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Toxicological Review of Ammonia

FEBRUARY 2012

Toxicological Review of Ammonia

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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists	58	UF _A	interspecies uncertainty factor
ALP	alkaline phosphatase	59	UF _H	intraspecies uncertainty factor
ALT	alanine aminotransferase	60	UF _L	LOAEL to NOAEL uncertainty factor
AST	aspartate aminotransferase	61	UF _S	subchronic-to-chronic uncertainty factor
ATSDR	Agency for Toxic Substances and Disease Registry	62	UF _D	database deficiencies uncertainty factor
ATSG	acid-treated silica gel	63		
BAL	bronchioalveolar lavage	64		
BMD	benchmark dose			
BMI	body mass index			
BrDU	5-bromo-2-deoxyuridine			
BUN	blood urea nitrogen			
CAC	cumulative ammonia concentration			
CASRN	Chemical Abstracts Service Registry Number			
CI	confidence interval			
EPA	Environmental Protection Agency			
EU	endotoxin unit			
FEF	forced expiratory flow			
FEV ₁	forced expiratory volume in 1 second			
FVC	forced vital capacity			
GABA	γ-amino butyric acid			
IgE	immunoglobulin E			
IgG	immunoglobulin G			
IRIS	Integrated Risk Information System			
LOAEL	lowest-observed-adverse-effect level			
MAO	monoamine oxidase			
MMEF	mean midexpiratory flow			
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine			
MRM	murine respiratory mycoplasmosis			
NH ₃	ammonia			
NH ₄ ⁺	ammonium ion			
NIOSH	National Institute for Occupational Safety and Health			
NOAEL	no-observed-adverse-effect level			
NO _x	nitrogen oxide			
NRC	National Research Council			
OR	odds ratio			
PBPK	physiologically based pharmacokinetic			
PEF	peak expiratory flow			
PEFR	peak expiratory flow rate			
PHA	phytohemagglutinin			
POD	point of departure			
PPD	purified protein derivative			
RfC	inhalation reference concentration			
RfD	oral reference dose			
RNA	ribonucleic acid			
SD	standard deviation			
SIFT-MS	selected ion flow tube mass spectrometry			
TLV	threshold limit value			
TWA	time-weighted average			
UF	uncertainty factor			

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

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

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

8
9 The remainder of this Preamble addresses step 1,
10 the development of a draft Toxicological Review. IRIS
11 assessments follow standard practices of evidence
12 evaluation and peer review, many of which are
13 discussed in EPA guidelines (U.S. EPA, 1986a,
14 1986b, 1991, 1996, 1998, 2000a, 2005a, 2005b,
15 2006a) and other descriptions of "best practices" (U.S.
16 EPA, 1994, 2000b, 2002, 2006b, 2011a). Transparent
17 application of scientific judgment is of paramount
18 importance. To provide a harmonized approach across
19 IRIS assessments, this Preamble summarizes concepts
20 from these guidelines and emphasizes principles of
21 general applicability.

22 **3. Identifying and selecting pertinent** 23 **studies**

24 **3.1 Identifying studies**

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

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

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

49 In assessments of chemical mixtures, mixture
50 studies are preferred for their ability to reflect
51 interactions among components (U.S. EPA, 1986a,
52 2000a). The literature search seeks, in decreasing
53 order of preference:

54 – Studies of the mixture being assessed.

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

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

62 **3.2 Selecting pertinent epidemiologic studies**

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

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

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

74 – Ecologic studies (geographic correlation studies)
75 relate exposures and disease by geographic area.
76 They can provide strong evidence if there are
77 large exposure contrasts between geographic
78 areas, relatively little exposure variation within
79 study areas, and population migration is limited.

80 – Case reports of high or accidental exposure lack
81 definition of the population at risk and the
82 expected number of cases. They can provide
83 information about a rare disease or about the
84 relevance of analogous results in animals.

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

89 **3.3 Selecting pertinent experimental studies**

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

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

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

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

- 1 – Studies of effects from chronic exposure are most
- 2 pertinent to lifetime human exposure.
- 3 – Studies of effects from subchronic exposure are
- 4 pertinent but less preferred than studies of chronic
- 5 exposure.
- 6 – Short-term and acute studies are less pertinent but
- 7 are useful for obtaining toxicokinetic or
- 8 mechanistic information. The assessment reviews
- 9 short-term and acute studies if they suggest
- 10 distribution or effects at a site not identified by
- 11 longer-term studies.
- 12 – For developmental toxicity and reproductive
- 13 toxicity, irreversible effects may result from a
- 14 brief exposure during a critical period of
- 15 development. Accordingly, specialized study
- 16 designs are used for these effects (U.S. EPA,
- 17 1991, 1996, 1998).
- 18 **4. Evaluating the quality of individual**
- 19 **studies**
- 20 **4.1 Evaluating the quality of epidemiologic**
- 21 **studies**
- 22 The assessment evaluates design and
- 23 methodologic aspects that can increase or decrease the
- 24 weight given to each epidemiologic study in the
- 25 overall evaluation (U.S. EPA, 1991, 1994, 1996a,
- 26 1998, 2005a):
- 27 – Documentation of study design, methods,
- 28 population characteristics, and results.
- 29 – Definition and selection of the study and
- 30 comparison populations.
- 31 – Ascertainment of exposure and the potential for
- 32 misclassification.
- 33 – Ascertainment of disease or effect and the
- 34 potential for misclassification.
- 35 – Duration of exposure and follow-up and adequacy
- 36 for assessing the occurrence of effects, including
- 37 latent effects.
- 38 – Characterization of exposure during critical
- 39 periods for the development of effects.
- 40 – Sample size and statistical power to detect
- 41 anticipated effects.
- 42 – Participation rates and the resulting potential for
- 43 selection bias.
- 44 – Potential confounding and other sources of bias
- 45 are identified and addressed in the study design or
- 46 in the analysis of results. The basis for
- 47 consideration of confounding is a reasonable
- 48 expectation that the confounder is prevalent in the
- 49 population and is related to both exposure and
- 50 outcome.

51 For developmental toxicity, reproductive toxicity,

52 neurotoxicity, and cancer there is further guidance on

53 the nuances of evaluating epidemiologic studies of

54 these effects (U.S. EPA, 1991, 1996, 1998, 2005a).

55 **4.2 Evaluating the quality of experimental**

56 **studies**

57 The assessment evaluates design and

58 methodologic aspects that can increase or decrease the

59 weight given to each experimental study in the overall

60 evaluation (U.S. EPA, 1991, 1994, 1996, 1998,

61 2005a):

- 62 – Documentation of study design, animals or study
- 63 population, methods, basic data, and results.
- 64 – Relevance of the animal model or study
- 65 population and the experimental methods.
- 66 – Characterization of the nature and extent of
- 67 impurities and contaminants of the administered
- 68 chemical or mixture.
- 69 – Characterization of dose and dosing regimen
- 70 (including age at exposure) and their adequacy to
- 71 elicit adverse effects, including latent effects.
- 72 – Sample sizes and statistical power to detect dose-
- 73 related differences or trends.
- 74 – Ascertainment of survival, vital signs, disease or
- 75 effects, and cause of death.
- 76 – Control of other variables that could influence the
- 77 occurrence of effects.

78 The assessment uses statistical tests to evaluate

79 whether the observations may be due to chance. The

80 standard for determining statistical significance of a

81 response is a trend test or comparison of outcomes in

82 the exposed groups against those of concurrent

83 controls. In some situations, examination of historical

84 control data from the same laboratory within a few

85 years of the study may improve the analysis. For an

86 uncommon effect that is not statistically significant

87 compared with concurrent controls, historical controls

88 may show that the effect is unlikely to be due to

89 chance. For a response that appears significant against

90 a concurrent control response that is unusual, historical

91 controls may offer a different interpretation (U.S.

92 EPA, 2005a).

93 For developmental toxicity, reproductive toxicity,

94 neurotoxicity, and cancer there is further guidance on

95 the nuances of evaluating experimental studies of

96 these effects (U.S. EPA, 1991, 1996, 1998, 2005a). In

97 multi-generation studies, agents that produce

98 developmental effects at doses that are not toxic to the

99 maternal animal are of special concern. Effects that

100 occur at doses associated with mild maternal toxicity

101 are not assumed to result only from maternal toxicity.

102 Moreover, maternal effects may be reversible, while

103 effects on the offspring may be permanent (U.S. EPA,

104 1991, 1998).

1 4.3 Reporting study results

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

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

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

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

24 5. Weighing the overall evidence of 25 each effect

26 5.1 Weighing epidemiologic evidence

27 For each effect, the assessment evaluates the
28 evidence from the epidemiologic studies as a whole to
29 determine the extent to which any observed
30 associations may be causal. Positive, negative, and
31 null results are given weight according to study
32 quality. This evaluation considers aspects of an
33 association that suggest causality, discussed by Hill
34 (1965) and elaborated by Rothman and Greenland
35 (1998) (U.S. EPA, 1994, 2002, 2005a; DHHS, 2004).

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

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

51 **Specificity of association:** As originally intended, this
52 refers to one cause associated with one disease.
53 Current understanding that many agents cause
54 multiple diseases and many diseases have

55 multiple causes make this a less informative
56 aspect of causality, unless the effect is rare or
57 unlikely to have multiple causes.

58 **Temporal relationship:** A causal interpretation
59 requires that exposure precede development of the
60 disease.

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

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

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

77 **“Natural experiments”:** A change in exposure that
78 brings about a change in disease frequency
79 provides strong evidence of causality.

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

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

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

100 5.2 Weighing experimental evidence

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

1 experimental results: consistency of response, dose- 56
2 response relationships, strength of response, biologic 57
3 plausibility, and coherence (U.S. EPA, 1994, 2002, 58
4 2005a). 59

5 In weighing evidence from multiple experiments, 60
6 EPA (2005a) distinguishes 61

7 **Conflicting evidence** (that is, mixed positive and 62
8 negative results in the same sex and strain using a 63
9 similar study protocol) from 64

10 **Differing results** (that is, positive results and negative 65
11 results are in different sexes or strains or use 66
12 different study protocols). 67

13 Negative or null results do not invalidate positive 68
14 results in a different experimental system. EPA 69
15 regards all as valid observations and looks to 70
16 mechanistic information, if available, to reconcile 71
17 differing results. 72

18 It is well established that there are critical periods 73
19 for some developmental and reproductive effects. 74
20 Accordingly, the assessment determines whether 75
21 critical periods have been adequately investigated 76
22 (U.S. EPA, 1991, 1996, 1998, 2005a, 2005b). 77
23 Similarly, the assessment determines whether the 78
24 database is adequate to evaluate other critical sites and 79
25 effects. 80

26 **5.3 Characterizing modes of action**

27 For each effect, the assessment discusses the 81
28 available information on its *modes of action* and 82
29 associated *key events* (*key events* being empirically 83
30 observable, necessary precursor steps or biologic 84
31 markers of such steps; *mode of action* being a series of 85
32 key events involving interaction with cells, operational 86
33 and anatomic changes, and resulting in disease). 87
34 Pertinent information may also come from studies of 88
35 metabolites or of compounds that are structurally 89
36 similar or that act through similar mechanisms. The 90
37 assessment addresses several questions about each 91
38 hypothesized mode of action (U.S. EPA, 2005a). 92

39 (a) **Is the hypothesized mode of action sufficiently** 93
40 **supported in test animals?** Strong support for a 94
41 key event being necessary to a mode of action can 95
42 come from experimental challenge to the 96
43 hypothesized mode of action, where suppressing a 97
44 key event suppresses the disease. Support for a 98
45 mode of action is meaningfully strengthened by 99
46 consistent results in different experimental 100
47 models, but not by replicate experiments in the 101
48 same model. The assessment may consider 102
49 various aspects of causality in addressing this 103
50 question. 104

51 (b) **Is the hypothesized mode of action relevant to** 105
52 **humans?** The assessment reviews the key events 106
53 to identify critical similarities and differences 107
54 between the test animals and humans. Site 108
55 concordance is not assumed between animals and 109

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).

95 **5.4 Characterizing the overall weight of the evidence**

96 After weighing the epidemiologic and 97
98 experimental studies pertinent to each effect, the 99
assessment may select a standard descriptor to 100
characterize the overall weight of the evidence. For 101
example, the following standard descriptors combine 102
epidemiologic, experimental, and mechanistic 103
evidence of carcinogenicity (U.S. EPA, 2005a). 104

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

1 animal evidence, identification of key precursor
2 events in animals, and strong evidence that they
3 are anticipated to occur in humans.

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

11 **Suggestive evidence of carcinogenic potential:** The
12 data raise concern for effects in humans but are
13 not sufficient for a stronger conclusion. This
14 descriptor covers a range of evidence, from a
15 positive result in the only available study to a
16 single positive result in an extensive database that
17 includes negative results in other species.

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

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

35 6. Selecting studies for derivation of 36 toxicity values

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

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

- 49 – Epidemiologic studies are preferred over animal
50 studies, if quantitative measures of exposure are
51 available and effects can be attributed to the
52 agent.

- 53 – Among experimental animal models, those that
54 respond most like humans are preferred, if the
55 comparability of response can be determined.

- 56 – Studies by a route of human environmental
57 exposure are preferred, although a validated
58 toxicokinetic model can be used to extrapolate
59 across exposure routes.

- 60 – Studies of longer exposure duration and follow-up
61 are preferred, to minimize uncertainty about
62 whether effects are representative of lifetime
63 exposure.

- 64 – Studies with multiple exposure levels are
65 preferred for their ability to provide information
66 about the shape of the exposure-response curve.

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

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

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

87 7. Deriving toxicity values

88 7.1 General framework for dose-response 89 analysis

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

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

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

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

6 **7.2 Modeling dose**

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

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

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

29 – Doses are standardized to equivalent human terms
30 to facilitate comparison of results from different
31 species.

32 – Oral doses are scaled allometrically using
33 $\text{mg/kg}^{3/4}\text{-d}$ as the equivalent dose metric
34 across species. As allometric scaling is
35 typically based on adult body weight, it is not
36 used for early-life exposure or for
37 developmental effects (U.S. EPA, 2005a,
38 2011a).

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

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

49 **7.3 Modeling response in the range of 50 observation**

51 Toxicodynamic (“biologically based”) modeling
52 can incorporate data on biologic processes leading to a
53 disease. Such models require sufficient data to
54 ascertain a mode of action and to quantitatively

55 support model parameters associated with its key
56 events. Because different models may provide
57 equivalent fits to the observed data but diverge
58 substantially at lower doses, critical biologic
59 parameters should be measured from laboratory
60 studies, not by model fitting. Confidence in the use of
61 a toxicodynamic model depends on the robustness of
62 its validation process and on the results of sensitivity
63 analyses. Peer review of the scientific basis and
64 performance of a model is essential (U.S. EPA,
65 2005a).

66 Because toxicodynamic modeling can require
67 many parameters and more knowledge and data than
68 are typically available, EPA has developed a standard
69 set of empirical (“curve-fitting”) models ([http://
70 www.epa.gov/ncea/bmnds/](http://www.epa.gov/ncea/bmnds/)) that can be applied to
71 typical data sets, including those that are nonlinear.
72 EPA has also developed guidance on modeling dose-
73 response data, assessing model fit, selecting suitable
74 models, and reporting modeling results (U.S. EPA,
75 2000b). Additional judgment or alternative analyses
76 are used when the procedure fails to yield reliable
77 results, for example, if the fit is poor, modeling may
78 be restricted to the lower doses, especially if there is
79 competing toxicity at higher doses (U.S. EPA, 2005a).

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

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

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

93 – If nonlinear extrapolation will be used, both
94 statistical and biologic factors are considered
95 (10% response for minimally adverse effects,
96 5% or lower for more severe effects or for
97 developmental toxicity data on individual
98 offspring).

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

104 **7.4 Extrapolating to lower doses**

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

1 (1) If a biologically based model has been developed 54
2 and validated for the agent, extrapolation may use 55
3 the fitted model beyond the observed range if 56
4 significant model uncertainty can be ruled out 57
5 with reasonable confidence. Below the range 58
6 where confidence bounds on the predictions are
7 reasonably precise, extrapolation may continue
8 using a linear model.

9 (2) Linear extrapolation is used if the dose-response 62
10 curve is expected to have a linear component
11 below the point of departure. This includes:

- 12 – Agents or their metabolites that are DNA- 65
13 reactive and have direct mutagenic activity. 66
- 14 – Agents or their metabolites for which human 68
15 exposures or body burdens are near doses
16 associated with key events leading to an
17 effect. 69

18 Linear extrapolation is also used if the evidence is
19 insufficient to establish a mode of action. 72

20 The result of linear extrapolation is described by
21 an *oral slope factor* or an *inhalation unit risk*,
22 which is the slope of the dose-response curve at
23 lower doses. 76

24 (3) Nonlinear extrapolation is used if there are
25 sufficient data to ascertain the mode of action and
26 to conclude that it is not linear at lower doses, and
27 the agent does not demonstrate mutagenic or other
28 activity consistent with linearity at lower doses. If
29 nonlinear extrapolation is appropriate but no
30 model is developed, a default is to calculate
31 reference values. 77

32 If linear extrapolation is used, the assessment
33 develops a candidate slope factor or unit risk for each
34 suitable data set. These results are arrayed, using
35 common dose metrics, to show the distribution of
36 relative potency across various effects and
37 experimental systems. The assessment then derives an
38 overall slope factor and an overall unit risk for the
39 agent, considering the various dose-response analyses,
40 the study preferences discussed in section 6, and the
41 possibility of basing a more robust result on multiple
42 data sets. 84

43 7.5 Considering susceptible populations and 44 life-stages

45 The assessment analyzes the available information
46 on populations and life-stages that may be particularly
47 susceptible to each effect. A tiered approach is used
48 (U.S. EPA, 2005a). 103

49 (1) If an epidemiologic or experimental study reports
50 quantitative results for a susceptible population or
51 life-stage, these data are analyzed to derive
52 separate toxicity values for susceptible
53 individuals. 107

(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

1 are most susceptible to the effect. This factor is 56
2 reduced only if the point of departure is derived 57
3 specifically for susceptible individuals (not for a 58
4 general population that includes both susceptible 59
5 and non-susceptible individuals) (U.S. EPA, 1991, 60
6 1994, 1996, 1998, 2002).

7 **Animal-to-human extrapolation.** A factor of 10 is 62
8 applied if animal results are used to make 63
9 inferences about humans. This factor is often 64
10 regarded as comprising toxicokinetics and 65
11 toxicodynamics in equal parts. Accordingly, if the 66
12 point of departure is based on toxicokinetic 67
13 modeling, dosimetry modeling, or allometric 68
14 scaling across species, a factor of $10^{1/2}$ (rounded 69
15 to 3) is applied to account for the remaining 70
16 uncertainty involving toxicodynamic differences. 71
17 An animal-to-human factor is not applied if a 72
18 biologically based model adjusts fully for 73
19 toxicokinetic and toxicodynamic differences and 74
20 residual uncertainty across species (U.S. EPA, 75
21 1991, 1994, 1996, 1998, 2002).

22 **Adverse-effect level to no-observed-adverse-effect**
23 **level.** If a point of departure is based on a lowest-
24 observed-adverse-effect level, the assessment
25 must infer a dose where such effects are not
26 expected. This can be a matter of great
27 uncertainty, especially if there is no evidence
28 available at lower doses. A factor of 10 is applied
29 to account for the uncertainty in making this
30 inference. A factor other than 10 may be used,
31 depending on the magnitude and nature of the
32 response and the shape of the dose-response curve
33 (U.S. EPA, 1991, 1994, 1996, 1998, 2002).

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

46 **Incomplete database.** If an incomplete database 100
47 raises concern that further studies might identify a 101
48 more sensitive effect, organ system, or life-stage, 102
49 the assessment may apply a database uncertainty 103
50 factor (U.S. EPA, 1991, 1994, 1996, 1998, 2002). 104
51 EPA typically follows the suggestion that a factor 105
52 of 10 be applied if both a prenatal toxicity study 106
53 and a two-generation reproduction study are 107
54 missing, and a factor of $10^{1/2}$ if either is missing 108
55 (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),
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2 This assessment was provided for review to scientists in EPA’s Program and Regional Offices.
3 Comments were submitted by the Programs and Regions listed below.

4
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5
6 This assessment was provided for review to other federal agencies and White House offices. The
7 federal agencies and White House offices that commented are listed below. Comments
8 submitted by the agencies listed below are available on the IRIS website.

9
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

10
11 A public listening session was held by EPA on [month] [date], [year]. Attendees external to the
12 EPA are listed below.

13
NAME Affiliation
NAME Affiliation
NAME Affiliation
NAME Affiliation

14
15 This assessment was released for public comment on [month] [date], 2012; the public comment
16 period ended on [month] [date], [year]. Comments were received from the following entities.

17
NAME Affiliation, Location
NAME Affiliation, Location
NAME Affiliation, Location
NAME Affiliation, Location

18
19 This assessment was peer-reviewed by independent expert scientists external to EPA (specify
20 SAB or NAS panel) and a peer review meeting was held on [month] [date], [year]. The external
21 peer review comments are available on the IRIS website. EPA’s response to the external peer
22 review and public comments is included in Appendix C and is also available on the IRIS
23 website.

24
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25
26
27

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 (NO_x) 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 (NH₃) 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 (NH₄⁺) (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 NH₃ 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 NO_x 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

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

3

4 **Other Agency and International Assessments**

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

10

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	NOAEL _{ADJ} : 3.1 mg/m ³	10	0.3 mg/m ³
Occupational epidemiology study			
Holness et al., 1989			

*Because the POD (NOAEL = 8.8 mg/m³) involved workplace exposure conditions, the NOAEL was adjusted for continuous exposure based on the ratio of VE_{no} (human occupational default minute volume of 10 m³ breathed during an 8-hour workday) to VE_h (human ambient default minute volume of 20 m³ 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/m³ (adjusted for continuous exposure from 8.8 mg/m³; 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/m³ 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

1 sample size and identification of a NOAEL only) and medium confidence in the database, which
2 includes occupational and volunteer studies and studies in animals that are mostly of subchronic
3 duration. Although there are no studies of developmental toxicity and studies of reproductive
4 and other systemic endpoints are limited, reproductive, developmental, and other systemic
5 effects are not expected at the RfC because it is well documented that ammonia is endogenously
6 produced in humans and animals, ammonia concentrations in blood are homeostatically
7 regulated to remain at low levels, and ammonia concentrations in air at the POD are not expected
8 to alter homeostasis.

10 **Evidence for human carcinogenicity**

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

20 **Susceptible Populations and Life Stages**

21 Hyperammonemia is a condition of elevated levels of circulating ammonia that can occur
22 in individuals with severe diseases of the liver or kidney or with hereditary urea cycle disorders.
23 These elevated ammonia levels can predispose an individual to encephalopathy due to the ability
24 of ammonia to cross the blood-brain barrier; these effects are especially marked in newborn
25 infants. Thus, individuals with disease conditions that lead to hyperammonemia may be more
26 susceptible to the effects of ammonia from external sources, but there are no studies that
27 specifically support this susceptibility. Studies of the toxicity of ammonia in children or young
28 animals compared to other life stages that would support an evaluation of childhood
29 susceptibility have not been conducted.

31 **Key issues addressed in assessment**

32 *Endogenous ammonia*

33 Ammonia, which is produced endogenously, has been detected in the expired air of
34 healthy volunteers. Higher and more variable ammonia concentrations are reported in breath
35 exhaled from the mouth or oral cavity (0.09 to 2.1 mg/m³). These levels are largely attributed to
36 the production of ammonia via bacterial degradation of food protein in the oral cavity or
37 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/m³) and more likely reflect levels
3 of ammonia circulating in the blood. These levels are lower than the ammonia RfC of 0.3 mg/m³
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

^aThe 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.

^bThe search using search terms related to concentrations of ammonia in exhaled breath was conducted for the period 1/1/2002–11/11/2011.

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

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

12

¹HERO (Health and Environmental Research On-line) is a database of scientific studies and other references used to develop EPA’s risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA’s Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

1 **Study Evaluation for Hazard Identification**

2 This document is not intended to be a comprehensive treatise on the chemical or
3 toxicological nature of ammonia. In general, the quality and relevance of health effects studies
4 were evaluated as outlined in the Preamble to this assessment. In addition, *A Review of the*
5 *Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for*
6 *Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*
7 (U.S. EPA, 1994) were consulted for guidance in evaluating the scientific quality of the available
8 studies.

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

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

31

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/m³ (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/m³ 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/m³ [25 ppm] compared with workers exposed to levels below the TLV. Distribution of respiratory irritation effects by cumulative ammonia concentration (CAC, mg/m³-years) also showed significantly higher relative risk for these respiratory irritation effect among workers with higher CAC (>50 mg/m³-years) compared to those with a lower CAC (< 50 mg/m³-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/m³, medium = 4.4–8.8 mg/m³, or low = <4.4 mg/m³. Although respiratory irritation prevalence was similar across groups, the exposed workers reported that exposure in the plant aggravated some

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/m³) than workers in the ammonia plant (arithmetic mean ammonia concentration of 4.9 mg/m³). 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/m³ 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/m³ 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/m³ ammonia
13 [Holness et al., 1989] and 4.9 mg/m³ [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/m³ 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/m³ ammonia or 35 days of exposure to 177 mg/m³ ammonia, with respiratory and olfactory
10 epithelium thickness increased three- to four times that of normal thickness (Broderon et al.,
11 1976). Thickening of nasal and tracheal epithelium (50 to 100%) was observed in pigs exposed
12 to 71 mg/m³ 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/m³ ammonia for 90 days and rats and guinea
15 pigs intermittently exposed to 770 mg/m³ ammonia (or 183 mg/m³, adjusted to continuous
16 exposure³) (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/m³ 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/m³ ammonia for 90 days (Coon et al., 1970).

22 At lower concentrations, approximately 50 mg/m³ 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/m³ 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/m³ 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/m³
30 ammonia for 114 days or after intermittent exposure to 155 mg/m³ ammonia (or 36.9 mg/m³,
31 adjusted to continuous exposure) for 6 weeks (Coon et al., 1970).

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

³C_{adjusted} = C × 8 hours/24 hours × 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 (FEV₁) in the high-exposure worker group (mean ammonia
3 concentration of 18.5 mg/m³) 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, $p \leq 0.002$); FEV₁ 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/m³-years had
8 significantly lower FVC% predicted (5.4% decrease, $p \leq 0.030$) and FEV₁% predicted (7.4%
9 decrease, $p < 0.006$) than workers with cumulative exposure ≤ 50 mg/m³-years, but similar
10 FEV₁/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/m³. 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/m³ to >8.8 mg/m³
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, FEV₁, and PEF [peak expiratory flow rate]) in
20 workers exposed to a mean concentration of 4.9 mg/m³ 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 FEV₁, 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/m³ but not at 71 mg/m³. 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/m³ (Douglas and Coe, 1987); however, there were no bronchial symptoms reported in
36 volunteers exposed to ammonia at concentrations of up to 35 mg/m³ 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 FEV₁) were reported in healthy

1 volunteers exposed to ammonia at concentrations up to 18 mg/m³ for 1.5 hours during exercise
2 (Sundblad et al., 2004). There were no changes in lung function as measured by FEV₁ in 25
3 healthy volunteers and 15 mild/moderate persistent asthmatic volunteers exposed to ammonia
4 concentrations up to 354 mg/m³ 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/m³ 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.

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

Health Effect	Study Design and Reference	Results	NOAEL/ LOAEL ^a (mg/m ³)																																												
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	No statistically significant differences in subjective symptomology relative to the control.	NOAEL: 3.1 LOAEL: not identified																																												
	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	<table border="1"> <thead> <tr> <th></th> <th>Control</th> <th>Exposed</th> <th>p-value</th> </tr> </thead> <tbody> <tr> <td>Flu</td> <td>3</td> <td>7</td> <td>0.6299</td> </tr> <tr> <td>Cough</td> <td>10</td> <td>16</td> <td>0.5289</td> </tr> <tr> <td>Sputum</td> <td>16</td> <td>22</td> <td>0.9770</td> </tr> <tr> <td>Bronchitis</td> <td>19</td> <td>22</td> <td>0.6938</td> </tr> <tr> <td>Wheeze</td> <td>10</td> <td>10</td> <td>0.9068</td> </tr> <tr> <td>Chest tightness</td> <td>6</td> <td>3</td> <td>0.6221</td> </tr> <tr> <td>Dyspnea</td> <td>13</td> <td>7</td> <td>0.0470</td> </tr> <tr> <td>Chest pain</td> <td>6</td> <td>2</td> <td>0.1563</td> </tr> <tr> <td>Rhinitis</td> <td>19</td> <td>10</td> <td>0.1185</td> </tr> <tr> <td>Throat</td> <td>3</td> <td>7</td> <td>0.5296</td> </tr> </tbody> </table>		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	
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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	<p>Exposure-related increase in respiratory symptoms.</p> <p>Respiratory symptom prevalence (%):</p> <table border="1"> <thead> <tr> <th></th> <th>Control (admin)</th> <th>Ammonia plant</th> <th>Urea plant</th> </tr> </thead> <tbody> <tr> <td>Cough</td> <td>8</td> <td>17 (0.42)^a</td> <td>28 (0.05, 0.41)^b</td> </tr> <tr> <td>Chest tightness</td> <td>8</td> <td>17 (0.42)^a</td> <td>33 (0.02, 0.19)^b</td> </tr> <tr> <td>Stuffy nose</td> <td>4</td> <td>12 (0.35)^a</td> <td>16 (0.17, 1.0)^b</td> </tr> <tr> <td>Runny nose</td> <td>4</td> <td>4 (1.0)^a</td> <td>16 (0.17, 0.28)^b</td> </tr> <tr> <td>Sneeze</td> <td>8</td> <td>0 (0.49)^a</td> <td>22 (0.22, 0.01)^b</td> </tr> </tbody> </table> <p>^ap-value for ammonia plant compared to control ^bp-value for urea plant compared to control and urea plant compared to ammonia plant</p>		Control (admin)	Ammonia plant	Urea 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	NOAEL: 1.8 LOAEL: 6.6																					
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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/m ³ Mean of employment duration: 51.8 mo (exposed workers) and 73.1 mo (controls) Ballal et al., 1998	<p>Higher relative risks for those exposed to ammonia at concentrations >TLV as compared to those exposed at levels ≤TLV:</p> <p>Cough: 4-fold Phlegm: 4.7-fold Wheezing: 2.2-fold Dyspnea: 4-fold Chronic bronchitis: 1.6-fold Asthma: 3.7-fold</p>	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	<p>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</p> <p>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</p> <p>Average exposure: 12 y</p> <p>Holness et al., 1989</p>	<p>No statistically significant differences in lung function relative to the control.</p> <table border="1"> <thead> <tr> <th></th> <th>Exposed</th> <th>Control</th> <th>p value</th> </tr> </thead> <tbody> <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>FEV₁</td> <td>94.1</td> <td>95.1</td> <td>0.3520</td> </tr> <tr> <td>FEV₁/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>FEV₁ 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> </tbody> </table>		Exposed	Control	p value	Lung function (% predicted values):				FVC	96.8	98.6	0.0944	FEV ₁	94.1	95.1	0.3520	FEV ₁ /FVC	97.1	96.5	0.4801	Change in lung function over work shift:				FVC day1	-0.8	-0.9	0.9940	day 2	-0.0	+0.1	0.8378	FEV ₁ day 1	-0.2	-0.2	0.9363	day 2	+0.7	+0.5	0.8561	NOAEL: 3.1 LOAEL: not identified
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day 2	+0.7	+0.5	0.8561																																								
	<p>Cross-sectional occupational study of urea fertilizer factory in Bangladesh; 63 ammonia plant workers, 77 urea plant workers, and 25 controls (from administration building)</p> <p>Ammonia plant: 4.9 mg/m^{3 d} (1.8 mg/m³ adjusted^c)</p> <p>Urea plant: 18.5 mg/m^{3 d} (6.6 mg/m³ adjusted^c)</p> <p>Mean employment duration: 16 y</p> <p>Rahman et al., 2007</p>	<p>Dose-related decrease in lung function parameters.</p> <table border="1"> <thead> <tr> <th></th> <th>Pre-shift</th> <th>Post-shift</th> <th>p-value</th> </tr> </thead> <tbody> <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>FEV₁</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>FEV₁</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> </tbody> </table>		Pre-shift	Post-shift	p-value	Ammonia plant				FVC	3.308	3.332	0.67	FEV ₁	2.627	2.705	0.24	PEFR	8.081	8.313	0.22	Urea plant				FVC	3.362	3.258	0.01	FEV ₁	2.701	2.646	0.05	PEFR	7.805	7.810	0.97	NOAEL: 1.8 LOAEL: 6.6				
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	<p>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</p> <p>Exposures were stratified < or > the ACGIH TLV of 18 mg/m³</p> <p>Mean of employment duration: not reported</p> <p>Ali et al., 2001</p>	<p>Lung function results based on exposure concentration and cumulative exposure:</p> <table border="1"> <thead> <tr> <th></th> <th>Control</th> <th>Exposed</th> <th>p-value</th> </tr> </thead> <tbody> <tr> <td>FVC₁% 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>FEV₁/FVC%</td> <td>83.0</td> <td>84.2</td> <td>NS</td> </tr> <tr> <td></td> <td>≤50 mg/m³-y</td> <td>>50 mg/m³-y</td> <td>p-value</td> </tr> <tr> <td>FVC₁% 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>FEV₁/FVC%</td> <td>84.7</td> <td>83.4</td> <td>NS</td> </tr> </tbody> </table> <p>NS = not significant</p>		Control	Exposed	p-value	FVC ₁ % predicted	96.6	98.1	NS	FVC% predicted	101.0	105.6	0.002	FEV ₁ /FVC%	83.0	84.2	NS		≤50 mg/m ³ -y	>50 mg/m ³ -y	p-value	FVC ₁ % predicted	100.7	93.4	0.006	FVC% predicted	105.6	100.2	0.03	FEV ₁ /FVC%	84.7	83.4	NS	NOAEL and LOAEL values were not identified because exposures were not adequately characterized								
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^aThe NOAEL and LOAEL values presented were identified by EPA.

^bAt 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.

^cAdjusted to continuous exposure based on the ratio of VE_h (human occupational default minute volume of 10 m³ breathed during an 8-hour workday) to VE_a (human ambient default minute volume of 20 m³ breathed during the entire day) and an exposure of 5 days out of 7 days (i.e., measured concentration × 10/20 × 5/7).

^dExposure 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/m³, respectively; using the Dräger PAC III method, ammonia concentrations were 4.9 and 18.5 mg/m³ (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.

^eThe 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 (<i>Saimiri sciureus</i>); 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 (<i>Saimiri sciureus</i>); 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

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	<p>0, 155, or 770 mg/m³ 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m³ adjusted^b)</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); 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</p> <p>Coon et al., 1970</p>	No nasal irritation observed.	NOAEL: 183 LOAEL: not identified
	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>Beagle dog; male; 2/group</p> <p>Coon et al., 1970</p>	Nasal discharge.	NOAEL: 40 LOAEL: 470
	<p>0 or 40 mg/m³ for 114 d or 127, 262 or 470 mg/m³ for 90 d or 455 mg/m³ for 65 d</p> <p>Sprague-Dawley or Long-Evans rat; male and female; 15-51/group</p> <p>Coon et al., 1970</p>	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
	<p>7 or 106 mg/m³ from bedding for 75 d</p> <p>Sherman rat; 5/sex/group</p> <p>Broderson et al., 1976</p>	Nasal lesions at 106 mg/m ³ .	NOAEL: 7 LOAEL:106
	<p>0 or 177 mg/m³ in an inhalation chamber for 35 d</p> <p>F344 rat; 6/sex/group</p> <p>Broderson et al., 1976</p>	Nasal lesions at 177 mg/m ³ .	NOAEL: not identified LOAEL:177
	<p>Ammonia vapor of 0 or 12% ammonia solution for 15 min/d, 6 d/wk, for 8 wks</p> <p>White albino mouse; male; 50</p> <p>Gaafar et al., 1992</p>	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.

^aThe NOAEL and LOAEL values presented were identified by EPA.

^bAdjusted 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).

^cThe 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

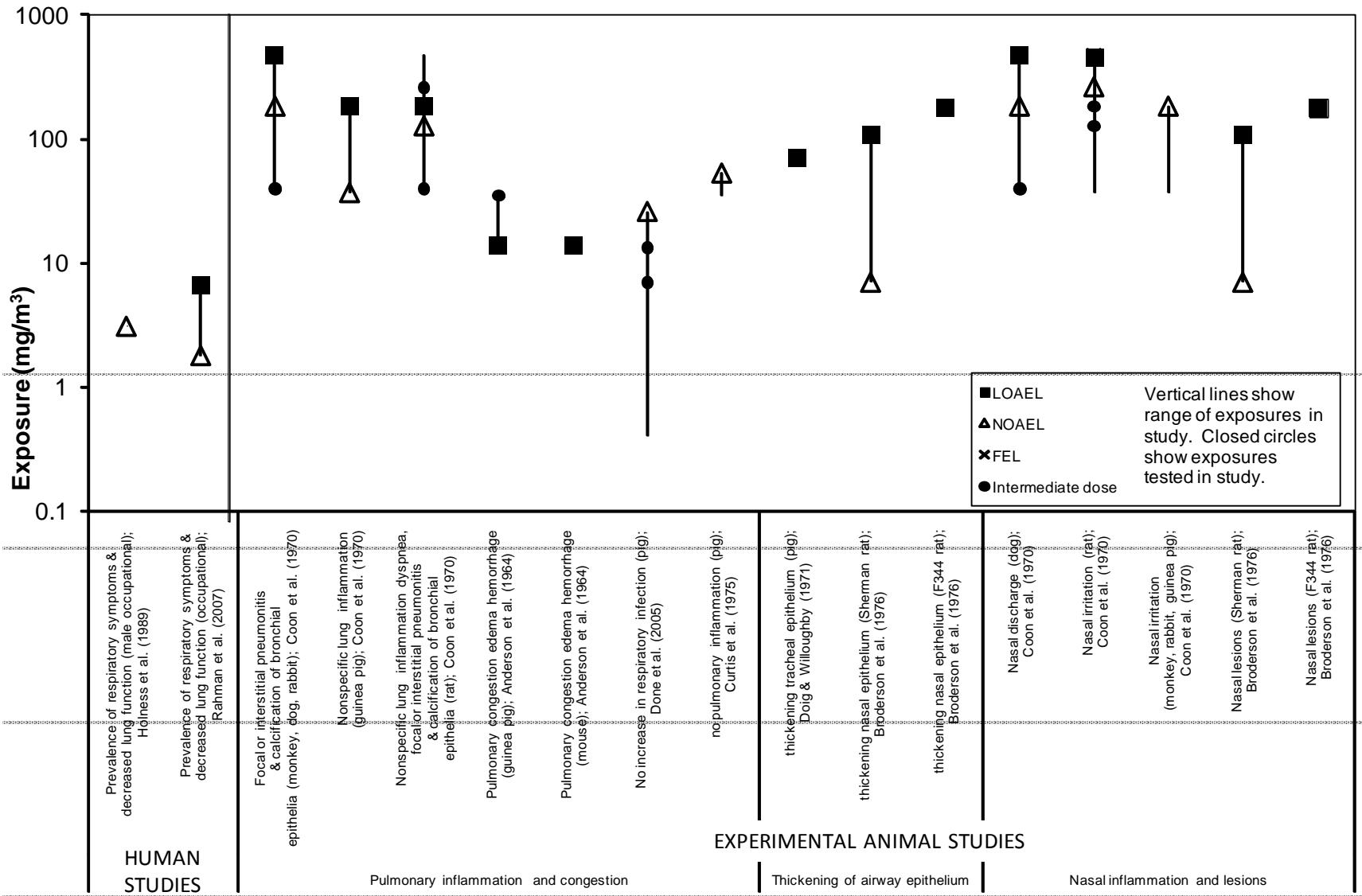


Figure 1-1. Exposure-response array for respiratory effects following inhalation exposure.

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➤ Mode of Action Analysis – Respiratory Effects

Data regarding the potential mode of action for respiratory effects associated with chronic exposure to ammonia are limited. However, it is well established that injury to respiratory tissues resulting from acute exposure to inhaled ammonia is primarily due to its alkaline properties and its solubility. Given its high solubility, ammonia readily dissolves in the moisture on the mucous membranes, forming ammonium hydroxide, which causes liquefaction necrosis of the tissues. Ammonia directly denatures tissue proteins due to the production of alkaline proteinates. Specifically, ammonium hydroxide causes saponification of cell membrane lipids that leads to cell disruption and death (necrosis). As cell proteins break down, water is extracted, resulting in an inflammatory response, which further damages the surrounding tissues (Amshel et al, 2000; Mellea, 1989; Jarudi and Golden, 1973).

1.1.2. Gastrointestinal Effects

Reports of gastrointestinal effects of ammonia in humans are limited to case reports involving intentional or accidental ingestion of household cleaning solutions or ammonia inhalant capsules (Dworkin et al., 2004; Rosenbaum et al., 1998; Christesen, 1995; Wason et al., 1990; Lopez et al., 1988; Klein et al., 1985; Klendshoj and Rejent, 1966). Clinical signs reported in these case studies include stomachache, nausea, dizziness, diarrhea, drooling, erythematous and edematous lips, reddened and blistered tongues, dysphagia, vomiting, oropharyngeal burns, laryngeal and epiglottal edema, erythematous esophagus with severe corrosive injury, and hemorrhagic esophago-gastro-duodeno-enteritis.

In animals following oral exposure, statistically significant decreases of 40–60% in the thickness of the gastric mucosa were reported in Sprague-Dawley rats administered 0.01% ammonia in drinking water for durations of 2–8 weeks (Tsuji et al., 1993; Kawano et al., 1991); estimated doses were 22 mg/kg-day (Kawano et al., 1991) and 33 mg/kg-day (Tsuji et al., 1993). These studies were designed to investigate the hypothesis that the bacterium *Helicobacter pylori*, which produces a potent urease that increases ammonia production, plays a significant role in the etiology of chronic atrophic gastritis. Kawano et al. (1991) reported that the magnitude of the decrease in gastric mucosal thickness increased with dose and duration of exposure and that the effect was more prominent in the mucosa of the antrum region of the stomach than in the body region of the stomach.⁴ As discussed further under Mode of Action – Gastrointestinal Effects (see below), the difference in response to ammonia in drinking water in the two regions of the rat stomach may be a function of differences in pH in these regions and resulting differences in the extent of ionization of ammonia to NH_4^+ . Parietal cell number per oxyntic gland also decreased in a statistically significant dose- and time-dependent fashion, up to approximately 35% at 0.01%

⁴The 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 (Tsuji 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 (Tsuji 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
20

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. <i>Thickness of mucosa relative to control:</i> 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. <i>Thickness of mucosa relative to control (d 3, wk 1, 2, 4, 8):</i> 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

^aThe NOAEL and LOAEL values presented were identified by EPA.

^bAmmonia 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.

^cAmmonia 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.

^dStatistically 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.

1 This literature suggests that the alkalinity of the ammonia solution does not play a direct
2 role in the gastric effects associated with ammonia. An ammonia solution (pH 10.3) produced
3 dose-related acute macroscopic mucosal lesions, whereas a glycine-sodium hydroxide buffer (pH
4 10.3) or ammonium chloride (pH 4.5) did not (Tsuji et al., 1992b). Rather, the ability of
5 ammonia to damage the gastric mucosa may be related to its ionization state. Ammonia (NH₃)
6 can easily penetrate cell membranes, subsequently reacting to form NH₄⁺ and OH⁻ in the interior
7 of the membrane (Tsuji et al., 1992b). The finding that antral and body regions of the rat
8 stomach mucosa responded differently following administration of 33 mg/kg-day ammonia in
9 drinking water for 8 weeks (Tsuji et al., 1993) is consistent with the influence of ionization on
10 toxicity. The hydrogen chloride secreted by the mucosa in the body of the stomach resulted in a
11 decrease in pH and a corresponding decrease in the ratio of ammonia to ammonium ion. In
12 contrast, in the antral mucosa (a nonacid-secreting area), pH is higher, the ratio of ammonia to
13 ammonium ion is increased, and measures of gastric atrophy were increased compared to those
14 observed in the stomach body where there was relatively higher exposure to NH₄⁺.

15 Several specific events have been identified that may contribute to the induction of
16 gastric lesions by ammonia. Increased cell vacuolation and decreased viability of cells in vitro
17 were associated with increasing ammonia concentration in an in vitro system (Mégraud et al.,
18 1992); the effect was not linked to pH change because of the high buffering properties of the
19 medium. Using an in situ rat stomach model, hemorrhagic mucosal lesions induced by ammonia
20 were associated with the rapid release and activation of cathepsins, mammalian cysteine
21 proteases that are released from lysosomes or activated in the cytosol and that can be damaging
22 to cells, tissues, or organs (Nagy et al., 1996). Ammonia also appears to inhibit cellular and
23 mitochondrial respiration, possibly by elevating intracellular or intraorganelle pH or by
24 impairing adenosine triphosphate (ATP) synthesis (Tsuji et al., 1992b). Mori et al. (1998)
25 proposed a role for increased release of endothelin-1 and thyrotropin releasing hormone from the
26 gastric mucosa in ammonia-induced gastric mucosal injury based on findings in rats given
27 ammonia intragastrically. Regardless of the specific mechanism(s) by which ammonia induces
28 cellular toxicity, gastric injury appears to accelerate mucosal cell desquamation and stimulate
29 cell proliferation via a compensatory mechanism (Tsuji et al., 1992a).

31 **1.1.3. Reproductive and Developmental Effects**

32 No statistically significant differences in reproductive or developmental endpoints were
33 found between two groups of female pigs (crossbred gilts) exposed to ammonia for 6 weeks at
34 mean concentrations of 5 or 25 mg/m³ and then mated (Diekman et al., 1993) in the only study of
35 the reproductive and developmental toxicity potential of ammonia (see Table 1-4). Age at
36 puberty did not differ significantly between the two groups. Gilts exposed to 25 mg/m³ ammonia
37 weighed 7% less (p < 0.05) at puberty than those exposed to 5 mg/m³; however, body weights of
38 the two groups were similar at gestation day 30. Conception rates in the mated females were

1 similar between the two groups (94.1 versus 100% in low- versus high-exposure groups). At
 2 sacrifice on day 30 of gestation, there were no significant differences between the two exposed
 3 groups in body weights of the pregnant gilts, number of corpora lutea, number of live fetuses, or
 4 weight and length of the fetuses. The strength of the findings from this study are limited by the
 5 absence of a control group and possible confounding by exposures to bacterial and mycoplasma
 6 pathogens. The evidence of reproductive and developmental effects in experimental animals
 7 exposed to ammonia is provided in Table 1-4.

8

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

^aThe NOAEL and LOAEL values presented were identified by EPA.

^bA 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.

9

10 **1.1.4. Immune System Effects**

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

20 Animal studies that examined ammonia immunotoxicity were conducted using short-term
 21 inhalation exposures and three general types of immune assays. Immunotoxicity studies of
 22 ammonia using measures of host resistance provide the most relevant data for assessing immune
 23 function since they directly measure the immune system's ability to control microorganism
 24 growth. Other available studies of ammonia employed assays that evaluated immune function.

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

4 Evidence of immunosuppression was observed in several host resistance studies utilizing
5 lung pathogens to measure reduced bacterial clearance following ammonia exposure.
6 Inoculation with the respiratory pathogen *Mycoplasma pulmonis* causes murine respiratory
7 mycoplasmosis (MRM) characterized by lung lesions. Lung lesions, both gross and
8 microscopic, were positively correlated with ammonia concentration in F344 rats continuously
9 exposed to ammonia in an inhalation chamber for 7 days prior to inoculation with *M. pulmonis*
10 (10^8 colony forming units [CFU]) followed by up to 42 days of ammonia exposure post
11 inoculation (Broderick et al., 1976). The incidence of lesions was significantly increased at
12 ammonia concentrations ≥ 35 mg/m³, and suggests that ammonia exposure decreased bacterial
13 clearance resulting in the development of *M. pulmonis*-induced MRM. However, the increasing
14 ammonia concentration was not associated with increased CFU of *M. pulmonis* isolated from the
15 respiratory tract. The high number of inoculating CFU could have overwhelmed the immune
16 response and elicited a maximal response that could not be further magnified in
17 immunocompromised animals. Conversely, significantly increased CFU of *M. pulmonis* bacteria
18 isolated in the trachea, nasal passages, lungs, and larynx was observed in F344 rats continuously
19 exposed to 71 mg/m³ ammonia for 7 days prior to *M. pulmonis* (10^4 – 10^6 CFU) inoculation and
20 continued for 28 days post inoculation (Schoeb et al., 1982). This increase in bacterial
21 colonization indicates a reduction in bacterial clearance following exposure to ammonia.
22 Lesions were not assessed in this study. OF1 mice exposed to 354 mg/m³ ammonia for 7 days
23 prior to inoculation with a 50% lethal dose (LD₅₀) of *Pasteurella multocida* significantly
24 increased mortality compared to controls (86% versus 50%, respectively); however, an 8-hour
25 exposure was insufficient to affect mortality (Richard et al., 1978a). The authors suggested that
26 the irritating action of ammonia destroyed the tracheobronchial mucosa and caused inflammatory
27 lesions thereby increasing sensitivity to respiratory infection with prolonged ammonia exposure.

28 Suppressed cell-mediated immunity and decreased T cell proliferation was also observed
29 following ammonia exposure. Using a delayed-type hypersensitivity (DTH) test to evaluate cell-
30 mediated immunity, Hartley guinea pigs were vaccinated with *Mycobacterium bovis* BCG and
31 exposed to ammonia followed by intradermal challenge with purified protein derivative (PPD).
32 Dermal lesion size was reduced in animals exposed to 64 mg/m³ indicating immunosuppression
33 (Targowski et al., 1984). Blood and bronchial lymphocytes harvested from naïve guinea pigs
34 treated with the same 3 week ammonia exposure and stimulated with phytohaemagglutinin or
35 concanavalin A demonstrated reduced T cell proliferation (Targowski et al., 1984). Bactericidal
36 activity in alveolar macrophages isolated from ammonia-exposed guinea pigs was not affected.
37 Lymphocytes and macrophages isolated from unexposed guinea pigs and treated with ammonia
38 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.

6

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	<p>≤3.5 (control), 18, 35, 71, 177 mg/m³, 7 days (continuous exposure) pre inoculation/28-42 days post inoculation with <i>M. pulmonis</i></p> <p>F344 rat; male and female; 11-12/sex/group</p> <p>Broderson et al., 1976</p>	<p>Increased incidence of gross lung lesions; no effect on CFU.</p> <p><i>% of animals with gross lesions: 16 (control), 46, 66^b, 33, 83%</i></p>	NOAEL: 18 LOAEL: 35 ^c
	<p><1.4 (control) or 71 mg/m³, 7 days (continuous exposure) pre inoculation/ 28 days post inoculation with <i>M. pulmonis</i></p> <p>F344 rat; 5-15/group (sex unknown)</p> <p>Schoeb et al., 1982</p>	<p>Increased bacterial colonization (as a result of reduced bacterial clearance).</p> <p>No quantitative data available.</p>	NOAEL: not identified LOAEL: 71
	<p>0 or 354 mg/m³, 8 hours or 7 days (continuous exposure), prior to infection with <i>P. multocida</i></p> <p>OF1 mouse; male; 99/group</p> <p>Richard et al., 1978</p>	<p>Increased mortality.</p> <p><i>Mouse mortality: 50% (control) and 86%^b</i></p>	NOAEL: not identified LOAEL: 354
T cell proliferation	<p><11 (control), 35 or 64 mg/m³, 3 weeks (continuous exposure)</p> <p>Hartley guinea pig; 8/group (sex unknown)</p> <p>Targowski et al., 1984</p>	<p>Reduced proliferation in blood and bronchial T cells.</p> <p>No quantitative data available.</p>	NOAEL: 35 LOAEL: 64
	<p><11 (control), 35 or 64 mg/m³, 3 weeks (continuous exposure) followed by PPD challenge in BCG immunized</p> <p>Hartley guinea pig; 8/group (sex unknown)</p> <p>Targowski et al., 1984</p>	<p>Reduced dermal lesion size.</p> <p><i>Mean diameter (mm):12 (control), 12.6 and 8.7^b</i></p>	NOAEL: 35 LOAEL: 64

^aThe NOAEL and LOAEL values presented were identified by EPA.

^bStatistically significant from controls.

^cStudy did not find statistical significance despite a large increase in the response at the lowest dose measured.

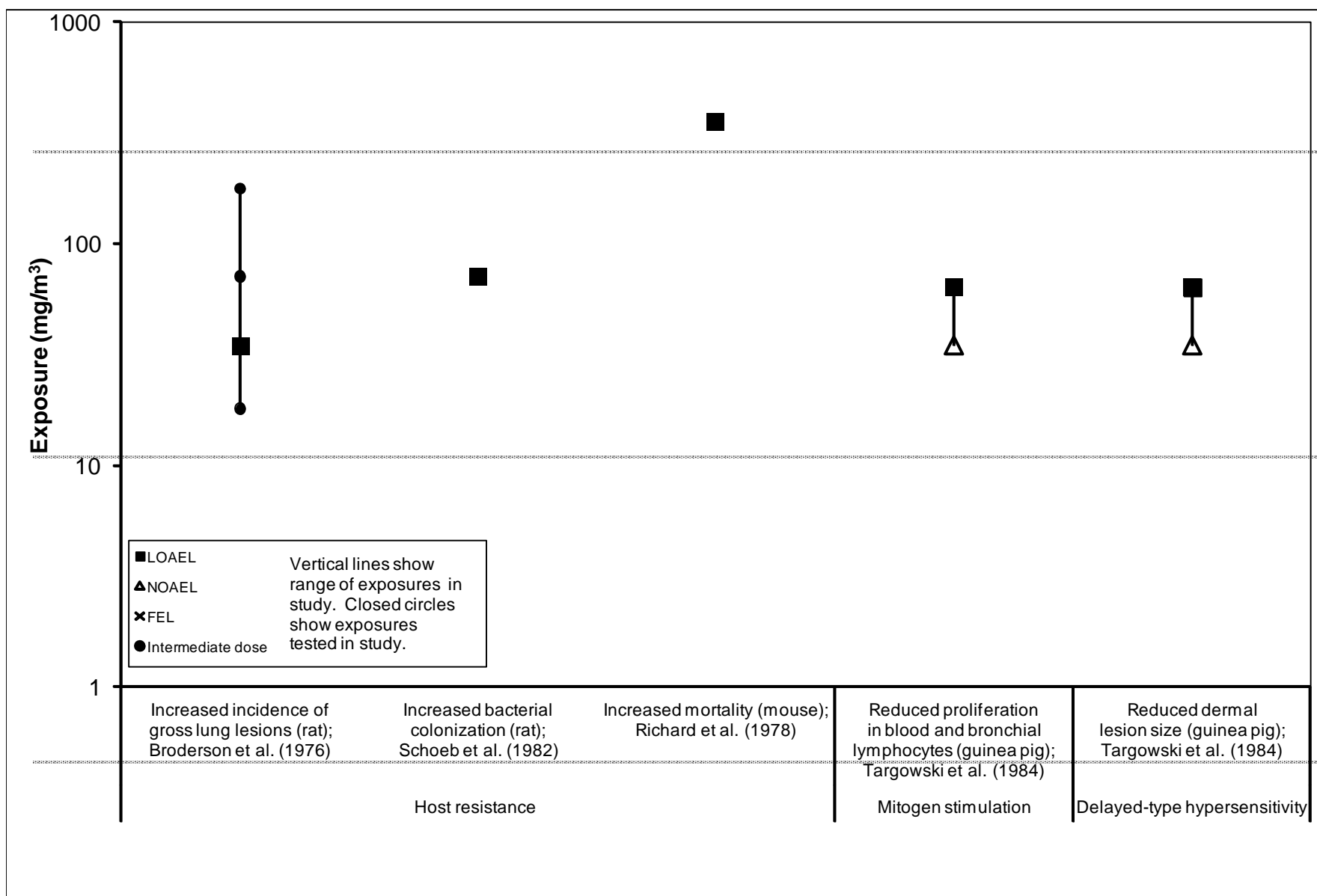


Figure 1-2. Exposure-response array for immune system effects following inhalation exposure.

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1 **1.1.5. Other Systemic Effects**

2 Although the majority of information for ammonia suggests that ammonia induces effects
3 in and around the portal of entry, there is limited evidence that ammonia can produce effects on
4 organs distal from the portal of entry, including the liver, adrenal gland, kidney, spleen, and
5 heart. Alterations in liver function, based on elevated mean levels of aspartate aminotransferase
6 (AST), alanine aminotransferase (ALT), and blood urea, decreased hemoglobin, and inhibition of
7 catalase and monoamine oxidase (MAO) activities were observed in workers exposed to
8 ammonia over an average exposure duration of 12 years at an Egyptian urea production plant;
9 measurements of workplace exposure concentrations were not provided (Hamid and El-Gazzar,
10 1996). The authors suggested that inhibition of catalase can affect electrical stability,
11 permeability, and fluidity of membranes, which may lead to hepatotoxicity in occupationally
12 exposed workers (Hamid and El-Gazzar, 1996).

13 Evidence of hepatotoxicity in animals comes from observations of histopathological
14 alterations in the liver. Fatty changes in the liver were consistently reported at concentrations
15 $\geq 470 \text{ mg/m}^3$ ammonia in rats, guinea pigs, rabbits, dogs, and monkeys following identical
16 subchronic inhalation exposure regimens (Coon et al., 1970). Congestion of the liver was
17 observed in guinea pigs following subchronic and short-term inhalation exposure to 35 and
18 120 mg/m^3 (Anderson et al., 1964; Weatherby, 1952); no liver effects were observed in similarly
19 exposed mice at 14 mg/m^3 (Anderson et al., 1964; Weatherby, 1952). No histopathological or
20 hematological effects were observed in rats, guinea pigs, rabbits, dogs, or monkeys when these
21 animals were repeatedly, but not continuously, exposed to ammonia even at high concentrations
22 (e.g., 770 mg/m^3 for 8 hours/day, 5 days/week), suggesting that mammals can recover from
23 short-term exposure to elevated ammonia levels (Coon et al., 1970). In addition, no effects were
24 observed in mice exposed to 14 mg/m^3 for up to 6 weeks (Anderson et al., 1964).

25 Adrenal effects were observed in animals following subchronic and short-term exposure
26 to ammonia; data in humans were not found. Increased mean adrenal weights and fat content of
27 the adrenal gland, as well as histological changes in the adrenal gland (enlarged cells of the zona
28 fasciculata of the adrenal cortex that were rich in lipid) were observed in rabbits exposed orally
29 via gavage to ammonium hydroxide for durations ranging from 5.5 days to 17 months (Fazekas,
30 1939). While the strength of these findings is limited by inadequate reporting and study design,
31 a separate study identified early degenerative changes in the adrenal glands of guinea pigs
32 exposed to 120 mg/m^3 ammonia by inhalation for 18 weeks (Weatherby, 1952), providing
33 additional limited evidence for effects on the adrenal gland.

34 Evidence that inhaled ammonia can affect the kidney and spleen is limited to studies in
35 experimental animals. Nonspecific degenerative changes in the kidneys (not further described)
36 of rats exposed to 262 mg/m^3 were reported (Coon et al., 1970). Histopathological evaluation of
37 other animal species in the same study exposed to 470 mg/m^3 , a concentration that induced a
38 high rate of mortality in rats, consistently showed alterations in the kidneys (calcification and

1 proliferation of tubular epithelium; incidence not reported). Exposure of guinea pigs to inhaled
2 ammonia at a concentration of 120 mg/m³ for 18 weeks (but not 6 or 12 weeks) resulted in
3 histopathological alterations (congestion) of the kidneys and spleen, although incidence was not
4 reported (Weatherby, 1952). Enlarged and congested spleens were reported in guinea pigs
5 exposed to 35 mg/m³ ammonia for 6 weeks in a separate study (Anderson et al., 1964).

6 Myocardial fibrosis was observed in monkeys, dogs, rabbits, guinea pigs, and rats
7 following subchronic, inhalation exposure to 470 mg/m³ ammonia; no changes were observed at
8 lower concentrations (Coon et al., 1970). At the same concentration, ocular irritation
9 (characterized as heavy lacrimation, erythema, discharge and ocular opacity of the cornea) was
10 also reported by Coon et al. (1970) in dogs and rabbits, but not observed in similarly treated
11 monkeys and rats. Additionally, there is limited evidence of biochemical or metabolic effects of
12 acute or short-term ammonia exposure. Acidosis, as evidenced by a decrease in blood pH and an
13 increase in arterial blood carbon dioxide partial pressure (pCO₂), occurred in rats exposed to
14 212 mg/m³ ammonia for 5–15 days (Manninen et al., 1988). Blood pH and pCO₂ did not change
15 in rats exposed to ≤818 mg/m³ for up to 24 hours, although statistically significant increases in
16 oxygen partial pressure (pO₂) were reported in rats exposed to 10.6 and 22.6 mg/m³ ammonia,
17 but not at 219 and 818 mg/m³ over the same time period (Schaerdel et al., 1983).

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

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

30

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. <i>Response relative to control (adrenal weight): 95% increase</i> <i>Response relative to control (fat): 4.5-fold increase</i>	Not identified.

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

3

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 (<i>Saimiri sciureus</i>); 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 (<i>Saimiri sciureus</i>); 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	<p>0, 155, or 770 mg/m³ 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m³ adjusted^b)</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); 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</p> <p>Coon et al., 1970</p>	No histopathologic changes observed.	NOAEL: 183 LOAEL: not identified
	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); 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</p> <p>Coon et al., 1970</p>	Calcification and proliferation of renal tubular epithelium.	NOAEL: 40 LOAEL: 470
	<p>0 or 40 mg/m³ for 114 d or 127, 262 or 470 mg/m³ for 90 d;</p> <p>Sprague-Dawley or Long-Evans rat; male and female; 15-51/group</p> <p>Coon et al., 1970</p>	Calcification and proliferation of renal tubular epithelium.	NOAEL: 262 LOAEL: 470
	<p>0 or 120 mg/m³ 6 h/day, 5 days/week for 6, 12 or 18 weeks; (24.1 mg/m³ adjusted^b)</p> <p>Guinea pig (strain not specified); male; 6-12/group</p> <p>Weatherby, 1952</p>	Congestion of the spleen and kidneys.	NOAEL: not identified LOAEL: 24.1
	<p>0 or 14 for 7-42 days or 35 mg/m³ for 42 days</p> <p>Guinea pig (strain not specified); male and female; 2/group</p> <p>Anderson et al., 1964</p>	Enlarged and congested spleens.	NOAEL: 14 LOAEL: 35
	<p>0 or 14 mg/m³ for 7-42 days</p> <p>Swiss albino mouse; male and female; 4/group</p> <p>Anderson et al., 1964</p>	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	<p>0, 155, or 770 mg/m³ 8 hrs/d, 5 d/wk for 6 wks; (36.9, 183 mg/m³ adjusted^b)</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); 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</p> <p>Coon et al., 1970</p>	No histopathologic changes observed.	NOAEL: 183 LOAEL: not identified
	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); 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</p> <p>Coon et al., 1970</p>	Myocardial fibrosis.	NOAEL: 40 LOAEL: 470
	<p>0 or 40 mg/m³ for 114 d or 127, 262 or 470 mg/m³ for 90 d;</p> <p>Sprague-Dawley or Long-Evans rat; male and female; 15-51/group</p> <p>Coon et al., 1970</p>	Myocardial fibrosis.	NOAEL: 262 LOAEL: 470
Ocular Irritation	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>Beagle dog; male; 2/group</p> <p>Coon et al., 1970</p>	Heavy lacrimation.	NOAEL: 40 LOAEL: 470
	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>New Zealand albino rabbit; male; 3/group</p> <p>Coon et al., 1970</p>	Erythema, discharge and ocular opacity over ¼ to ½ of cornea.	NOAEL: 40 LOAEL: 470
	<p>0 or 40 mg/m³ for 114 d or 470 mg/m³ for 90 d</p> <p>Squirrel monkey (<i>Saimiri sciureus</i>); male; 3/group and 3/group and Princeton-derived guinea pig; male and female; 15/group</p> <p>Coon et al., 1970</p>	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 (<i>Saimiri sciureus</i>); 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)”. <i>Response difference from control: 0.09^c and 0.07^c</i>	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 . <i>Response relative to control^d: 16, 6, 20% at 10.6 mg/m³ and at 8, 12, 24 hrs; 18, 26, 17% at 22.6 mg/m³ and at 8, 12, 24 hrs</i>	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 neurotransmitter 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). <i>Response relative to control:</i> 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. <i>Response relative to control at 212 mg/m³:</i> 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

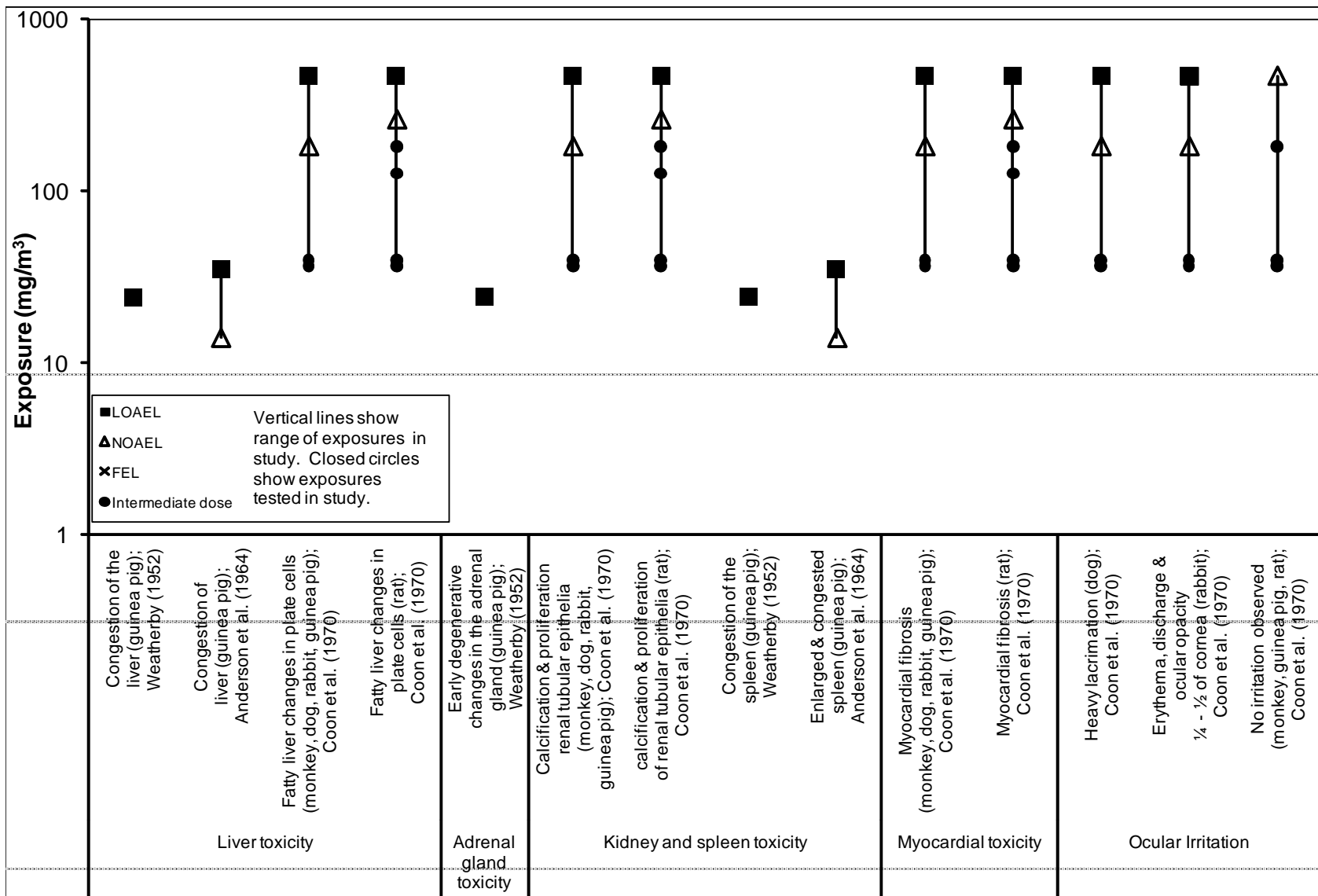
^aThe NOAEL and LOAEL values presented were identified by EPA.

^bAdjusted 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).

^cStatistically significant difference from controls.

^dMeasurements 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 **1.1.6. Cancer**

2 No information is available regarding the carcinogenic effects of ammonia in humans
3 following oral or inhalation exposure. The carcinogenic potential of ammonia by the inhalation
4 route has not been assessed in animals, and animal carcinogenicity data by the oral route of
5 exposure are limited. Toth (1972) concluded that tumor incidence was not increased in Swiss
6 mice exposed for their lifetime (not further specified) to ammonium hydroxide in drinking water
7 at concentrations up to 0.3% (equivalent to 410 and 520 mg/kg-day in female and male mice,
8 respectively) or in C₃H mice exposed to ammonium hydroxide in drinking water at a
9 concentration of 0.1% (equivalent to 214 and 191 mg/kg-day in female and male mice,
10 respectively). With the exception of mammary gland tumors in female C₃H mice (a tumor with a
11 high background incidence), concurrent control tumor incidence data were not reported and
12 comparison of tumor incidence in exposed and control mice could not be performed. The
13 general lack of concurrent control data limits the ability to interpret the findings of this study.

14 The incidence of gastric cancer and the number of gastric tumors per tumor-bearing rat
15 were statistically significantly higher in rats exposed to 0.01% ammonia solution in drinking
16 water (equivalent to 10 mg/kg-day) for 24 weeks following pretreatment (for 24 weeks) with the
17 initiator N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) compared with rats receiving only
18 MNNG and tap water (Tsuji et al., 1992a). In an almost identically designed study, reported by
19 Tsuji et al. (1995), similar increases in the incidence of gastric tumors were observed in rats
20 following exposure to MNNG and 10 mg/kg-day ammonia. Additionally, the size and
21 penetration to deeper tissue layers of the MNNG-initiated gastric tumors were enhanced in the
22 rats treated with ammonia (Tsuji et al., 1995). The investigators suggested that ammonia
23 administered in drinking water may act as a cancer promoter (Tsuji et al., 1995, 1992a);
24 however, in the absence of an ammonia-only exposure group in these studies, it is not possible to
25 distinguish between possible promotion and initiator activity.

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

28

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 ₃ H 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. <i>Mammary gland adenocarcinoma: 76, 60%</i>
	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 Tsuji 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 <i>Gastric tumor incidence: 31, 70^d% # of gastric tumors/tumor-bearing rat: 1.3, 2.1^d</i>
	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 Tsuji 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 <i>Gastric tumor incidence: 30, 66^d% Penetrated muscle layer or deeper: 12, 22^d% Size (mm): 4.4, 5.3^d</i>

^aAmmonium 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).

^bAmmonium 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).

^cAmmonia 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).

^dStatistically 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 8 **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 29 **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.

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.

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 (Tsuji 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 (Tsuji et al., 1993; Kawano
36 et al., 1991); Tsuji 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

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

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

12 13 ***Reproductive/Developmental Effects***

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

21 Ammonia is endogenously produced in humans and animals during fetal and adult life
22 and concentrations in blood are homeostatically regulated to remain at low levels. Studies in
23 humans and animals demonstrate that ammonia is present in fetal circulation. In vivo studies in
24 several animal species and in vitro studies of human placenta suggest that ammonia is produced
25 within the uteroplacenta and released into the fetal and maternal circulations (Bell et al., 1989;
26 Johnson et al., 1986; Haugel et al., 1983; Meschia et al., 1980; Remesar et al., 1980; Holzman et
27 al., 1979, 1977; Rubaltelli and Formentin, 1968; Luschinsky, 1951). Józwick et al. (2005)
28 reported that ammonia levels in human fetal blood (specifically, umbilical arterial and venous
29 blood) at birth were 1.0–1.4 µg/mL, compared to 0.5 µg/mL in the mothers' venous blood.
30 DeSanto et al. (1993) similarly collected human umbilical arterial and venous blood at delivery
31 (range of 25–43 weeks of gestation). Ammonia was present in blood samples, with umbilical
32 arterial ammonia concentrations significantly higher than venous concentrations; there was no
33 correlation between umbilical ammonia levels and gestational age. In sheep, uteroplacental
34 tissues are the main site of ammonia production, with outputs of ammonia into both the uterine
35 and umbilical circulations (Józwick et al., 1999). In late-gestation pregnant sheep that were
36 catheterized to allow measurement of ammonia exposure to the fetus, concentrations of ammonia
37 in umbilical arterial and venous blood and uterine arterial and venous blood ranged from about
38 0.39 to 0.60 µg/mL (Józwick et al., 2005, 1999). Thus, the developing fetus and reproductive

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

4 ***Immune System Effects***

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

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

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

26 ***Systemic Effects***

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

33 Effects on various organs, including liver, adrenal gland, kidney, spleen, and heart, were
34 observed in several studies that examined responses to ammonia exposure in a number of
35 laboratory species. While effects on many of these organs were observed in multiple species,
36 including monkey, dog, rabbit, guinea pig, and rat, effects were not consistent across exposure
37 protocols. For example, Coon et al. (1970) reported fatty liver and calcification and proliferation
38 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/m³, 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/m³. 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

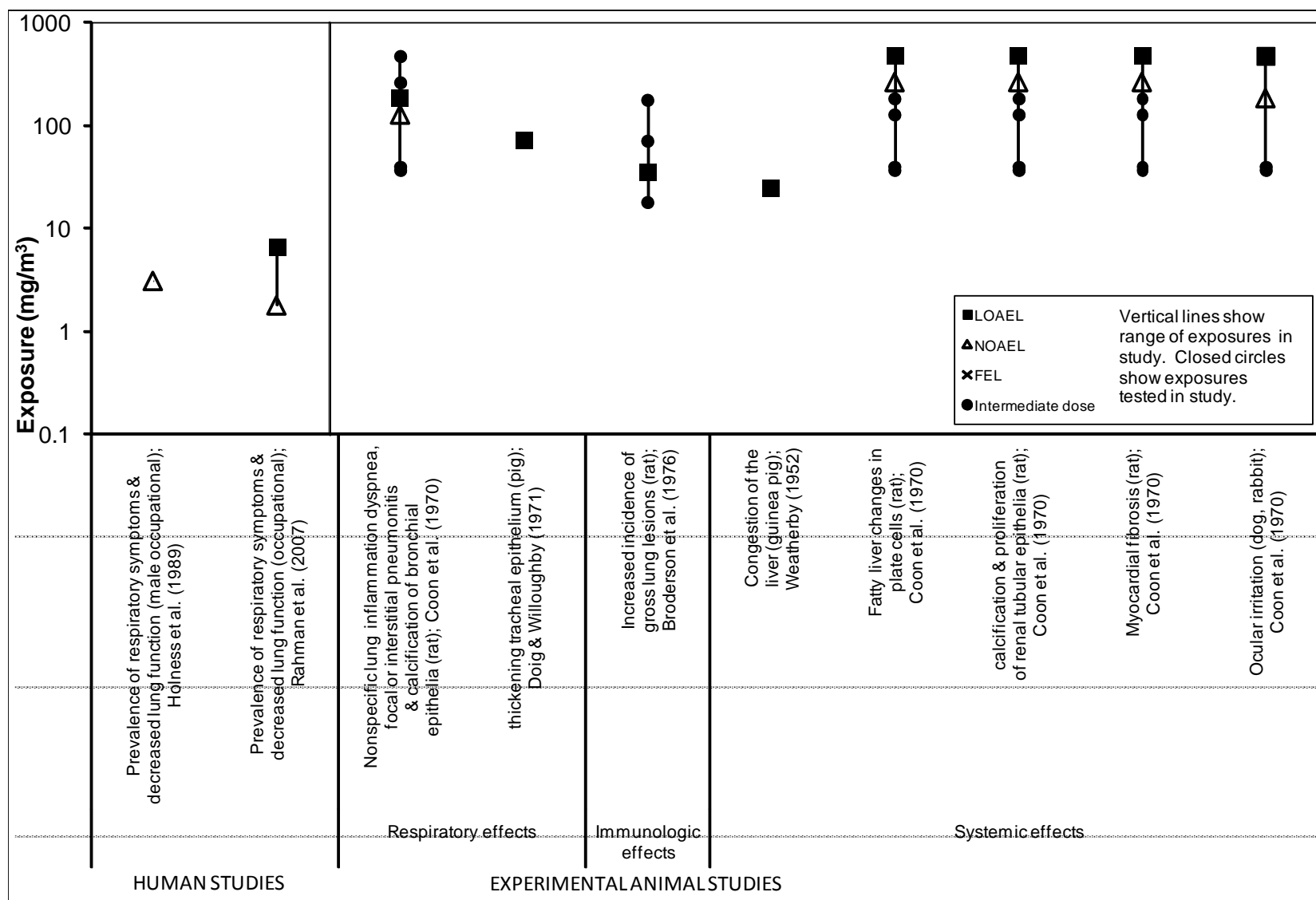


Figure 1-4. Exposure-response array for toxicological effects following inhalation exposure.

1
2
3

1 ***Cancer***

2 The available information on carcinogenicity following exposure to ammonia is limited
3 to oral animal studies. There was no evidence of carcinogenicity in Swiss or C₃H mice
4 administered ammonium hydroxide in drinking water for a lifetime (Toth, 1972). There is
5 limited evidence that ammonia administered in drinking water may act as a cancer promoter
6 based on the findings of studies designed to examine *H. pylori*-induced gastric cancer (Tsuji et
7 al., 1995, 1992a). Additionally, the genotoxic potential cannot be characterized based on the
8 available genotoxicity information. Thus, under the *Guidelines for Carcinogen Risk Assessment*
9 (U.S. EPA, 2005a), there is “inadequate information to assess the carcinogenic potential” of
10 ammonia.

11

12

13

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 (Tsuji 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 $\frac{3}{4}$
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.

1 As discussed in Section 1.2, respiratory effects have been identified as a hazard following
2 inhalation exposure to ammonia. The studies in humans and animals examining inhalation
3 exposure to ammonia provide evidence that inhaled ammonia is associated with toxicity to the
4 respiratory system. The experimental toxicology literature for ammonia also provides evidence
5 that inhaled ammonia may be associated with toxicity to target organs other than the respiratory
6 system, including the liver, adrenal gland, kidney, spleen, heart, and immune system. The
7 weight of evidence for these effects is less robust than for respiratory effects. Therefore, the
8 respiratory system is the primary and most sensitive target of inhaled ammonia toxicity in
9 humans and experimental animals.

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

18 Of the available human data, two occupational studies—Rahman et al. (2007) and
19 Holness et al. (1989)—provide information useful for examining the relationship between
20 chronic ammonia exposure and respiratory irritation and decreased lung function (quantitative
21 dose-response analysis of ammonia respiratory tract toxicity data). Both studies reported the
22 presence or absence of respiratory effects in workers exposed to ammonia over a range of
23 concentrations (approximately 1 to 7 mg/m³). Both studies provide consistent estimates of the
24 effect level for ammonia, with the NOAEL_{ADJ} of 3.1 mg/m³ identified from the Holness et al.
25 (1989) study falling between the NOAEL_{ADJ} and LOAEL_{ADJ} values (1.8 and 6.6 mg/m³,
26 respectively) from the Rahman et al. (2007) study. These studies are considered as candidate
27 principal studies for RfC derivation. Other occupational epidemiology studies (Ali et al., 2001;
28 Ballal et al., 1998) did not provide exposure information adequate for dose-response analysis and
29 thus were not useful for RfC derivation.

30 Consideration of analytical methods suggests that higher confidence is associated with
31 the exposure measures reported by Holness et al. (1989) than Rahman et al. (2007). Rahman et
32 al. (2007) used two analytical methods for measuring ammonia concentrations in workplace air
33 (Dräger PAC III and Dräger tube); concentrations measured by the two methods differed by
34 four- to fivefold, indicating some uncertainty in these measurements, although ammonia
35 concentrations measured by the two methods were strongly correlated. In contrast, the Holness
36 et al. (1989) study used an established analytical method for measuring exposure to ammonia
37 recommended by NIOSH that involved the collection of air samples on acid-treated silica gel
38 (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

13 **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 $\text{NOAEL}_{\text{ADJ}}$ 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 **$\text{NOAEL}_{\text{ADJ}}$ 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:

$$\begin{aligned} \text{NOAEL}_{\text{ADJ}} &= \text{NOAEL} \times \text{VE}_{\text{ho}}/\text{VE}_{\text{h}} \times 5 \text{ days}/7 \text{ days} \\ &= 8.8 \text{ mg/m}^3 \times 10 \text{ m}^3/20 \text{ m}^3 \times 5 \text{ days}/7 \text{ days} \\ &= 3.1 \text{ mg/m}^3 \end{aligned}$$

31 Where:

32 VE_{ho} = human occupational default minute volume (10 m^3 breathed during the 8-hour workday,
33 corresponding to a light to moderate activity level [U.S. EPA, 2011b])

34 VE_{h} = human ambient default minute volume (20 m^3 breathed during the entire day)
35
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/m³) 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

1 maternal blood (Jóźwik et al., 2005), providing some assurance that developmental
2 toxicity would not be associated with concentrations of ammonia at or below the POD.
3 DeSanto et al. (1993) reported that human fetal umbilical blood levels of ammonia at
4 birth were not influenced by gestational age based on deliveries ranging from gestation
5 week 25–43. Finally, evidence in animals (Manninen et al., 1988; Schaerdel et al., 1983)
6 suggests that exposure to ammonia at concentrations up to 18 mg/m³ does not alter blood
7 ammonia levels (see Appendix A, Section A.3, for a more detailed discussion of
8 ammonia distribution and elimination). Accordingly, exposure at the POD (3.1 mg/m³)
9 would not be expected to alter ammonia homeostasis or result in measureable increases in
10 blood ammonia concentrations. Thus, the concentration of ammonia at the POD for the
11 RfC would not be expected to result in systemic toxicity, including reproductive or
12 developmental toxicity.
13

14 The RfC for ammonia was calculated as follows:

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

20 **2.2.3. Uncertainties in the Derivation of the RfC**

21 As presented earlier in this section and in the Preamble, EPA standard practices and RfC
22 guidance (U.S. EPA, 2002, 1995, 1994a, b) were followed in applying a UF approach to a POD
23 to derive the RfC. Specific uncertainties were accounted for by the application of UFs (i.e., in
24 the case of the ammonia RfC, a factor to address the absence of data to evaluate the variability in
25 response to inhaled ammonia in the human population). The following discussion identifies
26 additional uncertainties associated with the quantification of the RfC for ammonia.
27

28 *Use of a NOAEL as a POD*

29 Data sets that support BMD modeling are generally preferred for reference value
30 derivation because the shape of the dose-response curve can be taken into account in establishing
31 the POD. For the ammonia RfC, no decreases in lung function or respiratory irritation were
32 observed in the worker population studied by Holness et al. (1989), i.e., the principal study used
33 to derive the RfC, and as such the data from this study did not support dose-response modeling.
34 Rather, a NOAEL from the Holness et al. (1989) study was used to estimate the POD. The
35 availability of dose-response data from a single study of ammonia would increase the confidence
36 in the estimation of the POD.
37

38 *Endogenous ammonia*

39 Ammonia, which is produced endogenously, has been detected in the expired air of
40 healthy volunteers at levels generally ranging from 0.013 to 2.1 mg/m³ (Boshier et al., 2010;
41 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/m³ (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/m³; 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/m³ 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.

22 **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

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

3 4 **2.2.5. Previous IRIS Assessment: Reference Concentration**

5 The previous IRIS assessment for ammonia (posted to the database in 1991) presented an
6 RfC of 0.1 mg/m³ based on co-principal studies—the occupational exposure study of workers in
7 a soda ash plant by Holness et al. (1989) and the subchronic study by Broderon et al. (1976) that
8 examined the effects of ammonia exposure in F344 rats inoculated on day 7 of the study with the
9 bacterium *M. pulmonis*. The NOAEL of 6.4 mg/m³ (estimated as the mean concentration of the
10 entire exposed group) from the Holness et al. (1989) study (duration adjusted: NOAEL_{ADJ} =
11 2.3 mg/m³) was used as the POD.⁵

12 The previous RfC was derived by dividing the POD by a composite UF of 30: 10 to
13 account for the protection of sensitive individuals and 3 for database deficiencies to account for
14 the lack of chronic data, the proximity of the LOAEL from the subchronic inhalation study in the
15 rat (Broderon et al., 1976) to the NOAEL, and the lack of reproductive and developmental
16 toxicology studies. A UF_D of 3 (rather than 10) was applied because studies in rats (Schaerdel et
17 al., 1983) showed no increase in blood ammonia levels at an inhalation exposure to 32 ppm (22.6
18 mg/m³) and only minimal increases at 300–1,000 ppm (212–707 mg/m³), suggesting that no
19 significant distribution is likely to occur at the human equivalent concentration. In this
20 document, a UF_D of one was selected because a more thorough investigation of the literature on
21 ammonia homeostasis and literature published since 1991 on fetoplacental ammonia levels
22 provides further support that exposure to ammonia at the POD would not result in a measurable
23 increase in blood ammonia, including fetal blood levels.

24 **2.3. Cancer Risk Estimates**

25 The carcinogenicity assessment provides information on the carcinogenic hazard
26 potential of the substance in question and quantitative estimates of risk from oral and inhalation
27 exposure may be derived. Quantitative risk estimates may be derived from the application of a
28 low-dose extrapolation procedure. If derived, and unless otherwise stated, the oral slope factor is
29 a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an
30 inhalation unit risk is a plausible upper bound on the estimate of risk per µg/m³ air breathed.

31 As discussed in Section 1.2, there is **“inadequate information to assess the**
32 **carcinogenic potential”** of ammonia. Therefore, **a quantitative cancer assessment was not**
33 **conducted and cancer risk estimates were not derived for ammonia.** The previous IRIS
34 assessment did not include a carcinogenicity assessment.

⁵In this document, the lower bound of the high exposure category from the Holness et al. (1989) study (8.8 mg/m³, adjusted for continuous exposure to 3.1 mg/m³) 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.

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