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 EPA's National Center for Environmental Assessment 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 the toxicity of ammonia/ammonium.

EPA asked the SAB 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's 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 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, with several areas still requiring further refinement. There is some duplication across the main assessment and the detailed study summaries in the appendices. 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 descriptions of the supporting studies in the appendices is appropriate, but the principal study should be given a more detailed description and evaluation in the main assessment. Some of these

issues will likely be resolved as EPA develops and adopts a standard systematic review approach for evaluating and selecting key studies.

The rationale for excluding ammonium salts from the assessment should be expanded. The SAB also notes that a more detailed evaluation of the chemical reactions and pathways of ammonia generation that may impact gastrointestinal endpoints is required, particularly as it relates to the conclusion of not deriving a reference dose (RfD).

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

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

The description of endogenous ammonia production appears to be generally appropriate, but the SAB recommends expanding this section. While there is no doubt that ammonia in expired breath is increased in pathological conditions (such as liver disease) that give rise to hyperammonemia, there is no evidence to suggest that the concentration of ammonia in the oral cavity is a major contributor to either the systemic or inhaled concentrations of ammonia. In order to provide further context for the potential contributions of endogenously-generated ammonia to inhaled doses, it is recommended that EPA considers including concentration ranges for typical indoor and ambient concentrations of ammonia. Ultimately, it is important to recognize that exhalation is a clearance mechanism of an otherwise toxic contaminant.

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

Sincerely,

Enclosure

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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1. EXECUTIVE SUMMARY

The Science Advisory Board was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft Toxicological Review of Ammonia (August 2013 Draft)* (also referred to as the assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. 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 public comments received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information to the Toxicological Review.

EPA asked the SAB to conduct a review of the appropriateness and scientific soundness of the conclusions presented in the draft IRIS ammonia assessment. In addition, the SAB was asked to comment on the modification of the overall structure of the assessment as recommended by the National Research Council (NRC) in 2011. The panel charged with conducting the review included members of the SAB Chemical Assessment Advisory Committee augmented with additional toxicological experts. An overview of the SAB's recommendations and advice on how to improve the clarity, transparency and utility of the assessment are presented below and discussed in greater depth in the body of the report.

<u>Implementation of the NRC's recommendations</u>

Clarity of the Preamble

The SAB commends the agency for the progress made thus far in implementing the NRC's recommendations for the IRIS program. The SAB expects that further refinements and modifications to future assessments will be made based on feedback from external reviewers, users and other stakeholders. Recognizing that 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. It also would be helpful to clarify the reasoning behind the Preface being separate from the Preamble and having such an extended section prior to the Executive Summary. It appears that the Preamble is intended to describe the general approach/methods used by the agency and will be included with all IRIS assessments; since the Preface would presumably summarize issues that are specific to a particular chemical assessment, it should be placed after the Preamble.

IRIS assessment structure

The new format is a refreshing and long overdue improvement. The ammonia assessment is one of the first since the NRC made its recommendations in 2011. 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 does not reflect fully the systematic review envisioned by the NRC. The new structure of the assessment will take some getting used to for those who are familiar with the old structure. It is a clear improvement, but additional refinements should be forthcoming in subsequent chemical assessments. There does seem to be some duplication across the main assessment and the detailed study summaries in the appendices, but this is hard to avoid and may serve to emphasize the

importance of key publications. The use of tables and figures is particularly helpful and the EPA needs to continue to work on efficiently summarizing and presenting data using this approach.

Transparency of integrative approaches

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

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

Adequacy of response to public comments

EPA has adequately and appropriately addressed the scientific issues raised by public commenters. With regard to some of the comments where EPA disagreed with the commenters, the SAB concluded that EPA has provided adequate scientific justification for the agency's conclusions. The toxicological assessment is ultimately an EPA document and the agency must be responsible for its content. Given that the assessment is being reviewed by the SAB and may yet undergo additional agency reviews, the current approach provides adequate opportunity for public feedback and oversight.

Draft IRIS Ammonia Assessment

Executive Summary

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

Literature Search Strategy/Study Selection and Evaluation

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

Although the narrative provides an evaluation of the studies according to preselected criteria, not all criteria recommended by the NRC (2011) are incorporated (e.g., precision of the effect) and there is no specific overall study quality indicator. While it is understood that this is an area still under development, the application of the study quality criteria for the selection and evaluation of key non-cancer experimental animal studies that were included in the assessment is unclear. A clear description of even a minimal set of quality criteria for acceptability of specific studies included in the ammonia assessment (as opposed to those included as supportive information) would improve transparency of the process for selecting key studies. Further clarification of inclusion/exclusion criteria may provide some insight as to why some apparently relevant publications were not included or cited. In addition, the SAB also encourages the EPA to reconsider its decision not to include publications beyond the March 2013 deadline.

Hazard Identification

Synthesis of Evidence

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

Summary and Evaluation

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

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

Oral Reference Dose (RfD)

Although there is a fairly extensive literature on the systemic and organ-specific effects of ammonia (e.g., liver, brain, and kidney) with inhalation exposure, there are no controlled animal studies of the systemic effects of ammonia (not ammonium salts) through the ingestion route of exposure. Reports of systemic effects in humans with ingestion are confined to case reports of poisonings and accidental

ingestion. The EPA intentionally excluded from consideration studies of gastrointestinal effects (or the lack thereof) with oral administration of ammonium (NH_4^+) salts. This decision was based on concerns that the possible adverse effects of ammonia in such studies could not be separated from adverse effects resulting from the associated anion. Therefore, the EPA did not attempt to derive an RfD for such systemic effects. The SAB noted that while a possible independent gastrointestinal toxicity of the anion may be a valid concern, the dichotomy between ammonia and ammonium salts in the consideration of an RfD is not because ammonia in solution (i.e., in an aqueous delivery medium and/or in stomach fluid) is present as the free ammonium (NH_4^+) ion. Given this reasoning, the SAB concluded that the agency should evaluate the relevant toxicity studies that use ammonium salts to determine if they can offer valuable information for the derivation of an RfD. If the effects of the anion cannot be discerned, the decision to exclude ammonium salts will be buttressed by the evaluation of these studies. The SAB also noted that a decision to address ammonium salts would require further evaluation of the inhalation of ammonium salt particulate matter and the impact on the RfC.

Inhalation Reference Concentration (RfC)

Evaluation of Studies

The evaluation of studies is clearly described in the supplementary materials, and concisely summarized in the main assessment. The EPA has indicated that the Holness et al. (1989) study has the strongest exposure assessment, and provides a clear argument in support of that judgment. Although the selection of studies and effects for the RfC is mostly clear, and the Holness et al. (1989) study is the most appropriate for RfC derivation, exclusion of the controlled human exposure studies (Mirabelli et al., 2007) is not well explained. These studies have several methodological strengths, such as well-characterized exposures and resistance to confounding factors. Clarification as to why they are excluded as candidates for RfC derivation is needed. It is unclear if the quality of exposure assessment overrides the other factors listed in the Preamble for selection of a key study. The SAB also recommends expansion of the discussion of the potential implications of factors such as reversibility and long-term attenuation of effects through acclimatization and/or the healthy worker effect (e.g., self-selected attrition due to respiratory symptoms) as they are confounders that may lead to underestimating effects.

Deriving an RfC

The approach for deriving the RfC is reasonable and clearly described, but it is not clear to what extent the EPA considered continuous dose-response modeling. The EPA should attempt to obtain individual-level data and/or the mean/median exposure concentrations for the high dose group from Dr. Holness in order to determine if an alternative point of departure (POD) could be identified, overcoming the limitation of having only the upper exposure range in the published manuscript. If individual data are unavailable, EPA should consider whether there is sufficient information available in the Holness publication to estimate the mean concentration for the high exposure group—perhaps assuming a lognormal or other skewed distribution for the measured concentrations. For the POD derived from the Holness study, a dose conversion factor was used to convert the observed workplace ammonia concentration to an ammonia concentration that would provide an equivalent cumulative dose with continuous 24/7 exposure. Evidence of a cumulative effect of ammonia exposure is important to consider, especially if corroborated by other studies. The selection of the uncertainty factor was appropriate, clearly described, and consistent with the 2002 EPA guidance.

Quantitative Cancer Assessment

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

Endogenous Production of Ammonia

The description of endogenous ammonia production appears to be generally appropriate, but the SAB recommends expanding this section to describe all sources of endogenous ammonia. The SAB also notes that the effects described in these studies are at levels over and above endogenous levels.

There is no doubt that ammonia in expired breath is increased in pathological conditions that give rise to hyperammonemia, such as liver disease. There is also some evidence of ammonia exhalation in kidney disease, but this may have been secondary to increased circulating urea as a result of decreased urea excretion (Narasimhan et al., 2001). Studies suggest that absorption of ammonia in lungs occurs in a compartment that does not readily mix with the metabolic pool of ammonia. The amount of ammonia that equilibrates between the endogenous lung metabolic pool and alveolar air is likely to be quite small even under hyperammonemic conditions. The concentration of ammonia in oral cavity air is an indicator of the exhaled concentration (including the contribution from the bacterial digest of residual food particles in the mouth). However, because of confounding problems with "contaminating" ammonia in the expired air and difficulties associated with its actual measurement, it may be challenging to correlate prior ***chronic*** exposure of individuals to ammonia with alveolar ammonia concentrations. Additionally, the concentration of ammonia in the oral cavity reflects neither the endogenous inhaled ammonia (which is closely related to the alveolar ammonia concentrations), nor the concentration of ammonia in inhaled air (since mouth air is diluted with external air on inhalation). Thus, the concentration of ammonia in the oral cavity is not a major contributor to either the systemic or inhaled concentration of ammonia.

As a means of providing further context for the potential contributions of endogenously-generated ammonia to NH_3 inhalation doses, it is recommended that the EPA consider including concentration ranges for typical indoor and ambient concentrations of ammonia. These data need not be comprehensive (i.e., the result of a systematic review) but will be helpful for also placing the RfC in the context of expected concentrations in non-industrial, residential, and office indoor environments, and in outdoor air (for example, data collected by EPA's Passive Ammonia Monitoring Network). These concentration ranges could then be included as part of the Executive Summary.

2. INTRODUCTION

2.1. 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.

2.2. 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. Two subsequent public teleconferences were held on December 17 and 19, 2014 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, the 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. The full charge to the SAB is provided as Appendix A.

3. RESPONSE TO THE CHARGE

3.1. Enhancements to IRIS Assessments

3.1.1. Preamble

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.*

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 the International Agency for Research on Cancer (IARC) and the National Toxicology Program (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 should be clarified. 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. EPA should clearly state that the Preamble is generic and not necessarily applicable to the ammonia 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 these factors are not among the six key factors listed in the Preamble Section 6.

3. EPA should verify that all the relevant EPA guidance documents are included (e.g., U.S. EPA 2002, 2014).
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 the NRC (2001, 2014) studies in the process of IRIS protocol development.

3.1.2. IRIS Document Structure

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.

The new format is a refreshing, and long overdue improvement, but remains a work in progress. The ammonia assessment was 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 is evidence of the agency's commitment to implement the NRC's recommendations including devoting the necessary 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 implemented the systematic review as envisioned by the NRC. However, 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 is some duplication across the main assessment and the detailed study summaries in the appendices in the supplemental information, 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.

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

3.1.3. Process for Evaluating Critical Studies

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

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

NRC (2011) anticipated that the evolution of IRIS would be a multiyear process. The assessment demonstrates significant strides toward the goals outlined by the NRC. However gaps exist. For example, description of study quality remains incomplete. It would be useful to develop overall qualifiers to the studies in the summary tables as per NRC's recommendations.

Recommendation:

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

3.1.4. EPA's Response to Public Comments

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

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

Recommendations:

1. One specific comment (p. G-8) deserves greater attention by the EPA, namely that EPA attempt to obtain the study data from Dr. Holness in order to determine a representative exposure concentration for the NOAEL study group, rather than using the least exposed person in that study group. This is a good suggestion and EPA's response needs elaboration. Did the agency try to obtain the data but was refused? Or does it feel that the original data are irrelevant because the least exposed person in that exposure group is the most appropriate basis for the RfC?
2. Public comments suggested the use of studies upon which other exposure guidelines (e.g., Threshold Limit Values (TLV); Acute Exposure Guideline Levels (AEGL-1)) were established. This is an issue which is likely to occur repeatedly. These values serve a different purpose and EPA might consider expanding the section of the assessment that covers such U.S. and international guidelines. Table A-1 in Appendix A of the supplemental information for the assessment should be modified to include additional exposure guidelines for ammonia, their definition and purpose, and provide links to the assessments that explain the rationale for the guidelines and the chemical-specific documentation that supports them.

3.2. Toxicological Review of Ammonia

3.2.1. Executive Summary

Charge Question A1. Please comment on whether the conclusions have been clearly and sufficiently described for purposes of condensing the Toxicological Review information into a concise summary.

The Executive Summary is a very concise summary that highlights many of the important conclusions made in the EPA's assessment. Determination of whether the conclusions have been clearly and

sufficiently described should take into consideration the potential audience and purposes of this summary. According to EPA representatives, a future goal for Executive Summaries is to use them with minimal editing for the IRIS website. In addition, the EPA indicated that it is not uncommon for state regulators and other risk assessors to focus primarily, if not exclusively, on the Executive Summary as a source of information for the toxicological assessment. From this perspective, the EPA's effort to be concise results in a summary that is too vague and unclear in some of the subsections. The following general recommendations are offered to improve the utility of the Executive Summary, accuracy in describing key toxicity endpoints, and transparency in the EPA's evaluation and decisions. More specific detailed comments are included in Appendix B of this report.

Recommendations:

1. A section should be included at the beginning of the Executive Summary that provides information on the chemistry of ammonia[1], ammonium and ammonium salts and a rationale for excluding or including ammonium salts as oral exposure to ammonia results in exposure to ammonium. Otherwise, the EPA's discussion in the Executive Summary of non-cancer effects following oral exposure will not be credible if it appears to ignore published literature on toxicity of repeated oral exposures to ammonium salts without any explanation. As discussed in Appendix B in more detailed comments, it appears that toxicity of ammonium salts may be dependent, in part, on the anion. Therefore, there is good reason to exercise caution when reviewing those studies that do not control for the effect of the anion in deriving an oral RfD for ingested ammonia. However, toxicology studies on ammonium salts, especially negative results, potentially can provide supportive evidence for the absence of adverse effects. In addition, studies in which the anion is chloride may not result in a meaningful increase in chloride exposure given the large concentration of endogenous chloride normally present in the stomach and thus may allow for consideration of ammonium toxicity independent of the effect of the anion.
2. The sections should be rearranged so that the discussion on non-cancer effects of inhalation exposure comes before the discussion of oral exposures (if an oral RfD is not derived).
3. The first sentence 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.

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[1] Note on terminology: The word "ammonia" (unless specified otherwise) is used loosely to mean the total of ammonia free base (NH_3) plus ammonium ion (NH_4^+) when used in the context of physiological fluids and tissues. NH_3 is a weak base

$NH_3 + H^+ \leftrightarrows NH_4^+$

Since the pK_a 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.

5. The description of evidence that ammonia may act as a cancer promoter is vague and needs additional explanation. There is limited evidence that ammonia may act as a cancer promoter in the stomach when administered to rats orally following pretreatment with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MMNG) (Tsujii et al., 1992;1995); however, chronic studies with ammonium chloride did not produce stomach (or other) tumors when administered via the diet or water (Barzel and Jowsey, 1969; Lina and Kuijpers, 2004).
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 gray 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 the 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 the agency to focus on deriving an inhalation RfC.

3.2.2. Literature Search Strategy/Study Selection and Evaluation

Charge Question B1. 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.

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

As indicated by the EPA in the background materials and presentations to the SAB, the ammonia assessment does not include the problem formulation step that will be incorporated in future assessments. The SAB discussed this issue briefly and agreed with the agency's decision not to include

problem formulation for the current draft assessment since that step will necessarily occur much earlier in the development of an assessment.

The EPA should expand the list of databases included in the literature search. The list of databases included in the literature search strategy is appropriate but not sufficiently comprehensive for the purpose of systematic review. It appears mainly to be derived from prior practice at EPA and, as stated in the assessment, from the literature review supporting the ATSDR's Toxicological Profile for Ammonia (ATSDR, 2004). The SAB agrees with EPA's objective to reduce unnecessary duplication of efforts across agencies. However, since it is unclear if and to what extent the ATSDR's toxicological profiles incorporate principles of systematic review to generate their literature search results, they should not be deemed directly transferable to the EPA's assessment without further clarification. In addition, further explanation is needed as to why only these databases (Table LS-1, page xxxvii) were deemed sufficient for the purposes of a systematic literature review. There are additional relevant databases potentially suitable for the ammonia assessment, in particular important toxicology-specific databases such as EPA's Office of Pesticide Programs (OPP), Organisation of Economic Co-operation and Development's (OECD) High Production Volume (HPV) Chemicals, EPA's HPV database, the Registry of Toxic Effects of Chemical Substances compiled by NIOSH/Health Canada and European Chemical Agency (ECHA), among others, that were not considered. The SAB recommends that they be incorporated in the search strategy.

The process of selecting potentially relevant studies needs to be clarified. The initial broad literature search and some of the operational aspects of the strategy (e.g., timeline, search keywords, search strings, forward and backward, and forward searches) are fairly well described, consistent with Section 3.1 of the Preamble and with systematic review principles, and reflect progress towards implementing NRC's (2011) recommendations. However, the SAB noted that the process for conducting the subsequent more targeted follow-up searches was unclear. There are no evident relationships between the series of questions that guided the follow-up searches (as described in NRC, 2011, page 158, and further expanded in NRC, 2014) and the results of the search sequences depicted in Figure LS-1 on page xxxviii of the assessment. The narrative description of the selection process presented on page xxxvi is useful but too general. As a result, the process leading to study selection shown in Figure LS-1 is not sufficiently informative and leads to confusion. The explanation for the list of secondary keywords (Table L-S1, footnote c) used to include/exclude publications following the initial broad search does not provide adequate information on the follow-up queries and the corresponding inclusion/exclusion criteria. Consequently, the rationale for many of the inclusion/exclusion criteria used following the secondary keyword searches is difficult to follow. For example, one of the criteria for excluding publications following the secondary keyword search is "Co-exposure to other chemicals", but co-exposures are also present in many of the human studies included and used in the assessment, so this exclusion criterion must have had some specific target(s) at a specific stage in the search that is not discernable by the SAB. Another example is exclusion because the "Exposure route not relevant" or "Non-standard animal model" which, taken at face value, could exclude publications potentially relevant for understanding mechanisms of action. While, as indicated earlier, the SAB realizes that standard protocols for IRIS-specific systematic review of the literature have not yet been developed, transparency in the ammonia assessment could be enhanced by adding a table listing key queries with links to Figure 1 and Table LS-1. This list could be added to Appendix D of the supplemental information as a new table or, alternatively, Table D-1 could be modified to include these questions with links to the search strings in Table D, keywords in Table LS-1 and inclusion/exclusion criteria in Figure LS-1.

Inclusion/exclusion criteria for studies should be made more transparent. Additional clarification of inclusion/exclusion criteria may provide some insight as to why some apparently relevant publications were not included or cited. For example, it is unclear why an epidemiologic study by Mirabelli and co-authors (2007) that utilized the ECHRS II cohort and included exposure of hospital nurses to cleaning solutions was either not found in the search or found but excluded. This study evaluated the risk of new onset asthma in a large cohort of workers that were occupationally exposed to cleaning solutions containing ammonia. In addition, the SAB encourages EPA to reconsider the inclusion of publications beyond the March 2013 deadline (e.g., Hovland et al., 2014).

Exclusion of ammonium salts should be supported by a thorough review of the relevant literature. EPA excluded ammonium salts from consideration because of uncertainty about the influence of different anions on reported effects. The appropriateness of this exclusion is discussed later in detail in relationship to the RfD derivation. However, it appears that Table C-1 was compiled based on whatever studies on ammonium salts happened to be readily available, rather than based on a systematic search for studies. It is possible that this is an incorrect perception, and that Table C-1 includes oral toxicity studies on ammonium salts based on a systematic search and, if so, EPA should indicate this clearly in the description of search criteria and in Appendix C of the supplemental information. The rationale for excluding ammonium salts could also be buttressed by adding data on LC50's and LD50's for various ammonium salts to show their variability.

The description of studies needs to be made uniform across all types of studies. Apart from the limitations mentioned above, the selection and evaluation of key studies is fairly well supported. The summaries in the tables provided in Appendix E-2 of the supplemental information are well designed and informative (it would be useful to provide hyperlinks between citations and E-2 summaries in electronic versions of the assessment and supporting information). EPA identifies the criteria used in the evaluation process [lines 19-21 in page xxxix (which correspond to most of the criteria summarized on page 158 of NRC, 2011)] and addresses each criterion separately across categories of studies. Description of key study characteristics according to the criteria and major limitations are likewise listed in appendices D-2 to D-4. The same outline is applied to the health care/cleaning and livestock farming settings in pages xlii-xliii, but not to the industrial studies, so it is recommended that the outline for the narrative be made uniform, with particular attention paid to describing the range of different co-exposures presents in the various types of study settings.

The potential contribution to ammonia exposure from tobacco smoke should be described. Smoking is a possible confounder in many of these studies since it contributes to ammonia exposure as well as to other co-exposures impacting health outcomes. For example, Seeman and Carchman (2008) reports on ammonia exposure from cigarette smoke exposure. This study is highly suggestive that habituated cigarette smokers need to be separated from non-smoking groups, not just for respiratory effects of smoking, but additionally due to potential supplemental exposure to ammonia due to its contamination within tobacco products, as well as the likelihood of differential smoking rates across occupational exposure groups (without which there is no confounding). Therefore, there should be some mention of the relevant literature on the varying levels of ammonia in tobacco and its presence in cigarette smoke (in addition to intrinsic levels, tobacco can also be treated with ammonia so ammonia concentrations in cigarette smoke can vary significantly).

EPA should clarify the criteria by which it determines the significance of specific limitations in studies. A clarification (or citations to relevant guidelines) as to how EPA judges a potential limitation to be a major one or not should be added. An overall summary of the consistency of exposures, confounders and outcomes across categories of studies (including relevant findings from studies summarized in Appendix E.2.3.) would help to further support this section of the assessment.

The criteria by which a study is judged acceptable for assessment purposes should be clearly stated. Although the narrative provides an evaluation of the studies according to preselected criteria, not all criteria recommended by the NRC (2011) are incorporated (e.g., precision of the effect) and there is no specific overall study quality indicator. While it is understood that this is an area still under development, the application of the study quality criteria for the selection and evaluation of key non-cancer experimental animal studies that were included in the assessment is unclear. For example, in the *Hazard Identification* section, some of the studies summarized in Table 1-3 and in Figure 1-1 have inadequate sample sizes and/or substandard reporting of results. The Preamble indicates that the quality of each individual study is assessed (page xvi), but it does not explicitly state the criteria on which a study is deemed unacceptable or of low quality for assessment purposes. Although, studies of low or inadequate quality could be included in the supplementary tables, selection of pertinent studies for assessment purposes in Table 1-3 and Figure 1-1 should be based on specific minimal standard criteria for acceptability for assessment purposes. Alternatively, a score for the quality of the studies presented in Table 1-3 and Figure 1-1 could be included. However, removing studies that are of inadequate or low quality might streamline EPA's assessment.

In developing criteria for ranking the quality of studies, EPA might determine that good quality studies are those which satisfy minimal EPA guideline requirements for sample size, dose selection, and control for bias for subjective measures (i.e., OPPTS 870 guidelines; OPPTS, 1998). EPA's Preamble (section 4.2 and 6) should include reference to these guidelines and not just the guidance documents as points of comparison. The cited EPA guidance documents do not include guidance for evaluating the quality of the studies. Other approaches may include expert judgment or the use of a Klimisch scoring approach (Klimisch et al., 1997) which is widely used by European authorities to evaluate the quality of studies or other similar schemes, such as, an older approach used by EPA's Office of Pesticide Programs. Expert judgment is required in these and other scoring approaches, due to the complexity of research protocols, outcomes, and casual inference.

Finally, it is not clear why EPA did not extend requests for additional data from the public beyond 2009 (lines 17-18, page xxxvi); this should be clarified.

Recommendations:

1. The SAB recommends that the EPA expand the list of databases included in the literature search
2. The SAB recommends that the process for selecting studies be clarified and the inclusion/exclusion criteria for studies be more transparent. Additional clarification may provide some insight as to why some apparently relevant publications were not included or cited.
3. The SAB recommends that the exclusion of ammonium salts be supported by a thorough review of the relevant literature.
4. The SAB recommends that the description of studies be made uniform across all types of studies.
5. The SAB recommends that the potential contribution to ammonia exposure from tobacco smoke be described.

6. The SAB recommends that the EPA clarify the criteria by which it determines the acceptability of studies and the significance of specific limitations in studies.

3.2.3. Hazard Identification: Synthesis of Evidence

Charge Question C1. A synthesis of the evidence for ammonia toxicity is provided in Chapter 1, Hazard Identification. Please comment on whether the available data have been clearly and appropriately synthesized for each toxicological effect. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically supported.

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

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

The SAB also recommends that the biological bases for tolerance/adaptation be considered as part of the evaluation, and that gastrointestinal effects of ammonia be re-examined as part of a more integrated evaluation of the *in vivo* biological properties of ammonia. As noted elsewhere in this report the pK_a of ammonia is 9.2. Thus, in the highly acidic environment of the stomach ammonia will exist completely as the form of ammonium ion (NH_4^+) whether the ammonia is administered to the gastrointestinal tract as ammonium hydroxide or as ammonium salts, such as ammonium chloride or ammonium acetate. In one study, at intervals over a period of 90 days ammonium acetate was administered in standard chow to rats at a concentration of 20% wt/wt (and simultaneously in the drinking water at a concentration of 5 mM) (Bodega et al., 1993). Initially, this treatment resulted in moderate hyperammonemia, but within a few days, the blood ammonia levels were normalized. This finding suggests an adaptive biochemical response to the high ammonia levels in the gastrointestinal tract, such as decreased gastrointestinal transport of NH_4^+, increased removal of NH_4^+ as urea in the liver, increased removal of NH_4^+ in extrahepatic tissues through incorporation into glutamine, and/or increased excretion of NH_4^+ via the kidneys. Tolerance should also be discussed in the context of exposure to ambient ammonia (NH_3) gas. For instance, the definition of a no adverse effect level (NOAEL) level based on the responses of workers chronically exposed to ammonia fumes as described in Holness et al. (1989) should take into account the tolerance known to occur in humans and animals exposed repeatedly to irritant gases such as ammonia. Tolerance may lead to underestimation of risk of injury in the nasal and lower respiratory

tracts in humans, if as suggested by animals studies, induction of tolerance to sensory irritation compromises perception of the presence of injurious concentrations of inhalable irritants (Barrow and Steinhagen, 1982). The integration of principles of tolerance into the evaluation should be differentiated from "healthy worker" issues or independent host factors, such as genetics, also known to influence the response and sensitivity to inhalable irritants.

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

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, Brand A, Landrau VJ, Wirtshafter D, Park TJ. (2009). Extreme tolerance to ammonia fumes in African naked mole-rats: animals that naturally lack neuropeptides from 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.

Recommendations:

1. The SAB recommends that documentation on how the evaluation criteria were applied to individual studies and ultimately integrated into the weight of the evidence analysis be presented and captured in the tabular summaries.
2. Tolerance may lead to underestimation of risk of injury in the nasal and lower respiratory tracts in humans; therefore, the SAB recommends it should be considered as part of the evaluation.
3. The SAB recommends that gastrointestinal effects of ammonia be re-examined as part of a more integrated evaluation of the *in vivo* biological properties of ammonia.

3.2.4. **Hazard Identification: Summary and Evaluation**

Charge Question C2. 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?

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.

Recommendation:

1. The SAB recommends an expansion of the discussion relating to possible gastrointestinal effects, as well as their impact on the decision not to derive an RfD.

Charge Question C3. 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?

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. There is limited evidence that ammonia may act as a cancer promoter in the stomach when rats are exposed orally following pretreatment with the initiator N-methyl-N'-nitro-N-nitrosoguanidine (MMNG) (Tsujii et al., 1992;1995); however, chronic studies with ammonium chloride did not produce stomach (or other) tumors when administered via the diet or water (Barzel and Jowsey, 1969; Lina and Kuijpers, 2004). 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 two potentially relevant lines of evidence should be considered as part of the evaluation as shown below.

Recommendations:

1. The SAB recommends further discussion of an epidemiologic study regarding promoter influences (Fang et al., 2011).
2. The SAB recommends an expanded evaluation of an early animal study reporting increased numbers of adenocarcinomas following delivery of ammonium acetate via intra-rectal infusions (Clinton et al., 1988).

3.2.5. Oral Reference Dose (RfD)

Charge Question D1. 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.

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

There are, however, studies suggesting gastrointestinal effects (gastric mucosal thinning, compensatory cell replication of the cells of the gastric mucosa). The EPA confined its consideration of ammonia-related gastrointestinal effects to three studies —Tsujii et al. (1993); Kawano et al. (1991); and Hata et al. (1994)— based on the criterion that these were the only controlled (and non-acute) studies of gastrointestinal effects that employed ammonia (i.e., NH_3) *per se*. The EPA intentionally excluded from consideration studies of gastrointestinal effects (or the lack thereof) with oral administration of ammonium (NH_4^+) salts. This decision was based on concerns that the possible adverse effects of ammonia in such studies could not be separated from adverse effects resulting from the associated anion. This rationale needs further clarification, evaluation and justification than is currently provided in Appendix C of the supplemental information appended to the ammonia assessment.

The SAB concluded this dichotomy between ammonia *per se* and ammonium salts with respect to ingestion was based, in part, on a misunderstanding of the chemistry of ammonia and ammonium (the basic chemistry of ammonia needs to be addressed in a separate introductory section). Ingested ammonia almost instantaneously becomes the ammonium ion in an aqueous solution, such as that present in the stomach. In the three studies that the EPA considered as a possible basis for an RfD based on effects on the gastric mucosa, it is not clear whether ammonia gas was bubbled through water to make the dilute "ammonia" solution that the rats consumed, or whether ammonium salts (most likely NH_4Cl) were dissolved in the water. The SAB recommends that the EPA attempt to contact the investigators to clarify this question. In either case, however, the resulting aqueous solution likely contained predominantly NH_4^+, and a much smaller concentration, typically 50+-fold lower, of NH_3. Furthermore, even if the solution was made by the addition of ammonium salts, such as NH_4Cl, the NH_4^+ would be present as the free ion in the gastric fluid of the stomach. Thus, while a possible independent gastrointestinal (GI) toxicity of the anion may be a valid concern, the dichotomy between ammonia and ammonium in the consideration of an RfD is not.

The SAB agrees that there is evidence that the anion (chloride, acetate, sulfate) can impact toxicity of the ammonium salt on organs such as the kidney, liver and adrenal gland based on the published studies (e.g., Ota et al., 2006). This supports EPA's decision not to include toxicity of ammonium salts to derive reference values for ammonia or ammonium hydroxide. However, the EPA needs to strengthen the rationale presented in the assessment to support their decision. The main report references Appendix C of the supplemental information for evidence that the anion impacts the toxicity of the ammonium salt, yet there is insufficient information in Appendix C to make comparisons across ammonium salts because the negative findings of key organs are not reported. Reporting the negative results is also necessary to support EPA's discussion of ammonium's effect on the thickness of the gastric mucosal layer. The EPA draft assessment correctly states that there are no effects in the stomach or other parts of the GI tract following chronic exposure to either ammonium chloride or ammonium sulfate, and references Appendix C, Table C-1. Yet Appendix C and Table C-1 do not report the negative results. Table C-1 can be improved so that the comparisons across ammonium salts are more systematic and transparent by adding (a) the dose of the salt expressed as mmole per kg body weight, (b) the sample size, and (c) positive or negative results for key organs that were affected by at least one ammonium salt (or indication that the organ was not examined).

The gastric mucosal thinning observed by Tsjuii et al. (1993) and Kawano et al. (1991) with sub-chronic exposure was not clearly progressive and was not accompanied by observed micropathology. The SAB discussed the nature of this effect at some length and particularly whether there was a biological basis for assuming that this effect could be progressive with chronic exposure. This question could not be resolved with studies on ammonia alone given the lack of knowledge about the nature of the effect, itself and the lack of chronic exposure data. However, the SAB noted that EPA had included in Appendix C a description of the study of Lina and Kuijpers (2004), a robust rat study of chronic exposure to NH_4Cl with comprehensive pathology evaluation in which the dose was somewhat higher than those in the three studies that the EPA more formally considered. In addition, the study design also included a Cl^- control group. The SAB obtained a copy of this paper and noted that the results do not appear to show

gastric pathology. Given both the chemistry of ammonia/ammonium as discussed above and the presence of a control group in this study that appears to address potential concerns about the toxicity of the anion, this study does support EPA's conclusions (1-20 and 2-1) that the data does not support deriving an oral RfD based on GI findings. It is not clear why the EPA did not more fully address this study. Furthermore, there appears to be a number of animal studies of oral exposure to ammonium salts that, if EPA decides to address the ammonium ion along with ammonia in this assessment, could additionally inform consideration of gastrointestinal effects.

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

Recommendations:

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

Charge Question D2. As described in the Preface, data on ammonia salts were not considered in the identification of effects of the derivation of an RfD for ammonia and ammonium hydroxide because of concerns about the potential impact of the counter ion on toxicity outcomes. Please comment on whether the rationale for this decision is scientifically supported and clearly described.

Due to the lack of clarity about the chemistry of ammonia/ammonium, and given the existence of at least one study of ammonium that appears to have adequately controlled for the possible toxicity of the counter ion (Lina and Kuijpers, 2004), the SAB concluded that the rationale for the decision not to derive an RfD for ammonia should be further expanded (refer to the response to Question D1 for further information). As EPA considers this SAB response, it should also contemplate additional studies possibly relevant to the selection of the RfD as further described in Appendix C of this report.

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

Recommendation:

1. The SAB recommends that the rationale for the decision not to derive an RfD should be better supported and more clearly described.

3.2.6. Inhalation Reference Concentration (RfC)

Charge Question E1. Please comment on whether the evaluation and selection of studies and effects for the derivation of the RfC is scientifically supported and clearly described (see Section 2.2.1). Please identify and provide the rationale for any other studies or effects that should be considered.

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

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

Some panel members expressed concern about the selection of self-reported respiratory symptoms and small subclinical changes in lung function measures as "adverse" health outcomes, requesting that the EPA elaborate on its rationale. For example, coughing and sneezing are relatively mild compared to many other adverse health outcomes used in RfC derivation for other chemicals; e.g., decreased lymphocyte count for benzene (U.S. EPA, 2003); hand tremor, memory disturbance, autonomic dysfunction for mercury (U.S. EPA, 1995); and fetal cardiac malformations and decreased thymus weight for trichloroethylene (U.S. EPA, 2011). In addition, the use of pre-shift to post-shift comparisons in some of the studies suggests that these health outcomes may be reversible overnight, at least in part. The SAB therefore recommends that further discussion of the potential implications of reversibility and long-term attenuation of effects through acclimatization and/or the healthy worker effect (e.g., self-selected attrition due to respiratory symptoms) be added. The different studies have different goals and thus different designs. These are issues that need to be mentioned, and could lead to underestimates of effect (e.g., a healthy worker effect).

Additional comments and information that is relevant to the selection studies for the derivation of an RfC can be found in Appendix C of this report. The SAB also noted a News and Analysis article that appeared in a recent issue of Science (Stoktad, 2014). According to the authors of the original study (Paulot and Jacob, 2014), ammonia gas emanating from farming practices can form aerosols that

adversely affect human health. The assessment only considered worker exposure to ***gaseous*** ammonia; a brief discussion of the possible deleterious effects of air-borne ***particulate*** ammonia should be added.

Recommendations:

1. The SAB recommends that a better description of the controlled human studies and the rationale for their exclusion should be strengthened.
2. The SAB recommends that further discussion of the potential implications of reversibility and long-term attenuation of effects through acclimatization and/or the healthy worker effect (e.g., self-selected attrition due to respiratory symptoms) be added.

Charge Question E2. The NOAEL/LOAEL approach was used to identify the point of departure (POD) for derivation of the RfC (see Section 2.2.2). Please comment on whether this approach is scientifically supported and clearly described.

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

Considering that no significant adverse effects were found in any dose group in the Holness (1989) study, the highest exposure concentration at which effects do not occur is most likely greater than the minimum ammonia concentration in the highest exposure group (8.8 mg/m^3). One reason to prefer a central estimate (i.e., mean or median) of the high exposure group ammonia concentration rather than the minimum is that the reported range of concentrations is only for a single 8.5-hour work shift. Because day-to-day exposure concentrations can vary extensively in occupational settings, one should expect the minimum concentration (the basis of EPA's NOAEL) to be highly unstable and to be different if the study were conducted on a different day. In contrast, the arithmetic mean exposure concentration would be much more stable with repetition of the study due to the central limit theorem and shrinkage of each participant's estimated chronic exposure level towards the grand mean. If individual data are unavailable, EPA should consider whether there is sufficient information available in the Holness publication to estimate the mean concentration for the high exposure group--perhaps assuming a lognormal or other skewed distribution for the measured concentrations. The Holness study should be used whether the individual data are obtained or not.

For the point of departure (POD) derived from the Holness (1989) study, a dose conversion factor was used to convert the observed workplace ammonia concentration to an ammonia concentration that would provide an equivalent cumulative dose with continuous 24/7 exposure. This presumes that the reported respiratory and lung function effects of ammonia are due to cumulative exposure rather than acute exposure, but it is not clear to what extent that assumption is supported by evidence. There is some support provided in Table 3 of the Ballal et al. (1998) study, which indicates significant effects of exposure duration on risks of cough, phlegm, and wheezing (even when accounting for the exposure concentration). This evidence of a cumulative effect of ammonia exposure is important, especially if

corroborated by other studies.

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

Recommendations:

1. The SAB recommends that EPA attempt to obtain individual-level data and/or the mean/median exposure concentrations for the high dose group from Dr. Holness in order to identify a better-supported point of departure.
2. The SAB recommends that the evidence supporting the presumption that the reported respiratory and lung function effects of ammonia result from cumulative exposure rather than acute exposure should be clarified and strengthened. The evidence of a cumulative effect of ammonia exposure is important, especially if corroborated by other studies.
3. The SAB recommends that the source of the exposure values used in the assessment and the rationale for their use should be clarified.

Charge Question E3. Please comment on the rationale for the selection of the uncertainty factors (UFs) applied to the POD for the derivation of the RfC (see Section 2.2.3). Are the UFs appropriate based on the recommendations described in Section 4.4.5 of A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002), and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

The selection of the uncertainty factor was appropriate, clearly described, and consistent with the 2002 EPA recommendations. There was some discussion on whether the critical effect was related more to irritation rather than inflammation (suggesting a smaller uncertainty factor for human variability because of less variation in toxicokinetics). The SAB ultimately judged that the critical effect was not clearly irritation, supporting EPA's choice of the default uncertainty factor of 10. Moreover, the healthy worker effect, perhaps operating in the Holness study, would also support this default value.

While inhalation studies do not provide evidence for the neurotoxicity of ammonia in humans, there is considerable evidence that systemic administration of ammonia (intraperitoneal or arterial infusion) produces a neurological response in experimental animals (Hindfelt et al., 1977; Voorhies et al., 1983). However, the overall weight of evidence suggests that oral exposure to ammonium salts does not produce evidence of neuropathy. EPA is referred to Appendix C of this report for additional information that might clarify aspects of this toxicity relevant to the development of risk assessment values.

3.2.7. Quantitative Cancer Assessment

Charge Question F1. 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.

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

3.2.8. Endogenous Production of Ammonia

Charge Question G1. 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.

The description of endogenous ammonia production appears to be generally appropriate, but the SAB recommends expanding this section to describe all sources of endogenous ammonia. There is no doubt that ammonia in expired breath is increased in pathological conditions that give rise to hyperammonemia. Studies suggest that absorption of ammonia in lungs occurs in a compartment that does not readily mix with the metabolic pool of ammonia. The amount of ammonia that equilibrates between the endogenous lung metabolic pool and alveolar air is likely to be quite small even under hyperammonemic conditions. The concentration of ammonia in oral cavity air is an indicator of the exhaled concentration (including the contribution from the bacterial digest of residual food particles in the mouth). However, because of confounding problems with "contaminating" ammonia in the expired air and difficulties associated with its actual measurement, it may be challenging to correlate prior ***chronic*** exposure of individuals to ammonia with alveolar ammonia concentrations. Additionally, the concentration of ammonia in oral cavity reflects neither the endogenous inhaled ammonia (which is closely related to the alveolar ammonia concentrations), nor the concentration of ammonia in inhaled air (since mouth air is dilated with external air on inhalation). Thus, the concentration of ammonia in the mouth is not a major contributor to either the systemic or inhaled concentration of ammonia. Furthermore, it should be noted that exhaled ammonia concentrations are likely higher than inhaled concentrations even for mouth breathers, much as exhaled CO_2 is higher than inhaled CO_2. There is no reason to assume that exhaled air is safe for continuous inhalation—indeed, continuous inhalation of exhaled air, as eventually occurs in a small enclosed space, is deadly.

As a means of providing further context for the potential contributions of endogenously generated ammonia to NH_3 inhalation doses, the EPA should consider including concentration ranges for typical indoor and ambient concentrations of ammonia. These data need not be comprehensive (i.e., the result of a systematic review) but also will be helpful for placing the RfC in the context of expected concentrations in non-industrial, residential, and office indoor environments, and in outdoor air (for example, data collected by EPA's Passive Ammonia Monitoring Network). These concentration ranges could then be included as part of the Executive Summary. Along with this information, the assessment should clearly state that exhalation of air and ammonia, like other excretion processes, is a clearance mechanism of an otherwise toxic contaminant.

In addition, there are several areas in the assessment, the supplemental information and in other materials provided that should be revisited and may need to be modified. Specific instances in the documents (including page and line references) are provided where the SAB finds that a change or clarification of the provided materials could be beneficial (see Appendix VI of this report). One general suggestion for EPA's section on endogenous ammonia, and also a general comment for the assessment, is to more explicitly discuss the different chemical aspects of the two different molecular forms of ammonia, NH_3 and NH_4^+.

Additional Comments for consideration on the endogenous ammonia section.

There are very many enzyme-catalyzed reactions by which ammonia can be generated *in vivo*. For example, Cooper and Plum (1987) list at least seventeen enzyme-catalyzed reactions that can generate ammonia from amino acids and nucleotides in the brain. Considerable ammonia is generated in the gut from the action of bacteria on nitrogenous substance. In humans, a large portion of this ammonia is derived from the hydrolysis of urea by urease-containing bacteria in the colon (Gibson et al., 1976). The kidney is also a net producer of ammonia (Weiner et al., 2014). Tracer studies suggest that in human volunteers 15-30% of urea synthesized in the kidney is converted to ammonia by intestinal bacteria (Walser and Bodenlos, 1959). Intestinal cells utilize glutamine as a major energy source (Pinkus and Windmueller 1977; Roediger 1982; Mallet et al., 1986). As a result of the bacterial action and endogenous production of ammonia from glutamine by intestinal cells the concentration of ammonia in the portal vein can be quite high (0.2 mM – 0.3 mM, rising to ≥1 mM in hyperammonemia (Häussinger and Sies, 1979). In contrast, because of the efficient removal of ammonia by liver periportal cells as urea and to a lesser extent removal of ammonia as glutamine in perivenous cells (see Appendix C of this report) the concentration of ammonia in the systemic circulation is much lower than that of portal blood. For example, the clinical reference for the upper limit of normal concentration of ammonia in human blood is 40 μM. These and other related reactions lead to the exhalation of a small amount of ammonia gas from the lungs under normal human metabolism. Additional information on the endogenous production of ammonia by humans can be found in Appendix C of this report.

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

Recommendations:

1. The SAB recommends that the discussion of endogenous ammonia be expanded.

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APPENDIX A. Charge to the Science Advisory Board for the IRIS Toxicological Review of Ammonia

August 2013 (Updated March 2014[2], June 2014[3] and July 2014[4])

Introduction

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

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

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

- a *Preamble* to describe the methods used to develop IRIS assessments;
- an *Executive Summary* to concisely summarize the major conclusions of the assessment;
- a *Literature Search Strategy/ Study Selection and Evaluation* section to describe the process for identifying and evaluating the evidence for consideration in developing the assessment;

[2] 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.
[3] The charge questions were modified (as shown in bold font) as a result of panel discussions during the June 2, 2014 preliminary teleconference.
[4] The charge questions were modified to refer reviewers to specific sections in the assessment.

- a *Hazard Identification* chapter to systematically synthesize and integrate the available evidence of organ/system-specific hazards; and
- a *Dose-Response Analysis* chapter to describe the selection of studies for consideration in calculating toxicity values and to describe the analysis and methodology in deriving and selecting toxicity values.

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

Charge Questions

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

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

General Charge Questions:

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.[5]

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 assessment structure and whether it will increase the ability for the assessments to be more clear, concise, and easy to follow.

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

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

Chemical-Specific Charge Questions:

A. Executive Summary

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

B. Literature Search Strategy/Study Selection and Evaluation

1. The process for identifying and selecting pertinent studies for consideration in developing the

[5] Whether such guidance and methods were used in this ammonia IRIS assessment will be the focus of chemical-specific questions.

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.

C. Hazard Identification

Synthesis of Evidence

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

Summary and Evaluation

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 **or systemic toxicity through other routes** (see Section 1.2.1)?

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* (Section 2.5 of U.S. EPA), there is "inadequate information to assess the carcinogenic potential" of ammonia (see Section 1.2.2)?

D. Oral Reference Dose (RfD)

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

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.

2. As described in the *Preface*, data on ammonium salts were not considered in the identification of effects or the derivation of an RfD for ammonia and ammonium hydroxide because of

concerns about the potential impact of the counter ion on toxicity outcomes. Please comment on whether the rationale for this decision is scientifically supported and clearly described.

E. Inhalation Reference Concentration (RfC)

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

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

2. The NOAEL/LOAEL approach was used to identify the point of departure (POD) for derivation of the RfC (see Section 2.2.2). Please comment on whether this approach is scientifically supported and clearly described.

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

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.

APPENDIX B. Specific Comments on the Toxicological Assessment

Comments on the Executive Summary

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

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

1. Brief description of the chemistry of ammonia, ammonium and ammonia salts and rationale for excluding ammonium salts (to support current EPA assessment) or to include ammonium salts (if EPA decides to include). This is very important to provide adequate background to understand (a) EPA's inclusion/exclusion criteria for literature, and (b) EPA's decision on whether or not to derive an oral RfD based on ammonium salts.
    a. Based on comparisons of papers on ammonium salts summarized in Appendix C and more recent repeat-dose paper on ammonium acetate (e.g., comparing Lina and Kuijpers, (2004) with Satpute et al. (2012)), EPA's conclusion that the anion appears to influence the dose response and target organ toxicity seems appropriate.
    b. The table in Appendix C comparing different ammonium salts can be improved by adding dose concentration in mmoles ammonia/kg body weight, adding sample size, and including both negative and positive results for key organs affected.
    c. Although data from ammonium salts should not be used to characterize dose-response relationships for ingested ammonia, the absence of findings can contribute to the weight of evidence for hazard identification. A statement to that effect may be useful for later discussions about the oral RfD.

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

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

Page xxx, Line 38: Strengthen the discussion immediately following p. xxx line 38 that chronic studies on ammonium salts (Lina and Kuijpers, 2004; Ota et al., 2006) indicate that measured decreases in thickness of gastric mucosa (Hata et al., 1994; Tsujii et al., 1992; 1993) do not appear to progress to adverse effects following chronic exposures. EPA's discussion of this on page 1-20 regarding absence of histopathology in the stomach following chronic exposure to ammonium salts should be briefly summarized here in the Executive Summary so that EPA's rationale for not deriving an oral RfD is strengthened and made more transparent.

Page xxxii, Line 14 to Page xxxiii Line 6: The EPA can strengthen their selection of the higher control levels of exposure from the Holness et al. (1989) by including a brief description and explanation of the severity or magnitude of change in FEV1 and FVC relative to the clinical level of concern (i.e. difference of 200 ml – Murray et al. (ed. 1994) in the Rahman et al. 2007 study,

and clarification that the increased prevalence of respiratory symptoms (e.g. cough and chest tightness) are self-reported.

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

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

Comments on the Toxicological Review of Ammonia

Page xi, Line 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.

Page xxx, Line 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.

Page 1-26, Line 35-37: Kidney disease does not cause high plasma ammonia levels.

Page xxx, Line 19: We recommend using the word "indicate or demonstrate" instead of "suggest" in this sentence. There are numerous animal studies showing that ammonia at high concentrations can affect the respiratory system.

Comments on the Supplemental Information

Page E-2, Line 27: We believe that the better quality data suggests/supports that the small intestine also contributes to intestinal ammoniagenesis, 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.

Page E-2, Line 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.

Page E-2, Line 30: The term "active transport" has specific biological meaning and is perhaps inappropriate in this context. Removing the term "active" would correct this issue.

Page E-3, Line 9-10: The older ammonia concentration data were generated using outdated assays for plasma ammonia and are no longer generally considered appropriate for use.

Page E-3, Line 11: The proportion of "total ammonia" present, at pH 7.40 (normal physiologic blood pH), as NH_4+ is ~98.3% and as NH_3 is ~1.7%. The relative amount of each is determined by pH. For every 0.3 pH unit change, the amount of NH_3 changes in parallel by 100% (i.e., with pH 7.70, 3.4%, pH 7.10, 0.85%). The amount of NH4+ 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, *Comp Physiol* 3:201-220, 2013). This issue of the relative amounts of NH_3 and NH_4+ in body fluids comes up multiple times in the assessment. For simplicity sake, this issue is not discussed again.

Page E-4, Line 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.

Page E-6, Line 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.

Page E-6, Line 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.

Page E-6, Line 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.

Page E-6, Line 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, *Am J Physiol Renal Physiol* 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.

Additional Comments on Selection of an RfC

1. Hemoptysis (coughing up blood) was also seen in the Ballal et al. (1998) study, which should be mentioned in EPA's study description.
2. In Table 3 in the Ali et al. (2001) study, the direct comparison between the exposed and the control groups, only the FEV1 % (Forced Expiratory Volume) is higher in the exposed group than in the controls – this appears to be a beneficial effect and should be noted in

EPA's study description, though the authors noted that it could be a result of different smoking rates among the controls and the exposed workers.

3. The small sample size (N=2 and 4) should be highlighted for the Anderson et al. (1964) study. Because of this, less weight should be placed on the results of this study.
4. The study by LD Calvert et al. (2009) might be a helpful starting point if there is merit in characterizing baseline plasma levels of ammonia expected across different populations at risk.
5. There is virtually no difference in pre-shift values between the ammonia and urea plants in the Rahman et al. (2007) study, indicating that the effects that were measured in this study are primarily acute effects of exposure that appear to be reversed overnight. This provides additional evidence supporting EPA's selection of the NOAEL, because the effects reported in the Rahman et al. LOAEL appear to be acute effects of exposure.
6. The addition of an evidence table to Section 2.2.1 is recommended (see Table 2 below for example).
7. EPA could strengthen the justification for selection of the higher control levels of exposure from Holness et al. (1989) by adding a brief description and explanation of outcomes in the Rahman et al. (2007) study. For example, the magnitude of change in FEV1 and FVC (Forced Vital Capacity) are relatively small compared to changes that might be of concern in a clinical setting (i.e., difference of 200 ml). In addition, clarification that the respiratory symptoms (e.g. cough and chest tightness) are self-reported adds perspective. It might also be useful to include a brief discussion of potential co-exposures to other materials/chemicals with similar respiratory effects (e.g. formaldehyde, particulate dust, sodium carbonate).

Other Comments:

In the future, protocols for literature searches should consider the likelihood of revisiting the literature to address specific issues not previously foreseen. Not unrelated to the issues discussed above is the need for revisiting the literature search or undertaking a new one when unforeseen but important questions arise during the review of key and/or supportive publications. An example, admittedly not an ideal one, is the evaluation of the findings by Rahman et al. (2007) because of differences between ammonia concentrations measurements using the PAC III and Dräeger diffusion tubes. EPA contacted Draeger Safety Inc. about the reliability of the two methods in order to select the relatively more trustworthy set of measurements and followed Dräeger's recommendation. Aside from expediency, it is not clear whether or not EPA revisited the literature search for publications of ammonia measurement method comparisons, or indoor and outdoor ammonia measurements, or scripted activity studies of exposure to ammonia which may have identified suitable publications for resolving this specific question. These types of publications are not likely to be captured with the search schemes depicted in Figure LS-1and Table LS-1 or Appendix D. In addition, it would not be unusual to find these types of comparisons in the grey literature or in master degree theses. Protocols for literature searches should incorporate standard approaches to address additional information needs when this type of situation arises.

APPENDIX C. Additional Studies for Consideration

This appendix suggests additional studies relevant to: (1) selection of the RfD, (2) neurotoxic effects from inhalation exposure of ammonia; and (3) endogenous production of ammonia.

Additional Studies Relevant to Selection of the RfD

There are additional reports in the literature in which chronic gastrointestinal administration of ammonia to experimental animals resulted in elevated levels of circulating ammonia. It is of interest that the concentration of ammonia in the colon lumen is extraordinarily high (15 – 44 mM) (Worrell et al. 2008). This value is much higher than that normally present in the portal vein (0.2 – 0.3 mM) and in the blood (<40 μM). The colonocytes also possess NH_4^+ transporters. Thus, the colon is well suited to process relatively large amounts of ammonia. Nevertheless, excessive ammonia can be damaging to parts of the gastrointestinal system. For example, excess ammonia can cause apoptosis in rat gastric mucosal epithelium, possibly as a result of a drain on ATP resulting from excessive conversion of ammonia to glutamine in these cells (Kubota et al. 2004). It is also well known that *Helicobacter pylori* produces copious amounts of ammonia (in part as a result of the action of urease) to counteract the acidity of the stomach; this excess ammonia may contribute to the gastritis often associated with *H. pylori* (Lichtenberger et al., 1995).

As noted in the assessment, accidental or deliberate ingestion of ammonia-containing solutions/foods in humans has occasionally been reported in the literature. These studies have described deleterious gastrointestinal effects. However, the draft assessment does not consider the possibility that ingested ammonia may result in elevated blood ammonia levels and possible neurological consequences. Nevertheless, because of the diffusibility of ammonia and the presence of NH_4^+ transport systems in the colon (Worrell et al., 2008), it is probable that at least some of the ingested ammonia enters the circulation in humans orally exposed to ammonia. Thus, in humans who have ingested ammonia solutions reported nausea, drooling and erythematous/edematous effects on the lips could be considered as systemic effects.

Pilbeam et al. (1983a, b) gavage-fed ammoniated cation exchange resin to normal rats and rats with a portacaval anastomosis (PCA; a model of chronic liver disease). The slow release of ammonia from the resin simulates chronic hyperammonemia. Marked hyperammonemia was noted in the animals administered the ammoniated resin, especially in the PCA rats. Severe neurological symptoms were noted in the PCA rats administered the ammoniated resin. Damage not only to astrocytes, but also to some oligodendrocytes and neurons, was noted with nuclear and cytoplasmic swelling (Pilbeam et al. 1983a). Rats with a PCA fed ammoniated resin showed increased chloride content and Na^+:K^+ ratio in the brainstem, and an increased chloride space in the brainstem (Pilbeam et al.. 1983b). In other studies Grisolía and colleagues administered ammonium acetate (20% w/w) in the diet of rats to generate a simple model of chronic hyperammonemia (Azorin et al., 1989). The concentration of ammonia in the blood of these animals was increased threefold and there were marked increases of ammonia in brain, liver and muscle. Urea excretion increased two fold and brain glutamine increased twofold. In other studies from this group it was shown that chronic ammonium acetate in the diet of rats altered the mitochondrial ratio NAD^+/NADH in the brain (Kosenko et al.. 1993).

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

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

Additional Studies on Neurotoxic Effects from Inhalation Exposure to Ammonia

While inhalation studies do not provide evidence for the neurotoxicity of ammonia in humans (IRIS assessment, p I-27) there is considerable evidence that systemic administration of ammonia produces a neurotoxic response in experimental animals. As mentioned earlier, the ammonia assessment suggests that administration of ammonium salts can be problematic due to the confounding effects of the counter anion. For example, if ammonium chloride is administered systemically the chloride ion may have a deleterious effect on acid-base balance that is directly related to the ammonium ion. For this reason, researchers studying the neurotoxic effects of ammonia in experimental animals usually administer ammonium acetate on the assumption that the acetate is rapidly metabolized to CO_2. The ammonium acetate is most often administered by an intraperitoneal route (e.g. Hindfelt et al., 1977), but sometimes by arterial infusion (Voorhies et al., 1983). A few studies have also been reported in which ammonium acetate was administered to rats via a gastrointestinal route. For example, in a recent study, Satpute et al. (2014) administered 100 mg/kg daily of ammonium acetate to rats by gavage feeding for 4 months and noted toxicity toward liver, kidney and brain. However, the results of this study must be interpreted cautiously. Blood ammonia levels were reported both as μM/ml (an impossible unit) and as μmol/ml. Assuming the latter, then the baseline levels of blood ammonia reported in this study would be 60

mM. If one assumes that there was a typographical error and that the authors meant 60 μM (instead of 60 mM) then this would be an acceptable level for ammonia in control rat blood. The increase in blood ammonia after four months of gavage feeding would then be modest – from 60 μM to ~100 μM. This level of ammonia in blood is unlikely to produce neuropathy in rats. A confounding problem is that no analysis of the GI tract was performed by Satpute et al. (2014). It is the opinion of the committee that damage to the GI tract resulting from four months of force feeding may have contributed to the reported damage to various organs. This conclusion is bolstered by the findings, mentioned above, of Bodega et al. (1993). These authors reported blood ammonia levels of ~300 μM following administration of ammonium acetate via the food/drinking water for 90 days) (Bodega et al., 1993) or for 15 days (Boyano-Adanez et al., 1996) resulting in inconsequential changes in the brain – a transient, but non-significant change in brain glial fibrilliary acidic protein and a decrease in somatostain binding, respectively. No overt encephalopathy was noted in either study. The findings of Bodega et al. (1993) and Boyano-Adanez et al. (1996) are also consistent with findings reported by Lina and Kuijpers (2004). These authors administered a long-term diet supplemented with 1% or 2.1% NH_4Cl to rats. The authors also investigated the effect of long term administration of 3% KCl (which may be regarded as a control for chloride in the NH_4Cl-treated group) to a separate group of rats. NH_4Cl treatment resulted in metabolic acidosis. Both treatments were associated with hypertrophy of the adrenal zona glomerulosa, but no effect on the brain was noted (see Table 1). In summary, the weight of evidence suggests that ammonium salts in the form of ammonium acetate or ammonium chloride chronically administered to the gastrointestinal tract of experimental animals (rats) at moderate concentrations do not cause major neuropathy.

It has long been known that acute (or subacute) hyperammonemia) in experimental animals is associated with stupor, seizures and coma (e.g. Navazio et al., 1961). For example, Voorhies et al. (1983) infused 0.19 – 1.5 M ammonium acetate into monkeys (*Macaca mullata*) by means of an indwelling arterial catheter to achieve a maximal blood ammonia level of ~0.9 mM by 24 h, at which point the animals were comatose. Brain section of sacrificed animals showed marked astrocytic swelling. Once the insult was removed astrocyte swelling abated and the animals returned to normal. In another example, rats were infused intravenously with ammonium acetate for 24 hours to raise the level of blood ammonia from ~30 μM to 400 μM as a model of acute/subacute hyperammonemia (Tanigami et al., 2005). In the same study it was noted that inhibition of brain glutamine synthetase with methionine sulfoximine reduced astrocyte swelling and ameliorated some of the reactive astroglial cytoskeletal alterations seen at 24 h of hyperammonemia. The most commonly used model of chronic hyperammonemia appears to be the portacaval shunted rat in which the contents of the portal vein bypass the liver and are shunted into the systemic circulation. For example, blood ammonia levels were shown to rise from ~85 μM to ~225 μM after 12 weeks of portacaval shunting (Cruz and Duffy 1983). Rats subjected to portacaval shunting are subject to increased cerebral sensitivity to an acute ammonia challenge compared to controls (Hindfelt et al., 1977; Gjedde et al., 1978).

By the 1970s it was realized that inborn errors of the urea cycle result in elevated levels of blood ammonia that are devastating to the infant human brain (Shih 1976). The longer the period of neonatal hyperammonemia in children with defects of the urea cycle, the greater the neurological impairment in the surviving infant (Msall et al., 1984). Normal levels of blood ammonia are <40 μM (typically ~25 μM) in children and adults (Brusilow et al. (2010), but may rise to 1 mM in newborns with severe defects of the urea cycle (e.g. Kuhara et al., 2011).

Why is hyperammonemia so deleterious to the brain? In EPA's draft assessment (p. I-27), it was suggested that glutamate and γ-aminobutyrate play a role in ammonia-induced toxicity. A role for GABA in ammonia-induced HE has been suggested where increased brain ammonia increases the GABA-induced chloride channel current and affects the benzodiazepine receptors in neurons and astrocytes. According to a more recent review by Jones and Mullen (2012) "Evidence of increased GABAergic tone in models of HE has accumulated; potential mechanisms include increased synaptic availability of GABA and accumulation of natural benzodiazepine receptor ligands with agonist properties. Pathophysiological concentrations of ammonia associated with HE, have the potential of enhancing GABAergic tone by mechanisms that involve its interactions with the GABAa receptor complex". Other studies have suggested that hyperammonemia associated with liver disease may compromise energy metabolism, but the changes appear to be subtle (reviewed by Ott and Vilstrup 2014). However, most recent studies of ammonia-induced neurotoxicity have focused on excessive production of glutamine in the brain. Since ammonia enters the brain largely by diffusion (Cooper et al., 1979; Lockwood et al., 1980; Raichle and Larson, 1981) increased circulating ammonia in hyperammonemic syndromes is expected to result in increased entry of ammonia into the brain. This has been confirmed in PET studies with [^{13}N]ammonia in hyperammonemic patients with cirrhosis of the liver (Keiding and Pavese, 2013). As discussed by Cooper and Plum (1987) brain glutamine synthetase is probably not saturated with ammonia. Thus the rate of synthesis of cerebral glutamine is likely to increase in hyperammonemic syndromes with pathological consequences (as outlined below).

A clue implicating excess glutamine production in the neurotoxic response in hyperammonemic encephalopathy is the finding that, unlike most neurological diseases, hyperammonemia results in damage that is largely confined to the astrocytes and not to neurons (Norenberg 1976). Interestingly, in the brain, glutamine synthetase is confined almost exclusively to astrocytes (Martinez-Hernandez et al.. 1977; Norenberg and Martinez-Hernandez 1979). Thus, astrocyte end feet are uniquely poised to "intercept" ammonia entering the brain by diffusion across the blood-brain barrier and to incorporate this ammonia into glutamine. However, this arrangement comes at a price. Most investigators now believe that a major contributor to the neurotoxicity of excess ammonia is the associated increased levels of glutamine in astrocytes. Increased glutamine in these cells results from stimulation of glutamine synthetase perhaps coupled to an ammonia-induced decrease in glutamine egress from astrocytes via the SNAT-5 transporter (Desjardins et al., 2012). Persuasive evidence for a role of excess cerebral glutamine in ammonia-induced encephalopathy is the finding that methionine sulfoximine, a potent inhibitor of glutamine synthetase, protects rodents against neurotoxic doses of ammonia (reviewed by Brusilow et al., 2010). Brusilow and colleagues have argued that the major insult to the brain in hyperammonemic syndromes is excess production of glutamine producing an osmotic stress in astrocytes (the osmotic gliopathy theory) (Brusilow et al. 2010 and references cited therein). Certainly, neural swelling as a result of osmotic stress occurs during hyperammonemia, especially during acute liver failure, and this swelling can be detected in the brains of hyperammonemic HE patients by magnetic resonance (MR) imaging techniques. For example, Mardini et al. (2011) used MR to investigate the cerebral water content of 13 cirrhotic patients confronted with an ammonia challenge. The authors concluded that ammonia can directly drive changes in brain water distribution as a mechanism for cerebral edema development. Since cerebral astrocytes contain glutamine synthetase, the MR data suggest intracerebral formation of glutamine from ammonia. The authors also noted a rapid decrease in

myo-inositol indicating that this organic osmolyte plays a protective role in HE via its release from astrocytes in order to maintain cell volume. Other MR studies have suggested that not only does hyperammonemia induce low grade swelling in astrocytes but edema can also be detected in white matter (Keiding and Pavese 2013).

While there seems to be little doubt that cerebral edema, resulting from excessive accumulation of glutamine in astrocytes, is a major contributing factor to hyperammonemia-induced encephalopathy (especially in acute liver failure) other factors may also contribute. For example, there is considerable evidence for an ammonia-induced neuroinflammatory response in hyperammonemic liver disease. The evidence includes activation of microglia, together with increased synthesis *in situ* of the proinflammatory cytokines TNF, IL-1β and IL-6 (reviewed by Butterworth 2013). In addition, according to Häussinger and et al. (1998) once the astrocytes lose their capacity to self-regulate volume during hyperammonemia and excessive glutamine accumulates, low grade edema sets in, resulting in triggering of "a complex signaling cascade which relies on NMDA receptor activation, elevation of intracellular Ca^{2+} concentration and prostanoid-driven glutamate exocytosis, which result in increased formation of reactive nitrogen and oxygen species (RNOS) through activation of NADPH oxidase and nitric oxide synthase. Since RNOS in turn promote astrocyte swelling, a self-amplifying signaling loop between osmotic- and oxidative stress ensues, which triggers a variety of downstream consequences" (Görg et al. 2013).

Additional Studies on Endogenous Production of Ammonia

An important source of endogenous ammonia is derived from the metabolism of amino acids. A major route for conversion of amino acid nitrogen to ammonia involves coupling of an aminotransferase (transaminase) to the glutamate dehydrogenase reaction. The amino acid is transaminated with α-ketoglutarate to the corresponding α-keto acid and glutamate. The glutamate is then converted back to α-ketoglutarate with the concomitant formation of ammonia in a reaction catalyzed by glutamate dehydrogenase. This ammonia is mainly incorporated into urea in the liver or into glutamine in extrahepatic tissues (see below). Nitrogen transferred from an amino acid to glutamate via a transaminase reaction can be further transferred to aspartate via the aspartate aminotransferase reaction. In the muscle this aspartate nitrogen is a source of ammonia via the purine nucleotide cycle (PNC). For a recent discussion of these pathways see Cooper (2012). The role of the PNC is relatively little studied, but the few studies tended to support the notion that the purine nucleotide cycle is important for the production of muscle ammonia. Other studies suggest that amino acids, particularly the branched chain amino acids are important source of ammonia in exercising muscle (Graham and MacLean 1992). However, because of rapid nitrogen exchange catalyzed by aminotransferases, the arguments may be moot. N flow: (1) branched chain amino acids ⇆ glutamate ⇆ ammonia; (2) branched chain amino acids ⇆ glutamate ⇆ aspartate → ammonia.] It is interesting to note that during exercise muscle is a net source of ammonia. During extreme exercise, such as ultramarathon running, muscle can release pathologically high levels of ammonia that result in disruption of brain function (Wilkinson et al., 2010). On the other hand, during rest muscle appears to be a net sink for removal of ammonia.

The main route for removal of ammonia carried to the liver by the portal vein is incorporation into urea by enzymes of the urea cycle in the periportal hepatocytes. Glutamine synthetase is located in

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

Because extrahepatic tissues do not contain a functioning urea cycle ammonia generated by the breakdown of nitrogenous substances in these tissues must be removed by another mechanism. In most tissues this removal is accomplished by incorporation of ammonia into the amide position of glutamine via a reaction catalyzed by glutamine synthetase. For example, it has been shown, using an intracarotid bolus of [^{13}N]ammonia, that >95% of blood-derived ammonia entering the rat brain (and also, presumably, endogenously derived ammonia) is very rapidly incorporated (in seconds) into glutamine (amide) in a distinct metabolic compartment (astrocytes) (Cooper et al.. 1979). The major route for cerebral metabolism of blood-derived [^{13}N]ammonia in hyperammonemic rats is also via the glutamine synthetase reaction (Cooper et al.. 1985). Ammonia enters the brain mostly by diffusion as the free base (NH_3) (Cooper et al.. 1979; Lockwood et al.. 1980) although a small portion may cross the blood-brain barrier as ammonium ion (NH_4^+) (Raichle and Larson 1981). It should be noted that astrocytes have a remarkable ability to take up ammonia by diffusion of NH_3 and by active transport of NH_4^+ (Nagaraja and Brookes 1998). Because astrocyte end feet underlie the blood-brain barrier and contain high levels of glutamine synthetase (see below) these cells are in a unique position to metabolize blood-derived ammonia and metabolically derived ammonia in the brain (Cooper and Plum, 1987)

Freed and Gelbard (1982) determined the disposition of label in 14 major organs of anesthetized rats following intravenous (femoral vein) bolus injection of [^{13}N]ammonia. They found that most of the administered dose was extracted by the musculature, kidneys and lungs. It was noted that labeled metabolites were rapidly lost from the lungs and kidney. Whole body imaging after administration of [^{13}N]ammonia was previously used to show that skeletal muscle in human volunteers is a major sink for removal of circulating ammonia (Lockwood et al.. 1979). Cachexia is a major risk factor for patients with liver disease and hyperammonemic encephalopathy. Lockwood et al. (1979) concluded that muscle atrophy may thereby contribute to the development of hyperammonemic encephalopathy with an associated increase in the brain ammonia utilization rate. However, in portacaval shunted rats (a model of chronic liver disease) muscle glutamine synthetase is upregulated presumably in an attempt to counteract the loss of liver enzymes responsible for removing ammonia (Girard and Butterworth 1992).

The finding of Freed and Gelbard (1982) that lungs may be important for the removal of circulating ammonia is interesting given the fact that high levels of inhaled ammonia are toxic to the lungs. In later studies it was shown by Cooper and Freed (2005), using [^{13}N]ammonia, that rat lungs contain glutamine synthetase and that a considerable portion of [^{13}N]ammonia passing through the lungs is removed as L-[*amide*-^{13}N]glutamine. Evidently, however, the presence of

glutamine synthetase in the lungs is ineffective at preventing damage to these organs at high levels of *inspired* ammonia.

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

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

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

Some comment here may be appropriate on the relationship between endogenous ammonia in the lungs and alveolar air. In 1959, two groups suggested that ammonia in alveolar air reflects the concentration of ammonia in the lungs (Robin et al.. 1959; Jacquez et al., 1959). With the techniques available at the time it was not possible to measure ammonia in expired breath of normal experimental animals. However, Robin et al. (1959) were able to measure ammonia in alveolar air of anesthetized dogs administered ammonium acetate, and Jacquez et al. (1959) were able to measure ammonia in alveolar air of anesthetized dogs made chronically hyperammonemic by a portacaval shunt. In later studies, Reinyk et al. (2007) noted that comatogenic doses of sodium thiopental in rats produced hyperammonemia that was associated with an increased exhalation of ammonia. Breath ammonia analysis has also been carried out on patients with kidney disease as a potential estimator of the severity of the associated uremia (Davies et al., 2014). Thus, hyperammonemic syndromes (e.g. liver disease and kidney disease) result in increased lung

ammonia that in turn is reflected in increased expiration of ammonia. However, there are caveats regarding interpretation of these studies that need to be discussed.

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

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

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