environ Health 47(4):309–313.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NRC. (2007) Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 6. Committee on Acute Exposure Guideline Levels, Committee on Toxicology, National Research Council. Washington, DC: National Academies Press. Avialable online at: http://www.nap.edu/catalog/12018.html.

NRC (2011) Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington: National Academies Press.

NSF (National Science Foundation). (1999) Environmental science and engineering for the 21st century. Interim report. National Science Board Task Force on the Environment.

O'Connor, EA; Parker, MO; McLeman, MA; et al. (2010) The impact of chronic environmental stressors on growing pigs, *Sus scrofa* (Part 1): stress physiology, production and play behaviour. Animal 4(11):1899–1909.

O'Kane, GJ. (1983) Inhalation of ammonia vapour. Anaesthesia 38:1208–1213.

O'Neil, MJ; Heckelman, PE; Koch, CB; et al. (2006) The Merck index. 14th edition. Whitehouse Station, NJ: Merck & Co., Inc., pp. 83–89.

OSHA (Occupational Safety & Health Administration). (2011). Occupational Safety and Health Standards; Toxic and Hazardous Substances; Table Z-1, Limits for Air Contaminants. 29 CFR 1910.1000 Table Z-1. Available online at: http://www.osha.gov/dts/chemicalsampling/data/CH_218300.html. Accessed December 22, 2011.

Osmond, AH; Tallents, CJ. (1968) Ammonia attacks. Br Med J 3:740.

Ota, Y; Hasumura, M; Okamura, M; et al. (2006) Chronic toxicity and carcinogenicity of dietary administered ammonium sulfate in F344 rats. Food Chem Toxicol 44(1):17–27.

Petrova, M; Diamond, J; Schuster, B; et al. (2008) Evaluation of trigeminal sensitivity to ammonia in asthmatics and healthy human volunteers. Inhal Toxicol 20(12):1085–1092.

Phillips, CJ; Pines, MK; Latter, M; et al. (2010) The physiological and behavioral responses of steers to gaseous ammonia in simulated long-distance transport by ship. J Anim Sci 88(11):3579–3589.

Pinson, DM; Schoeb, TR; Lindsey, JR; et al. (1986) Evaluation by scoring and computerized morphometry of lesions of early mycoplasma pulmonis infection of ammonia exposure in F344/N rats (erratum in Vet Pathol 23(6):785–786). Vet Pathol 23(5):550–555.

Pirjavec, A; Kovic, I; Lulic, I; et al. (2009) Massive anhydrous ammonia injury leading to lung transplantation. J Trauma 67(4):E93–E97.

Pitts, RF. (1971) The role of ammonia production and excretion in regulation of acid-base balance. N Engl J Med 284:32–38.

Preller, L; Heederik, D; Boleij, JSM; et al. (1995) Lung function and chronic respiratory symptoms of pig farmers: focus on exposure to endotoxins and ammonia and use of disinfectants. Occup Environ Med 52:654–660.

Price, S; Watts, JC. (2008) Ammonia gas incident. Anaesthesia 63(8):894–895.

Price, SK; Hughes, JE; Morrison, SC; et al. (1983) Fatal ammonia inhalation: a case report with autopsy findings. S Afr Med J 64:952–955.

Prudhomme, JC; Shusterman, DJ; Blanc, PD. (1998) Acute-onset persistent olfactory deficit resulting from multiple overexposures to ammonia vapor at work. J Am Board Fam Pract 11(1):66–69.

Qin, C; Foreman, RD; Farber, JP. (2007a) Afferent pathway and neuromodulation of superficial and deeper thoracic spinal neurons receiving noxious pulmonary inputs in rats. Auton Neurosci 131(1–2):77–86.

Qin, C; Foreman, RD; Farber, JP. (2007b) Inhalation of a pulmonary irritant modulates activity of lumbosacral spinal neurons receiving colonic input in rats. Am J Physiol Regul Integr Comp Physiol 293(5):R2052–2058.

Rahman, MH; Bratveit, M; Moen, BE. (2007) Exposure to ammonia and acute respiratory effects in a urea fertilizer factory. Int J Occup Environ Health 13(2):153–159.

Read, AJ. (1982) Ionization constants of aqueous ammonia from 25 to 250°C and to 2000 bar. J Solution Chem 11(9):649–664.

Rejniuk, VL; Schafer, TV; Ovsep'yan, RV; et al. (2007) Effect of atmospheric ammonia on mortality rate of rats with barbiturate intoxication. Bull Exp Biol Med 143(6):692–694.

Rejniuk, VL; Schafer, TV; Ivnitsky, JJ. (2008) Ammonia potentiates the lethal effect of ethanol on rats. Bull Exp Biol Med 145(6):741–743.

Remesar, K; Arola, L; Palou, A; et al. (1980) Activities of enzymes involved in amino acid metabolism in developing rat placenta. Eur J Biochem 110:289–293.

Reynolds, SJ; Donham, KJ; Whitten, P; et al. (1996) Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. Am J Ind Med 29(1):33–40.

Richard, D; Bouley, G; Boudene, C. (1978a) Effects of continuous inhalation of ammonia in the rat and mouse. Bull Eur Physiopathol Respir 14:573–582. (French)

Richard, D; Jouany, JM; Boudene, C. (1978b) Acute toxicity of ammonia gas in the rabbit by inhalation. C R Hebd Seances Acad Sci 287:375–378. (French)

Rosenbaum, AM; Walner, DL; Dunham, ME; et al. (1998) Ammonia capsule ingestion causing upper aerodigestive tract injury. Otolaryngol Head Neck Surg 119(6):678–680.

Rosenfeld, M. (1932) Experimental modification of mitosis by ammonia. Arch Exp Zellforsch Besonders Gewebezuecht 14:1–13. (German)

Rosswall, T. (1981) The biogeochemical nitrogen cycle. In: Likens, GE, ed. Some perspectives of the major biogeochemical cycles. Chichester, England: John Wiley & Sons, pp. 25–49.

Rothman, KJ; Greenland, S. (1998) Modern Epidemiology. Philadelphia: Lippincott Williams and Wilkins.

Rubaltelli, FF: Formentin, PA. (1968) Ammonia nitrogen, urea and uric acid blood vessels in the mother and in both umbilical vessel at delivery. Biol Neonate 13(3):147–154.

Sabuncuoglu, N; Coban, O; Lacin, E; et al. (2008) Effect of barn ventilation on blood gas status and some physiological traits of dairy cows. J Environ Biol 29(1):107–110.

Sadasivudu, B; Murthy, RK. (1978) Effects of ammonia on monoamine oxidase and enzymes of GABA metabolism in mouse brain. Arch Int Physiol Biochim 86:67–82.

Sadasivudu, B; Rao, TI; Radhakrishna, M. (1979) Chronic metabolic effects of ammonia in mouse brain. Arch Int Physiol Biochim 87:871–885.

Schaerdel, AD; White, WJ; Lang, CM; et al. (1983) Localized and systemic effects of environmental ammonia in rats. Lab Anim Sci 33(1):40–45.

Schoeb, TR; Davidson, MK; Lindsey, JR. (1982) Intracage ammonia promotes growth of micoplasma pulmonis in the respiratory tract of rats. Infect Immun 38:212–217.

Schubiger, G; Bachmann, C; Barben, P; et al. (1991) N-acetylglutamate synthetase deficiency: diagnosis, management and follow-up of a rare disorder of ammonia detoxication. Eur J Pediatr 150(5):353–356.

Shimizu, H; Suzuki, Y; Takemura, N; et al. (1985) Results of microbial mutation test for forty-three industrial chemicals. Sangyo Igaku 27(6):400–419.

Sigurdarson, ST; O'Shaughnessy, PT; Watt, JA; et al. (2004) Experimental human exposure to inhaled grain dust and ammonia: towards a model of concentrated animal feeding operations. Am J Ind Med 46(4):345–348.

Silver, SD; McGrath, FP. (1948) A comparison of acute toxicities of ethylene imine and ammonia to mice. J Ind Hyg Toxicol 30:7–9.

Silverman, L; Whittenberger, JL; Muller, J. (1949) Physiological response of man to ammonia in low concentrations. J Ind Hyg Toxicol 31:74–78.

Sjoblom, E; Hojer, J; Kulling, PE; et al. (1999) A placebo-controlled experimental study of steroid inhalation therapy in ammonia-induced lung injury. J Toxicol Clin Toxicol 37(1):59–67.

Slot, GMJ. (1938) Ammonia gas burns. Lancet (Dec 10):1356–1357.

Smeets, MAM; Bulsing, PJ; van Rooden, S; et al. (2007) Odor and irritation thresholds for ammonia: a comparison between static and dynamic olfactometry. Chem Senses 32(1):11–20.

Smith, D; Spanel, P; Davies, S. (1999) Trace gases in breath of healthy volunteers when fasting and after a protein-calorie meal: a preliminary study. J Appl Physiol 87(5):1584–1588.

Smith, D; Wang, T; Pysanenko, A; et al. (2008) A selected ion flow tube mass spectrometry study of ammonia in mouth- and nose-exhaled breath and in the oral cavity. Rapid Commun Mass Spectrom 22(6):783–789.

Sobonya, R. (1977) Fatal anhydrous ammonia inhalation. Hum Pathol 8:293–299.

Socolow, RH. (1999) Nitrogen management and the future of food: lessons from the management of energy and carbon. Proc Natl Acad Sci (USA) 6:6001–6008.

Sorensen, M; Munk, OL; Keiding, S. (2009) Backflux of ammonia from brain to blood in human subjects with and without hepatic encephalopathy. Metab Brain Dis 24(1):237–242.

Sotiropoulos, G; Kilaghbian, T; Dougherty, W; et al. (1998) Cold injury from pressurized liquid ammonia: a report of two cases. J Emerg Med 16(3):409–412.

Souba, WW. (1987) Interorgan ammonia metabolism in health and disease: a surgeon's view. J Parenter Enteral Nutr 11(6):569–579.

Spanel, P; Dryahina, K; Smith, D. (2007a) The concentration distributions of some metabolites in the exhaled breath of young adults. J Breath Res 1(2):026001. Available online at http://iopscience.iop.org/1752-7163/1/2/026001/pdf/1752-7163_1_2_026001.pdf (accessed December 22, 2010).

Spanel, P; Dryahina, K; Smith, D. (2007b) Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4–83 years. J Breath Res 1(1):011001 Available online at http://iopscience.iop.org/1752-7163/1/1/011001/pdf/1752-7163_1_1_011001.pdf (accessed December 22, 2010).

Stabenau, JR; Warren, KS; Rall, DP. (1958) The role of pH gradient in the distribution of ammonia between blood and cerebro-spinal fluid, brain and muscle. J Clin Invest 38:373–383.

Stombaugh, DP; Teague, HS; Roller, WL. (1969) Effects of atmospheric ammonia on the pig. J Anim Sci 28:844–847.

Stroud, S. (1981) Ammonia inhalation: a case report. Crit Care Nurse 1:23–26.

Summerskill, WHJ; Wolpert, E. (1970) Ammonia metabolism in the gut. Am J Clin Nutr 23:633–639.

Sundblad, BM; Larsson, BM; Acevedo, F; et al. (2004) Acute respiratory effects of exposure to ammonia on healthy persons. Scand J Work Environ Health 30(4):313–321.

Suzuki, H; Yanaka, A; Nakahara, A; et al. (2000) The mechanism of gastric mucosal apoptosis induced by ammonia. Gastroenterology 118(Suppl 2):A734.

Takagaki, G; Berl, S; Clarke, DD; et al. (1961) Glutamic acid metabolism in brain and liver during infusion with ammonia labelled with nitrogen-15. Nature 189:326.

Takeuchi, K; Ohuchi, T; Harada, H; et al. (1995) Irritant and protective action of urea-urease ammonia in rat gastric mucosa. Dig Dis Sci 40(2):274–281.

Taplin, GV; Chopra, S; Yanda, RL; et al. (1976) Radionucliaic lung-imaging procedures in the assessment of injury due to ammonia inhalation. Chest 69:582–586.

Targowski, SP; Klucinski, W; Babiker, S; et al. (1984) Effect of ammonia on in vivo and in vitro immune response. Infect Immun 43(1):289–293.

Tepper, A; Constock, GW; Levine, M. (1991) A longitudinal study of pulmonary function in fire fighters. Am J Ind Med 20(3):307–316.

Tonelli, AR; Pham, A. (2009) Bronchiectasis a long-term sequela of ammonia inhalation: a case report and review of the literature. Burns 35(3):451–453.

Toth, B. (1972) Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in Swiss mice. failure of ammonium hydroxide to interfere in the development of tumors. Int J Cancer 9:109–118.

Tsujii, M; Kawano, S; Tsuji, S; et al. (1992a) Ammonia: a possible promotor in *Helicobacter pylori*-related gastric carcinogenesis. Cancer Lett 65(1):15–18.

Tsujii, M; Kawano, S; Tsuji, S; et al. (1992b) Mechanism of gastric mucosal damage induced by ammonia. Gastroenterology 102:1881-1888.

Tsujii, M; Kawano, S; Tsujii, S; et al. (1993) Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. Gastroenterology 104(3):796–801.

Tsujii, M; Kawano, S; Tsujii, S; et al. (1995) Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. Carcinogenesis 16(3):563–566.

Turner, C; Spanel, P; Smith, D. (2006) A longitudinal study of ammonia, acetone and propanol in the exhaled breath of 30 subjects using selected ion flow tube mass spectrometry, SIFT-MS. Physiol Meas 27(4):321–337.

Urbain, B; Gustin, P; Prouvost, JF; et al. (1994) Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of the nasal lavage method in pigs. Am J Vet Res 55(9):1335–1340.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA . (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6-87/008. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798–63826. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (1992) Draft report: a cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}$/day. Federal Register 57(109): 24152-24173.

U.S. EPA. (1994) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8-90/066F. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available online at http://www.epa.gov/raf/publications/pdfs/BENCHMARK.PDF (accessed March 11, 2011).

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2000a) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2000b) Benchmark dose technical guidance document. External review draft. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2006a) A framework for assessing health risk of environmental exposures to children. National Center for Environmental Assessment, Washington, DC, EPA/600/R-05/093F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363 (accessed March 14, 2011).

U.S. EPA (2006b) Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment. EPA/600/R-05/043F.

U.S. EPA. (2006c) Science policy council handbook: peer review. Third edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available online at http://www.epa.gov/iris/backgrd.html (accessed April 20, 2010).

U.S. EPA. (2007) Integrated Risk Information System (IRIS); Announcement of 2008 Program. Fed Reg 72(245): 72715-72719, December 21, 2007.

U.S. EPA. (2009) IRIS Process. http://www.epa.gov/iris/process.htm.

U.S. EPA. (2011a) Recommended use of body weight$^{3/4}$ as the default method in derivation of the oral reference dose. Risk Assessment Forum, Washington, DC; EPA/100/R11/0001. http://www.epa.gov/raf/publications/interspecies-extrapolation.htm (accessed October 27, 2011).

U.S. EPA. (2011b) Exposure factors handbook 2011 edition (final). National Center for Environmental Assessment, Washington, DC, EPA/600/R-09/052F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=236252 (accessed October 27, 2011).

van de Poll, MC; Ligthart-Melis, GC; Olde Damink, SW; et al. (2008) The gut does not contribute to systemic ammonia release in humans without portosystemic shunting. Am J Physiol Gastrointest Liver Physiol 295(4):G760–G765.

Van Slyke, DD; Phillips, RA; Hamilton, PB; et al. (1943) Glutamine as source material of urinary ammonia. J Biol Chem 150:481–482.

Verberk, MM. (1977) Effects of ammonia in volunteers. Int Arch Occup Environ Health 39:73–81.

Verschueren, K. (2001) Ammonia. In: Handbook of environmental data on organic chemicals. New York, NY: John Wiley & Sons, Inc., pp. 21–24, 188–189.

Vogelzang, PFJ; van der Gulden, JWJ; Preller, L; et al. (1997) Bronchial hyperresponsiveness and exposure in pig farmers. Int Arch Occup Environ Health 70(5):327–333.

Vogelzang, PFJ; van der Gulden, JWJ; Folgering, H; et al. (1998) Longitudinal changes in lung function associated with aspects of swine-confinement exposure. J Occup Environ Med 40:1048–1052.

Vogelzang, PFJ; van der Gulden, JWJ; Folgering, H; et al. (2000) Longitudinal changes in bronchial responsiveness associated with swine confinement dust exposure. Chest 117(5):1488–1495.

Vollmuth, TA; Schlesinger, RB. (1984) Measurement of respiratory tract ammonia in the rabbit and implications to sulfuric acid inhalation studies. Fundam Appl Toxicol 4:455–464.

Walton, M. (1973) Industrial ammonia gassing. Br J Ind Med 30:78–86.

Wands, RC. (1981) Alkaline materials. In: Clayton, GD; Clayton, FE; eds. Patty's industrial hygiene and toxicology. 4th edition. New York, NY: John Wiley & Sons, Inc., pp. 3045–3069.

Ward, K; Costello, GP; Murray, B. (1983) Acute and long-term pulmonary sequelae of acute ammonia inhalation. Irish Med J 76(6):279–281.

Wason, S; Stepman, M; Breide, C. (1990) Ingestion of aromatic ammonia smelling salts capsules. Am J Dis Child 144(2):139–140.

Weatherby, JH. (1952) Chronic toxicity of ammonia fumes by inhalation. Proc Soc Exp Biol Med 81:300–301.

Weiser, JR; Mackenroth, T. (1989) Acute inhalatory mass ammonia intoxication with fatal course. Exp Pathol 37(1–4):291–295.

Welch, A. (2006) Exposing the dangers of anhydrous ammonia. Nurse Pract 31(11):40–45.

White, CE; Park, MS; Renz, EM; et al. (2007) Burn center treatment of patients with severe anhydrous ammonia injury: case reports and literature review. J Burn Care Res 28(6):922–928.

White, ES. (1971) A case of near fatal ammonia gas poisoning. J Occup Med 13:543–550.

Whittaker, AG; Love, S; Parkin, TD; et al. (2009) Stabling causes a significant increase in the pH of the equine airway. Equine Vet J 41(9):940–943.

WHO (World Health Organization). (1986) Ammonia. Environmental Health Criteria 54. Available online at http://www.inchem.org/documents/ehc/ehc/ehc54.htm (accessed August 18, 2009).

Yadav, JS; Kaushik, VK. (1997) Genotoxic effect of ammonia exposure on workers in a fertility factory. Indian J Exp Biol 35(5):487–492.

Yang, GY. (1987) An industrial mass ammonia exposure. Vet Hum Toxicol 29:476–477.

Zejda, JE. (1994) Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. J Occup Med 36(1):49–56.