

Science Advisory Board (SAB) Draft Report (November 14, 2014) to Assist Meeting Deliberations

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has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

DATE

EPA-SAB-15-xxx

The Honorable Gina McCarthy  
Administrator  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, D.C. 20460

Subject: SAB Review of the EPA’s Draft Toxicological Review of Ammonia

Dear Administrator McCarthy:

The Integrated Risk Information System (IRIS) program under the auspices of the EPA’s National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review the draft assessment titled, *Draft IRIS Toxicological Review of Ammonia* (“the assessment”). The assessment consists of a review of publicly available scientific literature on ammonia/ammonium.

EPA asked the SAB to conduct a review to assess the appropriateness and scientific soundness of the conclusions presented in the IRIS Ammonia assessment. EPA also asked the SAB to comment on the adequacy of EPA’s implementation of the NRC recommendations for changes to the format and structure of the IRIS assessments. In response to EPA’s request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with chemical-specific experts to conduct the review. The panel held two public meetings (a teleconference on June 2, 2014 and a face-to-face on July 14-16, 2014) to discuss and deliberate on the charge questions and consider public comments. The enclosed report provides the SAB’s consensus advice and recommendations. This letter briefly conveys the major findings.

The SAB commends the agency’s efforts in addressing the NRC’s recommendations for developing a clear, consistent format for the IRIS toxicological reviews. Overall, the SAB notes that the agency has made significant improvements but there are several areas that still require further refinement. There does seem to be some duplication across the main assessment and the detailed study summaries in the appendix. The use of tables and figures is particularly helpful and the EPA needs to continue to refine their presentation to allow users to efficiently navigate between the main assessment and the supplementary information.

The selection and evaluation of key studies is well supported apart from a few deficiencies. The inclusion and exclusion criteria should be more transparently presented. The placement of the

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1 descriptions of the supporting studies in the appendix is appropriate but the principal study should be  
2 given a more detailed description and evaluation in the main assessment. Some of these issues will  
3 likely be resolved as EPA develops and adopts a standard systematic review approach for evaluating and  
4 selecting key studies. The rationale for excluding ammonium salts from the assessment should be  
5 expanded. The SAB also notes that a more detailed evaluation of the chemical reactions and ammonia  
6 generation that may impact gastrointestinal endpoints is required, particularly as it relates to the  
7 conclusion of not deriving a reference dose (RfD).

8  
9 The SAB agrees that the scientific evidence is sufficiently robust to support the conclusion that  
10 ammonia induces significant respiratory effects in humans and animals and the use of this endpoint as a  
11 point of departure for derivation of the reference concentration (RfC). The SAB recommends that  
12 further discussion of the potential implications of reversibility and long-term attenuation of effects  
13 through acclimatization and/or the healthy worker effect that may lead to an underestimation of risk be  
14 added.

15  
16 The SAB concludes that the use of the Holness et al. (1989) study is appropriate but recommends that  
17 EPA contact the author in order to determine if alternative points of departure could be identified.  
18 Evidence of a cumulative effect of ammonia exposure is important to consider, especially if  
19 corroborated by other studies. Additionally, the SAB agrees with the conclusion that there is inadequate  
20 information to assess the carcinogenic potential of ammonia. The rationale for not deriving quantitative  
21 cancer risk estimates is described clearly and is well supported scientifically.

22  
23 The description of endogenous ammonia production appears to be generally appropriate, but the SAB  
24 recommends expanding this section to describe all sources of endogenous ammonia. While there is no  
25 doubt that ammonia in expired breath is increased in pathological conditions (such as liver disease and  
26 kidney disease) that give rise to hyperammonemia, there is no evidence to suggest that the concentration  
27 of ammonia in the oral cavity is a major contributor to either the systemic or inhaled concentration of  
28 ammonia. To provide further context for the RfC, the SAB recommends that EPA consider including  
29 ranges and citations for typical [?] indoor and ambient concentrations of ammonia.

30  
31 The SAB appreciates this opportunity to review EPA's Draft Toxicological Review of Ammonia and  
32 looks forward to the EPA's response to these recommendations.

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35 Sincerely,

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44  
45 Enclosure

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**NOTICE**

This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to the problems facing the agency. This report has not been reviewed for approval by the agency and, hence, the contents of this report do not represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA website at <http://www.epa.gov/sab>.

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2  
3 **U.S. Environmental Protection Agency**  
4 **Science Advisory Board**  
5 **Chemical Assessment Advisory Committee Augmented**  
6 **for the Review of the Draft IRIS Ammonia Assessment**  
7

8  
9  
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**U.S. Environmental Protection Agency  
Science Advisory Board  
BOARD**

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[To be added]

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**TABLE OF CONTENTS**

**[To be added]**

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1  
2  
3 I. EXECUTIVE SUMMARY  
4  
5

6 The Science Advisory Board was asked by the EPA Integrated Risk Information System (IRIS) program  
7 to review the agency's *Draft Toxicological Review of Ammonia (August 2013 Draft)* (also referred to as  
8 the assessment). EPA's IRIS is a human health assessment program that evaluates information on health  
9 effects that may result from exposure to environmental contaminants. The assessment consists of a  
10 review of publicly available scientific literature on ammonia (gaseous) and ammonium hydroxide  
11 (ammonia dissolved in water). It does not include an evaluation of the literature on ammonium salts. The  
12 assessment was revised in the August 2013 and a summary of EPA's disposition of the public comments  
13 received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information  
14 to the Toxicological Review.

15  
16 EPA asked the SAB to conduct a review of the appropriateness and scientific soundness of the  
17 conclusions presented in the draft IRIS ammonia assessment. In addition, the SAB was asked to  
18 comment on the modification of the overall structure of the assessment as recommended by the National  
19 Research Council (NRC) in 2011. The panel charged with conducting the review included the SAB  
20 Chemical Assessment Advisory Committee members augmented with additional toxicological experts.  
21 The charge questions are included in Appendix A of this report. The SAB offers a brief overview of  
22 their recommendations and advice on how to improve the clarity, transparency and utility of the  
23 assessment here and in greater depth in the body of the report.  
24  
25

26 Implementation of the NRC recommendations  
27

28 **Clarity of the Preamble**

29 The SAB commends the agency for the progress made thus far in implementing the NRC  
30 recommendations. The SAB expects that further refinements and modifications will be made based on  
31 feedback from external reviewers, users and other stakeholders. Recognizing that the Preamble is a  
32 "work in progress," this current iteration of the Preamble goes a long way to providing a clear, concise,  
33 useful, and objective summary of the complex set of guidance and methods that EPA uses in developing  
34 IRIS assessments. Citation of EPA guidance documents and links to the documents are particularly  
35 helpful but must be closely checked for accuracy. It also would be helpful to clarify the reasoning  
36 behind the Preface being separate from the Preamble and having such an extended section prior to the  
37 Executive Summary. It appears that the Preamble is intended to describe the general approach/methods  
38 used by the agency and will be included with all IRIS assessments; since the Preface would presumably  
39 summarize issues that are specific to a particular chemical assessment, it should be placed after the  
40 Preamble.  
41

42 **IRIS assessment structure**

43 The new format is a refreshing and long overdue improvement. The ammonia assessment is one of the  
44 first since the NRC made its recommendations. The EPA has clearly begun a stepwise implementation  
45 of the recommendations for systematic review but, as indicated in the more recent 2014 NRC review,  
46 this assessment has not fully conducted the systematic review as envisioned by the NRC. The new

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1 structure of the assessment will take some getting used to for those who are familiar with the old  
2 structure. It is a clear improvement but additional refinements will certainly be forthcoming in  
3 subsequent chemical assessments. There does seem to be some duplication across the main assessment  
4 and the detailed study summaries in the appendices, but this is hard to avoid and may serve to emphasize  
5 the importance of key publications. The use of tables and figures is particularly helpful and the EPA  
6 needs to continue to work on efficiently summarizing and presenting data using this format.  
7

### 8 **Transparency of integrative approaches**

9 The ammonia assessment is an excellent first step in the direction suggested by the NRC, but there is  
10 still more that needs to be done. It provides greater emphasis on the integration of studies and a more  
11 transparent discussion of the weight of evidence than previous assessments. Study evaluation is  
12 generally well done in the ammonia assessment, but does not appear to consistently follow a  
13 standardized approach.  
14

15 EPA has indicated that it is working on adopting systematic review principles and other standardized  
16 approaches for evidence gathering and evaluation as it moves forward with IRIS program enhancements.  
17 The EPA has made a good start in improving their evaluations of the critical studies. In general, the key  
18 studies were adequately evaluated and the key features of the evaluations were well described. It would  
19 be useful to develop overall qualifiers for the studies included in the summary tables as per NRC  
20 recommendations.  
21

### 22 **Adequacy of response to public comments**

23 EPA has adequately and appropriately addressed the scientific issues raised by public commenters.  
24 With regards to some of the comments where EPA disagreed with the commenters, the SAB concluded  
25 that EPA has provided adequate scientific justification for their conclusions. Furthermore, it must be  
26 remembered that this is ultimately an EPA document and the agency must be responsible for its content.  
27 Given that the assessment is being reviewed by this committee, has been reviewed by the NRC  
28 committee and may yet undergo additional agency reviews, the current approach provides adequate  
29 opportunity for public feedback and oversight.  
30  
31

## 32 Draft IRIS Ammonia Assessment

### 34 **Executive Summary**

35 The Executive Summary is a very concise summary that highlights many of the important conclusions  
36 made in the EPA's assessment. To improve the utility of the Executive Summary, accuracy in  
37 describing key toxicity endpoints, and transparency in EPA's evaluation and decisions, the SAB offers  
38 the following general recommendations. A section should be included at the beginning of the Executive  
39 Summary that provides information on the chemistry of ammonia, ammonium and ammonium salts and  
40 the rationale for excluding or including ammonium salts. The sections should be rearranged so that the  
41 discussion on non-cancer effects of inhalation exposure comes before the discussion of oral exposures  
42 (if an oral reference dose (RfD) is not derived). A discussion of the weight of evidence of critical  
43 epidemiology studies is missing from the Executive Summary and a brief synopsis should be included.  
44  
45  
46

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1 **Literature Search Strategy/Study Selection and Evaluation**

2 Overall, the literature search approach for screening, evaluation, and selection of studies to include in  
3 the assessment are fairly well described and supported. EPA is encouraged to incorporate and implement  
4 recommendations from both NRC 2011 and 2014 reports in future assessments. In particular, the NRC  
5 recommended the development of a standardized, detailed literature search and evaluation protocol  
6 specific to IRIS objectives. Many of the components of such protocols are described in the Preamble of  
7 the ammonia assessment, but the extent and mechanisms for their application to the ammonia  
8 assessment are not sufficiently clear.

9  
10 Although the narrative provides an evaluation of the studies according to preselected criteria, not all  
11 criteria recommended by the NRC (2011) are incorporated (e.g., precision of the effect) and there is no  
12 specific overall study quality indicator. While it is understood that this is an area still under  
13 development, the application of the study quality criteria for the selection and evaluation of key non-  
14 cancer experimental animal studies that were included in the assessment is unclear. Additional  
15 clarification of inclusion/exclusion criteria may provide some insight as to why some apparently  
16 relevant publications were not included or cited. In addition, the SAB also encourages EPA to  
17 reconsider the inclusion of publications beyond the March 2013 deadline.

18  
19 **Hazard Identification**

20 *Synthesis of Evidence*

21 The SAB concluded that in general terms the data included in the assessment have been clearly and  
22 appropriately synthesized for each toxicological endpoint, and that the weight of the evidence for hazard  
23 identification has been adequately described and documented. It should be noted, however, that the  
24 published scientific data available on ammonia toxicity is rather limited for most endpoints. The  
25 scientific evidence is, however, sufficiently robust for respiratory effects to support the conclusion that  
26 ammonia induces these effects in humans and animals. Thus, the SAB concluded that the weight of the  
27 evidence for respiratory effects supports its use as a point of departure for the reference concentration  
28 (RfC). While the synthesis of the evidence for ammonia toxicity included in Chapter 1 was presented in  
29 an objective, systematic and concise manner, a clearer explanation of how the evaluation criteria were  
30 applied to individual studies and ultimately integrated into the weight of the evidence analysis is needed.  
31 Importantly, these revisions should be captured in the tabular summaries included in the chapter. The  
32 SAB also recommends that the biological bases for tolerance/adaptation that may lead to  
33 underestimation of risk be considered as part of the evaluation, and that gastrointestinal effects of  
34 ammonia be re-examined as part of a more integrated evaluation of the chemistry of ammonia,  
35 ammonium hydroxide, and ammonium salts as a function of pH.

36  
37 *Summary and Evaluation*

38 The scientific evidence supporting the conclusion that ammonia poses a potential hazard to the  
39 respiratory system is well-integrated. As noted above, a more detailed evaluation of the chemical  
40 reactions and ammonia generation that may impact gastrointestinal endpoints is required, particularly as  
41 it relates to the conclusion of not deriving an RfD.

42  
43 In general, the conclusion that there is inadequate information to assess the carcinogenic potential of  
44 ammonia is supported by the scientific evidence reviewed. While the SAB agrees that the evidence  
45 presented by Tsujii et al, (1993) suggesting ammonia exhibits tumor-promoting properties is weak and  
46 insufficient, the strengths and weaknesses of other potentially relevant lines of evidence should be

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1 considered and discussed as part of the evaluation.  
2  
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### 5 **Oral Reference Dose (RfD)**

6 Although there is a fairly extensive literature on the systemic and organ-specific effects of ammonia  
7 (e.g., liver, brain, and kidney) with inhalation exposure, there are no controlled animal studies of the  
8 systemic effects of ammonia [not ammonium salts] through the ingestion route of exposure. Reports of  
9 systemic effects in humans with ingestion are confined to case reports of poisonings and accidental  
10 ingestion. EPA intentionally excluded from consideration studies of gastrointestinal effects (or the lack  
11 thereof) with oral administration of ammonium (NH<sub>4</sub><sup>+</sup>) salts. This decision was based on concerns that  
12 the possible adverse effects of ammonia in such studies could not be separated from adverse effects  
13 resulting from the associated anion. Therefore, EPA did not attempt to derive an RfD for such systemic  
14 effects. The SAB noted that while a possible independent gastrointestinal toxicity of the anion may be a  
15 valid concern, the dichotomy between ammonia and ammonium salts in the consideration of an RfD is  
16 not because ammonia in solution (i.e., in an aqueous delivery medium and/or in stomach fluid) is present as the  
17 free ammonium (NH<sub>4</sub><sup>+</sup>) ion. Given this reasoning, the SAB concluded that EPA should evaluate the  
18 relevant toxicity studies that use ammonium salts to determine if they can offer valuable information for  
19 the derivation of an RfD. If the effects of the anion cannot be discerned, the decision to exclude  
20 ammonium salts will be buttressed by the evaluation of these studies. The SAB also noted that a decision  
21 to address ammonium salts would also require further evaluation of the inhalation of ammonium salt  
22 particulate matter and the impact on the RfC.  
23

### 24 **Inhalation Reference Concentration (RfC)**

#### 25 *Evaluation of Studies*

26 The evaluation of studies is clearly described in the supplementary materials, and concisely summarized  
27 in the main assessment. EPA has indicated that the Holness et al. (1989) study has the strongest  
28 exposure assessment, and provides a clear argument in support of that judgment. Although the selection  
29 of studies and effects for the RfC is mostly clear and the Holness et al. (1989) study is the most  
30 appropriate for RfC derivation, exclusion of the controlled human exposure studies [references] is not  
31 well explained. These studies have several methodological strengths such as, well-characterized  
32 exposures and resistance to confounding factors. Clarification as to why they are excluded as candidates  
33 for RfC derivation is needed. It is unclear if the quality of exposure assessment overrides the other  
34 factors listed in the Preamble for selection of a key study. The SAB also recommends expansion of the  
35 discussion of the potential implications of factors such as, reversibility and long-term attenuation of  
36 effects through acclimatization and/or the healthy worker effect (e.g., self-selected attrition due to  
37 respiratory symptoms) as they are confounders that may lead to underestimating effects.  
38

#### 39 *Deriving an RfC*

40 The approach for deriving the RfC is reasonable and clearly described, but it is not clear to what extent  
41 EPA considered continuous dose-response modeling. EPA should attempt to obtain individual-level  
42 data and/or the mean/median exposure concentrations for the high dose group from Dr. Holness in order  
43 to determine if an alternative point of departure (POD) could be identified, overcoming the limitation of  
44 having only the upper exposure range in the published manuscript. If individual data are unavailable,  
45 EPA should consider whether there is sufficient information available in the Holness publication to  
46 estimate the mean concentration for the high exposure group--perhaps assuming a lognormal or other

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1 skewed distribution for the measured concentrations. For the POD derived from the Holness study, a  
2 dose conversion factor was used to convert the observed workplace ammonia concentration to an  
3 ammonia concentration that would provide an equivalent cumulative dose with continuous 24/7  
4 exposure. Evidence of a cumulative effect of ammonia exposure is important to consider, especially if  
5 corroborated by other studies. The selection of the uncertainty factor was appropriate, clearly described,  
6 and consistent with the 2002 EPA guidance.

### 8 **Quantitative Cancer Assessment**

9 The SAB agrees with EPA’s conclusion that the existing data in the literature are inadequate to reach a  
10 conclusion on the carcinogenicity of ammonia, and thus it would not be scientifically justified to  
11 develop quantitative cancer risk estimates for this chemical. The rationale for not deriving these  
12 estimates is described clearly and is well supported scientifically.

### 14 **Endogenous Production of Ammonia**

15 The description of endogenous ammonia production appears to be generally appropriate, but the SAB  
16 recommends expanding this section to describe all sources of endogenous ammonia, [add examples].  
17 One important consideration for EPA is that the production of endogenous ammonia should be used  
18 [explain how] in the interpretation of all epidemiologic studies (as well as human and animal controlled  
19 exposure studies not used for RfC derivation). The SAB also notes that the effects described in these  
20 studies are at levels over and above endogenous levels.

22 There is no doubt that ammonia in expired breath is increased in pathological conditions (such as liver  
23 disease and kidney disease) that give rise to hyperammonemia. Studies suggest that absorption of  
24 ammonia in lungs occurs in a compartment that does not readily mix with the metabolic pool of  
25 ammonia. The amount of ammonia that equilibrates between the endogenous lung metabolic pool and  
26 alveolar air is likely to be quite small even under hyperammonemic conditions. The concentration of  
27 ammonia in oral cavity air is an indicator of the exhaled concentration (including the contribution from  
28 the bacterial digest of residual food particles in the mouth). However, because of confounding problems  
29 with “contaminating” ammonia in the expired air and difficulties associated with its actual measurement,  
30 it may be challenging to correlate prior *chronic* exposure of individuals to ammonia with alveolar  
31 ammonia concentrations. Additionally, the concentration of ammonia in oral cavity reflects neither the  
32 endogenous inhaled ammonia (which is closely related to the alveolar ammonia concentrations), nor the  
33 concentration of ammonia in inhaled air (since mouth air is diluted with external air on inhalation).  
34 Thus, the concentration of ammonia in the oral cavity is not a major contributor to either the systemic or  
35 inhaled concentration of ammonia.

37 As a means of providing further context for the RfC, it is recommended that EPA consider including  
38 ranges and citations for typical [?] indoor and ambient concentrations of ammonia. These data need not  
39 be comprehensive but will be helpful for placing the RfC in the context of expected concentrations in  
40 non-industrial, residential, and office indoor environments, and in outdoor air (for example, data  
41 collected by EPA’s Passive Ammonia Monitoring Network). These ranges could also be included as part  
42 of the Executive Summary of the assessment.

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## II. INTRODUCTION

### Background

In June 2012, the Environmental Protection Agency (EPA) released the Integrated Risk Information System (IRIS) "Draft Toxicological Review of Ammonia" (henceforth referred to as "the assessment"). The assessment consists of a review of publicly available scientific literature on ammonia (gaseous) and ammonium hydroxide (ammonia dissolved in water). It does not include an evaluation of the literature on ammonium salts. The assessment was revised in August 2013 and a summary of EPA's disposition of the comments received from the public was added in Appendix G of the Supplemental Information to the Toxicological Review.

### Charge to the SAB

EPA asked the SAB to conduct a review to assess the appropriateness and scientific soundness of the conclusions presented in the IRIS Ammonia assessment. In response to EPA's request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with chemical-specific experts to conduct the review. The panel held two public meetings (a teleconference on June 2, 2014 and a face-to-face meeting on July 14-16, 2014) to discuss and deliberate on the charge questions and consider public comments. A subsequent public teleconference was held on [INSERT DATE] to discuss the panel's report. The SAB panel's draft report was then considered and [INSERT DISPENSATION] by the chartered SAB on a [INSERT DATE]. Oral and written public comments have been considered throughout the advisory process.

In addition to providing advice and recommendations on how to improve the ammonia assessment, EPA also asked four general charge questions and sought feedback on its new *Preamble* that provides a description of the guidance and methods that EPA uses in developing IRIS assessments. In addition, EPA asked for comments on the new IRIS assessment structure, the clarity and transparency of the discussions of weight of evidence and the adequacy of the response to public comments.

This report is organized to follow the order of the charge questions. The report responds to the general charge questions first and then addresses the chemical-specific questions.

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### III. RESPONSE TO THE CHARGE

#### General Charge Questions

*General Charge Question 1: NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to describe more fully the methods of the assessment. NRC stated that they were “not recommending the addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise statements of criteria used to exclude, include, and advance studies for derivation of [toxicity values].” Please comment on whether the new Preamble provides a clear, concise, \*useful and objective\* description of the guidance and methods that EPA uses in developing IRIS assessments.*

#### **Response**

The EPA has made a concerted effort to respond to the NRC’s recommendation for an expanded methods introductory section. The use of a Preamble that summarizes EPA’s guidance and methods is a step forward and will be useful for future IRIS assessments. It must be made clear that this is a brief description of the policies and procedures already adopted by EPA and that the original guidance documents are controlling, not the abbreviated description in the Preamble. The Preamble does not establish new policy. From the public comments, some appeared to over interpret the Preamble statements. Preparing a Preamble that can be used in multiple IRIS assessments is an efficient approach and is similar to approaches used by IARC and the NTP. Although the Preamble is a “work in progress”, this current iteration of the Preamble goes a long way to providing a clear, concise, useful, and objective summary of the complex set of guidance and methods that EPA uses in developing IRIS assessments. Citation of EPA guidance documents and links to the documents are particularly helpful but must be closely checked for accuracy.

To a first time reader it is a bit awkward to find a rather long Preface before the Executive Summary. The reasoning behind the Preface being separate from the Preamble and having such an extended section prior to the Executive Summary could use some clarification. However, there is logic in the approach and once familiar with that organizational structure, it seems a useful way to organize the chemical-specific portions of the assessment.

#### **Recommendations:**

1. Since the Preamble is a complex, “stand alone” document, at some future date (not for this ammonia assessment) it would be advisable to have it separately examined and reviewed in detail.
2. Section 6 (Selection of studies for derivation of toxicity values) is less clear than the other sections of the Preamble and would benefit from elaboration and citation of any relevant EPA guidance document. Six clear preferences are stated in Section 6, but it is not clear how they are balanced against each other or against other factors not listed to determine which study to select for derivation of toxicity values. For example, the quality of exposure measurement, multiple outcomes, and highest NOAEL were the primary factors used to select the Holness et al. (1989) study for the ammonia assessment, but are not listed among the six key factors in the Preamble Section 6.
3. EPA should verify that all the relevant EPA’s guidance documents are included.

Science Advisory Board (SAB) Draft Report (November 14, 2014) to Assist Meeting Deliberations

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4. Briefly describe the mechanism employed to perform peer review of important and relevant articles that have not been peer reviewed (Page xiv, lines 12-24)
5. Clarify which “ethical standards” are considered (Page xvi, lines 3-5). It is likely that older studies may not have been conducted with strict adherence to current criteria for the use of human subjects in research. Are ethical uses of vertebrate animals also considered?
6. Consider whether assessments should provide ranges for typical levels of exposure or intake for comparison purposes to estimated doses or concentrations.
7. The statement in Page XX, lines 26-30 needs to be revised; the scientific quality of studies should be foremost in assessing credibility.
8. The Preamble should include a mention to the role played by NRC, 2001 and 2014 in the process of IRIS protocol development.
9. Consider specific suggestions from various SAB members to revise the text in the Preamble (e.g. definitions of terms).

*General Charge Question 2: NRC (2011) provided comments on ways to improve the presentation of steps used to generate IRIS assessments and indicated key outcomes at each step, including systematic review of evidence, hazard identification, and dose-response assessment. Please comment on the new IRIS document structure and whether it will increase the ability for the assessments to be more clear, concise, and easy to follow.*

**Response**

The new format is a refreshing, and long overdue improvement, but is a work in progress. The ammonia assessment was very easy to follow and will be a good template as future assessments evolve. The IRIS program is to be commended for not delaying release of this assessment and others begun before the NRC report until the IRIS program response was perfected. These assessments do not need to be masterpieces but rather concise sources of systematically reviewed reference materials. While IRIS assessments have evolved over time, this is the first major overhaul and EPA is to be commended for its commitment to implement the NRC recommendations and committing the resources to do so in a systematic but progressive manner.

The ammonia assessment is one of the first since the NRC made its 2011 recommendations. The EPA has clearly begun a stepwise implementation of the recommendations for systematic review but, as indicated in the more recent 2014 NRC review, this assessment has not fully conducted the systematic review as envisioned by the NRC. However, we should note that the NRC/IOM approach is not a directive and should be expected to need modifications in order to address some of the issues that EPA faces as implementation progresses. The structure of the assessment is somewhat unusual and will take some getting used to for those familiar with the old structure. It is a clear improvement but additional refinements (of future assessments) will certainly be forthcoming in subsequent chemical-specific assessments.

**Recommendations:**

1. There does seem to be some duplication across the main assessment and the detailed study summaries in the appendix, but that is hard to avoid and may serve to emphasize the importance of some publications. A clearer statement of how the main text reviews are intended to be different from the appendix summaries would benefit the user.

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- 1 2. Summaries of key publications and outcomes are informative but concise, with the bulk of study  
2 descriptions presented in appendix summaries. It is somewhat more cumbersome at this stage  
3 because of the need to refer backwards and forwards between main text and appendix materials  
4 when looking for specific details. This could be simplified in electronic versions of the  
5 assessment by adding hyperlinks between the main text and appendix materials.
- 6 3. The main assessment does an excellent job of summarizing the key information, but does invite  
7 some clarifying questions. The assessment is generally effective in providing the information  
8 needed to evaluate the studies but not as good at getting to the core of the assessment of the  
9 information. The tables and appendix materials are an improvement over the more laborious  
10 descriptions in earlier IRIS reviews. However, since the Holness et al., 1989 study is the basis of  
11 the RfC, it should be described concisely but in more detail in the assessment itself. It is fine to  
12 have the descriptions of the supporting studies in the appendix but the principal study should be  
13 given a more detailed description and evaluation in the main assessment.
- 14 4. The EPA needs to continue to work on efficiently summarizing and presenting data through the  
15 use of tables and figures. Providing connections between the information in the text, tables and  
16 figures is a worthwhile goal. It would also be helpful to provide some indication of study quality  
17 in the tables and figures or alternatively, only present studies that met minimal criteria, which are  
18 clearly stated. For example, the Anderson et al. (1964) studies in Figure 1-1 should have been  
19 tagged in some way as weak studies or else omitted from the Figure.
- 20 5. The discussion of kinetics is key to many risk assessment documents. EPA should consider  
21 moving appropriate kinetic information into the main text from the appendix if it is used in  
22 selection and weighing of studies, derivation of RfC/RfD, or any other key steps in the  
23 assessment.

24  
25  
26 *General Charge Question 3: NRC (2011) states that “all critical studies need to be thoroughly*  
27 *evaluated with standardized approaches that are clearly formulated” and that “strengthened, more*  
28 *integrative and more transparent discussions of weight of evidence are needed.” NRC also indicated*  
29 *that the changes suggested would involve a multiyear process. Please comment on EPA’s success*  
30 *thus far in implementing these recommendations.*

### 31 32 **Response**

33 The ammonia assessment is an excellent first step in the direction suggested by NRC, but there is still  
34 terrain to cover. It provides a greater emphasis on integration of studies and a more transparent  
35 discussion of the weight of evidence than previous assessments. Study evaluation is generally well done  
36 in the ammonia assessment, but does not appear to consistently follow a standardized approach. EPA  
37 has indicated that it is working on adopting systematic review principles and other standardized  
38 approaches to evidence gathering and evaluation as it moves forward with IRIS program revisions. That  
39 remains to be accomplished. The EPA has made a good start in improving their evaluations of the  
40 critical studies. In general, the key studies were adequately evaluated and the key features of the  
41 evaluations were described. As indicated in the response to Question 2, a more detailed description and  
42 critique of the key Holness et al. 1989 study in the main text would strengthen the assessment.

43  
44 NRC (2011) anticipated the evolution of IRIS would be a multiyear process. The assessment  
45 demonstrates significant strides toward the goals outlined by NRC. However gaps exist. For example,

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1 description of study quality remains incomplete. It would be useful to develop overall qualifiers to the  
2 studies in the summary tables as per NRC recommendations.

3  
4 **Recommendations:**

- 5 1. Some of the gaps identified during this review may well be because, despite the many policies  
6 and procedures outlined in the Preamble, existing EPA procedures and policies do not adequately  
7 cover all necessary contingencies. While in some circumstances professional expert judgment is  
8 needed, a systematic approach should be adopted to provide more transparency and clarity.

9  
10  
11 *General Charge Question 4: EPA solicited public comments on the draft IRIS assessment of*  
12 *ammonia and has revised the assessment to respond to the scientific issues raised in the comments.*  
13 *A summary of the public comments and EPA's responses are provided in Appendix G of the*  
14 *Supplemental Information to the Toxicological Review of Ammonia. Please consider in your review*  
15 *whether there are scientific issues that were raised by the public as described in Appendix G that*  
16 *may not have been adequately addressed by EPA.*

17  
18 **Response**

19 EPA has adequately and appropriately addressed the scientific issues raised by public commenters.  
20 With regards to some of the comments where EPA disagreed with the commenters, the SAB concluded  
21 that EPA has provided adequate scientific justification for their conclusions. Additionally, it must be  
22 remembered that this is ultimately an EPA document and the agency must be responsible for its content.  
23 Given that the assessment is being reviewed by this committee, has been reviewed by the NRC  
24 committee and may yet undergo additional Agency reviews, the current approach provides adequate  
25 opportunity for public feedback and oversight.

26  
27 **Recommendations:**

- 28 1. One specific comment (p. G-8) deserves greater attention by EPA. It was suggested that EPA  
29 attempt to obtain the study data from Dr. Holness in order to determine a representative exposure  
30 concentration for the NOAEL study group, rather than using the least exposed person in that  
31 study group. This is a good suggestion and EPA's response needs elaboration. Did the agency  
32 try to obtain the data but was refused? Or does it feel that the original data are irrelevant because  
33 the least exposed person in that exposure group is the most appropriate basis for the RfC? A  
34 committee member was able to provide Dr. Holness' contact information:  
35 <http://www.stmichaelshospital.com/research/profile.php?id=holness&>  
36 2. Suggestions were made for using studies upon which other exposure guidelines (TLV, AEGL-1)  
37 were established. This is an issue which is likely to occur repeatedly. These values serve a  
38 different purpose and EPA might consider expanding the section of the assessment that covers  
39 such US and international guidelines. Table A-1 in Appendix A should be modified to include  
40 additional exposure guidelines for ammonia, their definition and purpose, and provide links to  
41 the assessments that explain the rationale for the guidelines and the chemical-specific  
42 documentation that supports them.

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1 Ammonia Assessment-Specific Charge Questions

2  
3 **A. Executive summary**

4  
5 *Charge Question A.1.: Please comment on whether the conclusions have been clearly and*  
6 *sufficiently described for purposes of condensing the Toxicological Review information into a*  
7 *concise summary.*

8  
9 **Response**

10 The Executive Summary is a very concise summary that highlights many of the important conclusions  
11 made in the EPA’s assessment. Determination of whether the conclusions have been clearly and  
12 sufficiently described should take into consideration the potential audience and purposes of this  
13 summary. According to EPA representatives, a future goal for Executive Summaries is to use them with  
14 minimal editing for the IRIS website. In addition, EPA indicated that it is not uncommon for state  
15 regulators and other risk assessors to focus primarily, if not exclusively, on the Executive Summary as  
16 source of information for the toxicological assessment. From this perspective, the EPA’s effort to be  
17 concise results in a summary that is too vague and unclear in some of the subsections. The following  
18 general recommendations are offered to improve the utility of the Executive Summary, accuracy in  
19 describing key toxicity endpoints, and transparency in EPA’s evaluation and decisions. More specific  
20 detailed comments are included in Appendix B of this report.

21  
22 **Recommendations:**

- 23 1. A section should be included at the beginning of the Executive Summary that provides  
24 information on the chemistry of ammonia<sup>1</sup>, ammonium and ammonium salts and a rationale for  
25 excluding or including ammonium salts as oral exposure to ammonia results in exposure to  
26 ammonium. Otherwise, EPA’s discussion of non-cancer effects following oral exposure in the  
27 Executive Summary will not be credible because it appears to ignore published literature on  
28 toxicity of repeated oral exposures to ammonium salts without any explanation. As discussed  
29 below in more detailed comments, it appears that toxicity of ammonium salts may be dependent,  
30 in part, on the anion. Therefore, there is good reason to ignore those studies that do not control  
31 for the effect of the anion in deriving an oral RfD for ingested ammonia. However, toxicology  
32 studies on ammonium salts, especially negative results, potentially can provide supportive  
33 evidence for the absence of adverse effects. In addition, studies in which the anion is chloride  
34 may not result in a meaningful increase in chloride exposure given the large concentration of  
35 endogenous chloride normally present in the stomach and thus, may allow for consideration of  
36 ammonium toxicity independent of the effect of the anion.
- 37 2. The sections should be rearranged so that the discussion on non-cancer effects of inhalation  
38 exposure comes before the discussion of oral exposures (if an oral RfD is not derived).

---

<sup>1</sup> Note on terminology: The word “ammonia” (unless specified otherwise) is used loosely to mean the total of ammonia free base (NH<sub>3</sub>) plus ammonium ion (NH<sub>4</sub><sup>+</sup>) when used in the context of physiological fluids and tissues. NH<sub>3</sub> is a weak base



Since the pK<sub>a</sub> is 9.2, about 98-99% of “ammonia” will exist as ammonium ion and only 1 – 2% as ammonia free base at physiological pH values (pH 7.2 – 7.4). The panel generally agreed that these terms should be defined at the outset.

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3. The first sentences of the non-cancer oral section should indicate that an oral RfD was not derived (assuming that EPA continues to conclude that an oral RfD should not be derived).
4. A brief discussion of the weight of evidence of critical epidemiology studies is missing from the Executive Summary. This can easily be done by adding descriptors for the nature of effects measured (e.g. self-report versus clinical exam; magnitude of change in lung function relative to clinical levels of concern) and a brief discussion of how each key epidemiology study cited as the basis for the RfC derivation controlled for potential confounding effects of co-exposures to other chemicals or particulate matter that might cause similar respiratory effects as those associated with ammonia.
5. The description of evidence that ammonia may act as a cancer promoter is vague and needs additional explanation.
6. In the section on susceptible populations, EPA did not include people with preexisting lung disease including asthma. EPA may want to consider including parts of the discussion on page 1-38 of the actual study data relevant for asthmatics as a susceptible population.
7. The grey summary box of the Executive Summary in the assessment should indicate that there is inadequate information to evaluate the carcinogenicity of ammonia or to derive an oral RfD for ammonia. If EPA has reliable data to indicate that exposure is primarily through air compared to other routes of exposure, then this should be emphasized as another reason for EPA to focus on deriving an inhalation RfC.

## B. Literature Search Strategy/Study Selection and Evaluation

*Charge Question B.1. The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection and Evaluation section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g., study quality, risk of bias) used for the selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of ammonia.*

### Response

Overall, the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are fairly well described and supported. However, while the search strategy incorporates elements of systematic review, there are several areas in need of additional clarification and further strengthening. The SAB understands that the NRC's recommendations (NRC, 2011) are yet to be fully implemented, and that adoption of past and more recent recommendations by the NRC (NRC, 2014) is an evolving process and thus not yet reflected in the current ammonia assessment. However, EPA is encouraged to incorporate and implement recommendations from both NRC reports as much as reasonably possible given time constraints. In particular, the NRC 2014 report provides additional advice and recommendations directly relevant to the development process and transparency of the literature search strategy of the draft ammonia assessment (which the NRC reviewed in the preparation of its 2014 report). In particular, one of the recommendations that the SAB also discussed is the need for accelerating the development of standardized, detailed literature search and evaluation protocols specific to IRIS objectives. Many of the components of such protocols are described in the Preamble of the

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1 ammonia assessment, but the extent and mechanisms for their application to the ammonia assessment  
2 are not sufficiently clear. Thus, some of the weaknesses identified by the SAB may be reflective of  
3 EPA's progress towards implementation of the NRC's past and more recent recommendations, and/or  
4 insufficient clarity as to how extant methods and procedures were actually applied.

5  
6 As indicated by EPA in the background materials and presentations to the SAB, the ammonia  
7 assessment does not include the problem formulation step that will be incorporated in future  
8 assessments. The SAB discussed this issue briefly and agreed with EPA's decision not to include  
9 problem formulation for the current draft assessment since that step will necessarily occur much earlier  
10 in the development of an assessment.

## 11 12 **Recommendations:**

- 13 1. The SAB recommends that the EPA expand the list of databases included in the literature search.  
14 The list of databases included in the literature search strategy is appropriate but not sufficiently  
15 comprehensive for the purpose of systematic review. It appears to mainly be derived from prior  
16 practice at EPA and, as stated in the assessment, from the literature review supporting the ATSDR's  
17 Toxicological Profile for Ammonia (ATSDR, 2004). The SAB agrees with EPA's objective to  
18 reduce unnecessary duplication of efforts across agencies. However, since it is unclear if and to what  
19 extent the ATSDR's toxicological profiles incorporate principles of systematic review to generate  
20 their literature search results, they should not be deemed directly transferable to EPA's assessment  
21 without further clarification. In addition, further explanation is needed as to why only these  
22 databases (Table LS-1, page xxxvii) were deemed sufficient for the purposes of a systematic  
23 literature review. There are additional relevant databases potentially suitable for the ammonia  
24 assessment, in particular important toxicology-specific databases such as EPA's Office of Pesticide  
25 Programs (OPP), Organisation of Co-operation and Development's High Production Volume (HPV)  
26 Chemicals, EPA's HPV database, the Registry of Toxic Effects of Chemical Substances compiled by  
27 NIOSH/Health Canada and European Chemical Agency (ECHA), among others, that were not  
28 considered. The SAB recommends that they be incorporated in the search strategy.
- 29 2. The process of selecting potentially relevant studies needs to be clarified. The initial broad literature  
30 search and some of the operational aspects of the strategy (e.g., timeline, search keywords, search  
31 strings, forward and backward and forward searches, etc.) are fairly well described, consistent with  
32 Section 3.1 of the Preamble and with systematic review principles, and reflect progress towards  
33 implementing NRC's (2011) recommendations. However, the SAB noted that the process for  
34 conducting the subsequent more targeted follow-up searches was unclear. There are no evident  
35 relationships between the series of questions that guided the follow-up searches (as described in  
36 NRC, 2011, page 158, and further expanded in NRC, 2014) and the results of the search sequences  
37 depicted in Figure LS-1 on page xxxviii of the assessment. The narrative description of the selection  
38 process presented on page xxxvi is useful but too general. As a result, the process leading to study  
39 selection shown in Figure LS-1 is not sufficiently informative and leads to confusion. The  
40 explanation for the list of secondary keywords (Table L-S1, footnote c) used to include/exclude  
41 publications following the initial broad search does not provide adequate information on the follow-  
42 up queries and the corresponding inclusion/exclusion criteria. Consequently, the rationale for many  
43 of the inclusion/exclusion criteria used following the secondary keyword searches is difficult to  
44 follow. For example, one of the criteria for excluding publications following the secondary keyword  
45 search is "Co-exposure to other chemicals", but co-exposures are also present in many of the human  
46 studies included and used in the assessment, so this exclusion criterion must have had some specific

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- 1 target(s) at a specific stage in the search that is not discernable by the SAB. Another example is  
2 exclusion because the “Exposure route not relevant” or “Non-standard animal model” which, taken  
3 at face value, could exclude publications potentially relevant for understanding mechanisms of  
4 action. While, as indicated earlier, the SAB realizes that standard protocols for IRIS-specific  
5 systematic review of the literature have not yet been developed, transparency in the ammonia  
6 assessment could be enhanced by adding a table listing key queries with links to Figure 1 and Table  
7 LS-1. This list could be added to Appendix D as a new table or, alternatively, Table D-1 could be  
8 modified to include these questions with links to the search strings in Table D, keywords in Table  
9 LS-1 and inclusion/exclusion criteria in Figure LS-1.
- 10 3. Inclusion/exclusion criteria for studies should be made more transparent. Additional clarification of  
11 inclusion/exclusion criteria may provide some insight as to why some apparently relevant  
12 publications were not included or cited. For example, it is unclear why an epidemiologic study by  
13 Mirabelli and co-authors (2007) that utilized the ECHRS II cohort and included exposure of hospital  
14 nurses to cleaning solutions was either not found in the search or found but excluded. This study  
15 evaluated the risk of new onset asthma in a large cohort of workers that were occupationally exposed  
16 to cleaning solutions containing ammonia. In addition, the SAB encourages EPA to reconsider the  
17 inclusion of publications beyond the March 2013 deadline (e.g., Hovland et al., 2014).
- 18 4. Protocols for literature searches should consider the likelihood of revisiting the literature to address  
19 specific issues not previously foreseen. Not unrelated to the issues discussed above is the need for  
20 revisiting the literature search or undertaking a new one when unforeseen but important questions  
21 arise during the review of key and/or supportive publications. An example, admittedly not an ideal  
22 one, is the evaluation of the findings by Rahman et al. (2007) because of differences between  
23 ammonia concentrations measurements using the PAC III and Draeger diffusion tubes. EPA  
24 contacted Draeger Safety Inc. about the reliability of the two methods in order to select the relatively  
25 more trustworthy set of measurements and followed Draeger’s recommendation. Aside from  
26 expediency, it is not clear whether or not EPA revisited the literature search for publications of  
27 ammonia measurement method comparisons, or indoor and outdoor ammonia measurements, or  
28 scripted activity studies of exposure to ammonia which may have identified suitable publications for  
29 resolving this specific question. These types of publications are not likely to be captured with the  
30 search schemes depicted in Figure LS-1 and Table LS-1 or Appendix D. In addition, it would not be  
31 unusual to find these types of comparisons in the grey literature or in master degree theses. Protocols  
32 for literature searches should incorporate standard approaches to address additional information  
33 needs when this type of situation arises.
- 34 5. Exclusion of ammonium salts should be supported by a systematic review of the relevant literature.  
35 EPA excluded ammonium salts from consideration because of uncertainty about the influence of  
36 different anions on reported effects. The appropriateness of this exclusion is discussed later in detail  
37 in relationship to the RfD derivation. However, it appears that Table C-1 was compiled based on  
38 whatever studies on ammonium salts happened to be readily available, rather than based on a  
39 systematic search for studies. It is possible that this is an incorrect perception, and that Table C-1  
40 includes oral toxicity studies on ammonium salts based on a systematic search and, if so, EPA  
41 should indicate this clearly in the description of search criteria and in Appendix C. The rationale for  
42 excluding ammonium salts could also be buttressed by adding data on LC50’s and LD50’s for  
43 various ammonium salts to show their variability.
- 44 6. The description of studies needs to be made uniform across all types of studies. Apart from the  
45 limitations mentioned above, the selection and evaluation of key studies is fairly well supported. The  
46 table summaries provided in Appendix E-2 are well designed and informative (it would be useful to

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- 1 provide hyperlinks between citations and E-2 summaries in electronic versions of the assessment and  
2 supporting information). EPA identifies the criteria used in the evaluation process [lines 19-21 in  
3 page xxxix (which correspond to most of the criteria summarized on page 158 of NRC, 2011)] and  
4 addresses each criterion separately across categories of studies. Description of key study  
5 characteristics according to the criteria and major limitations are likewise listed in appendices D-2 to  
6 D-4. The same outline is applied to the health care/cleaning and livestock farming settings in pages  
7 xlii-xliii, but not to the industrial studies, so it is recommended that the outline for the narrative be  
8 made uniform, with particular attention paid to describing the range of different co-exposures  
9 presents in the various types of study settings.
- 10 7. The potential contribution to ammonia exposure from tobacco smoke should be described. Smoking  
11 is a typical confounder in many of these studies since it contributes to ammonia exposure as well as  
12 to other co-exposures impacting health outcomes. Therefore, there should be some mention of the  
13 relevant literature on the varying levels of ammonia in tobacco and its presence in cigarette smoke  
14 (in addition to intrinsic levels, tobacco can be treated with ammonia as a means of increasing  
15 nicotine release so ammonia concentrations in cigarette smoke can vary significantly).
- 16 8. EPA should clarify the criteria by which it determines the significance of specific limitations in  
17 studies. A clarification (or citations to relevant guidelines) as to how EPA judges a potential  
18 limitation to be a major one or not should be added. An overall summary of the consistency of  
19 exposures, confounders and outcomes across categories of studies (including relevant findings from  
20 studies summarized in Appendix E.2.3.) would help to further support this section of the assessment.
- 21 9. The criteria by which a study is judged acceptable for assessment purposes should be clearly stated.  
22 Although the narrative provides an evaluation of the studies according to preselected criteria, not all  
23 criteria recommended by the NRC (2011) are incorporated (e.g., precision of the effect) and there is  
24 no specific overall study quality indicator. While it is understood that this is an area still under  
25 development, the application of the study quality criteria for the selection and evaluation of key non-  
26 cancer experimental animal studies that were included in the assessment is unclear. For example, in  
27 the *Hazard Identification* section, some of the studies summarized in Table 1-3 and in Figure 1-1  
28 have inadequate sample sizes and/or substandard reporting of results. The Preamble indicates that  
29 the quality of each individual study is assessed (page xvi), but it does not explicitly state the criteria  
30 on which a study is deemed unacceptable or of low quality for assessment purposes. Although,  
31 studies of low or inadequate quality could be included in the supplementary tables, selection of  
32 pertinent studies for assessment purposes in Table 1-3 and Figure 1-1 should be based on specific  
33 minimal standard criteria for acceptability for assessment purposes. Alternatively, a score for the  
34 quality of the studies presented in Table 1-3 and Figure 1-1 could be included. However, removing  
35 studies that are of inadequate or low quality might streamline EPA's assessment.
- 36
- 37 In developing criteria for ranking the quality of studies, EPA might determine that good quality  
38 studies are those which satisfy minimal EPA guideline requirements for sample size, dose selection,  
39 and control for bias for subjective measures (i.e., OPPTS 870 guidelines; OPPTS, 1998). EPA's  
40 Preamble (section 4.2 and 6) should include reference to these guidelines and not just the guidance  
41 documents as points of comparison. The cited EPA guidance documents do not include guidance for  
42 evaluating the quality of the studies. Other approaches may include the use of a Klimisch scoring  
43 approach (Klimisch et al., 1987) which is widely used by European authorities to evaluate the  
44 quality of studies or other similar schemes.
- 45 10. Finally, it is not clear why EPA did not extend requests for additional data from the public beyond  
46 2009 (lines 17-18, page xxxvi); this should be clarified.

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1  
2 **C. Hazard Identification**

3  
4 Synthesis of Evidence

5 *Charge Question C.1. A synthesis of the evidence for ammonia toxicity is provided in Chapter 1,*  
6 *Hazard Identification. Please comment on whether the available data have been clearly and*  
7 *appropriately synthesized for each toxicological effect. Please comment on whether the weight of*  
8 *evidence for hazard identification has been clearly described and scientifically supported.*

9  
10 **Response**

11 The SAB concluded that in general terms the data included in the assessment have been clearly and  
12 appropriately synthesized for each toxicological endpoint, and that the weight of the evidence for hazard  
13 identification has been adequately described and documented. It should be noted, however, that the  
14 published scientific data available on ammonia toxicity is rather limited for most endpoints. Due to the  
15 age and quality of many of the studies on ammonia, their utility for risk assessment is limited. Having  
16 said that, the scientific evidence for respiratory effects is sufficiently robust to support the conclusion  
17 that ammonia induces these effects in humans and animals. Thus, the SAB concluded that the weight of  
18 the evidence for respiratory effects supports its use as a point of departure for the RfC.

19  
20 While the synthesis of the evidence for ammonia toxicity included in Chapter 1 was presented in an  
21 objective, systematic and concise manner, the core elements considered in the evaluation of the evidence  
22 should be better define. Precise documentation on how the evaluation criteria were applied to individual  
23 studies and ultimately integrated into the weight of the evidence analysis is needed. Importantly, these  
24 revisions should be captured in the tabular summaries included in the chapter. Within this context, a  
25 more detailed description of the Holness et al. (1989) study is warranted in support of the RfC approach,  
26 along with the inclusion of a brief summary statement of the acute and short-term studies in both  
27 animals and humans that identify ammonia as an irritant and toxicant to the upper respiratory tract (and  
28 the eye).

29  
30 The SAB also recommends that the biological bases for tolerance/adaptation be considered as part of the  
31 evaluation, and that gastrointestinal effects of ammonia be re-examined as part of a more integrated  
32 evaluation of the chemistry of ammonia, ammonium hydroxide, and ammonium salts as a function of  
33 pH. For instance, the definition of a no adverse effect level (NOAEL) level based on the responses of  
34 workers chronically exposed to ammonia fumes as described in Holness et al. (1989) should take into  
35 account the tolerance known to occur in humans and animals exposed repeatedly to irritant gases such as  
36 ammonia. Tolerance may lead to underestimation of risk of injury in the nasal and lower respiratory  
37 tracts in humans, if as suggested by animals studies, induction of tolerance to sensory irritation  
38 compromises perception of the presence of injurious concentrations of inhalable irritants (Barrow and  
39 Steinhagen, 1982). The integration of principles of tolerance into the evaluation should be differentiated  
40 from “healthy worker” issues or independent host factors, such as genetics, also known to influence the  
41 response and sensitivity to inhalable irritants.

42  
43 Three additional studies using either animal models or a small size occupational cohort of ammonia  
44 tolerance should be considered for inclusion as part of the analysis, namely:

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- 1) Von Essen, S and Romberger, D. (2003). The respiratory inflammatory response to the swine confinement building environment: the adaptation to respiratory exposures in the chronically exposed worker. *J Agric Saf Health*, 9, 185-196
- 2) LaVinka, PC and Brand, A. (2009). Extreme tolerance to ammonia fumes in African naked mole-rats: animals that naturally lack neuropeptides form trigeminal chemosensory nerve fibers. *J Comp Physiol A*, 195, 419-427.
- 3) Petrova, M, Diamond, J. Schuster, B. and Dalton, P. (2008). Evaluation of trigeminal sensitivity to ammonia in asthmatics and healthy human volunteers, *Inhal Toxicol*, 20, 1085-1092.

### Summary and Evaluation

*Charge Question C.2. Does EPA’s hazard assessment of noncancer human health effects of ammonia clearly integrate the available scientific evidence (i.e., human, experimental animal, and mechanistic evidence) to support the conclusion that ammonia poses a potential hazard to the respiratory system?*

#### **Response**

The scientific evidence supporting the conclusion that ammonia poses a potential hazard to the respiratory system is well-integrated. As noted earlier in this report, a more detailed evaluation of the chemical reactions and ammonia generation that may impact gastrointestinal endpoints is required, particularly as it relates to the decision of not deriving an RfD.

*Charge Question C.3. Does EPA’s hazard assessment of the carcinogenicity of ammonia clearly integrate the available scientific evidence to support the conclusion that under EPA’s Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is “inadequate information to assess the carcinogenic potential” of ammonia?*

#### **Response**

In general, the conclusion that there is inadequate information to assess the carcinogenic potential of ammonia is supported by the scientific evidence reviewed. It should be noted that the dated study by Toth et al. (1972) reporting no increases in tumor incidence in Swiss or CH3 mice administered ammonium hydroxide was significantly limited by the lack of adequate controls and insufficient experimental details. While the SAB agrees that the evidence presented by Tsujii et al. (1993) suggesting ammonia exhibits tumor-promoting properties is insufficient, the strengths and weaknesses of three potentially relevant lines of evidence should be considered as part of the evaluation. These are: 1) an epidemiologic study regarding promoter influences (Fang et al., 2011), 2) an early animal study reporting increased numbers of adenocarcinomas following delivery of ammonium acetate via intra-rectal infusions (Clinton et al., 1988); and 3) the clinical experience of high serum levels of ammonia not associated with cancer (Cooper, personal communication).

### **D. Oral Reference Dose (RfD)**

*Charge Question D.1. Please comment on whether the rationale for not deriving an RfD is scientifically supported and clearly described (see Section 2.1). Please comment on whether data are available to support the derivation of an RfD for ammonia. If so, please identify these data.*

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1 **Response**

2 Although there is a fairly extensive literature on the systemic and organ-specific effects of ammonia  
3 (e.g., liver, brain, and kidney) with inhalation exposure, there are no controlled animal studies of the  
4 systemic effects of ammonia [not ammonium salts] through the ingestion route of exposure, and reports  
5 of systemic effects in humans with ingestion are confined to case reports of poisonings and accidental  
6 ingestion. Only studies using various ammonium salts have been performed as it may be that ammonia  
7 itself cannot be feasibly studied via ingestion. Therefore, EPA appropriately did not attempt to derive an  
8 RfD for such systemic effects.

9  
10 There are, however, studies suggesting gastrointestinal effects (gastric mucosal thinning, compensatory  
11 cell replication of the cells of the gastric mucosa). EPA confined its consideration of ammonia-related  
12 gastrointestinal effects to three studies (Tsuji et al. (1993); Kawano et al., (1991); and Hata et al.  
13 (1994)) based on the criterion that these were the only controlled (and non-acute) studies of  
14 gastrointestinal effects that employed ammonia (i.e., NH<sub>3</sub>) *per se*. EPA intentionally excluded from  
15 consideration studies of gastrointestinal effects (or the lack thereof) with oral administration of  
16 ammonium (NH<sub>4</sub><sup>+</sup>) salts. This decision was based on concerns that the possible adverse effects of  
17 ammonia in such studies could not be separated from adverse effects resulting from the associated anion.  
18 This rationale needs further clarification, evaluation and justification than is currently provided in  
19 Appendix C of the ammonia assessment.

20  
21 The SAB concluded this dichotomy between ammonia *per se* and ammonium salts with respect to  
22 ingestion was based, in part, on a misunderstanding of the chemistry of ammonia and ammonium (the  
23 basic chemistry of ammonia needs to be addressed in a separate introductory section). Ingested ammonia  
24 almost instantaneously becomes the ammonium ion in an aqueous solution, such as that present in the  
25 stomach. In the three studies that EPA considered as a possible basis for an RfD based on effects on the  
26 gastric mucosa, it is not clear whether ammonia gas was bubbled through water to make the dilute  
27 “ammonia” solution that the rats consumed, or whether ammonium salts (most likely NH<sub>4</sub>Cl) were  
28 dissolved in the water. The SAB recommended that EPA attempt to contact the investigators to clarify  
29 this question. In either case, however, the resulting aqueous solution likely contained predominantly  
30 NH<sub>4</sub><sup>+</sup>, and a much smaller concentration, typically 50+-fold lower, of NH<sub>3</sub>. Furthermore, even if the  
31 solution was made by the addition of ammonium salts, such as NH<sub>4</sub>Cl, the NH<sub>4</sub><sup>+</sup> would be present as the  
32 free ion in the gastric fluid of the stomach. Thus, while a possible independent gastrointestinal (GI)  
33 toxicity of the anion may be a valid concern, the dichotomy between ammonia and ammonium salts in  
34 the consideration of an RfD is not.

35  
36 The SAB agreed that there is evidence that the anion (chloride, acetate, sulfate) can impact toxicity of  
37 the ammonium salt on organs such as the kidney, liver and adrenal gland based on the published papers  
38 available to EPA SAB (e.g. Lina and Kulpers, 2012, Satpute et al. 2014, Ota et al. 2006). This supports  
39 EPA’s decision not to include toxicity of ammonium salts to derive reference values for ammonia or  
40 ammonium hydroxide. However, the EPA’s report needs to be strengthened to support this conclusion.  
41 The main report references Appendix C for evidence that the anion impacts the toxicity of the  
42 ammonium salt, yet there is insufficient information in Appendix C to make comparisons across  
43 ammonium salts because the negative findings of key organs are not reported. Reporting the negative  
44 results is also necessary to support EPA’s discussion of ammonium’s effect on the thickness of the  
45 gastric mucosal layer. The EPA draft assessment correctly states that there are no effects in the stomach  
46 or other parts of the GI tract following chronic exposure to either ammonium chloride or ammonium

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1 sulfate, and references Appendix C, Table C-1. Yet Appendix C and Table C-1 does not report the  
2 negative results. Table 1 below provides an example of how results could be presented so that it is clear  
3 if the organs were examined and the outcomes, which will allow the comparisons across ammonium  
4 salts to be more systematic and transparent.

5  
6 The gastric mucosal thinning observed by Tsjui et al. (1993) and Kawano et al. (1991) with sub-chronic  
7 exposure was not clearly progressive and was not accompanied by observed micropathology. The SAB  
8 discussed the nature of this effect at some length and particularly whether there was a biological basis  
9 for assuming that this effect could be progressive with chronic exposure. This question could not be  
10 resolved with studies on ammonia alone given the lack of knowledge about the nature of the effect, itself  
11 and the lack of chronic exposure data. However, the SAB noted that EPA had included in Appendix C a  
12 description of the study of Lina and Kuijpers (2004), a robust rat study of chronic exposure to NH<sub>4</sub>Cl  
13 with comprehensive pathology evaluation in which the dose was somewhat higher than those in the  
14 three studies that EPA more formally considered. In addition, the study design also included a Cl<sup>-</sup>  
15 control group. The SAB obtained a copy of this paper and noted that the results do not appear to show  
16 gastric pathology. Given both the chemistry of ammonia/ammonium as discussed above and the  
17 presence of a control group in this study that appears to address potential concerns about the toxicity of  
18 the anion, this study does support EPA's conclusions (1-20 and 2-1) that the data does not support  
19 deriving an oral RfD based on GI findings. It is not clear why EPA did not more fully address this study.  
20 Furthermore, there appears to be a number of animal studies of oral exposure to ammonium salts that, if  
21 EPA decides to address the ammonium ion along with ammonia in this assessment, could additionally  
22 inform consideration of gastrointestinal effects.

23  
24 The negative findings that contribute to the weight of evidence are not adequately described in  
25 Appendix C or in the weight-of-evidence evaluation in section 1.2.1 of the ammonia assessment.  
26 Appendix C Table C-1 can be improved by expressing concentration of the ammonium salts as molar  
27 concentrations of ammonia compound and by reporting both negative and positive findings for key  
28 organs of concern.

29  
30 In conclusion, the SAB agrees that EPA should evaluate the publications more completely to determine  
31 if they should continue to exclude ammonium salts from the IRIS assessment, or explicitly expand the  
32 scope of the assessment to include the ammonium ion with ammonia. In either case, EPA's rationale  
33 and presentation of data to support their conclusions need to be strengthened. The SAB concluded that  
34 EPA should evaluate the relevant toxicity studies that use ammonium salts to determine if they can offer  
35 valuable information for the derivation of an RfD. If the effects of the anion cannot be discerned, the  
36 decision to exclude ammonium salts will be buttressed by the evaluation of these studies. The SAB also  
37 noted that a decision to address ammonium salts would also require further evaluation of the RfC and  
38 the impact of the inhalation of ammonium particulate.

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1

2 Table 1. Example Table of effect of ammonium chloride on selected non-neoplastic histopathology for Appendix C

	<b>Exposure level and duration</b>	<b>mmol/kg body weight/day</b>	<b>Adrenals</b>	<b>Liver</b>	<b>Kidney</b>	<b>Stomach</b>	<b>Brain</b>
<b>Ammonium chloride Diet (Lina and Kuijpers, 2004)</b>	2.1% and 4% 4 week N=10/sex	M: 41, 80 F: 42, 78	Zona glomerulosa hypertrophy (a) 0%: 1/20 4%: 3/20 KCl: not examined	No effect	No effect on severe or (total) tubular nephrosis (b) 0%: 0/20 severe (6/20 total) 2.1%: 0/10 (1/10) – F only 4%: 0/20 (3/20) KCl: not examined	Not examined	Not examined
and KCl as “neutral diet”, which had no effect on acid-base balance	2.1% and 4% 13 week N=10/sex	M: 30, 57 F: 34, 69	Zona glomerulosa hypertrophy (a) 0%: 1/20 2.1%: 2/20 4%: 14/20** KCl: 5/20	No effect	No effect on severe or (total) tubular nephrosis (b) 0%: 0/20 severe (13/20 total) 2.1%: 0/10 (7/20) 4%: 0/20 (9/20) KCl: 0/20 (12/20)	No effect	No effect
Mmol/kg based on measurement of food consumption	1% and 2.1% 18 month N=15/sex	M: 9, 19 F: 11, 26	Zona glomerulosa hypertrophy (a) 0%: 0/30 1%: 0/30 2.1%: 4/30 KCl: 7/30	No effect	No effect on severe or (total) tubular nephrosis (b) 0%: 0/30 severe (20/30 total) 1%: 0/30 (17/30) 2.1%: 1/30 (23/30) KCl: 1/30 (17/30)	No effect	No effect
	1% and 2.1% 30 month N=50/sex	M: 9, 19 F: 20, 23	Zona glomerulosa hypertrophy (a) 0%: 0/100 1%: 21/100* 2.1%: 65/100** KCl: 54/100**	No effect	↓ incidence severe; no effect (total) tubular nephrosis (b) 0%: 25/100 severe (72/100 total) 1%: 22/100 (80/100) 2.1%: 15/100* (72/100) KCl: 18/100 (75/100)	No effect	No effect

3

(a) Incidence for males and females were combined, \* P<0.05; \*\* P<0.01 compared to concurrent control reported if either male or female were significant

4

(b) Incidence for severe or very severe nephrosis. In brackets the total incidence of tubular nephrosis is presented. \* P<0.05; \*\* P<0.01 compared to concurrent control reported if either male or female were significant

5

6

7

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1  
2 *Charge Question D.2. As described in the Preface, data on ammonia salts were not considered*  
3 *in the identification of effects of the derivation of an RfD for ammonia and ammonium hydroxide*  
4 *because of concerns about the potential impact of the counter ion on toxicity outcomes. Please*  
5 *comment on whether the rationale for this decision is scientifically supported and clearly*  
6 *described.*

7  
8 **Response**

9 Due to the lack of clarity about the chemistry of ammonia/ammonium, and given the existence of at least  
10 one study of ammonium that appears to have adequately controlled for the possible toxicity of the  
11 counter ion (Lina and Kuijpers, 2004), the SAB concluded that the rationale for the decision not to  
12 derive an RfD for ammonia should be further expanded.

13  
14 **Other studies for consideration relevant to the selection of the RfD**

15 There are additional reports in the literature in which chronic gastrointestinal administration of ammonia  
16 to experimental animals resulted in elevated levels of circulating ammonia. It is of interest that the  
17 concentration of ammonia in the colon lumen is extraordinarily high (15 – 44 mM) (Worrell et al. 2008).  
18 This value is much higher than that normally present in the portal vein (0.2 – 0.3 mM) and in the blood  
19 (<40 µM). The colonocytes also possess NH<sub>4</sub><sup>+</sup> transporters. Thus, the colon is well suited to process  
20 relatively large amounts of ammonia. Nevertheless, excessive ammonia can be damaging to parts of the  
21 gastrointestinal system. For example, excess ammonia can cause apoptosis in rat gastric mucosal  
22 epithelium, possibly as a result of a drain on ATP resulting from excessive conversion of ammonia to  
23 glutamine in these cells (Kubota et al. 2004). It is also well known that *Helicobacter pylori* produces  
24 copious amounts of ammonia (in part as a result of the action of urease) to counteract the acidity of the  
25 stomach; this excess ammonia may contribute to the gastritis often associated with *H. pylori*  
26 (Lichtenberger et al. 1995).

27  
28 As noted in the assessment, accidental or deliberate ingestion of ammonia-containing solutions/foods in  
29 humans has occasionally been reported in the literature. These studies have described deleterious  
30 gastrointestinal effects. However, the draft assessment does not consider the possibility that ingested  
31 ammonia may result in elevated blood ammonia levels and possible neurological consequences.  
32 Nevertheless, because of the diffusibility of ammonia and the presence of NH<sub>4</sub><sup>+</sup> transport systems in the  
33 colon (Worrell et al.. 2008) it is probable that at least some of the ingested ammonia enters the  
34 circulation in humans orally exposed to ammonia (as was noted in the study of Satpute et al. (2014) in  
35 rats alluded to above). Thus, in humans who have ingested ammonia solutions reported nausea, drooling  
36 and erythematous/edematous effects on the lips could be considered as systemic effects.

37  
38 Pilbeam et al. (1983a, b) gavage-fed ammoniated cation exchange resin to normal rats and rats with a  
39 portacaval anastomosis (PCA; a model of chronic liver disease). The slow release of ammonia from the  
40 resin simulates chronic hyperammonemia. Marked hyperammonemia was noted in the animals  
41 administered the ammoniated resin, especially in the PCA rats. Severe neurological symptoms were  
42 noted in the PCA rats administered the ammoniated resin. Damage not only to astrocytes, but also to  
43 some oligodendrocytes and neurons, was noted with nuclear and cytoplasmic swelling (Pilbeam et al.  
44 1983a). Rats with a PCA fed ammoniated resin showed increased chloride content and Na<sup>+</sup>:K<sup>+</sup> ratio in  
45 the brainstem, and an increased chloride space in the brainstem (Pilbeam et al.. 1983b). In other studies  
46 Grisolia and colleagues administered ammonium acetate (20% w/w) in the diet of rats to generate a

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1 simple model of chronic hyperammonemia (Azorin et al., 1989). The concentration of ammonia in the  
2 blood of these animals was increased threefold and there were marked increases of ammonia in brain,  
3 liver and muscle. Urea excretion increased two fold and brain glutamine increased twofold. In other  
4 studies from this group it was shown that chronic ammonium acetate in the diet of rats altered the  
5 mitochondrial ratio  $NAD^+/NADH$  in the brain (Kosenko et al., 1993).

6  
7 In an interesting corollary, Bosoi et al. (2011) showed that gavage administration of a spherical carbon  
8 absorbent (AST-120) to hyperammonemic rats with ligated bile-ducts resulted in a *decrease* of  
9 circulating ammonia levels and attenuation of brain edema. This study is consistent with the hypothesis  
10 that intestinal-derived ammonia can be a contributing factor to hyperammonemia.

11  
12 Thus, there is strong evidence that following *chronic* oral/intestinal administration of ammonia, levels  
13 of ammonia in the blood and glutamine in the brain are greatly increased in experimental animals and  
14 people with impaired liver function. Elevation of brain glutamine in humans via chronic exposure to  
15 oral ammonia may be of potential concern. However, there are no published case histories where a  
16 patient has been subjected to chronic ammonia administration via the oral route beyond studies where  
17 people are exposed for several days (i.e., acid-loading) to determine how the kidney responds to  
18 acidosis. On the other hand, since there are many documented cases of acute ammonia exposure through  
19 the oral route, it would be important to determine whether *acute* oral exposure to ammonia will result in  
20 elevated ammonia in the circulation that has the potential to deleteriously alter brain nitrogen  
21 homeostasis in humans. But there appear to be no relevant published animal studies, and the published  
22 human studies have been more concerned with the gastrointestinal effects than with possible  
23 neurological outcomes. There are studies described in the literature where acute liver failure results in  
24 elevated blood ammonia and encephalopathy. For example, acetaminophen overdose leads to rapid  
25 increases in blood ammonia, followed by coma (e.g. Brusilow and Cooper 2011). Valproate is a widely  
26 used, generally safe drug used in the treatment of epilepsy and some neuropsychiatric disorders. On rare  
27 occasions, however, the drug can precipitate acute hyperammonemic encephalopathy (reviewed by  
28 Lewis et al., 2012). The point to be made here is that acute elevations of blood ammonia can induce  
29 coma in humans. Thus, if acutely ingested ammonia is sufficiently concentrated the possibility exists  
30 that enough ammonia will enter the circulation to deleteriously affect the brain.

31  
32 In conclusion, acute oral administration of ammonia is well documented to cause gastrointestinal effects  
33 in humans and experimental animals. However, there are no detailed studies of the effect of acute oral  
34 ammonia dosing on brain function. Nevertheless, it is likely that acute oral dosing will lead to increased  
35 blood ammonia in individuals with abnormal liver function. It is well documented that acute  
36 hyperammonemia from whatever cause can lead to brain dysfunction in those individuals.

### 37 38 **E. Inhalation Reference Concentration (RfC)**

39  
40 *Charge Question E.1. Please comment on whether the evaluation and selection of studies and*  
41 *effects for the derivation of the RfC is scientifically supported and clearly described (see Section*  
42 *2.2.1). Please identify and provide the rationale for any other studies or effects that should be*  
43 *considered.*

### 44 45 **Response**

46 The evaluation of studies is clearly described in the supplementary materials, and concisely summarized

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1 in the main assessment. Although the selection of studies and effects for the RfC is mostly clear and the  
2 Holness et al. (1989) study appears to be the most appropriate for RfC derivation, exclusion of the  
3 controlled human exposure studies is not well explained. These studies have several methodological  
4 strengths such as, well-characterized exposures and resistance to confounding. Clarification as to why  
5 they are excluded as candidates for RfC derivation is needed. For example, it may be that they were  
6 excluded due to the use of short-term exposures.

7  
8 EPA has indicated that the 1989 Holness study has the strongest exposure assessment, and provides a  
9 clear argument in support of that judgment. Other studies are available but it is unclear if the quality of  
10 exposure assessment overrides the other factors listed in the Preamble for selection of a key study. For  
11 example, the Ballal et al. (1998) and Rahman et al. (2007) studies appear to report results from  
12 epidemiological models for exposure on a continuous scale; these studies could be used to derive BMDs,  
13 which the Preamble indicates is preferred over the NOAEL/LOAEL approach. The role in study  
14 selection of any differences in outcome measures and of confounding controls among these studies is  
15 also unclear.

16  
17 Some panel members expressed concern about the selection of self-reported respiratory symptoms and  
18 small subclinical changes in lung function measures as "adverse" health outcomes, requesting that EPA  
19 elaborate on its rationale. For example, coughing and sneezing are relatively mild compared to many  
20 other adverse health outcomes used in RfC derivation for other chemicals [add examples]. In addition,  
21 the use of pre-shift to post-shift comparisons in some of the studies suggests that these health outcomes  
22 may be reversible overnight, at least in part. The SAB therefore recommends that further discussion of  
23 the potential implications of reversibility and long-term attenuation of effects through acclimatization  
24 and/or the healthy worker effect (e.g., self-selected attrition due to respiratory symptoms) be added. The  
25 different studies have different goals and thus different designs. These are issues that need to be  
26 mentioned, but they cannot be used quantitatively and would lead to underestimates of effect (e.g., a  
27 healthy worker effect).

28  
29 The SAB also noted a News and Analysis article that appeared in a recent issue of Science (Stokstad,  
30 2014). According to the authors of the original study (Paulot and Jacob 2014), ammonia gas emanating  
31 from farming practices can form aerosols that adversely affect human health. The assessment only  
32 considered worker exposure to *gaseous* ammonia; a brief discussion of the possible deleterious effects  
33 of air-borne *particulate* ammonia should be added.

34  
35 *Charge Question E.2- The NOAEL/LOAEL approach was used to identify the point of departure*  
36 *(POD) for derivation of the RfC (see Section 2.2.2). Please comment on whether this approach is*  
37 *scientifically supported and clearly described.*

38  
39 **Response**

40 The approach is reasonable and clearly described, but it is not clear to what extent EPA considered  
41 continuous dose-response modeling for that study or for other available studies. EPA should attempt to  
42 obtain individual-level data and/or the mean/median exposure concentrations for the high dose group  
43 from Dr. Holness in order to identify a better-supported point of departure (overcoming the limitation of  
44 having only the exposure range in her published manuscript). Individual data would also allow for  
45 direct determination of the individual NOAEL via the NOSTASOT procedure (Tukey, 1985),  
46 combination of the various respiratory responses (e.g., the adverse outcome could be defined as one or

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1 more respiratory systems rather than modeling each symptom separately), and continuous dose-response  
2 modeling.

3  
4 Considering that no significant adverse effects were found in any dose group in the Holness study, the  
5 highest exposure concentration at which effects do not occur is most likely greater than the minimum  
6 ammonia concentration in the highest exposure group (8.8 mg/m<sup>3</sup>). One reason to prefer a central  
7 estimate (i.e., mean or median) of the high exposure group ammonia concentration rather than the  
8 minimum is that the reported range of concentrations is only for a single 8.5-hour work shift. Because  
9 day-to-day exposure concentrations typically vary extensively in occupational settings, one should  
10 expect the minimum concentration (the basis of EPA's NOAEL) to be highly unstable and to be different  
11 if the study were conducted on a different day. In contrast, the arithmetic mean exposure concentration  
12 would be much more stable with repetition of the study due to the central limit theorem and shrinkage of  
13 each participant's estimated chronic exposure level towards the grand mean. If individual data are  
14 unavailable, EPA should consider whether there is sufficient information available in the Holness  
15 publication to estimate the mean concentration for the high exposure group--perhaps assuming a  
16 lognormal or other skewed distribution for the measured concentrations.

17  
18 For the point of departure (POD) derived from the Holness study, a dose conversion factor was used to  
19 convert the observed workplace ammonia concentration to an ammonia concentration that would  
20 provide an equivalent cumulative dose with continuous 24/7 exposure. This presumes that the reported  
21 respiratory and lung function effects of ammonia are due to cumulative exposure rather than acute  
22 exposure, but it's not clear to what extent that assumption is supported by evidence. There is some  
23 support provided in Table 3 of the Ballal et al. (1998) study, which indicates significant effects of  
24 exposure duration on risks of cough, phlegm, and wheezing (even when accounting for the exposure  
25 concentration). This evidence of a cumulative effect of ammonia exposure is important, especially if  
26 corroborated by other studies.

27  
28 One panelist noted that it's unclear why EPA has assumed inhalation rates of 10 m<sup>3</sup> of air per 8-hour  
29 work shift (1.25 m<sup>3</sup>/hour) and 20 m<sup>3</sup> per 24-hr day by the general population. EPA cites the 2011 EPA  
30 Exposure Factors Handbook, but the recommended values differ in that document, e.g. means of 11.3  
31 m<sup>3</sup>/day for women, 15.2 m<sup>3</sup>/day for men, 1.0 m<sup>3</sup>/hr for light activities, and 1.6 m<sup>3</sup>/hr for moderate  
32 activities (Table 5-23). If 20 m<sup>3</sup>/day is meant to be an upper bound, it would be useful for EPA to cite  
33 its data source and to discuss whether incorporation of this aspect of interindividual pharmacokinetic  
34 variability at the NOAEL determination stage has implications for later selection of an uncertainty  
35 factor.

36  
37 *Charge Question E.3. Please comment on the rationale for the selection of the uncertainty*  
38 *factors (UFs) applied to the POD for the derivation of the RfC (see Section 2.2.3). Are the UFs*  
39 *appropriate based on the recommendations described in Section 4.4.5 of A Review of the*  
40 *Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), and clearly*  
41 *described? If changes to the selected UFs are proposed, please identify and provide scientific*  
42 *support for the proposed changes.*

43  
44 **Response**

45 The selection of the uncertainty factor was appropriate, clearly described, and consistent with the 2002  
46 EPA recommendations. There was some discussion on whether the critical effect was related more to

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1 irritation rather than inflammation (suggesting a smaller uncertainty factor for human variability because  
2 of less variation in toxicokinetics). The SAB ultimately judged that the critical effect was not clearly  
3 irritation, supporting EPA's choice of the default uncertainty factor of 10. Moreover, the healthy worker  
4 effect, perhaps operating in the Holness study, would also support this default value.  
5  
6

7 **Additional Comments for consideration on the selection of an RfC**

- 8 1. Hemoptysis (coughing up blood) was also seen in the Ballal et al. (1998) study, which should be  
9 mentioned in EPA's study description.
- 10 2. In Table 3 in the Ali et al. (2001) study, the direct comparison between the exposed and the  
11 control groups, only the FEV1 % (Forced Expiratory Volume) is higher in the exposed group  
12 than in the controls – this appears to be a beneficial effect and should be noted in EPA's study  
13 description, though the authors noted that it could be a result of different smoking rates among  
14 the controls and the exposed workers.
- 15 3. The small sample size (N=2 and 4) should be highlighted for the Anderson et al. (1964) study.  
16 Because of this, less weight should be placed on the results of this study.
- 17 4. Seeman and Carchman (2008) reports on ammonia exposure from cigarette smoke exposure.  
18 This study is highly suggestive that habituated cigarette smokers need to be separated from non-  
19 smoking groups, not just for respiratory effects of smoking, but additionally due to potential  
20 supplemental exposure to ammonia due to its contamination within tobacco products.
- 21 5. The study by LD Calvert et al. (2009) might be a helpful starting point if there is merit in  
22 characterizing baseline plasma levels of ammonia expected across different populations at risk.
- 23 6. There is virtually no difference in pre-shift values between the ammonia and urea plants in the  
24 Rahman et al. (2007) study, indicating that the effects that were measured in this study are  
25 primarily acute effects of exposure that appear to be reversed overnight. This provides  
26 additional evidence supporting EPA's selection of the NOAEL, because the effects reported at  
27 the Rahman et al. LOAEL appear to be acute effects of exposure.
- 28 7. The addition of an evidence table to Section 2.2.1 is recommended (see Table 2 below for  
29 example).
- 30 8. EPA could strengthen the justification for selection of the higher control levels of exposure from  
31 Holness et al. (1989) by adding a brief description and explanation of outcomes in the Rahman et  
32 al. (2007) study. For example, the magnitude of change in FEV1 and FVC (Forced Vital  
33 Capacity) are relatively small compared to changes that might be of concern in a clinical setting  
34 (i.e., difference of 200 ml). In addition, clarification that the respiratory symptoms (e.g. cough  
35 and chest tightness) are self-reported adds perspective. It might also be useful to include a brief  
36 discussion of potential co-exposures to other materials/chemicals with similar respiratory effects  
37 (e.g. formaldehyde, particulate dust, sodium carbonate).

**Science Advisory Board (SAB) Draft Report (November 14, 2014) to Assist Meeting Deliberations**

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Table 2. Example summary table for information useful to section 2.2.1 for deriving the Inhalation RfC

	Exposure and analytical methods	Sample size	EPA's selection of "NOAEL" or "LOAEL"	Outcome	Factors that can influence outcome	Factors that can influence outcome that were controlled for
Holness et al (1989)	<p>NIOSH collection of air samples for each individual on acid-treated silica gel absorption tubes – <i>medium confidence</i></p> <p>8.4 hr collection period over one shift</p> <p>Cumulative exposure=exposure multiplied by years of exposure</p>	<p>Control =31 plant workers (0.2 mg/m<sup>3</sup>)</p> <p>Exposed = 58 which were divided into low (&lt;4.4 mg/m<sup>3</sup>;n=34) medium (n=12 4.4-8.8) and high (&gt;8.8 n=12) exposure without explanation for cut-offs</p> <p><i>Low sample size when dividing into 3 groups</i></p>	<p>NOAEL = highest exposure group &gt;8.8 mg/m<sup>3</sup></p> <p>Individual values needed to calculate mean or median of the high exposure group</p>	<p>No effect on lung function and self-report symptoms using ATS questionnaire</p> <p>Lung function reported as percent predicted values rather than actual volume</p> <p>No clinical exam of worker</p>	<p>Sodium ash dust was considered an aggravating factor that was not controlled for.</p> <p>Fewer self-reports of hay fever (self-selected out of work place)</p>	
Rahman et al. (2007)	<p>Drager PACIII and Drager tube for 1 worker/day shift (so personal monitoring for only one person per shift group).</p> <p>PACIII considered to be more reliable but much lower (1/3 or 1/4) than Drager tube measures</p> <p><i>low confidence</i></p>	<p>Administration = 25 (&lt;LOD)</p> <p>Ammonia plant = 24 (4.9 mg/m<sup>3</sup> ; range 2.8-11.1 mg/m<sup>3</sup>)</p> <p>Urea plant =64 (18.5 mg/m<sup>3</sup> ; 9-31 mg/m<sup>3</sup>)</p> <p><i>Low sample size</i></p>	<p>NOAEL= 4.9 mg/m<sup>3</sup> from ammonia plant (range 2.8-11.1 mg/m<sup>3</sup>)</p> <p>LOAEL = 18.5 mg/m<sup>3</sup> (range 9-31 mg/m<sup>3</sup> )</p>	<p>Effect on 2/5 self-report acute respiratory symptoms (cough and chest tightness)</p> <p>Decrease of 100 ml and 55 ml for FVC and FEV1, respectively, when comparing post-shift with pre-shift in UREA PLANT.</p> <p>Decrease of 200 ml is considered clinically relevant on an individual basis.</p>	<p>The numerical differences in pre-shift lung function between urea and ammonia plant is small, suggesting that the effects measured are acute and not chronic effects of exposure.</p> <p>Nitrogen dioxide was monitored but not included as a factor in the statistical analysis</p> <p>Dust and Sulfur dioxide were discussed as potential factors but not measured</p> <p>Formaldehyde?</p>	<p>Lower percentage of smoker in urea group than ammonia group, but comparable to administration control group. Analysis of Lung function controlled for smoking in the linear regression model.</p> <p>Analysis of acute respiratory symptoms was conducted with and without current smokers and those with previous respiratory diseases excluded</p>

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### 1 **Additional studies on neurotoxic effects resulting from inhalation exposure of ammonia**

2 While inhalation studies do not provide evidence for the neurotoxicity of ammonia in humans (draft  
3 review, p I-27) there is considerable evidence that systemic administration of ammonia produces a  
4 neurotoxic response in experimental animals. As mentioned earlier, the ammonia assessment suggests  
5 that administration of ammonium salts can be problematic due to the confounding effects of the counter  
6 anion. For example, if ammonium chloride is administered systemically the chloride ion may have a  
7 deleterious effect on acid-base balance that is directly related to the ammonium ion. For this reason,  
8 researchers studying the neurotoxic effects of ammonia in experimental animals usually administer  
9 ammonium acetate on the assumption that the acetate is rapidly metabolized to CO<sub>2</sub>. The ammonium  
10 acetate is most often administered by an intraperitoneal route (e.g. Hindfelt et al., 1977), but sometimes  
11 by arterial infusion (Voorheis et al., 1983). However, in a study conducted by Satpute et al. (2014)  
12 experimental animals exposed to 100 mg/kg ammonium acetate exhibited significant signs of toxicity in  
13 liver, kidney and brain that are not observed in the same 1 month duration or much longer chronic  
14 exposures following oral exposure to ammonium chloride. This would also suggest that at some dose  
15 level the acetate is causing acid-base imbalance. Furthermore, a study by Luna and Kuijpers (2004)  
16 found no effects in the brain following 13 weeks of exposure to 2.1-4% solutions of ammonium chloride  
17 and 30 months of exposure to 1-2.1% solutions of ammonium chloride (see Table 1 above). This study  
18 also had a Cl<sup>-</sup> control group.

19  
20 [Dose, Route and duration of exposure needs to be included in the following paragraphs]

21 It has long been known that acute (or subacute) hyperammonemia in experimental animals is associated  
22 with stupor, seizures and coma (e.g. Navazio et al., 1961). By the 1970s it was realized that inborn  
23 errors of the urea cycle result in elevated levels of ammonia that are devastating to the infant human  
24 brain (Shih 1976). The longer the period of neonatal hyperammonemia in children with defects of the  
25 urea cycle, the greater the neurological impairment in the survivors (Msall et al., 1984).  
26 Hyperammonemia is now considered a major factor contributing to the encephalopathy associated with  
27 both acute and chronic liver disease (hepatic encephalopathy, HE). [

28  
29 Why is hyperammonemia so deleterious to the brain? In EPA's draft assessment (p. I-27), it was  
30 suggested that glutamate and  $\gamma$ -aminobutyrate play a role in ammonia-induced toxicity. A role for  
31 GABA in ammonia-induced HE has been suggested where increased brain ammonia increases the  
32 GABA-induced chloride channel current and affects the benzodiazepine receptors in neurons and  
33 astrocytes. According to a more recent review by Jones and Mullen (2012) "Evidence of increased  
34 GABAergic tone in models of HE has accumulated; potential mechanisms include increased synaptic  
35 availability of GABA and accumulation of natural benzodiazepine receptor ligands with agonist  
36 properties. Pathophysiological concentrations of ammonia associated with HE, have the potential of  
37 enhancing GABAergic tone by mechanisms that involve its interactions with the GABA<sub>A</sub> receptor  
38 complex". Other studies have suggested that hyperammonemia associated with liver disease may  
39 compromise energy metabolism, but the changes appear to be subtle (reviewed by Ott and Vilstrup  
40 2014). However, most recent studies of ammonia-induced neurotoxicity have focused on excessive  
41 production of glutamine in the brain. Since ammonia enters the brain largely by diffusion (Cooper et al.,  
42 1979; Lockwood et al., 1980; Raichle and Larson, 1981) increased circulating ammonia in  
43 hyperammonemic syndromes is expected to result in increased entry of ammonia into the brain. This  
44 has been confirmed in PET studies with [<sup>13</sup>N]ammonia in hyperammonemic patients with cirrhosis of  
45 the liver (Keiding and Pavese, 2013). As discussed by Cooper and Plum (1987) brain glutamine

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1 synthetase is probably not saturated with ammonia. Thus the rate of synthesis of cerebral glutamine is  
2 likely to increase in hyperammonemic syndromes with pathological consequences (as outlined below).

3  
4 A clue implicating excess glutamine production in the neurotoxic response in hyperammonemic  
5 encephalopathy is the finding that, unlike most neurological diseases, hyperammonemia results in  
6 damage that is largely confined to the astrocytes and not to neurons (Norenberg 1976). Interestingly, in  
7 the brain, glutamine synthetase is confined almost exclusively to astrocytes (Martinez-Hernandez et al..  
8 1977; Norenberg and Martinez-Hernandez 1979). Thus, astrocyte end feet are uniquely poised to  
9 “intercept” ammonia entering the brain by diffusion across the blood-brain barrier and to incorporate  
10 this ammonia into glutamine. However, this arrangement comes at a price. Most investigators now  
11 believe that a major contributor to the neurotoxicity of excess ammonia is the associated increased levels  
12 of glutamine in astrocytes. Increased glutamine in these cells results from stimulation of glutamine  
13 synthetase perhaps coupled to an ammonia-induced decrease in glutamine egress from astrocytes via the  
14 SNAT-5 transporter (Desjardins et al., 2012). Persuasive evidence for a role of excess cerebral  
15 glutamine in ammonia-induced encephalopathy is the finding that methionine sulfoximine, a potent  
16 inhibitor of glutamine synthetase, protects rodents against neurotoxic doses of ammonia (reviewed by  
17 Brusilow et al., 2010). Brusilow and colleagues have argued that the major insult to the brain in  
18 hyperammonemic syndromes is excess production of glutamine producing an osmotic stress in  
19 astrocytes (the osmotic gliopathy theory) (Brusilow et al. 2010 and references cited therein). Certainly,  
20 neural swelling as a result of osmotic stress occurs during hyperammonemia, especially during acute  
21 liver failure, and this swelling can be detected in the brains of hyperammonemic HE patients by  
22 magnetic resonance (MR) imaging techniques. For example, Mardini et al. (2011) used MR to  
23 investigate the cerebral water content of 13 cirrhotic patients confronted with an ammonia challenge.  
24 The authors concluded that ammonia can directly drive changes in brain water distribution as a  
25 mechanism for cerebral edema development. Since cerebral astrocytes contain glutamine synthetase, the  
26 MR data suggest intracerebral formation of glutamine from ammonia. The authors also noted a rapid  
27 decrease in myo-inositol indicating that this organic osmolyte plays a protective role in HE via its  
28 release from astrocytes in order to maintain cell volume. Other MR studies have suggested that not only  
29 does hyperammonemia induce low grade swelling in astrocytes but edema can also be detected in white  
30 matter (Keiding and Pavese 2013).

31  
32 While there seems to be little doubt that cerebral edema, resulting from excessive accumulation of  
33 glutamine in astrocytes, is a major contributing factor to hyperammonemia-induced encephalopathy  
34 (especially in acute liver failure) other factors may also contribute. For example, there is considerable  
35 evidence for an ammonia-induced neuroinflammatory response in hyperammonemic liver disease. The  
36 evidence includes activation of microglia, together with increased synthesis *in situ* of the  
37 proinflammatory cytokines TNF, IL-1 $\beta$  and IL-6 (reviewed by Butterworth 2013). In addition,  
38 according to Häussinger and colleagues once the astrocytes lose their capacity to self-regulate volume  
39 during hyperammonemia and excessive glutamine accumulates, low grade edema sets in, resulting in  
40 triggering of “a complex signaling cascade which relies on NMDA receptor activation, elevation of  
41 intracellular Ca<sup>2+</sup> concentration and prostanoid-driven glutamate exocytosis, which result in increased  
42 formation of reactive nitrogen and oxygen species (RNOS) through activation of NADPH oxidase and  
43 nitric oxide synthase. Since RNOS in turn promote astrocyte swelling, a self-amplifying signaling loop  
44 between osmotic- and oxidative stress ensues, which triggers a variety of downstream consequences”  
45 (Görg et al. 2013).

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1 **F. Quantitative Cancer Assessment**

2  
3 *Charge Question F.1. Quantitative cancer estimates were not derived for ammonia because of*  
4 *inadequate information. Please comment on whether the rationale for not deriving quantitative*  
5 *cancer estimates for ammonia is scientifically supported and clearly described (see Section 2.3).*  
6 *Please comment on whether data are available to support a quantitative cancer assessment. If so,*  
7 *please identify these data.*

8  
9 **Response**

10 The existing data in the literature are inadequate to reach a conclusion on the carcinogenicity of  
11 ammonia, and thus it would not be scientifically justified to develop quantitative cancer risk estimates  
12 for this chemical. The rationale for not deriving these estimates is described clearly and is well  
13 supported scientifically.

14  
15 **G. Endogenous Production of Ammonia**

16  
17 *Charge Question G.1. Ammonia is produced endogenously and has been detected in the expired air*  
18 *of healthy volunteers. Please comment on whether the discussion of endogenous ammonia in*  
19 *Section 2.2.4 of the Toxicological Review is scientifically supported and clearly described.*

20  
21 **Response**

22 The description of endogenous ammonia production appears to be generally appropriate, but the  
23 committee recommends expanding this section to describe all sources of endogenous ammonia [such  
24 as?]. One important consideration for EPA is that the production of endogenous ammonia should be  
25 used in the interpretation of all epidemiologic studies [how?] (as well as human and animal controlled  
26 exposure studies not used for RfC derivation), as stated in lines 9-10 of page 2-8.

27  
28 There is no doubt that ammonia in expired breath is increased in pathological conditions (such as liver  
29 disease and kidney disease) that give rise to hyperammonemia. Studies suggest that absorption of  
30 ammonia in lungs occurs in a compartment that does not readily mix with the metabolic pool of  
31 ammonia. The amount of ammonia that equilibrates between the endogenous lung metabolic pool and  
32 alveolar air is likely to be quite small even under hyperammonemic conditions. The concentration of  
33 ammonia in oral cavity air is an indicator of the exhaled concentration (including the contribution from  
34 the bacterial digest of residual food particles in the mouth). However, because of confounding problems  
35 with “contaminating” ammonia in the expired air and difficulties associated with its actual measurement,  
36 it may be challenging to correlate prior *chronic* exposure of individuals to ammonia with alveolar  
37 ammonia concentrations. Additionally, the concentration of ammonia in oral cavity reflects neither the  
38 endogenous inhaled ammonia (which is closely related to the alveolar ammonia concentrations), nor the  
39 concentration of ammonia in inhaled air (since mouth air is diluted with external air on inhalation).  
40 Thus, the concentration of ammonia in the mouth is not a major contributor to either the systemic or  
41 inhaled concentration of ammonia. Furthermore, it should be noted that exhaled ammonia concentrations  
42 are likely higher than inhaled concentrations even for mouth breathers, much as exhaled CO<sub>2</sub> is higher  
43 than inhaled CO<sub>2</sub>. There is no reason to assume that exhaled air is safe for continuous inhalation--  
44 indeed, continuous inhalation of exhaled air, as eventually occurs in a small enclosed space, is deadly.

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1 As a means of providing further context for the RfC, it is recommended that EPA considers including  
2 ranges and citations for typical [?] indoor and ambient concentrations of ammonia. This data need not  
3 be comprehensive but will be helpful for placing the RfC in the context of expected concentrations in  
4 non-industrial, residential, and office indoor environments, and in outdoor air (for example, data  
5 collected by EPA's Passive Ammonia Monitoring Network). These ranges could then be included as  
6 part of the Executive Summary.

7  
8  
9 In addition, there are several areas in the assessment, the supplemental information and in other  
10 materials provided that should be revisited and may need to be modified. In order to simplify our  
11 discussion of these points, we have listed specific page numbers and lines where we believe that a  
12 change or clarification of the provided materials could be beneficial. We have organized our comments  
13 by specific document (see Appendix B). One general suggestion for EPA's section on endogenous  
14 ammonia, and also a general comment for the assessment, is to more explicitly discuss the different  
15 chemical aspects of the two different molecular forms of ammonia,  $\text{NH}_3$  and  $\text{NH}_4^+$ .

### 16 17 **Additional Comments for consideration on the endogenous ammonia section.**

18  
19 1). Discussion of endogenous production of ammonia: There are very many enzyme-catalyzed  
20 reactions by which ammonia can be generated *in vivo*. For example, Cooper and Plum (1987) list at  
21 least seventeen enzyme-catalyzed reactions that can generate ammonia from amino acids and  
22 nucleotides in the brain. Considerable ammonia is generated in the gut from the action of bacteria on  
23 nitrogenous substance. In humans, a large portion of this ammonia is derived from the hydrolysis of urea  
24 by urease-containing bacteria in the colon (Gibson et al., 1976). Tracer studies suggest that in human  
25 volunteers 15-30% of urea synthesized in the liver is converted to ammonia by intestinal bacteria  
26 (Walser and Bodenlos 1959). Intestinal cells utilize glutamine as a major energy source (Pinkus and  
27 Windmueller 1977; Roediger 1982; Mallet et al., 1986). As a result of the bacterial action and  
28 endogenous production of ammonia from glutamine by intestinal cells the concentration of ammonia in  
29 the portal vein can be quite high (0.2 mM – 0.3 mM, rising to  $\geq 1$  mM in hyperammonemia (Häussinger  
30 and Sies 1979). In contrast, because of the efficient removal of ammonia by liver periportal cells as urea  
31 and to a lesser extent removal of ammonia as glutamine in perivenous cells (see the next section) the  
32 concentration of ammonia in the systemic circulation is much lower than that of portal blood. For  
33 example, the clinical reference for the upper limit of normal concentration of ammonia in human blood  
34 is 40  $\mu\text{M}$ .

35  
36 An important source of endogenous ammonia is derived from the metabolism of amino acids. A major  
37 route for conversion of amino acid nitrogen to ammonia involves coupling of an aminotransferase  
38 (transaminase) to the glutamate dehydrogenase reaction. The amino acid is transaminated with  $\alpha$ -  
39 ketoglutarate to the corresponding  $\alpha$ -keto acid and glutamate. The glutamate is then converted back to  
40  $\alpha$ -ketoglutarate with the concomitant formation of ammonia in a reaction catalyzed by glutamate  
41 dehydrogenase. This ammonia is mainly incorporated into urea in the liver or into glutamine in  
42 extrahepatic tissues (see below). Nitrogen transferred from an amino acid to glutamate via a  
43 transaminase reaction can be further transferred to aspartate via the aspartate aminotransferase reaction.  
44 In the muscle this aspartate nitrogen is a source of ammonia via the purine nucleotide cycle (PNC). For  
45 a recent discussion of these pathways see Cooper (2012). The role of the PNC is relatively little studied,  
46 but the few studies tended to support the notion that the purine nucleotide cycle is important for the

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1 production of muscle ammonia. Other studies suggest that amino acids, particularly the branched chain  
2 amino acids are important source of ammonia in exercising muscle (Graham and MacLean 1992).  
3 However, because of rapid nitrogen exchange catalyzed by aminotransferases, the arguments may be  
4 moot. N flow: (1) branched chain amino acids  $\rightleftharpoons$  glutamate  $\rightleftharpoons$  ammonia; (2) branched chain amino acids  
5  $\rightleftharpoons$  glutamate  $\rightleftharpoons$  aspartate  $\rightarrow$  ammonia.] It is interesting to note that during exercise muscle is a net  
6 source of ammonia. During extreme exercise, such as ultramarathon running, muscle can release  
7 ammonia that can result in pathological levels of ammonia resulting in disruption of brain function  
8 (reviewed by Wilkinson et al., 2010). On the other hand, during rest muscle appears to be a net sink for  
9 removal of ammonia.

10  
11 2). Endogenous removal of ammonia: The main route for removal of ammonia carried to the liver  
12 by the portal vein is incorporation into urea by enzymes of the urea cycle in the periportal hepatocytes.  
13 Glutamine synthetase is located in the perivenous hepatocytes downstream in the sinusoid. This enzyme  
14 acts as a backup system to remove ammonia that is not removed as urea by the periportal cells  
15 (Häussinger 1998). This two system backup arrangement is very effective. For example, Cooper et al..  
16 (1987) showed that ~93% of tracer quantities of [ $^{13}\text{N}$ ]ammonia ( $^{13}\text{N}$  is a positron-emitting isotope with a  
17  $t_{1/2}$  of 9.96 min) injected into the portal vein of anesthetized rats is removed in a single pass through the  
18 liver. Of the [ $^{13}\text{N}$ ]ammonia taken up by the liver about 93% is incorporated into urea and about 7% is  
19 incorporated into the amide position of glutamine. Despite the fact that the urea cycle consists of five  
20 enzyme steps and two mitochondrial transport processes the process is remarkably effective. It was  
21 estimated that the  $t_{1/2}$  for conversion of ammonia to urea in the rat liver is about 11 sec (Cooper et al..  
22 1987).

23  
24 Because extrahepatic tissues do not contain a functioning urea cycle ammonia generated by the  
25 breakdown of nitrogenous substances in these tissues must be removed by another mechanism. In most  
26 tissues this removal is accomplished by incorporation of ammonia into the amide position of glutamine  
27 via a reaction catalyzed by glutamine synthetase. For example, it has been shown, using an intracarotid  
28 bolus of [ $^{13}\text{N}$ ]ammonia, that >95% of blood-derived ammonia entering the rat brain (and also,  
29 presumably, endogenously derived ammonia) is very rapidly incorporated (in seconds) into glutamine  
30 (amide) in a distinct metabolic compartment (astrocytes) (Cooper et al.. 1979). The major route for  
31 cerebral metabolism of blood-derived [ $^{13}\text{N}$ ]ammonia in hyperammonemic rats is also via the glutamine  
32 synthetase reaction (Cooper et al.. 1985). Ammonia enters the brain mostly by diffusion as the free base  
33 ( $\text{NH}_3$ ) (Cooper et al.. 1979; Lockwood et al.. 1980) although a small portion may cross the blood-brain  
34 barrier as ammonium ion ( $\text{NH}_4^+$ ) (Raichle and Larson 1981). It should be noted that astrocytes have a  
35 remarkable ability to take up ammonia by diffusion of  $\text{NH}_3$  and by active transport of  $\text{NH}_4^+$  (Nagaraja  
36 and Brookes 1998). Because astrocyte end feet underlie the blood-brain barrier and contain high levels  
37 of glutamine synthetase (see below) these cells are in a unique position to metabolize blood-derived  
38 ammonia and metabolically derived ammonia in the brain (Cooper and Plum 1987)

39  
40 Freed and Gelbard (1982) determined the disposition of label in 14 major organs of anesthetized rats  
41 following intravenous (femoral vein) bolus injection of [ $^{13}\text{N}$ ]ammonia. They found that most of the  
42 administered dose was extracted by the musculature, kidneys and lungs. It was noted that labeled  
43 metabolites were rapidly lost from the lungs and kidney. Whole body imaging after administration of  
44 [ $^{13}\text{N}$ ]ammonia was previously used to show that skeletal muscle in human volunteers is a major sink for  
45 removal of circulating ammonia (Lockwood et al.. 1979). Cachexia is a major risk factor for patients  
46 with liver disease and hyperammonemic encephalopathy. Lockwood et al. (1979) concluded that

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1 muscle atrophy may thereby contribute to the development of hyperammonemic encephalopathy with an  
2 associated increase in the brain ammonia utilization rate. However, in portacaval shunted rats (a model  
3 of chronic liver disease) muscle glutamine synthetase is upregulated presumably in an attempt to  
4 counteract the loss of liver enzymes responsible for removing ammonia (Girard and Butterworth 1992).

5  
6 The finding of Freed and Gelbard (1982) that lungs may be important for the removal of circulating  
7 ammonia is interesting given the fact that high levels of inhaled ammonia are toxic to the lungs. In later  
8 studies it was shown by Cooper and Freed (2005), using [<sup>13</sup>N]ammonia, that rat lungs contain glutamine  
9 synthetase and that a considerable portion of [<sup>13</sup>N]ammonia passing through the lungs is removed as L-  
10 [amide-<sup>13</sup>N]glutamine. Evidently, however, the presence of glutamine synthetase in the lungs is  
11 ineffective at preventing damage to these organs at high levels of *inspired* ammonia.

12  
13 It is noted in the assessment that ammonia can be detected in the breath of humans. However, in the  
14 study of Cooper and Freed (2005) it was shown that very little <sup>13</sup>N could be detected in the exhaled rat  
15 breath after intravenous administration of [<sup>13</sup>N]ammonia despite a considerable first pass extraction of  
16 [<sup>13</sup>N]ammonia (~30% of the dose administered via the femoral vein) by the lungs. This finding supports  
17 the notion that the major source of ammonia in the breath does not originate from endogenous ammonia  
18 in the lung tissue *per se*, but rather is formed by bacterial action on nitrogenous substances in the oral  
19 and nasal cavities (see below).

20  
21 3. Comments on the draft assessment section on ammonia in the exhaled air: Further development  
22 of the discussion in the assessment regarding measurements of ammonia in exhaled air is needed and  
23 especially how it may impact the RfC. For instance, what is the relevance to hyperammonemia, ingested  
24 ammonia or to long term exposure to gaseous ammonia? The discussion in the draft includes three  
25 references to Spaněl et al. who have measured ammonia in the expired air of human volunteers. In the  
26 latest cited study by Spaněl et al.. (2013) the authors measured exhaled ammonia after *acute* inhalation  
27 of ambient ammonia. About 70% of the inhaled ammonia was recovered in the exhaled air. However,  
28 endogenous ammonia is rapidly converted to glutamine in rat lungs (Cooper and Freed 2005) and  
29 presumably also in human lungs. Thus, the findings of Spaněl et al. (2013) suggest that absorption of  
30 ammonia in lungs occurs in a compartment that does not readily mix with the metabolic pool of  
31 ammonia. This compartment is presumably mucous. In this context it has long been known that cave-  
32 dwelling bats can tolerate levels of ambient ammonia that would quickly overcome and kill most  
33 mammals. It is thought that mucous in the respiratory tract of bats affords protection (Studier 1966). The  
34 ammonia is absorbed by the mucous to be released later in “ammonia-less” air. Presumably, humans  
35 have less mucous in the respiratory tract than do bats and protection against ambient ammonia by  
36 respiratory tract mucous is more limited.

37  
38 Another interesting paper studied naked mole rats. These animals can tolerate extremely higher levels  
39 of ammonia in their burrows – levels that other mammals would try to avoid (LaVinka et al. 2009). The  
40 lack of nocifensive behavior in these animals to high ammonia concentrations may be due to unique  
41 chemosensory nerve fibers.

42  
43 Some comment here may be appropriate on the relationship between endogenous ammonia in the lungs  
44 and alveolar air. In 1959, two groups suggested that ammonia in alveolar air reflects the concentration  
45 of ammonia in the lungs (Robin et al.. 1959; Jacquez et al., 1959). With the techniques available at the  
46 time it was not possible to measure ammonia in expired breath of normal experimental animals.

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1 However, Robins et al. (1959) were able to measure ammonia in alveolar air of anesthetized dogs  
2 administered ammonium acetate, and Jacquez et al. (1959) were able to measure ammonia in alveolar air  
3 of anesthetized dogs made chronically hyperammonemic by a portacaval shunt. In later studies, Reinyk  
4 et al. (2007) noted that comatogenic doses of sodium thiopental in rats produced hyperammonemia that  
5 was associated with an increased exhalation of ammonia. Breath ammonia analysis has also been carried  
6 out on patients with kidney disease as a potential estimator of the severity of the associated uremia  
7 (Davies et al., 2014). Thus, hyperammonemic syndromes (e.g. liver disease, kidney disease) result in  
8 increased lung ammonia that in turn is reflected in increased expiration of ammonia. However, there are  
9 caveats regarding interpretation of these studies that need to be discussed.

10  
11 The amount of ammonia that equilibrates between the endogenous lung metabolic pool and alveolar air  
12 is likely to be quite small even under hyperammonemic conditions. For example, in the study by Cooper  
13 and Freed (2005) mentioned above, the authors measured the amount of label in exhaled air of  
14 anesthetized rats administered an intravenous dose of tracer quantities of [<sup>13</sup>N]ammonia. Despite the fact  
15 that at least 30% of the dose administered to anesthetized rats must have passed through the lungs within  
16 seconds, very little label (~1 part in 1,000,000 of the administered dose) could be detected in the expired  
17 breath over a five minute period.

18  
19 As pointed out in the draft assessment, ammonia measured in exhaled air can vary considerably  
20 depending on the route of exhalation. Ammonia exhaled from the mouth or oral cavity is largely  
21 attributed to the production of ammonia via bacterial degradation of food protein in the oral cavity or  
22 gastrointestinal tract and can be influenced by such factors as diet, oral hygiene and age. In contrast,  
23 ammonia concentrations in breath exhaled from the nose and trachea are lower and appear to better  
24 represent levels at the alveolar interface of the lung or tracheo-bronchal region and are thought to be  
25 more relevant to understanding systemic levels of ammonia in breath exhaled from the mouth. [In  
26 addition to the references quoted in the draft assessment (i.e. Schmidt et al., 2013; Smith et al., 2008;  
27 Larson et al., 1977) a recent article by Solga et al. (2013) should also be quoted. These authors found  
28 that the amount of ammonia in expired air depends heavily on temperature of the breath sample and  
29 breath analyzer, the pH of a mouth rinse and mode of breathing (mouth open versus mouth closed).]

30  
31 In conclusion to this section, there is no doubt that ammonia in expired breath is increased in  
32 pathological conditions (such as liver disease) that give rise to hyperammonemia. However, because of  
33 confounding problems with “contaminating” ammonia in the expired air and difficulties associated with  
34 its actual measurement, it may be challenging to correlate prior *chronic* exposure of individuals to  
35 ammonia with alveolar ammonia concentrations.

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1 **APPENDIX A**

2  
3  
4 **Charge to the Science Advisory Board for the**  
5 **IRIS Toxicological Review of Ammonia**

6  
7 **August 2013 (Updated March 2014<sup>2</sup>, June 2014<sup>3</sup> and July 2014<sup>4</sup>)**

8  
9 **Introduction**

10  
11 The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis  
12 supporting the draft Toxicological Review of Ammonia that will appear on the Agency's online database,  
13 the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center  
14 for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). An existing  
15 assessment for ammonia, which includes an inhalation reference concentration (RfC), was posted on IRIS in  
16 1991.

17  
18 IRIS is a human health assessment program that evaluates scientific information on effects that may result  
19 from exposure to specific chemical substances in the environment. Through IRIS, EPA provides high  
20 quality science-based human health assessments to support the Agency's regulatory activities and  
21 decisions to protect public health. IRIS assessments contain information for chemical substances that can  
22 be used to support the first two steps (hazard identification and dose-response assessment) of the human  
23 health risk assessment process. When supported by available data, IRIS provides health effects  
24 information and toxicity values for chronic health effects (including cancer and effects other than cancer).  
25 Government and others combine IRIS toxicity values with exposure information to characterize public  
26 health risks of chemical substances; this information is then used to support risk management decisions  
27 designed to protect public health.

28  
29 The external review draft Toxicological Review of Ammonia is based on a comprehensive review of the  
30 available scientific literature on the noncancer and cancer health effects in humans and experimental  
31 animals exposed to ammonia. Only data using ammonia or ammonium hydroxide were considered in this  
32 review; data developed using ammonium salts were not considered because of concerns that the effects of  
33 the counter ion might confound the study outcomes. This draft IRIS assessment includes:

- 34  
35 • a *Preamble* to describe the methods used to develop IRIS assessments;  
36 • an *Executive Summary* to concisely summarize the major conclusions of the assessment;  
37 • a *Literature Search Strategy/ Study Selection and Evaluation* section to describe the process for  
38 identifying and evaluating the evidence for consideration in developing the assessment;  
39 • a *Hazard Identification* chapter to systematically synthesize and integrate the available evidence of  
40 organ/system-specific hazards; and  
41 • a *Dose-Response Analysis* chapter to describe the selection of studies for consideration in calculating  
42 toxicity values and to describe the analysis and methodology in deriving and selecting toxicity values.  
43

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<sup>2</sup> The charge for ammonia was updated to include general charge question #4 requesting comment from the external peer review panel on the adequacy of EPA's assessment revisions and response to the public comments.

<sup>3</sup> The charge questions were modified (as shown in bold font) as a result of panel discussions during the June 2, 2014 preliminary teleconference.

<sup>4</sup> The charge questions were modified to refer reviewers to specific sections in the assessment.

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1 Additionally, appendices for chemical and physical properties, toxicity of ammonium salts, toxicokinetic  
2 information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental*  
3 *Information* (see Appendices A to G) to the draft Toxicological Review. The draft assessment was  
4 developed according to guidelines and technical reports published by EPA (see Preamble), and contains a  
5 qualitative characterization of the hazards for ammonia, including a cancer descriptor of the chemical's  
6 human carcinogenic potential and a noncancer toxicity value for chronic inhalation exposure (RfC). A  
7 chronic oral reference dose (RfD) was not derived and a quantitative cancer assessment for ammonia was  
8 not conducted due to inadequate data.

## 9 10 **Charge Questions**

11  
12 In April 2011, the National Research Council (NRC) released its *Review of the Environmental Protection*  
13 *Agency's Draft IRIS Assessment of Formaldehyde*. In addition to offering comments specifically about EPA's  
14 draft formaldehyde assessment, the NRC included comments and recommendations for improving the  
15 development of IRIS assessments. The IRIS Program's implementation of the NRC recommendations is  
16 following a phased approach. Phase 1 of implementation has focused on a subset of the short-term  
17 recommendations, such as editing and streamlining assessments, increasing transparency and clarity, and  
18 using more tables, figures, and appendices to present information and data in assessments. Phase 1 also  
19 focused on assessments that had been near the end of the development process and close to final posting.  
20 The IRIS Program is now in Phase 2 of implementation, which addresses all of the short-term NRC  
21 recommendations. The Program is implementing all of these recommendations but recognizes that  
22 achieving full and robust implementation of certain recommendations will be an evolving process with  
23 input and feedback from the public, stakeholders, and external peer review committees. This phased  
24 approach is consistent with the NRC's *Roadmap for Revision* as described in Chapter 7 of the formaldehyde  
25 review report. The NRC stated that "the committee recognizes that the changes suggested would involve a  
26 multi-year process and extensive effort by the staff at the National Center for Environmental Assessment  
27 and input and review by the EPA Science Advisory Board and others."

28  
29 Below is a set of charge questions that address scientific issues in the draft IRIS Toxicological Review of  
30 Ammonia. The charge questions also seek feedback on whether the assessment is clear and concise, a  
31 central concern expressed in the NRC report. Please provide detailed explanations for responses to the  
32 charge questions. EPA will also consider the Science Advisory Board review panel's comments on other  
33 major scientific issues specific to the hazard identification and dose-response assessment of ammonia.  
34 Please consider the accuracy, objectivity, and transparency of EPA's analyses and conclusions in your  
35 review.

## 36 37 **General Charge Questions:**

- 38
- 39 1. NRC (2011) indicated that the introductory section of IRIS assessments needed to be expanded to  
40 describe more fully the methods of the assessment. NRC stated that they were "not recommending the  
41 addition of long descriptions of EPA guidelines to the introduction, but rather clear, concise statements  
42 of criteria used to exclude, include, and advance studies for derivation of [toxicity values]." Please  
43 comment on whether the new *Preamble* provides a clear, concise, **useful and objective** description of  
44 the guidance and methods that EPA uses in developing IRIS assessments.<sup>5</sup>  
45
  - 46 2. NRC (2011) provided comments on ways to improve the presentation of steps used to generate IRIS  
47 assessments and indicated key outcomes at each step, including systematic review of evidence, hazard

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<sup>5</sup> Whether such guidance and methods were used in this ammonia IRIS assessment will be the focus of chemical-specific questions.

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1 identification, and dose-response assessment. Please comment on the new IRIS assessment structure  
2 and whether it will increase the ability for the assessments to be more clear, concise, and easy to follow.

3  
4 3. NRC (2011) states that “all critical studies need to be thoroughly evaluated with standardized  
5 approaches that are clearly formulated” and that “strengthened, more integrative, and more  
6 transparent discussions of weight of evidence are needed.” NRC also indicated that the changes  
7 suggested would involve a multiyear process. Please comment on EPA’s success thus far in  
8 implementing these recommendations.

9  
10 4. EPA solicited public comments on the draft IRIS assessment of ammonia and has revised the  
11 assessment to respond to the scientific issues raised in the comments. A summary of the public  
12 comments and EPA’s responses are provided in Appendix G of the Supplemental Information to the  
13 Toxicological Review of Ammonia. **Please consider in your review whether there are scientific  
14 issues that were raised by the public as described in Appendix G that may not have been  
15 adequately addressed by EPA.**

16  
17  
18 **Chemical-Specific Charge Questions:**

19  
20 **A. Executive Summary**

21  
22 1. The major conclusions of the assessment pertaining to the hazard identification and dose-response  
23 analysis have been summarized in the *Executive Summary*. Please comment on whether the  
24 conclusions have been clearly and sufficiently described for purposes of condensing the Toxicological  
25 Review information into a concise summary.

26  
27 **B. Literature Search Strategy/Study Selection and Evaluation**

28  
29 1. The process for identifying and selecting pertinent studies for consideration in developing the  
30 assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please  
31 comment on whether the literature search approach, screening, evaluation, and selection of studies for  
32 inclusion in the assessment are clearly described and supported. **Please comment on whether EPA  
33 has clearly identified the criteria (e.g., study quality, risk of bias) used for the selection of  
34 studies to review and for the selection of key studies to include in the assessment.** Please identify  
35 any additional peer-reviewed studies from the primary literature that should be considered in the  
36 assessment of noncancer and cancer health effects of ammonia.

37  
38 **C. Hazard Identification**

39  
40 ***Synthesis of Evidence***

41  
42 1. A synthesis of the evidence for ammonia toxicity is provided in Chapter 1, *Hazard Identification*. Please  
43 comment on whether the available data have been clearly and appropriately synthesized for each  
44 toxicological effect (see Sections 1.1.1 through 1.1.5). Please comment on whether the weight of  
45 evidence for hazard identification (see Summary of Respiratory Effects, p. 1-15; Summary of  
46 Gastrointestinal Effects, p. 1-20; Summary of Immune System Effects, p. 1-25; Summary of Other  
47 Systemic Effects, p. 1-33) has been clearly described and scientifically supported.

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1 **Summary and Evaluation**  
2

- 3 2. Does EPA’s hazard assessment of noncancer human health effects of ammonia clearly integrate the  
4 available scientific evidence (i.e., human, experimental animal, and mechanistic evidence) to support  
5 the conclusion that ammonia poses a potential hazard to the respiratory system **or systemic toxicity**  
6 **through other routes** (see Section 1.2.1)?  
7
- 8 3. Does EPA’s hazard assessment of the carcinogenicity of ammonia clearly integrate the available  
9 scientific evidence to support the conclusion that under EPA’s *Guidelines for Carcinogen Risk Assessment*  
10 (Section 2.5 of U.S. EPA), there is “inadequate information to assess the carcinogenic potential” of  
11 ammonia (see Section 1.2.2)?  
12

13 **D. Oral Reference Dose (RfD)**  
14

15 An RfD was not derived for ammonia based on insufficient data. Human data involving oral exposure to  
16 ammonia are limited to case reports involving intentional or accidental ingestion and repeat exposure  
17 animal studies are limited in scope and designed to investigate mechanisms by which ammonia can induce  
18 effects on the gastric mucosa of rats.  
19

- 20 1. Please comment on whether the rationale for not deriving an RfD is scientifically supported and clearly  
21 described (see Section 2.1). Please comment on whether data are available to support the derivation of  
22 an RfD for ammonia. If so, please identify these data.  
23
- 24 2. As described in the *Preface*, data on ammonium salts were not considered in the identification of effects  
25 or the derivation of an RfD for ammonia and ammonium hydroxide because of concerns about the  
26 potential impact of the counter ion on toxicity outcomes. Please comment on whether the rationale for  
27 this decision is scientifically supported and clearly described.  
28

29 **E. Inhalation Reference Concentration (RfC)**  
30

31 An RfC was derived for ammonia based on effects on the respiratory system, which was identified as the  
32 primary and most sensitive target of inhaled ammonia. An occupational epidemiology study by Holness et  
33 al. (1989), with the support of three other occupational epidemiology studies by Rahman et al. (2007), Ali  
34 et al. (2001), and Ballal et al. (1998), was selected as the principal study for RfC derivation. Decreased lung  
35 function and respiratory symptoms were selected as the critical effect.  
36

- 37 1. Please comment on whether the evaluation and selection of studies and effects for the derivation of the  
38 RfC is scientifically supported and clearly described (see Section 2.2.1). Please identify and provide the  
39 rationale for any other studies or effects that should be considered.  
40
- 41 2. The NOAEL/LOAEL approach was used to identify the point of departure (POD) for derivation of the  
42 RfC (see Section 2.2.2). Please comment on whether this approach is scientifically supported and  
43 clearly described.  
44
- 45 3. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD  
46 for the derivation of the RfC (see Section 2.2.3). Are the UFs appropriate based on the  
47 recommendations described in Section 4.4.5 of *A Review of the Reference Dose and Reference*  
48 *Concentration Processes* (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are  
49 proposed, please identify and provide scientific support for the proposed changes.  
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has not been reviewed or approved by the chartered SAB and does not represent EPA policy.

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**F. Quantitative Cancer Assessment**

1. Quantitative cancer estimates were not derived for ammonia because of inadequate information. Please comment on whether the rationale for not deriving quantitative cancer estimates for ammonia is scientifically supported and clearly described (see Section 2.3). Please comment on whether data are available to support a quantitative cancer assessment. If so, please identify these data.

**G. Endogenous Production of Ammonia**

1. Ammonia is produced endogenously and has been detected in the expired air of healthy volunteers. Please comment on whether the discussion of endogenous ammonia in Section 2.2.4 of the Toxicological Review is scientifically supported and clearly described.

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1 **APPENDIX B**

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4 **SPECIFIC COMMENTS ON THE EXECUTIVE SUMMARY**

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6 **“Occurrence and Health Effects”:**

7 Page xxx, Line 10: EPA indicates exposure is primarily through breathing air containing ammonia gas.  
8 Can EPA provide some perspective on whether inhalation exposure is much higher than oral exposure?  
9 A brief description of sources of exposure would be helpful. For example, EPA could reference the  
10 ammonia monitoring network (<http://nadp.sws.uiuc.edu/AMoN/>) as a possible source of information for  
11 ammonia gas concentrations.

12  
13 Page xxx, Line 24: Insert two brief new paragraphs/sections as follows:

- 14 1. Brief description of the chemistry of ammonia, ammonium and ammonia salts and rationale for  
15 excluding ammonium salts (to support current EPA assessment) or to include ammonium salts (if  
16 EPA decides to include). This is very important to provide adequate background to understand  
17 (a) EPA’s inclusion/exclusion criteria for literature, and (b) EPA’s decision on whether or not to  
18 derive an oral RfD based on ammonium salts.
- 19 a. Based on comparisons of papers on ammonium salts summarized in Appendix C and  
20 more recent repeat-dose paper on ammonium acetate (Satpute et al.. 2012 Tox and Ind  
21 Health 30:12-24), EPA’s conclusion that the anion appears to influence the dose response  
22 and target organ toxicity (compare ammonium acetate Satpute et al.. 2012 with  
23 ammonium chloride Lina and Kuijpers, 2004) seems appropriate.
  - 24 b. Although data from ammonium salts should not be used to characterize dose-response  
25 relationships for ingested ammonia, the absence of findings can contribute to the weight  
26 of evidence for hazard identification. A statement to that effect may be useful for later  
27 discussions about the oral RfD.
  - 28 c.

29 Page xxx, Line 25: If EPA decides to only derive an inhalation RfC, then consider beginning with  
30 “Effects other than cancer observed following inhalation exposure first (start with section beginning  
31 xxxi line 5).

32  
33 Page xxx, Line 26: State upfront that the oral reference dose was not derived.

34  
35 Page xxx, Line 38: Strengthen the discussion immediately following p. xxx line 38 that chronic studies  
36 on ammonium salts (Lina and Kuijpers, 2004; Ota et al., 2006) indicate that measured decreases in  
37 thickness of gastric mucosa (Hata et al., 1994; Tsujii et al., 1992; et al.. 1991) do not appear to progress  
38 to adverse effects following chronic exposures. EPA’s discussion of this on page 1-20 regarding  
39 absence of histopathology in the stomach following chronic exposure to ammonium salts should be  
40 briefly summarized here in the Executive Summary so that EPA’s rationale for not deriving an oral RfD  
41 is strengthened and made more transparent.

42  
43 Page xxxii, Line 14 to Page xxxiii Line 6: The EPA can strengthen their selection of the higher control  
44 levels of exposure from the Holness et al. (1989) by including a brief description and explanation of the  
45 severity or magnitude of change in FEV1 and FVC relative to the clinical level of concern (i.e.

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1 difference of 200 ml – Murray et al. (ed. 1994) in the Rahman et al. 2007 paper, and clarification that the  
2 increased prevalence of respiratory symptoms (e.g. cough and chest tightness) are self-reported.

3  
4 Section on “Evidence for Carcinogenicity” beginning Page xxxiii Line 35: This section could be  
5 strengthened by adding a few more details regarding the evidence that ammonia may act as a cancer  
6 promoter. For example, it would be helpful to put this finding into better perspective by indicating that  
7 ammonia may act as a cancer promoter in the stomach when administered to rats orally following pre-  
8 treatment with the initiator MMNG. In addition, the negative results from the chronic study with  
9 ammonium chloride (Lina and Kuijpers, 2004) are also useful taking into account the KCl control.  
10 However, the final discussion will depend on EPA’s reconsideration of all the relevant ammonium salt  
11 literature.

12  
13 Section on susceptible populations and lifestages beginning Page xxxiv Line 5: The SAB suggests  
14 including asthmatics since EPA’s draft discussion of this is already included on p. 1-38.

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20 **ADDITIONAL SPECIFIC COMMENTS ON THE ASSESSMENT**

21 **Toxicological Review of Ammonia**

22  
23

Page	Line	Comment
xi	5-8	While the WHO may have found this unclear, the majority of the medical research literature we believe would support that this is a direct response to the metabolic acidosis induced by ammonium chloride intake.
xxx	20-22	The following statement is incorrect - high levels of ammonia in air or water could have adverse effects on kidney and adrenal gland. The responses seen in these organs occurs only after ingestion of large amounts by the oral route and is considered a component of the normal physiologic response to the metabolic acidosis induced by hepatic ammonium metabolism, and is not an adverse effect.
1-26	35-37	Kidney disease does not cause high plasma ammonia levels.

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1 Supplemental Information

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Page	Line	Comment
E-2	27	We believe that the better quality data suggests/supports that the small intestine also contributes to intestinal ammoniogenesis, that this occurs through the use of amino acids as an energy source, and that it may contribute 60-70% of total intestinal ammonia production.
E-2	28	99% of intestinal ammonia/ammonium is absorbed, but this is not correct when considering renal ammonia production. In the kidneys, ~50% of the ammonia produced is excreted in the urine and ~50% is absorbed into to the systemic circulation.
E-2	30	The term “active transport” has specific biological meaning and is perhaps inappropriate in this context. Removing the term “active” would correct this issue.
E-3	9-10	The older ammonia concentration data were generated using outdated assays for plasma ammonia and are no longer generally considered appropriate for use.
E-3	11	<p>The proportion of “total ammonia” present, at pH 7.40 (normal physiologic blood pH), as NH<sub>4</sub><sup>+</sup> is ~98.3% and as NH<sub>3</sub> is ~1.7%. The relative amount of each is determined by pH. For every 0.3 pH unit change, the amount of NH<sub>3</sub> changes in parallel by 100% (i.e., with pH 7.70, 3.4%, pH 7.10, 0.85%). The amount of NH<sub>4</sub><sup>+</sup> changes in the opposite direct by an equivalent absolute amount (decreases 1.7% to 96.7% at pH 7.70 and increases 0.85% to 99.15% at pH 7.10) (Weiner and Verlander, <i>Comp Physiol</i> 3:201-220, 2013).</p> <p>This issue of the relative amounts of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> in body fluids comes up multiple times in the assessment. For simplicity sake, this issue is not discussed again.</p>
E-4	22	The gut (intestinal tract) generates substantial amounts of ammonia, ~200-250 mmol/d, which enters the portal vein. If liver function is normal, hepatic metabolism metabolizes all of this and there is no net change in plasma ammonia levels. If liver function is abnormal or if there are urea cycle enzymatic defects, then intestinal ammonia production can exceed hepatic metabolic capacity and lead to increased blood ammonia levels. In this case, the intestinal tract does contribute to systemic ammonia levels.
E-6	4-5	The statement that “abnormally elevated levels of ammonia are indicative of end-stage renal disease” is a reference to increased exhaled breath ammonia levels, and not to plasma ammonia levels.

Science Advisory Board (SAB) Draft Report (November 14, 2014) to Assist Meeting Deliberations

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Page	Line	Comment
E-6	7	Both acute and chronic liver failure can lead to decreased ureagenesis and ammonia metabolism and thereby to increased blood ammonia levels; this is not specific or limited to chronic liver failure. Also, fulminant hepatitis is a form of acute liver failure, not chronic liver failure.
E-6	21	Ammonia excreted by the kidneys derives almost completely from ammonia produced in the kidneys. In contrast to the implications of this statement, the kidneys actually add ammonia to the body, as renal vein ammonia content exceeds renal artery ammonia content.
	24-29	Ammonia excretion by the kidneys involves both Rh B Glycoprotein (Rhbg) and Rh C Glycoprotein (Rhcg) under basal conditions and in response to both metabolic acidosis and hypokalemia (reviewed in Weiner and Verlander, <i>Am J Physiol Renal Physiol</i> 306:F1107-F1120, 2014). A complete understanding of renal ammonia transport involves consideration of many other proteins and is probably beyond the scope of this summary.  The remainder of this paragraph is misleading and can be deleted.

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