UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

June 10, 2014

EPA-CASAC-14-002

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

Subject: CASAC Review of the EPA's *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (First External Review Draft – November 2013)*

Dear Administrator McCarthy:

The Clean Air Scientific Advisory Committee (CASAC) Oxides of Nitrogen Primary National Ambient Air Quality Standards (NAAQS) Review Panel met on March 12-13, 2014, and May 7, 2014, to peer review the EPA's *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (First External Review Draft – November 2013),* hereafter referred to as the First Draft ISA. The Chartered CASAC approved this report during a public teleconference on May 7, 2014. The CASAC's consensus responses to the agency's charge questions and the individual review comments from the CASAC Oxides of Nitrogen Review Panel are enclosed.

Overall, the CASAC finds the Draft ISA to be a good first draft. It is an impressive compilation of information and it is reasonably well organized. Recommendations for strengthening the document are highlighted below and detailed in the consensus responses.

The Executive Summary generally provides a synopsis of the key findings and conclusions of the Draft ISA, but can be improved by removing unnecessary jargon and clearly explaining scientific terms. The Executive Summary also could provide a brief discussion of what evidence is needed to go from one causal determination category to another.

The Integrated Summary summarizes each topic area with the rationale for the determination of causality, but it is difficult to get a clear overall picture. The organization and clarity can be improved by describing the major findings in each subsection and providing cohesive connections among the subsections, leading to conclusions from an integrated analysis. One way to help integrate the evidence on nitrogen dioxide (NO_2) health effects from epidemiological and toxicological studies (including controlled human studies) is to present a diagram showing possible biological pathways linking NO_2 exposure and various endpoints. This will support revised discussions about causal determination, as well as summarize the current state of knowledge regarding mode(s) of action for NO_2.

Summaries of monitored concentrations are, for the most part, appropriately covered. The discussion on exposure assessment and measurement error needs substantial revision. In particular, the section should be reorganized and begin with an introduction that discusses the relevance of exposure and dose to health effects and introduces the key relevant concepts and considerations. In addition, sufficient attention needs to be given to the role and impact of exposure assessment in epidemiological inference. The exposure section could be split into its own chapter. Potential confounding in epidemiological studies of NO_2 from co-emitted pollutants is still a major and mostly unresolved issue and needs to be better addressed in the ISA.

The Draft ISA provides numerous important points that help explain the mechanisms of NO_2 toxicity. The document states that the reactive nature of NO_2 makes it unlikely to pass beyond the epithelial lining fluid. Although this is largely true, a few points are oversimplified and require additional detail to better highlight the role of NO_2 in pathophysiology. The CASAC concurs that the existing dosimetric models for NO_2 are inadequate for cross-species comparisons and recommends that the major deficiencies and uncertainties associated with the lack of a validated NO_2 dosimetry model be explicitly described. The CASAC recommends development of a validated NO_2 dosimetry model for future NAAQS reviews and has recommendations on specific characteristics the model should have. The discussion on modes of action is valuable and well written, and includes extensive references to support the concepts. The modes of action section is generally well written, but the modes of action should be discussed with respect to the outcomes of interest.

The Draft ISA provides an excellent start towards summarizing the key results from the literature, but some recent studies are not considered. The material in the health effects chapters should be reorganized by potential health effects rather than type of study, to provide an overall assessment of the evidence for the various health endpoints.

Ambient NO_2 concentrations are highly correlated with concentrations of other pollutants from combustion sources in general and motor vehicles or traffic in particular, including strong correlations between ambient NO_2 and carbon monoxide, black carbon, organic species, some transition metals, and ultrafine particulates. In addition, other components of multi-pollutant mixtures such as some organic constituents, transition metals, ozone, and $PM_{2.5}$ can introduce positive or negative biases into the assessment of NO_2 health effects. This is particularly the case with ozone that often has a strong negative correlation with NO_2 and has in some studies been seen to induce positive confounding on the NO_2 effects. The issue of potential confounding by correlated copollutants is an important concern that is not adequately addressed. The discussion of the role of potential confounders in delineating and evaluating the evidence associated with various studies should be strengthened.

The CASAC does not find the causal framework to be applied with sufficient transparency. There needs to be better substantiation and better documentation of the evidence and lines of reasoning for the causal determinations. For causal determinations that have changed since the 2008 ISA, the evidence and lines of reasoning that have changed should be substantiated and documented.

The CASAC was asked to review a meta-analysis performed by the EPA that was previously not peer-reviewed. Overall, the analysis is reasonable and appropriate. The CASAC has specific recommendations for improving the analysis as detailed in the response to charge questions.

The discussion of at-risk factors that contribute to NO_2-associated health risks is generally clear and reflects the body of available evidence. A strength of the discussion is the presentation of the overall importance of the relevant at-risk category, including the overall size of the at-risk population at the start of each section. In addition, the summary table at the end of the genetics section is particularly useful and similar summary tables should be included for each of the other sections. The discussion would benefit from greater synthesis of the findings by risk factor, as sections often repeat study findings reported in the health effects chapters, without further elaboration on how these studies together inform our understanding of the at-risk factors for NO_2 exposures.

The CASAC appreciates the opportunity to provide advice on the ISA and looks forward to the EPA's response.

Sincerely,

/Signed/

Dr. H. Christopher Frey, Chair
Clean Air Scientific Advisory Committee

Enclosures

NOTICE

This report has been written as part of the activities of the EPA's Clean Air Scientific Advisory Committee (CASAC), a federal advisory committee independently chartered to provide extramural scientific information and advice to the Administrator and other officials of the EPA. The CASAC provides balanced, expert assessment of scientific matters related to issues and problems facing the agency. This report has not been reviewed for approval by the agency and, hence, the contents of this report do not necessarily represent the views and policies of the EPA, nor of other agencies within the Executive Branch of the federal government. In addition, any mention of trade names or commercial products does not constitute a recommendation for use. The CASAC reports are posted on the EPA website at: http://www.epa.gov/casac.

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U.S. Environmental Protection Agency
Clean Air Scientific Advisory Committee
Oxides of Nitrogen Primary NAAQS Review Panel

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Consensus Responses to Charge Questions on
EPA's Integrated Science Assessment for Oxides of Nitrogen – Health Criteria
(First External Review Draft – November 2013)

Executive Summary

The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions of the ISA for a broad range of audiences. Please comment on the clarity with which the Executive Summary communicates the key information from the ISA. Please provide recommendations on information that should be added or information that should be left for discussion in the subsequent chapters of the ISA.

The Executive Summary generally provides a synopsis of the key findings and conclusions of the Draft ISA, but can be improved by removing unnecessary jargon and clearly explaining scientific terms. The Executive Summary could also provide a brief rationale of what evidence is needed to go from one causal determination category to another. For the general community, a shorter (e.g., 5 to 7 page) Executive Summary would be useful perhaps organized around Table ES-1 or Table 1-1 with a brief rationale that focuses on what evidence is necessary to go from suggestive to causal (e.g., epidemiological results address confounders, epidemiological results are consistent across cities and across different NO_2 exposure metrics, human clinical results are consistent with epidemiology outcomes, and results from animal toxicology studies are consistent with both human clinical and epidemiology metrics).

Any revisions that are made to other sections of the ISA should be reflected in the corresponding summaries in the Executive Summary and Integrated Summary.

Chapter 1 – Integrated Summary

Chapter 1 summarizes key information from the Preamble about the process for developing an ISA. Chapter 1 also presents the integrative summary and conclusions from the subsequent detailed chapters of the ISA for Oxides of Nitrogen and characterizes available scientific information on policy-relevant issues.

a. Please comment on the usefulness and effectiveness of the summary presentation. Please provide recommendations on approaches that may improve the communication of key ISA findings to varied audiences and the synthesis of available information across subject areas.

The introductory sections of Chapter 1 provide a good presentation of the ISA's organization and scope, along with definitions of the categories of causality. The evaluation sections on health effects provide an in-depth collective summary of the material presented within the health effects chapters of the ISA. Although each topic area is nicely summarized with a concluding paragraph that provides the rationale for the determination of causality, it is difficult to get a clear overall picture. The organization and clarity can be improved by describing the major findings in each subsection and providing cohesive connections among the subsections, leading to the Conclusions from an integrated (rather than the

current fragmented) analysis. For example, on page 1-11, the last sentence of the 2nd paragraph, "however, the contribution of near-road exposure to … is not well characterized" as a concluding sentence of a concluding paragraph of this section is awkward. Such statements make the chapter fragmented. The Integrated Summary should also provide references to the relevant sections of the Draft ISA.

Table 1-1 is a useful summary table of the key evidence contributing to causal determinations for NO_2 exposure and health effects. The CASAC has recommendations on improving the transparency of the application of the causal framework, as detailed in responses to the charge questions for Chapters 4 and 5. More detail is needed regarding the key evidence supporting changes in the causal determinations from the 2008 ISA. Table 1-1 should fully reflect those key considerations that led to a change in the causal determination from the 2008 ISA.

b. What are the Panel's thoughts on the application of the Health and Environmental Research Online (HERO) system to support a more transparent assessment process?

The HERO system is very useful and is well described in this draft document.

c. To what extent does Chapter 1 communicate the key scientific information on sources, atmospheric chemistry, ambient concentrations, exposure, and health effects of oxides of nitrogen as well as at-risk lifestages and populations? What information should be added or is more appropriate to leave for discussion in the subsequent detailed chapters?

In general, Chapter 1 provides a good summary of the ISA. The content of Section 1.5 should be summarized here and the detail of the discussion should be moved into the exposure section or health effects chapters.

One way to help integrate the evidence on NO_2 health effects from epidemiological and toxicological studies (including controlled human studies), is to present a diagram showing possible biological pathways linking NO_2 exposure and various endpoints reviewed in the entire report (as an example, see Figure 3 in Brook et al., 2010). This will help support discussions about causal determinations and in summarizing the current state of knowledge regarding mode(s) of action for NO_2.

d. What are the Panel's thoughts on the rationale presented for forming causal determinations for NO_2 *exposure only and considering epidemiologic results for associations between* NO_X *and health effects in causal determinations for* NO_2 *(Sections 1.4.1 and 1.4.3)?*

The biological rationale supporting the idea that nitric oxide (NO) *per se* is not the toxic agent is reasonable. However, there is also an air quality rationale for not using NO_x ($NO + NO_2$) as a surrogate for NO_2, namely the variation in the NO_2/NO_x ratio as a function of distance from major roadways. This also needs to be emphasized in the Integrated Summary.

e. Based on individual Panel member recommendations from June 2013 on the Draft Plan for the Development of the Integrated Science Assessment for Nitrogen Oxides – Health Criteria (May 2013), Chapter 1 presents an integrated evaluation of various epidemiologic lines of evidence that inform the independent effects of NO_2 *exposure (Section 1.5). This section discusses available information that is not necessarily included in the health effect chapters on potential confounding by copollutants and other*

factors as well as the potential for NO_2 *to serve primarily as an indicator of traffic-related pollutants and traffic proximity. This discussion is in Chapter 1 because it integrates information across Chapters 2, 4, and 5. Please comment on the extent to which this discussion is informative in describing how the evidence of independent effects of* NO_2 *is evaluated in this ISA. Does the discussion accurately reflect the available evidence? If this discussion is informative, what information could be added or removed to improve the discussion. Should the discussion remain in Chapter 1 or should it be moved to another part of the ISA?*

This section is very informative and provides a more complete and in-depth discussion of the issues compared to that in the Executive Summary. The rationale for assessing confounding factors in the epidemiological studies still needs more emphasis.

The discussion about the differences in near-road gradients in NO_2 versus ultrafine particles (UFPs), carbon monoxide (CO), black carbon (BC), or organic carbon (OC) needs to be given further thought. Upwind values vary by pollutant (gradients are not normalized to on-road values prior to comparison) and epidemiological studies have relied on monitors placed away from the road where these gradient differences are not very pronounced. The panel studies with personal monitoring do not appear to have strong co-pollutant confounding, an important point made here. These latter studies should be referenced in Table 1-1 as additional supportive causal evidence.

f. Please comment on the extent to which the discussion of various policy-relevant considerations is clearly described and integrates relevant information (Section 1.6). Please identify any other relevant information that would be useful to include.

This is an excellent discussion. However, the statement on page 1-52, lines 7-11, that refers to "suggestive evidence" is puzzling. This seems to downplay the human clinical studies relative to epidemiology and, to the extent that it implies that epidemiological evidence is the most important, it violates the rules of evidence set out at the beginning of the document.

Chapter 2 – Atmospheric Chemistry and Exposure to Oxides of Nitrogen

Chapter 2 describes scientific information on sources, atmospheric chemistry, air quality characterization, and human exposure of oxides of nitrogen.

a. To what extent is the information presented regarding characteristics of sources, chemistry, monitoring concentrations, and human exposure accurate, complete, and relevant to the review of the NO_2 *NAAQS?*

Summaries of monitored concentrations are appropriately covered with some minor exceptions noted in individual panel member comments. There has been recent work regarding the complexity of near-road dispersion processes, such as the effect of vehicle movement on turbulence and the effect of sound barriers and near-road vegetation, and so on. Thus, although atmospheric chemistry is clearly important, physical transport processes are also important. Source characterization, oxides of nitrogen chemistry, and human exposures to oxides of nitrogen are complex topics; this chapter could benefit from changes described below on other sections of this charge question. Spatial gradients and non-ambient sources of exposure to NO_2 can lead to substantial uncertainties in estimates of personal exposures; this section of

the chapter needs substantial revisions as noted below. The EPA could consider whether Chapter 2 should be divided into a new Chapter 2 on "Air Quality" (to be inclusive of both physical and chemical processes) and a new Chapter 3 on "Exposure".

The simplified version of Figure 2-1 (page 4 of USEPA, 2014) should be included in Chapter 1. The text in Chapter 2 associated with Figure 2-1 should then reference the simplified figure inserted in Chapter 1. Potential confounding in epidemiological studies of NO_2 from co-emitted pollutants is still a major and mostly unresolved issue. Thus, the final phase of planned near-road sites that are required to monitor only NO_2 may have limited value in terms of health effect assessments relative to the multi-pollutant near-road sites. Section 2.4.2 (other NO_2 monitoring methods) mentions the cavity attenuated phase shift (CAPS) method for NO_2, which could be a practical and more accurate alternative (in terms of cost and operational effort) to the traditional chemiluminescence–molybdenum (CL-moly) converter Federal Reference Method (FRM) monitor. One consideration in routine network deployment of CAPS or other methods that only measure NO_2 (e.g., do not measure NO) is the potential loss of NO_x data, which is often the only widely available exposure surrogate for on-road pollutants.

b. To what extent are the analyses of air quality presented clearly conveyed, appropriately characterized, and relevant to the review of the NO_2 *NAAQS?*

The strength of associations between NO_2 and other mobile source co-pollutants in the near-road environment is a key topic that should be explored further. These relationships are influenced by averaging times - hourly, daily, seasonal, annual. This section would benefit from a brief discussion of Canadian or other NO_2 networks, especially those intended to characterize near-road exposures. If possible, the second draft ISA should include a short summary of available 1-hour maximum daily data from the new near-road network. The 1-hour maximum NO_2 concentrations in Table 2-1 should be revised or removed. If retained, the related (same hour) 1-hour maximum NO concentrations should be added to this table.

c. How effective are the source category groupings and the discussion of source emissions in understanding the importance and impacts of oxides of nitrogen from different sources on both national and local scales?

EPA should consider framing near-road chemistry as a secondary source, having different temporal and spatial scales from primary on-road emissions. The summary of non-U.S. background NO_2 could be shortened, because it is not much of an issue for exposure. Source groupings should focus on NO_2 emissions near where people live and key microenvironments where exposures are most likely to occur. Moreover, aged NO_2 emissions are transformed into other oxidized nitrogen species with very different and presumably lower health effects. The proposed revisions to major NO_x source groupings (figure 2-2) for the 2nd draft ISA shown on page 6 of EPA (2014) are appropriate, and the comparison of changes between the 2008 and 2011 national emissions inventory values are useful. If any 2014 national emissions inventory data are available, they should be used and incorporated.

d. Please comment on the extent to which available information on the spatial and temporal trends of ambient oxides of nitrogen at various scales has been adequately and accurately described.

There is substantial variability in spatial and temporal trends. For example, during the urban overnight/morning rush hour time period, primary sources usually dominate near-road NO_2 because

there is little to no ozone to titrate NO to NO_2 and no photochemistry. This section would benefit from more detail on such spatial and temporal patterns because there are substantial uncertainties and variation in near-road spatial scales over different time periods (e.g., pre-dawn versus mid-day). It would be helpful to have some additional discussion of how near-road is defined both in terms of monitor siting and exposures. Additional detail on long term spatial correlations between NO_2 and copollutants is needed to inform health studies. European near-road NO_2 monitors generally have different siting criteria, based on curbside of urban core streets, in contrast to the U.S. near-road network. A discussion should be included regarding how European and Canadian studies can provide perspective regarding the importance of monitor siting in evaluating results from epidemiological studies. Because the alignment of the monitoring network with epidemiological study subjects is emerging as an important consideration in estimation of health effects and correcting for exposure measurement error, understanding similarities and differences between the European and U.S. networks is worth some attention. A brief discussion of mobile source regulations that will reduce on-road NO_x emissions over the next several years would be useful; the 2001 heavy-duty diesel regulation (66 FR 5002) and the 2014 Tier 3 gasoline engine and fuel regulation (79 FR 23414) should result in substantial on-road mobile source NO_x reductions.

e. Please comment on the accuracy, level of detail, and completeness of the discussion regarding exposure assessment and the influence of exposure error on effect estimates in epidemiologic studies of the health effects of NO_2.

Considerable reworking of the exposure assessment section (2.6) is needed. In particular, the section should be reorganized and begin with an introduction that discusses the relevance of exposure and dose to health effects and introduces the key concepts and considerations. In addition, the exposure measurement error discussion in section 2.6.5 needs to be updated and expanded; see Dr. Sheppard's individual comments for more detail. The CASAC recommends the following topics be included:

- One important reason to discuss exposure assessment in this document is to inform judgments about estimated health effects from epidemiological studies. The discussion of exposure assessment should be put in proper context, including sufficient attention given to exposure assessment for use in epidemiological inference (e.g., as opposed to risk assessment).
- Directly consider study design in the exposure assessment and measurement error discussions. Exposures that can be used and their role in epidemiological inference are fundamentally different for panel studies, time series studies, and cohort studies. Measurement error considerations are different for time series designs (where temporal variation in pollution in paramount and aggregation has some important impacts) and cohort study designs (where spatial variation is crucial and prediction models are used to obtain exposure estimates for individuals).
- Address whether total or ambient personal exposure is (and should be) the relevant exposure of scientific interest. The health effect parameter being estimated (i.e., the target parameter for inference) in an epidemiological study depends on whether the exposure metric is total personal exposure, personal exposure from ambient sources, or ambient concentration.
- Distinguish two different impacts of exposure on inference: (1) whether or not the parameter being estimated is the scientifically motivated target parameter, and (2) given the parameter being estimated, the measurement error consequences of how exposure is measured and/or modeled.

- This section would benefit from some direct statements about the importance of the relatively high spatial variability of NO_x in the evaluation of exposure assessment for epidemiological study inference.
- There should be a discussion on the quality and validity of the epidemiological inferences that can be drawn from the diverse set of exposure modeling strategies used in the cited papers (e.g., from the nearest monitor, land use regression, dispersion modeling). How do the exposure modeling strategies and specific implementations of them affect judgments about causality of NO_2/NO_x health effects?

Chapter 3 – Dosimetry and Modes of Action for Inhaled Oxides of Nitrogen

Chapter 3 characterizes scientific evidence on the dosimetry and modes of action for NO_2 *and nitric oxide (NO). Dosimetry and modes of action are bridged by reactions of* NO_2 *with components of the extracellular lining fluid and by reactions of NO with heme proteins, processes that play roles in both uptake and biological responses.*

a. Given the ubiquity of reactive substrates and reaction rate of NO_2 *with these substrates, it appears unlikely* NO_2 *itself will penetrate through the lung lining fluid to the epithelium (see Table 3-1). Please comment of the adequacy of the discussion of* NO_2 *uptake and reactivity in the respiratory tract.*

Chapter 3 provides numerous important points that help explain the mechanisms of NO_2 toxicity. However, there is a need to better understand and describe mechanistically the spatiotemporal dynamics of NO_2 transport and reaction within the various microenvironments of the respiratory system. The discussion should take into account that the epithelial lining fluid (ELF) is not homogeneous, both throughout different levels of the respiratory system and even within particular microenvironments (such as the alveolar microenvironment). For example, the lining fluid in conducting airways is thicker and of different composition from that in alveolar spaces. The lining fluid in the alveolar region is thinner and is rich in surfactants, and there is limited evidence that small portions of the lung surface area may not even be covered by ELF. ELF thickness averages 0.14 μm over relatively flat portions of the alveolar walls, 0.89 μm at the alveolar wall junctions, and only 0.09 μm over the protruding features.

Although it is noted that the reactive nature of NO_2 makes it unlikely to pass beyond the ELF, a few points are oversimplified and require additional detail to better highlight the role of NO_2 in pathophysiology. The discussion of penetration does not adequately address the heterogeneous nature of the chemical composition and thickness of the lining fluid as a function of location in the respiratory tract. In dosimetric modeling for other reactive gases, this local variation has been shown to be important. Many models estimate that NO_2 can penetrate 0.6 μm, so NO_2 might be able to penetrate beyond the ELF to cell surfaces. The information in Table 3-1 could be expanded to separately discuss the chemistry of airway and alveolar lining fluids in the context of what fraction of inhaled NO_2 may potentially penetrate to those regions.

Furthermore, describing the interaction of NO_2 with the ELF in terms of classical (Fickian) diffusion processes and homogeneous chemical reactions would be an oversimplification that may be insufficient to describe actual *in vivo* ELF/NO_2 system dynamics. These observations should also apply to NO, which in fact is known to enter alveolar epithelial cells, but potentially through processes that are not diffusion-dependent (e.g., Brahmajothi et al., 2010).

Ultimately, it is true that much of inhaled NO_2 will react with surfactant. The basic conclusion of this section (3.2.2.1.3) that NO_2 does not penetrate deeply is correct, but should not be so dismissive. The section begins accurately noting that secondary/tertiary reactants must have a role – this section should end with a similar statement, so as not to suggest that the biochemistry does not support the plausibility of systemic pathophysiology. Additionally, the discussion of secondary species (section 3.3.2.1) is brief (reflecting scientific data gaps), but some further detail is warranted. Much of this section describes scavenging by antioxidants in the surfactant, but these are not described as secondary oxidation products. Rather than presenting them as secondary oxidation products, the manner in which they are presented makes it seem more akin to mechanisms of absorption, or defense. The CASAC recommends providing some detail as to the products of these reactions, especially as they may link to health outcomes or toxicity. Section 3.3.2.1 discusses nitrite in some detail, but then covers nitration of proteins and fatty acids/lipids in a very cursory way.

b. Since existing dosimetric models for NO_2 *do not consider the probability of oxidants/cytotoxic products reaching target sites, it was concluded that these models are inadequate for within or cross species comparisons. Please comment on the validity of this conclusion and identify and comment on the validity of any alternative conclusions.*

The CASAC concurs that the existing dosimetric models for NO_2 are inadequate for cross-species comparisons, which underscores the need for new models. Table 3.1 provides cross-species comparisons and is an interesting start to the discussion. More research is clearly needed related to the metabolites of NO_2 reaction. Recent studies in rodents and humans are conflicting in terms of short-term outcomes. Development of improved understanding of the complex reactions would benefit future review cycles and the general scientific community. Given limitations of data, it is not likely during this review cycle to be able to develop and implement a detailed mechanistic conceptual comprehensive NO_2 dosimetry model, including subsequent computational implementation, although such advancement is critically needed. Similar efforts have taken place in recent years for other chemical agents (e.g., Asgharian et al., 2011). Such a model would explicitly account for different life-stages and altered health states (development, obesity, aging, etc.) in a framework that takes into account existing hypotheses for NO_2/NO transport and transformation in the respiratory system. The process of developing such a model would provide a useful tool for hypothesis generation and rational design of future laboratory studies. Although complete development of such a model for NO_2 is not feasible at this time given lack of adequate data, the ISA can identify specific dosimetry modeling needs. Therefore, the ISA should summarize explicitly the major deficiencies and uncertainties associated with the lack of a validated NO_2 dosimetry model; such a summary could be included in the form of a brief table in Section 3.2, where these issues are discussed.

To the extent that NO_2 dosimetry models predict penetration of NO_2 to the alveolar region, given the relatively small volume of alveolar lining fluid, there might be some utility to examining potential cross-species effects on innate immunity functions mediated by the constituents of alveolar lining fluid.

c. Please comment on the adequacy of the discussion of endogenously occurring NO_2 *and NO and their reaction products in comparison to that derived from ambient inhalation.*

The chapter pulls in some background information on endogenous oxides of nitrogen creation and signaling, which adds some sophistication to the discussion. The section is appropriately broad and brief – there is far more recent research and publication activity in the field of biological roles of endogenous

oxides of nitrogen than in the field of air pollution, yet exceedingly little research on how these fields relate. Only a few concerns exist, detailed below:

1. Additional references could be included to support points made in this section. Several broader points can be covered with appropriate references:
   a) Oxides of nitrogen biochemistry in the wider context of "small molecule signaling agents" (e.g., Fukuto et al., 2012; Heinrich et al., 2013);
   b) Oxides of nitrogen biochemistry human microbiome dynamics; in particular in relation to the oral microbiome (e.g., Hezel and Weitzberg, 2013), that would also be exposed to exogenous inhaled oxides of nitrogen;
   c) Oxides of nitrogen biochemistry in relation to altered health states (e.g., obesity – Dai et al., 2013; Holguin, 2013)
2. Although endogenous oxides of nitrogen levels often may be higher than ambient levels, changes in ambient levels of oxides of nitrogen still alter the diffusion gradient for removal of excess oxides of nitrogen, which – in theory – may alter endogenous pathways. The sentence on page 3-18, lines 20-25 hints at this but is a bit unwieldy. Given its importance in finalizing the tenor of this section, it should be revised for clarity.
3. Additionally, although endogenous NO_2 may not be systemically distributed, there could potentially be an increase in reaction products in the tissues due to changes in levels of endogenous NO_2.
4. The discussion of endogenous NO and NO_2 should mention the possibility that endogenous production may be great enough in small selected spatial regions of the respiratory tract that the local anti-oxidant capacity is exhausted and thus exogenous oxidant insults could overbalance the system and increase the likelihood of an adverse effect.

It would probably be beyond the scope of the Draft ISA to further expand on the biology of endogenously occurring NO_2 and oxides of nitrogen and of their reaction products. It would, however, be useful to provide some additional references.

d. To what extent are the discussion and integration of the potential modes of action underlying the health effects of exposure to oxides of nitrogen presented accurately and in sufficient detail? Are there additional modes of action that should be included in order to characterize fully the underlying mechanisms of oxides of nitrogen?

The section on modes of action (MOA) is valuable and well written, providing extensive references to support the concepts. Several recommendations are given here related to the overall focus and direction of the section, which is important in setting the stage for the discussion in subsequent chapters. Many of the different MOA are not clearly discussed with respect to the outcome of interest. There may be some commonality of MOA that induce numerous outcomes, but deficiencies in the science make this conclusion difficult. Some of the MOA discussion could be grouped under topics such as "asthmatic outcomes," "chronic respiratory," "cardiovascular," etc. (as broad, non-binding examples). For instance, certain aspects of "neural" and "smooth muscle sensitization" could be combined. Discussions of the classical lung pathology outcomes related to centriacinar lesion development and epithelial hyperplasia would be of value. There may also be value in linking oxides of nitrogen outcomes and MOA with known outcomes and MOA of other pollutants, especially ozone (and maybe PM).

It appears that all (potential) vascular and systemic effects of NO_2 are grouped under "Transduction of extrapulmonary responses" (Section 3.3.2.8, pp. 3-43 to 3-46), which provides a brief but informative overview. The spectrum of these (potential) effects does not become clear either in the summary of page 3-59 or (even more) in the corresponding entry of Table 3.3 on page 3-57. The uncertainties regarding systemic effects (and the MOA involved in these) are very large; however, the range (and severity) of health effects that have been hypothesized to be related to NO_2 exposures is so wide that a more detailed listing of the biological mechanisms potentially associated with them would be justified.

Chapters 4 and 5 - Integrated Health Effects of Short-Term and Long-Term Exposure to Oxides of Nitrogen

Chapters 4 and 5 present assessments of the health effects associated with short-term and long-term exposure to oxides of nitrogen, respectively. The discussion is organized by health effect category, outcome, and scientific discipline.

a. To what extent do the discussions in this chapter accurately reflect the body of evidence from epidemiologic, controlled human exposure and toxicological studies?

The Draft ISA provides an excellent start towards summarizing the key results from the literature. Nevertheless, some tightening up of this draft is warranted. Some recent studies are not considered in the document. It is not always clear which and when confounders are considered in the described studies; statistical significance is not always indicated, and terminology such as "positive but imprecise" should be discarded in favor of numerical results. In other cases, the figures and tables present conflicting evidence or do not present results in comparable levels of detail.

b. Please comment on the balance of discussion of evidence from previous and recent studies in informing the causal determinations.

There is a good balance between discussion of evidence from previous and recent studies in informing the causal determinations. However, the strongest studies should be clearly identified along with the criteria that determine their strength.

c. Please comment on the adequacy of the discussion of the strengths and limitations of the evidence in the text and tables within Chapters 4 and 5 and in the evaluation of the evidence in the causal determinations.

There is particular concern about the treatment of potential copollutants in delineating and evaluating the evidence associated with various studies. (See response to Charge Question *g* below). The same level of consistency is not applied to the various endpoints assessed in the Draft ISA. More clarity on the criteria used to identify the level of evidence for a given endpoint would be helpful.

d. What are the views of the panel on the integration of epidemiologic, controlled human exposure, and toxicological evidence, in particular, on the balance of emphasis placed on each source of evidence? Please comment on the adequacy with which issues related to exposure assessment and mode of action are integrated in the health effects discussion. Please provide recommendations on information in other chapters of the ISA that would be useful to integrate with the health effects discussions in these chapters.

The organization of the material in the chapters is not as helpful as it could be in providing an overall assessment of the evidence for the various health endpoints. For example, asthma studies are described in several disparate sections of the document, organized largely by type of study rather than by potential health effect. An understanding of whether there is epidemiological evidence of exacerbations of asthma associated with short-term increases in ambient NO_2 concentration should be highlighted according to that outcome, rather than as now organized into "lung function," "respiratory symptoms and asthma medication use," and "respiratory hospital admissions and emergency department visits." The same could be said for many other outcomes that need to be considered.

There is also concern about the use of some subclinical outcomes in clinical studies as being considered of substantial importance in determining health effects; some of these subclinical outcomes, such as within-individual changes in heart rate variability, and to a lesser extent QT-interval changes and circulating inflammatory biomarkers, are not well-validated predictors of clinical outcomes associated with NO_2 exposure in populations. They likely provide more evidence regarding MOA than they do regarding clinical outcomes, and should be viewed as corroborative, rather than primary health effect findings. There should be a more extensive discussion of the exposure assessment results presented in Chapter 3 and how these findings would impact the interpretation of study results. Potential MOA also need to be considered for the potential copollutants. (See the response to Charge Question *g* below.)

e. Please comment on the appropriateness of using experimental and epidemiologic evidence for morbidity effects to inform the biological plausibility of total mortality associated with short-term (Section 4.4) and long-term (Section 5.5) NO_2 *exposure and in turn, to inform causal determinations.*

It is generally appropriate to use experimental and epidemiological evidence to inform the biological plausibility of the mortality effects, as part of the overall reasoning informing causal inference.

See the above comment; more organization along the lines of health impacts would be helpful. Also more discussion of the relationship between initiation and exacerbation of effects would inform this issue.

f. Section 4.2.2 discusses the effect of short-term NO_2 *exposure on airways responsiveness. This section focuses primarily on an EPA meta-analysis developed for this ISA of airway responsiveness data for individuals with asthma and secondarily on the potential of various factors to affect airways hyperresponsiveness independently or in conjunction with* NO_2 *exposure in controlled human exposure studies. This material presently is unpublished and we ask the Panel to provide the peer review for the analysis, in particular, to comment on the appropriateness of the methodology utilized for the meta-analysis, the conclusions reached based this analysis, and its use in the draft ISA. With regard to factors potentially affecting airways responsiveness, please comment on the adequacy of this discussion. Are there other modifying factors that should be considered?*

Overall, the limited original analysis described in this section of the ISA is reasonable and appropriate. This "meta-analysis" does not include pooling of individual level data beyond that which is available in the published studies. It would be helpful if the hypothesis to be addressed in the meta-analysis was explicitly stated at the beginning of the section. There are many sources of heterogeneity between the study protocols, and the Draft ISA separates individual subjects/studies according to whether the subjects were asthmatic and whether the experimental protocol involved exercise. It is inferred that the hypothesis (a reasonable one) is that responses to NO_2 would be most notable in asthmatics, and

responses would be attenuated with exercise. A detailed description of the meta-analysis could be included in an appendix. A more comprehensive analysis should discuss the role of asthmatic status and asthmatic sub-phenotype (if known), exercise, provocative agent, the temporal aspects of response, as well as definition and/or extent of adversity.

g. The 2008 ISA for Oxides of Nitrogen stated that one of the largest uncertainties was the potential for health effects observed in association with NO_2 *exposure to be confounded by correlated copollutants. To what extent has evidence that informs independent effects of* NO_2 *been adequately discussed in Chapters 4 and 5 and appropriately interpreted as reducing uncertainty (for example, evaluation of copollutant model results)? Has the current draft ISA appropriately considered recent epidemiologic findings regarding potential copollutant confounding in causal determinations? Please provide comments specifically for respiratory effects, cardiovascular effects, and total mortality of short-term* NO_2 *exposure.*

Ambient NO_2 concentrations are highly correlated with concentrations of other pollutants from combustion sources in general and motor vehicles or traffic in particular, including strong correlations between ambient NO_2 and CO, BC, and UFP. In addition, other components of multi-pollutant atmospheres such as some organic constituents, transition metals, ozone and $PM_{2.5}$ can introduce positive or negative biases into the assessment of NO_2 health effects. This is particularly the case with ozone that often has a strong negative correlation with NO_2 and has in some studies been seen to induce positive confounding on the NO_2 effects. This is true for both short-term and long term exposures. Given these covariance patterns, it is difficult to disaggregate effects attributed to NO_2 from these correlated co-pollutants in observational studies (i.e. estimate the effect of NO_2 while controlling for another pollutant). Although many epidemiologic studies approach this problem through adjustment in two- or multi-pollutant regression modeling, this approach is limited.

Among the key limitations of two-pollutant models, which serve as the basis for strengthening the causal determination for several NO_2 association in the Draft ISA, none consider non-linear relationships between NO_2 and its copollutants. Additionally, two-pollutant modeling has been used, almost exclusively, as a means of controlling for potential confounding. The potential for effect measure modification, expressed through joint effects model settings, are almost exclusively neglected in current two-pollutant modeling results (see Dr. Sarnat's individual comments, p. A-50, relating to model specification for further detail). There needs to be some discussion of the underlying toxicological evidence for the potential confounders as well as for NO_2, and how any toxicological differences could help the interpretation of results. There are also non-pollutant traffic risk factors, such as noise and stress that could be potential confounders in epidemiological studies, which are not discussed. In addition, there is the possibility that the mixture of pollutants, of which NO_2 is a component, is a better predictor of responses than any one component of the mixture. Other issues, such as pollutant interactions, mixture effects on dosimetry and mixture effects on biological outcomes should be more fully discussed. For many studies, there are limited data on copollutant exposures, particularly for some highly correlated traffic pollutants (e.g., organic carbon and metals). Thus, much of the observational data continues to suffer from potential confounding by these copollutants. Studies which address other copollutants jointly with NO_2 are less informative. At times, the Draft ISA does not clearly distinguish between the pollutants of greatest interest and others. Other considerations could aid in the discussion of this issue, including better description of the relationship between ambient and personal exposure metrics for NO_2 and potential copollutants, both temporally and spatially. Better integration of panel and indoor study results is needed, taking into account that the mixture of confounders could be substantially

different from those of other epidemiological approaches. Experimental studies of controlled exposures to NO_2 alone and with known levels of copollutants could be helpful; no such studies are identified in the document. However, there have been a few such studies reported, as summarized in the critical review of short-term NO_2 exposures by Hesterberg, et al. (2009) that should be discussed.

h. To what extent is the causal framework transparently applied to evidence for each of the health effect categories evaluated to form causal determinations? How consistently was the causal framework applied across the health effect categories? Do the text and tables in the summaries and causal determinations clearly communicate how the evidence was considered to form causal determinations?

Due to the deficiencies outlined above, the CASAC does not find the causal framework to be applied with sufficient transparency. There needs to be better substantiation and better documentation of the evidence and lines of reasoning for the causal determinations. For causal determinations that have changed since the 2008 ISA, the evidence and lines of reasoning that have changed should be substantiated and documented.

i. What are the views of the panel regarding the clarity and effectiveness of figures and tables in conveying information about the consistency of evidence for a given health endpoint? In particular, was the use of the tables and figures in both the text and online in the HERO database effective in providing additional information on the studies evaluated? Are there tables and figures in the ISA that would be more appropriate to include as a resource in the HERO database?

Some of the issues raised in this question are addressed above. A second draft will likely achieve greater consistency in the treatment of results across studies and endpoints. With respect to the HERO database, it is very helpful to have access to the papers cited in the ISA.

Chapter 6 - Populations Potentially at Increased Risk for Health Effects Related to Exposure to Oxides of Nitrogen

Chapter 6 evaluates scientific information and presents conclusions on factors that may modify exposure to NO_2*, physiological responses to* NO_2 *exposure, or risk of health effects associated with* NO_2 *exposure. Consistent with the ISAs for ozone and lead, conclusions on these at-risk factors inform at-risk lifestages and populations.*

a. How effective are the categories of at-risk factors in providing information on potential at-risk lifestages and populations? Is there information available on other key at-risk factors that is not included in the first draft ISA and should be added?

b. To what extent do the discussions in this chapter accurately reflect the body of available evidence from epidemiologic, controlled human exposure, and toxicological studies, including the extent to which evidence indicates that the effects of NO_2 *exposure are independent of other traffic-related copollutants?*

c. Please comment on the consistency and transparency with which the framework for drawing conclusions about at-risk factors has been applied in this ISA.

d. To what extent is available scientific evidence on factors that modify exposure to NO_2 *discussed in the chapter and adequately considered in conclusions for at-risk lifestages or populations?*

Chapter 6 generally presents clear information regarding at-risk factors for NO_2-associated health risks, reflecting the body of available evidence with some exceptions as noted in Dr. Jerrett's individual comments. Strengths of the section include its discussions at the start of each section of the overall importance of the relevant at-risk category, including the overall size of the at-risk population. In addition, the summary table at the end of the genetics section is particularly useful and should be repeated for each of the other sections. The chapter, however, would benefit from greater synthesis of the findings by risk factor, as sections often repeat study findings reported early in Chapters 4 and 5, without further elaboration on how these studies together inform our understanding of the at-risk factors for NO_2 exposures. This synthesis should have several goals, including:

(1) to characterize the relation (if any) of the at-risk factors to one another;
(2) for a particular at-risk factor, to show how findings for the often large number of health endpoints together inform at-risk causality determinations;
(3) to address other important considerations, including the impact of multiple co-occurring at-risk factors (e.g., obesity, diabetes, high occupational exposures, smoking) on NO_2-associated health risks; and
(4) to describe the relative strengths and limitations of the studies and how these strengths and limitations affect the causal determination.

In so doing, the Agency will better demonstrate consistency of findings, increase clarity and transparency for causal determinations, and streamline the organization of the chapter.

The categories of at-risk factors are appropriate. However, the list of specific at-risk factors should be expanded to include housing factors other than residential location (such as presence of indoor gas stoves and/or home ventilation), stress, traffic-related occupations, commuters, and children living or attending school in areas with high NO_2 concentrations.

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Appendix A

Individual Comments by CASAC Oxides of Nitrogen Primary NAAQS Review Panel Members on EPA's Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (First External Review Draft – November 2013)

Mr. George A. Allen

Comments on Chapter 2 – Atmospheric Chemistry andExposure to Oxides of Nitrogen

General Comments

Overall, this is a very thorough first draft document. For the sections I reviewed I did not find any major issues or omissions. It reads well and covers all aspects of the topics in sufficient detail.

Charge Questions

a. To what extent is the information presented regarding characteristics of sources, chemistry, monitoring concentrations, and human exposure accurate, complete, and relevant to the review of the NO_2 *NAAQS?*

Source characterization, NOx chemistry, and summaries of monitored concentrations are appropriately covered. Both NOx chemistry and human exposures to NOx are complex topics covered in this chapter; both are covered in sufficient detail. The issue of exposure mis-classification and the errors it introduces in analysis of NO_2 health effects is clearly explained. The spatial gradients and non-ambient sources of urban NO_2 can lead to substantial uncertainties in personal exposures; this is discussed in great detail.

b. To what extent are the analyses of air quality presented clearly conveyed, appropriately characterized, and relevant to the review of the NO_2 *NAAQS?*

The air quality analysis presented in this chapter is clearly presented and characterized in sufficient detail in ways that support the NO_2 NAAQS review. I would suggest that the 1-hour maximum NO_2 concentrations in Table 2-1 be reviewed or removed; a 1-hour value of 360 ppb NO_2 is inherently suspect and may be due to instrument calibrations or potential exceptional events that were not removed from the data set. The 1-hour NO_2 maximum example given for Boston of 197 ppb illustrates this point; NO for that hour (7 AM on a Saturday) was just 7 ppb and adjacent hours were not unusually elevated, implying a local source that was essentially all NO_2 -- an unlikely scenario. It might be helpful to include the related (same hour) 1-h max NO concentrations to this table (just one additional column), or simply remove the max 1-h column from this table.

c. How effective are the source category groupings and the discussion of source emissions in understanding the importance and impacts of oxides of nitrogen from different sources on both national and local scales?

The source category groupings and related emission data and discussion clearly show the relative contributions to NOx across different source types. Spatial scales are important for NO_2 given the very wide dynamic range of concentrations from elevated near-source urban concentrations to far rural locations where nearly all NOx has been either converted into other oxidized nitrogen species or removed from the atmosphere. The proposed revisions to major NOx source groupings (figure 2-2) for the 2nd draft ISA shown in the EPA presentation (page 6) are appropriate, and the comparison of changes between the 2008 and 2011 national emissions inventory values are useful.

d. Please comment on the extent to which available information on the spatial and temporal trends of ambient oxides of nitrogen at various scales has been adequately and accurately described.

Spatial and temporal trends of ambient NOx is appropriately discussed across the near-source (often near-road micro to mid spatial scales) to urban and rural scales.

e. Please comment on the accuracy, level of detail, and completeness of the discussion regarding exposure assessment and the influence of exposure error on effect estimates in epidemiologic studies of the health effects of NO_2.

This chapter is thorough in its discussion of exposure assessment. The issue of exposure error and its role in health effect estimates is discussed in detail. The discussion of Berkson and classical error types and the differences in effects these two error types have on health effect estimates is very well done.

Specific Comments

There are many discussions of the literature in this chapter that present results for NO, NO_2, or NOx in an inconsistent manner. In the same paragraph, for the same specific topic, study results are sometimes cited for NO, another study for NO_2, and a third for NOx, making it difficult to compare results across related studies. An example of this is pg. 2-40, lines 4-27. It may be that some studies only reported results for only one of these pollutants, but I suspect in many cases both NO and NO_2 data were reported. When only one pollutant was reported, it would be helpful if that was noted if the discussion includes references to the other pollutants.

NO_2 and NOx play very different roles in exposure assessment. The ISA does make it clear that NO_2 is the component of NOx shown to be of concern for health effects, and that NOx is preferred to NO_2 as a marker of exposure to a wide range of near-road pollutants that could be expected to have health effects, since it is mostly conserved at the neighborhood to small urban spatial scale. Thus both play important but very different roles in health effect assessments. This distinction gets lost in some of the discussion in this chapter.

Pg 2-4 lines 102: this discussion of HNO3 deposition reads like wet deposition dominates, but dry deposition is also a major sink.Pg 2-10 lines 9-11: it would be helpful to add the fraction of NO_2 in NOx for non-catalyzed diesel emissions for comparison. It could be noted here that CDPFs have not been allowed for several years now because of these increased NO_2 emissions.

Pg 2-11, Highway Vehicles. The recent final Tier 3 rule for gasoline engine emissions and lower S gasoline will provide a substantial reduction in NOx. Reductions of ~ 25% will rapidly be realized from just lower S (to 10 ppm from 30 ppm) gasoline, even with existing vehicles, starting in 2017. Further NOx emissions will be realized as Tier 3 gasoline vehicles penetrate into the on-road fleet. While this has not yet occurred, the regulation is now in place and it may be worth mentioning in this context. This, plus the SCR NOx controls required for diesel engines starting in 2010 also discussed on this page, will result in a substantial decline of on-road NOx emissions over the next several years.

Pg 2-12 lines 26-28: the HEI ACES phase 2 results were published in early December and thus should be included in the revised ISA. These results are summarized in the press release at: http://www.healtheffects.org/Pubs/ACES-Phase2-Final-Press-Release-120413.pdf

The full report is at:
http://crcao.org/reports/recentstudies2013/ACES%20Ph2/03-17124_CRC%20ACES%20Phase2-%20FINAL%20Report_Khalek-R6-SwRI.pdf
The report's results indicate that emission reductions substantially exceeded those required by the 2010 HDD engine rule.

One category of non-road NOx not included in section 2-3 is emergency generators, or "gensets". Every large building has one, and many of them are older totally uncontrolled engines with very high PM and NOx and VOC emissions. Normally they are only run for ~ 15 minutes each week for testing, but the potential for their use beyond this for grid-tied peak-period generation has been discussed.

Pg 2-21 and -22, section 2.4.2, Other Methods for Measuring NO_2. This discussion mentions the cavity attenuated phase shift (CAPS) method, which is sensitive and specific to NO_2. It is worth noting that one commercial CAPS NO_2 monitor now has FEM approval and a second commercial CAPS monitor is in the final stages of FEM approval at ORD. These methods are expected to be a practical alternative (in terms of cost and operational effort) to the traditional CL-moly converter FRM monitor. One consideration in routine network deployment of CAPS or any other method that only measures NO_2 (e.g., does not measure NO) is the potential loss of NOx data; NOx is often the only widely available exposure surrogate for on-road pollutants.

Pg 2-29 lines 10-12: the revised ISA should include specifics on the number of operational near-road NO_2 sites, and if at all possible, summaries of data from those sites.

Pg 2-40 lines 28-38 and next page: this discussion of the EPA NO_2 near-road pilot study should note that these were passive integrated samples of at least one-week duration and thus do not reflect short-term (e.g. hourly) concentration patterns.

Pg 2-41 lines 8-9: "near-road concentrations are typically 30% to 200% of urban background." It may not be correct to state that typical near-road concentrations can be 30% of urban background since it would be expected that near-road concentrations would be at least as high as urban background, and almost never lower.

Pg 2-80 and 81, section 2.6.4.3, Integrated Mobile Source Indicator. The discussion in this section is very helpful. Using the combination of three commonly available near-road pollutants (CO, EC or BC, and NOx) has the potential to improve exposure assessment to the broad category of near-road pollutants known or suspected to be drivers behind the observed substantial near-road health effects. This section doesn't mention BC as an alternative to EC measurements. BC is commonly measured at near-road sites using simple optical methods, while EC is usually not measured at near-road monitoring sites. EC and BC are almost always highly correlated although mass concentrations are sometimes different by substantial amounts.

Dr. Matthew Campen

Comments on Chapter 3

a) *Given the ubiquity of reactive substrates and reaction rate of* NO_2 *with these substrates, it appears unlikely* NO_2 *itself will penetrate through the lung lining fluid to the epithelium (see Table 3-1). Please comment of the adequacy of the discussion of* NO_2 *uptake and reactivity in the respiratory tract.*

This is an appropriate level of detail and information, however, the upshot of this section (3.2.2.1.3) is that NO_2 does not penetrate deeply, which has a dismissive note. The section begins accurately noting that secondary/tertiary reactants must have a role – I suggest ending this section with a similar statement, so as not to suggest that the biochemistry does not support the plausibility of systemic effects.

Additionally, there is then a gap where secondary species could be discussed. This is parallel to the scientific gap, so it is not surprising that is it brief, but some further detail in the discussion (3.3.2.1) seems warranted. Much of this section described scavenging by antioxidants in the surfactant, but these are not described as secondary oxidation products – they are, but the manner in which the discussion flows, this seems more akin to mechanisms of absorption, or defense. 3.3.2.1 discusses nitrite in some detail, but then covers nitration of proteins and fatty acids/lipids in a very cursory way.

b) *Since existing dosimetric models for* NO_2 *do not consider the probability of oxidants/cytotoxic products reaching target sites, it was concluded that these models are inadequate for within or cross-species comparisons. Please comment on the validity of this conclusion and identify and comment on the validity of any alternative conclusions.*

This is a reasonable choice, but underscored should be a need for such modeling to be conducted. Table 3.1 provides cross-species comparisons and is an interesting start to the discussion. More research is clearly needed related to the metabolites of NO_2 reaction. Recent studies in rodents and humans are conflicting in terms of short-term outcomes, thus understanding the complex reactions would benefit the review as well as the general scientific community.

c) *Please comment on the adequacy of the discussion of endogenously occurring* NO_2 *and NO and their reaction products in comparison to that derived from ambient inhalation.*

It is an interesting discussion and adds some sophistication to the dialogue from the EPA. Only a few concerns exist, however. For one, it seems to be scantily cited despite numerous interesting factual points. Second, while endogenous generation of NOx may often be higher than ambient, changes in ambient NOx still alter the diffusion gradient for removal of excess NOx, which – in theory – may alter endogenous pathways. The last sentence hints at this but is a bit unwieldy. Given its importance in finalizing the tenor of this section, I would consider revising for clarity.

d) *To what extent are the discussion and integration of the potential modes of action underlying the health effects of exposure to oxides of nitrogen presented accurately and in sufficient detail? Are there additional modes of action that should be included in order to characterize fully the underlying mechanisms of oxides of nitrogen?*

A few thoughts: discussion of the vagally-mediated bradycardia should probably be couched as either a species-specific effect or a profound toxicosis reaction that is unlikely to be seen in humans even in experimental exposure studies. This is probably akin to similar effects seen with ozone and PM. Furthermore, if the study design of Suzuki et al (1982 and 1981) assessed pulmonary injury in parallel with cardiac effects, it is not clear that one could conclude that the heart rate effects were "secondary" to lung injury – often ECG effects are seen very rapidly during exposures before pathological edema develops. It is true that pulmonary fluid accumulation can induce irritant receptor activity (might cite a paper for this claim), but I think the order of events (possibly due to study design limitations) does not permit this conclusion.

Conclusions for the neural pathway studies need to add caveats related to the concentrations discussed. Despite the indication that concentrations must be within 100x ambient levels to be considered, there are a number of 10ppm+ studies discussed in the mode of action section. The relevance really is questionable.

3.3.2.4 Epithelial Barrier Function

First paragraph – that "…ELF solutes of proteins that could diffuse down…" sentence… is this how it works? The hydrodynamic pressure leads from the capillary to the airway, so loss of barrier integrity should lead to fluid (first) moving into the airways, followed by larger molecules and proteins (second, and with more severe barrier loss). So, yes, ELF components become less concentrated and atelectasis is a risk with the loss of surfactant physicochemistry, and certainly alveolar proteinosis is a risk, but ELF factors moving into the blood is not something I am familiar with. Although, yes, Surfactant Protein D is a useful serum biomarker for COPD. A citation would be valuable here.

Next, discussions of LDH should clarify if this is a marker of epithelial barrier integrity or cellular injury.

Discussions of the Kleeburger et al 1997 paper (page 3-32, line 32) should also note the genes.

While exceedingly high exposures are often detailed, many times in discussions of human studies these facts are omitted. Channell et al and Huang used 500 ppm for 2 h and saw significant effects – this seems important information, in light of the studies where neural effects were not observed until mice were exposed to >10,000ppm. Moreover, by limiting the outcomes of Channell et al to "changes in blood lipids and increased levels of plasma soluble lectin-like receptor for oxidized low density lipoprotein", the upshot of observing inflammatory signaling resulting from the whole plasma is lost. These functional outcomes require some further consideration, given the low concentrations of NO_2 and that similar outcomes were seen with diesel emissions (which contain a comparable amount of NOx). 3.3.4 Perhaps examples in the literature could be used to show that NOx exposure leads to upregulation of NO_2/3, S-NOs and nitrated lipids? This section just seems a bit too academic.

Page 3-55, Transduction of extrapulmonary. The 3rd sentence really describes 3 options, not two, and should be worded to identify 1) neural 2) nitrated by-products and 3) inflammatory by-products – none of which are mutually exclusive. Also, there is a lot of attention to noting the high concentrations needed for neural pathways, and generally pulling back from this hypothesis, but the other options (which have stronger data) seem to merit as much treatment as the neural.

General comments

1. Discussion of the vagally-mediated bradycardia should probably be couched as either a species-specific effect or a profound toxicosis reaction that is unlikely to be seen in humans even in experimental exposure studies. This is probably akin to similar effects seen with ozone and PM. Furthermore, if the study design of Suzuki et al (1982 and 1981) assessed pulmonary injury in parallel with cardiac effects, it is not clear that one could conclude that the heart rate effects were "secondary" to lung injury – often ECG effects are seen very rapidly during exposures before pathological edema develops. It is true that pulmonary fluid accumulation can induce irritant receptor activity (might cite a paper for this claim), but I think the order of events (possibly due to study design limitations) does not permit this conclusion.

2. Conclusions for the neural pathway studies need to add caveats related to the concentrations discussed. Despite the indication that concentrations must be within 100x ambient levels to be considered, there are a number of 10ppm+ studies discussed in the mode of action section. The relevance really is questionable.

3. Given the very clear interaction between NO_2 and lung surfactant, are there lung diseases where dysfunctional surfactant chemistry plays an important role that are impacted by NO_2 exposure (either as an inducer or exacerbator)? For instance, individuals with acute respiratory distress syndrome may be more sensitive to NO_2 reactions with lung lining surfactants. Although it is likely such patients would be in an ICU setting, could NO_2 have contributed to the initiation of the syndrome or if of an infectious etiology, could NO_2 modification of surfactant chemistry have played a role? Very little is in the literature, although anecdotal evidence for pulmonary atelectasis was noted in rodent exposures to 340 ppb NO_2 (Sherwin, 1982).

4. Section 3.3 of the ISA document provides an informative and concise overview of potential Modes of Action underlying the health effects of inhalation exposure to oxides of nitrogen. Table 3.3 on pages 3-56 to 3-57 summarizes this overview; however the term "Modes of Action" would be more appropriate than the term "Biological Pathways," which appears in both the title and as the heading of the first column of Table 3.3. Of course Modes of Action (as well as pathways) can overlap and/or co-exist, and in fact alternative lists/classifications can be valid. It would probably be appropriate to include as a separate mode of action one that reflects changes in the dynamics of the ELF or even specifically of the lung surfactant. This can take place through a variety of processes (or "key events"), including modification by NOx or their metabolites of surfactant proteins (SP): SP-B and SP-C are involved in modulating the surface-active function of pulmonary surfactant while SP-A and SP-D (collectins) are associated with immune response. According to Atochina-Vasserman et al. (2010), "… research has highlighted the importance of SP-A and SP-D as targets of NO-mediated signaling events." Matalon et al. (2009) found that reactive nitrogen intermediates modify SP-D in a manner resulting to loss of aggregating activity and potential alterations of its structure and function at sites of inflammation.

5. 3.3.2.4 Epithelial Barrier Function - Discussions of LDH should clarify if this is a marker of epithelial barrier integrity or cellular injury.

6. Discussions of the Kleeberger et al 1997 paper (page 3-32, line 32) should also note the genes.

7. While exceedingly high exposures are often detailed, many times in discussions of human studies these facts related to concentration are omitted. Channell et al and Huang used 500 ppm for 2 h and saw significant effects – this seems important information, in light of the studies where neural effects were not observed until mice were exposed to >10,000ppm. Moreover, by limiting the outcomes of Channell et al to "changes in blood lipids and increased levels of plasma soluble lectin-like receptor for oxidized low density lipoprotein", the upshot of observing inflammatory signaling resulting from the whole plasma is lost. These functional outcomes require some further consideration, given the low concentrations of NO_2 and that similar outcomes were seen with diesel emissions (which contain a comparable amount of NOx).

8. 3.3.4 Perhaps examples in the literature could be used to show that NOx exposure leads to upregulation of NO_2/3, S-NOs and nitrated lipids? This section just seems a bit too academic.

9. Page 3-55, Transduction of extrapulmonary. The 3rd sentence really describes 3 options, not two, and should be worded to identify 1) neural 2) nitrated by-products and 3) inflammatory by-products – none of which are mutually exclusive. Also, there is a lot of attention to noting the high concentrations needed for neural pathways, and generally pulling back from this hypothesis, but the other options (which have stronger data) seem to merit as much treatment as the neural.

10. Section 3.2.1. This is more of a summary rather than an introduction to the scope of the Chapter.

11. p 3-6, lines 14-15. What is the reference for the statement about basal nitrite levels remaining unchanged?

12. p 3-10, line 31. Sentence should read "…and other factors."

13. p. 3-14, lines 4-17. This paragraph is redundant of material previously discussed

14. p 3-17, lines 3-4. What is the source for the comment about sensitivity to endogenously produced oxidants?

15. p 3-17, lines 21-26. This is aimed at indicating why endogenous NO_2 levels will not be affected by inhaled NO_2. However, while endogenous NO_2 may not be systemically distributed per the discussion, there could potentially be an increase in reaction products in the tissues due to changes in levels of endogenous NO_2.

16. p 3-18, lines 16-25. This part of the paragraph should be in Section 3.2.3. On page 3-17, it is noted that NO_2 reacts with some antioxidants resulting in production of nitrite, yet there is no indication of whether this would affect toxicity of inhaled NO_2. However, on p 3-18, it seems to be inferred that there may be toxicity of nitrite from NO or NO_2. In addition, the last sentences which indicate uncertainty about the relative contribution of endogenous NO_2 with low level inhalation exposure seem to contradict the comment noted in # 5 above that endogenous oxidants will likely not affect toxicity of inhaled oxidants.

17. p 3-17, lines 7-9. There are more recent references for the role of nitrite on muscle

18. p 3-18, lines 1-19. It is not clear why effects of such high levels are discussed.

19. p 3-29, lines 5-16. It is not clear why the discussion of gas partial pressures are in the section on neural reflexes.

20. p 3-13, lines 9-10. Where have these cells been demonstrated?

21. p 3-19, Endogenous NO_2. The discussion seems to be about NO rather than NO_2.

22. p 3-41. Section 3.3.2.6.3. This section should be part of the prior section, 3.3.2.6.2 and not a separate section.

23. p 3-43, line 14. Is it correct to say that the NO_2 exposure enhanced "..preexisting emphysema in animal models" or would it be better to say "preexisting emphysema-like conditions…."?

24. p 3-46, line 23-25. Here again it seems to contradict statements about the relative roles of endogenous and exogenous NO_2.

25. p 3-54, line 28-29. Sentence should read, "….may lead to development and exacerbation of…."

26. p 3-57. Summary. The last sentence noted that inhaled NO_2 may contribute to the endogenous body burden of NO_2 species, yet in many places earlier it is stated or inferred that this does not occur. There needs to be some consistency about this issue.

Comments on Chapter 5

Fig 5.1 could use a more descriptive caption.

The equations for RR on page 5-8 could use more explanation – why is this calculation spelled out specifically?

Dr. Ronald Cohen

Comments on Chapter 2

The chapter provides a useful overview. Chapter 2 would be improved and would provide a better basis for discussion in other chapters if was structured along the lines of a separation in time scales between the simple $NO/NO_2/O3$ triad which reach steady-state on time scales of 100 s and the more complex interaction with net ozone production, HNO3, organic nitrates etc which have time scales of hours.

Focus the chapter on key issues by more briefly summarizing regional background and global background.

With respect to the table of emissions, a source grouping that is population or area weighted would be more useful than simply summing the NEI.

A more thorough discussion of the observing system that supports an understanding of NO_2 effects as separate from co-emitted chemicals.

Detailed comments follow:

Section 2.2

Figure 2.1 could be more clear:

isoprene nitrates and Alkyl nitrates are subcategories of $RONO_2$; nitroaromatics and nitroPAHs are closely related and they are not directly related to $RONO_2$. They have direct C-N bonds.

pg 2-2 line 8: define rapidly and note that O3 is required.

pg 2-3 line 17-18. The statement is wrong. Total ANs, total PNs and HNO3 in the boundary layer are typical equal shares of the pie (see for example A.E. Perring, S.E. Pusede and R.C. Cohen, An Observational Perspective on the Atmospheric Impacts of Alkyl and Multifunctional Nitrates on Ozone and Secondary Organic Aerosol, Chemical Reviews, 113, 5848–5870, 2013 and references therein). The statement might be true if one explicitly noted that it is an average to 10km and over the continents and oceans and that that average is not a description of the continental surface layer.

pg 2-5 line 7-11 I think there is evidence and modeling indicating daytime vertical mixing within the PBL occurs on time scale of ~1 hr and conversion to higher oxides on times scales more like 4 hrs. So the statement about plumes aloft is only true at night and for stacks that are higher than the daytime PBL (if any).

pg 2-6 line 2-3 delete the words smaller amounts. I don't think the statement is correct and it is not important to the point of the section.

pg 2.6 line 8 recent research has shown the lifetime of INs with respect to ozone reactions is 100 times longer than indicated by Lockwood et al. L. Lee, A. Teng, P.O. Wennberg, J.D. Crounse, and R.C. Cohen, *On the Rates and Mechanisms of the Reactions of OH and O3 with Isoprene-derived Hydroxy Nitrates*, J. Phys. Chem. DOI: 10.1021/jp4107603, 2014.

I think the section should have separate sections for near source chemistry and far field chemistry--recognizing there is a transition region. The section should start with near source chemistry and treat it in more detail as it is essential to understanding the subjects of measurements of NOx near sources, the role of titration and the far-field chemistry is then mostly important (from the perspective of this assessment) to understanding the confounding factors of instrumentation with substantial positive artifacts.

Section 2.3

pg 2.9 Direct measurements of the overall trends in concentration should appear earlier, perhaps even before the inventory.

see for example:
A.R. Russell, L.C. Valin, and R.C. Cohen, *Trends in OMI* NO_2*observations over the United States: Effects of emission control technology and the economic recession*, Atmos. Chem. Phys. 12, 12197-12209, 2012. Note that many of the figures used in the report are also in this paper--but were peer reviewed unlike the ones in the report. There is not a significant difference in the point made by the images though.

Figure 2.2 The text should be a little more clear about the boundaries of the domain over which emissions are included and the extent to which biogenic sources are included.

From the point of view of the report, it would be useful to have the same figure with emissions only within 10km (or some similar distance) of cities with more than 10,000 people. That would help focus attention on the issues at hand and remove the distracting effect of integrals of small emissions that occur over very large land areas.

pg 2-17 line 3 should be energy released, not energy consumed.

pg 2-17 lines 16-24 references to papers by Jaegle and Hudman on soil NOx would be appropriate here. The Hudman ref is (R.C. Hudman, L.C. Valin, A.R. Russell and R.C. Cohen, *Interannual variation in soil NOx emissions observed from Space*, Atmos. Chem. Phys. 10, 9943-9952, 2010) and Jaegle is found within. There was also a follow on modeling paper by Hudman that is potentially useful reading.

Section 2.4

pg 2-19 lines 16-27

The best reference on the MoO convertors is Winer et al. 1974. After that paper it was widely accepted in the scientific community that the FRM for NO_2 should be interpreted as NOy. There is absolutely nothing new about the more recent papers. If you ask the authors of the 2007 papers why they wrote them (and I did)--the answer you get is that regulatory agencies in the US and Europe couldn't be made

to pay attention to the Winer et al. result without new measurements. I believe there was new attention because some people recognized a commercial opportunity for patentable technology.

There are at least a few published papers on near road gradients that are not referenced, I found 6 papers published since 2010 and an ARB report on the website of Suzanne Paulson, UCLA that are relevant to the near-road issues discussed in the Chapter. http://www.atmos.ucla.edu/~paulson/publications.html

I think the claim of variable sensitivity to positive interferences is too general. There is variable sensitivity to HNO3 based on inlet designs that fail to transmit HNO3 to the convertor and occasional materials issues prevent reduction of HNO3 to NO, however there is no variability in the sensitivity to $RO2NO_2$ (e.g. PAN) or $RONO_2$ (e.g. isoprene nitrate) molecules.

pg 2-19, line 28 The statement is not correct. There are numerous measurements prior to those referenced that make the same point--they just didn't label themselves as such because the scientific community had moved on to calling the FRM NO_2 method an NOy detector. For example there is an extensive literature attempted to close the NOy budget-comparing FRM measurements to the sum of distinct measurements of individual nitrogen species.

See for example:

Fahey, D. W., G. Hubler, D. D. Parrish, E. J. Williams, R. B. Norton, B. A. Ridley, H. B. Singh, S. C. Liu, and F. C. Fehsenfeld, Reactive nitrogen species in the troposphere: Measurements of NO, NO_2, HNO3, particulate nitrate, peroxyacetyl nitrate (PAN), O3, and total reactive odd nitrogen (NOy) at Niwot Ridge, Colorado, J. Geophys. Res., 91(D9), 9781 – 9793, 1986.

and a review of those issues in:

Day, D. A., M. B. Dillon, P. J. Wooldridge, J. A. Thornton, R. S. Rosen, E. C. Wood, and R. C. Cohen, On alkyl nitrates O3, and the ‘‘missing NOy,’’ J. Geophys. Res., 108(D16), 4501, doi:10.1029/2003JD003685, 2003.

pg 2-21 line 4 should read: "products, including HNO3, PAN and its analogues and total $RONO_2$.

pg 2-21 lines 5 and 6 should be deleted. A quite accurate estimate (+/-30% or better) of true NO_2 can be arrived at from NO and O3 measurements thus provided a good measure of the size of the interference to any FRM "NO_2" measurement.

pg 2-21 line 7-10 rewrite as "Concentrations of these higher oxides at the surface peak in the afternoon as a result of competition between photochemical production and losses to deposition and mixing out of the boundary layer.

Section 2.4.2

line 17-21: Expensive is not correct. It would be better to say these sensors have not been commercialized.

Section 2.4.3

pg 2-24 line 29 change the word "The current ..." to "One current ..." There are at least 3 competing algorithms.

pg 2-26 line 9 delete " from ...and since NO_2 is mainly a near surface pollutant ..." to the end of the sentence. The mixing heights are not directly related to the point being made. They only come in very indirectly as the NO_2 lifetime is longer at higher NO_2.

pg 2-27 line lines 4-14. It would be equally valid to use the mode as a transfer standard for any other time of day. The statement that the transfer from column to surface is only valid at the satellite overpass time is too strong.

pg 2-26 lines 15-27. The Russell et al. paper given above addresses the issues in this paragraph directly and more completely than many of the references used.

Section 2.4.4

It would be appropriate to acknowledge that the research community has developed multiple methods for observing NOy and its components and evaluated many of them in some detail.

For example, new chemical ionization mass spectrometric methods are especially good for HNO3 as are some methods based on transfer into liquids coupled to ion chromatography.

As a result of these methods, as applied in the lab and field, our understanding of the chemistry of odd-N is substantially more accurate than it was even 5 years ago.

Fine to say NO measurements in the networks are most reliable.

Section 2.5.1

page 2-37 line 1: define short; I think the answer is ~4hrs. also should read "to PANs, $RONO_2$ and HNO3" define highly variable; I think it would be correct to say concentrations of NOx decay on e-folding length scales of approximately 50km in summer and 200 km in winter. There is direct evidence for that in the satellite observations including the figures already in this report and also in L.C. Valin, A.R. Russell and R.C. Cohen, Variations of OH radical in an urban plume inferred from NO_2 column measurements, Geophys. Res. Lett. 40, 1856-1860, 2013. and references therein. Also in numerous other papers using the NOAA aircraft to fly downwind of urban and powerplant plumes and measurements along a transect of urban plumes such as the Sacramento one.

pg 2-37 lines 20-30 The satellite measurements are not reliable at a level of 10 ppt. They should be treated as +/- ~100 ppt.

I don't know of any direct observational evidence of a home heating effect on NOx.

Sections 2.5.2 and 2.5.3

These sections would be easier to read if the intro section had a separate discussion of $NO/NO_2/O3$ chemistry and how titration works. Specifically how the ratio of NOx to O3 affects the behavior.

pg 2-40 lines 26 and 27 the conclusion that NO_2 is freshly emitted is likely incorrect and is not substantiated. Simple analysis of the rate of conversion of NO to NO_2 indicates NO_2 would be 5 ppb 10 seconds after mixing out of the exhaust plane.

pg 2-41 line 9 should read "... 200% above urban ..."

pg 2-42 the figure is mislabeled NOx is in ppb not ppm

pg 2-43 The analysis presented on this page is somewhat confusing and convoluted. It would be more straightforward to present NOx first and then discuss partitioning of that NOx into NO and NO_2.

pg 2-43 line 7 delete the word "likely"

pg 2-43 lines 9-12 Absolute NO gradients are not evidence for the stated effect. The sentence should be deleted. The proper evidence would be NO/NO_2 ratios.

pg 2-44 it is incorrect to suggest the spatial extent of NO enhancements should be 100-300m. This is correct only if NO is substantially less than O3. If NO exceeds O3 then it is expected that NO will persist until the local plume mixes in sufficient O3. There are many examples of this effect in power plant plumes studied by aircraft and I think (although I can't recall a specific reference) some examples in modeling of NOx near roadways.

pg 2-44 paragraphs 1 and 3 on this page are repetitive.

pg 2-47 lines 21-22 satellite observations are not concentrations, they are columns. It would be correct to say satellite observations converted to concentration using a model of the vertical distribution of NO_2.

pg 2-48 It should be acknowledged that the figures imply the sensors sampled air where ozone was completed titrated as otherwise NO at night should be closer to zero.

pg 2-49 The discussion of O3-NOx relationships in this chapter is not well connected to the long standing understanding of those relationships. It will help if the chapter has a better introduction to the $NO/NO_2/O3$ chemistry as that chemistry explains a lot of the correlations discussed. Also, the larger spatial scale relationships between NOx and O3 are better understood that indicated in this document, see for examples S.E. Pusede and R.C. Cohen, On the observed response of O3 to NOx and VOC reductions in San Joaquin Valley California 1995-present, Atmos. Chem. Phys. 12, 8323-8339, 2012 and the references therein.

pg 2-51 line 12-15 It's not easy to see the stated conclusions in the figure referenced.

pg 2-51 line 12-15. Suggest deleting this sentence. There is no firm evidence for it that I am aware of.

pg 2-51 line 18 and rest of the paragraph. This level of detail is not all that relevant. The result should be summarized more briefly and without the figures. The summary statement is that transport of NOx from other continents is calculated to be less than 10% of the regional background and less than 0.01% of regulatory thresholds using models that reproduce observations of NOx and PAN in remote locations influenced by transport.

pg 2-65 lines 7-27 Since it has already been noted that the FRM has a positive bias due to sensitivity to PAN, $RONO_2$ and HNO3, it should be noted here that the agreement between the FRM and this other sensor implies similar biases in the other sensor.

pg 2-68 lines 8-9

NO_2 doesn't react with organic radicals to produce $RONO_2$--or at least such reactions are too slow to matter. The reactions that produce $RONO_2$ are NO3 and NO reactions.

pg 2-68 lines 33-36 Note NO_2 reacts with O3 to form NO3 (as discussed later in the text) I'm not sure how that fits into the analysis presented in the referenced papers, but it is an important consideration for interpreting the experiments described.

pg 2-70 The figure referenced should separately identify near roadway and other studies as we expect different correlations in the two regimes. In both NOx would be correlated with other primary pollutants but in the near field of emissions the reaction of NO with O3 results in increases in NO_2 while decreases in other primary pollutants are decreasing. As presented the figure suggests there is unexplained variability.

pg 2-71 lines 11-27 There are many, many studies describing why the relationships of ozone and NO_2 are expected to be nonlinear. One reason there are few studies describing a linear correlation is that attempts to do so are unlikely to survive peer review as they are presenting a model of the relationship that is known to be flawed. The Pusede and Cohen paper listed above include many relevant references to the issue--but it is by no means comprehensive or complete.

pg 2-20-2-71 and figure 2-19 also pg 2-78 line 5
The role of near road titration on observed correlations should be explicitly discussed. We expect in the near field that ozone and NO_2 will be anti-correlated. This issue should not be referred to as "complex chemistry." Then in the far field of a single plume, the two will be positively correlated. However, comparing two different plumes (or one plume at two different initial NOx) the increase in ozone will not be a linear function of NOx.

pg 2-79 an equally likely explanation is exposure to air where a mix of ratios of NOx to O3 is present.

pg 2-80 line 30 NO_2 is not prevalent in vehicle exhaust. NO is.

pg 2-82 and Fig 2-20.
I think the figure is misleading because the physically relevant parameter is not the increase in a pollutant divided by its background concentration but the absolute enhancement over the background. There are many analyses of plumes in atmospheric science that show that enhancement ratios defined in this way (e.g. Delta CO enhanced : Delta NOx enhanced) remain conserved during mixing with a

background while the ratios to the background vary. On the relevant times scales there are no known losses of NOx or CO, so an analysis that indicates the two behave differently is odd and should be treated with caution.

pg 2-102 lines 12-13 I do not think the diesel statement is relevant. If NOx is less than O3, then on time scales of 100 sec (e.g. 300m at 3m/s winds) NO/NO_2 and O3 approach a photostationary state independent of whether emission is as NO or NO_2.

pg 2-102 I think the observation that should be highlighted here is the dramatic drop on weekends in cities in the US (~50%) and the long term trend (~30% 2005-2012). Those large changes provide a significant opportunity for new epidemiological studies of the short term health response (weekdays vs weekends) and of the benefits of long term reductions (2005-2012). These issues are much more important to understanding the health effects of NO_2 than whether NO or NO_2 is emitted from tailpipes.

Dr. Douglas Dockery

First, I must commend the authors and editors of this *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria* for a very thoughtful, clear, and comprehensive synthesis of the information.

The body of new literature since the 2008 ISA for Oxides of Nitrogen has strengthened the evidence for causal associations with the health effects considered. Most of this evidence consists of epidemiologic studies. The 2008 ISA identified several generic concerns with the evidence for causality, particularly in the observational epidemiologic studies which still apply.

First, ambient NO_2 concentrations are highly correlated with concentrations of other pollutants from motor vehicles and traffic. The highest correlations are observed between ambient NO_2 and CO, BC, and UFP (Figure 2-19, page 2-77). This is true for both short-term and long term exposures. Thus it is difficult to separate out specific effects of NO_2 from correlated co-pollutants in observational studies. Most studies approach this problem through adjustment in two-pollutant regression modeling. New studies provide additional data, particularly for the short-term effects on respiratory conditions. However, for most studies, there is limited data on co-pollutant exposures, particularly for the highly correlated traffic pollutants (CO, BC, and UFP). Thus, most of the observational data continues to suffer from potential confounding by these co-pollutants.

Secondly, it is difficult to separate specific effects of ambient NO_2 from the air pollution mixture attributable to traffic. It is feasible that the associations with proximity to traffic may reflect the mixture rather than a specific component, such as NO_2. Studies to date have not been able to disentangle the mixture versus single component associations.

Thirdly, thirdly it is difficult to separate specific effects of ambient NO_2 from generic risk factors associated with proximity to traffic such as noise. There is increasing interest in attempting to separate ambient NO_2 effects from noise and other non-pollutant traffic risk factors. However, these potential alternative explanations are not considered in this ISA.

How do we disentangle the specific effects of NO_2 from those of traffic related co-pollutants and risk factors? Indoor NO_2 exposures may offer insights, as indoor NO_2 exposures represent a potentially different, informative mix of air pollutants. Thus, it is informative to consider the consistency of studies of indoor NO_2 with studies of outdoor ambient NO_2. Indoor NO_2 studies are given little attention in this ISA.

Ultimately, the most informative information will come from experimental studies which permit specific, controlled exposures to NO_2 alone or with fixed co-pollutants.

In this ISA, there is a clear enunciation of "weight of the evidence criteria causal determination" (Table 11, page l). Five levels of evidence are defined – **Causal** relationship, **Likely** to be a causal relationship, **Suggestive** of a causal relationship, **Inadequate** to infer causal relationship, and **Not Likely** to be a causal relationship. The ISA finds that the evidence has grown stronger for a causal relationship with ambient NO_2 compared to the 2008 ISA for all health end points considered.

The following Table is my attempt to summarize the evidence presented for most of the endpoints (except reproductive/development and cancer) compared to the issues noted above. It is clear that the strongest evidence is found for respiratory effects with short term exposure, and secondarily respiratory effects with long term exposure. This Table illustrates the gaps and inconsistencies in our understanding, either because of lack of studies, or because they were not included in the ISA review. It would be helpful to consider which is the case.

TABLE: Simplified summary of evidence for causality for ambient NO2 based on 2013 draft ISA

	SHORT-TERM NO_2 EXPOSURE			LONG-TERM NO_2 EXPOSURE		
	Respir-atory	Cardio-vascular	Mortal-ity	Respir-atory	Cardio-vascular	Mortal-ity
OBSERVATIONAL EVIDENCE						
NO_2 association	●	●	●	●	◑	◑
Exposure Response			●	●		
Adjusted for BC, CO, UFP, $PM_{2.5}$	●	◕		○	◕	
Adjust for Traffic indicators						
Coherence with Indoor NO_2	●			◑		
EXPERIMENTAL EVIDENCE						
Controlled Human	◑	○				
Toxicologic Mechanistic	●	○		●		
CLASSIFICATION(*Table ES-1*)	**CAUSAL**	**LIKELY**	**LIKELY**	**LIKELY**	**SUGGEST**	**SUGGEST**

● Consistent Evidence
◑ In-Consistent Evidence
○ Evidence does not support
◕ Evidence in opposite direction

Dr. Philip M. Fine

Comments on Chapter 2

Charge Question 3: Chapter 2 describes scientific information on sources, atmospheric chemistry, air quality characterization, and human exposure of oxides of nitrogen.

a. To what extent is the information presented regarding characteristics of sources, chemistry, monitoring concentrations, and human exposure accurate, complete, and relevant to the review of the NO_2 *NAAQS?*

The information presented is generally comprehensive, accurate, and relevant to the NAAQS review. Information on the changes in relative NO/NO_2 emissions from newer technology diesel vehicles (Page 2-10) is very important for near-road exposure considerations. While total NOx emissions are being reduced as the fleet turns over and new tailpipe standards are promulgated, NO_2 exposures may not decrease as rapidly in the near-road environment due to this phenomenon. Perhaps the projected trends and implications could be discussed in more detail.

b. To what extent are the analyses of air quality presented clearly conveyed, appropriately characterized, and relevant to the review of the NO_2 *NAAQS?*

The presentation of air quality data is brief, but the highlights are clearly conveyed on the tables and figures.

c. How effective are the source category groupings and the discussion of source emissions in understanding the importance and impacts of oxides of nitrogen from different sources on both national and local scales?

The discussion of sources is complete, properly grouped and informative. Some categories include a discussion of emissions trends or current or future controls, while others do not. It may be more consistent to discuss the history and future of controls in every appropriate category relative to NOx emissions trends.

d. Please comment on the extent to which available information on the spatial and temporal trends of ambient oxides of nitrogen at various scales has been adequately and accurately described.

<u>Page 2-47, second paragraph in Chapter 2.5.4</u>
The text states that while mean concentrations are highest in the first and fourth quarters, maximum concentrations are highest in the second and third quarters. Table 2-1 is cited for support of these seasonal trends, but the Table does not include seasonal data. Furthermore, much of the discussion in this chapter describes higher peak NO_2 concentrations in winter, as one would expect from meteorological considerations. The statement that higher maximums are seen in the spring/summer months should be corrected or supported with data.

Page 2-50, Figure 2-16

The significance of blue shaded range in Figure 2-16 is not explained. Is it the full range across all sites, percentile ranges, or standard deviations? It should have some explanation in the caption.

e. Please comment on the accuracy, level of detail, and completeness of the discussion regarding exposure assessment and the influence of exposure error on effect estimates in epidemiologic studies of the health effects of NO_2.

Not my primary area of expertise, but the discussion seems comprehensive and recognizes the challenges in NO_2 exposure assessment.

Dr. Panos G. Georgopoulos

Comments on Chapter 3

Chapter 3 characterizes scientific evidence on the dosimetry and modes of action for NO_2 *and nitric oxide (NO). Dosimetry and modes of action are bridged by reactions of* NO_2 *with components of the extracellular lining fluid and by reactions of NO with heme proteins, processes that play roles in both uptake and biological responses.*

a. *Given the ubiquity of reactive substrates and reaction rate of* NO_2 *with these substrates, it appears unlikely* NO_2 *itself will penetrate through the lung lining fluid to the epithelium (see Table 3-1). Please comment of the adequacy of the discussion of* NO_2 *uptake and reactivity in the respiratory tract.*

The assumption that it is unlikely for NO_2 itself to penetrate through the lung lining fluid to the epithelium appears generally reasonable. However, describing the interaction of NOx with the extracellular lining fluid (ECLF) in terms of classical (Fickian) diffusion processes and homogeneous chemical reactions would be an oversimplification that may be insufficient with respect to describing actual *in vivo* ECLF/NOx system dynamics. In fact, Bastacky et al. (1995) (a reference already cited in the ISA document) report that for the rat lung"[t]he thickness of the liquid layer averaged 0.14 μm over relatively flat portions of the alveolar walls, 0.89 μm at the alveolar wall junctions, and 0.09 μm over the protruding features (9 rats, 20 walls, 16 junctions, and 146 areas), for an area-weighted average thickness of 0.2 μm." Unfortunately, this reviewer is not aware of similar data for the human lung, but it is obvious that the local variation of ECLF thickness is significant and may challenge, under certain conditions the assumption that NO_2 cannot penetrate the ECLF. Also, it is known that different activity levels and associated inhalation rates result in changes to ECLF properties (such as thickness - see, e.g. Archie, 1973), whereas altered health (pathophysiological) states are expected to also cause changes (e.g. Albert & Jobe, 2012; Hobi et al., 2014).

So, there is a need to understand and describe mechanistically the spatiotemporal dynamics of NO_2 transport and reaction within the various microenvironments of the respiratory system, taking into account that the ECLF is far from homogeneous, both across the respiratory system and within particular microenvironments (such as the alveolar microenvironment). Furthermore, these dynamics have to be understood for different activity levels (and corresponding inhalation rates) and for altered health/pathophysiological states. These observations should also apply to NO, which in fact is known to enter alveolar epithelial cells, but potentially through processes that are not diffusion-dependent (e.g. Brahmajothi et al., 2010).

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b. *Since existing dosimetric models for* NO_2 *do not consider the probability of oxidants/cytotoxic products reaching target sites, it was concluded that these models are inadequate for within or cross species comparisons. Please comment on the validity of this conclusion and identify and comment on the validity of any alternative conclusions.*

The conclusion that existing dosimetric models for NO_2 are inadequate is in fact valid. Development of a detailed mechanistic conceptual comprehensive NO_2 dosimetry model, followed by subsequent computational implementation, is critically needed, along the lines of similar efforts that have taken place in recent years (e.g. Aberg et al., 2010; Asgharian et al., 2011). Such a model should explicitly account for different life-stages and altered health states (development, obesity, aging, etc.), in a framework that takes into account existing hypotheses for NO_2/NO transport and transformation in the respiratory system. Even during its development, this model would provide a useful tool for hypothesis generation and rational design of future laboratory studies. Of course, pursuing development of this model cannot take place as part of the current review process but it would be important for specific dosimetry modeling needs to be identified. It would also be important at the present time to summarize explicitly the major deficiencies and uncertainties associated with the lack of valid NO_2 dosimetry model; it is recommended to consider including such a summary in the form of a brief table in Section 3.2, where these issues are discussed.

References:

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Asgharian, B., Price, O.T., Schroeter, J.D., Kimbell, J.S., Jones, L., and Singal, M. 2011. Derivation of mass transfer coefficients for transient uptake and tissue disposition of soluble and reactive vapors in lung airways. *Ann Biomed Eng* 39 (6):1788-804. DOI:10.1007/s10439-011-0274-9

c. *Please comment on the adequacy of the discussion of endogenously occurring* NO_2 *and NO and their reaction products in comparison to that derived from ambient inhalation.*

It would probably be beyond the scope of the present ISA document to further expand on the biology of endogenously occurring NO_2 and NOx and of their reaction products. It would, however, be useful to, at least, provide some additional references with information regarding:

- NOx biochemistry in the wider context of "small molecule signaling agents" (e.g. Fukuto et al., 2012; Heinrich et al., 2013);
- NOx biochemistry human microbiome dynamics; in particular in relation to the oral microbiome (e.g. Hezel & Weitzberg, 2013), that would in fact be also exposed to exogenous inhaled NOx; and
- NOx biochemistry in relation to altered health states (e.g. obesity – see, for example Dai et al., 2013; Holguin, 2013)

References:

Dai, Z., Wu, Z., Yang, Y., Wang, J., Satterfield, M.C., Meininger, C.J., Bazer, F.W., and Wu, G. 2013. Nitric oxide and energy metabolism in mammals. BioFactors 39 (4):383-391. DOI:10.1002/biof.1099

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Heinrich, T.A., da Silva, R.S., Miranda, K.M., Switzer, C.H., Wink, D.A., and Fukuto, J.M. 2013. Biological nitric oxide signalling: chemistry and terminology. Br J Pharmacol 169 (7):1417-29. DOI:10.1111/bph.12217

Hezel, M., and Weitzberg, E. 2013. The oral microbiome and nitric oxide homoeostasis. Oral Dis. DOI:10.1111/odi.12157

Holguin, F. 2013. Arginine and nitric oxide pathways in obesity-associated asthma. *J Allergy (Cairo)* 2013:714595. DOI:10.1155/2013/714595

d. To what extent are the discussion and integration of the potential modes of action underlying the health effects of exposure to oxides of nitrogen presented accurately and in sufficient detail? Are there additional modes of action that should be included in order to characterize fully the underlying mechanisms of oxides of nitrogen?

Section 3.3 of the ISA document provides an informative and concise overview of potential Modes of Action underlying the health effects of inhalation exposure to oxides of nitrogen. Table 3.3 on pages 3-56 to 3-57 summarizes this overview; however the term "Modes of Action" would be more appropriate than the term "Biological Pathways," which appears in both the title and as the heading of the first column of Table 3.3.

Of course Modes of Action (as well as pathways) can overlap and/or co-exist, and in fact alternative lists/classifications can be valid. It would probably be appropriate to include as a separate mode of

action one that reflects changes in the dynamics of the Extracellular Lining Fluid (ECLF) or even specifically of the lung surfactant. This can take place through a variety of processes (or "key events"), including modification by NOx or their metabolites of surfactant proteins (SP): SP-B and SP-C are involved in modulating the surface-active function of pulmonary surfactant while SP-A and SP-D (collectins) are associated with immune response. According to Atochina-Vasserman et al. (2010), "… research has highlighted the importance of SP-A and SP-D as targets of NO-mediated signaling events." Matalon et al. (2009) found that reactive nitrogen intermediates modify SP-D in a manner resulting to loss of aggregating activity and potential alterations of its structure and function at sites of inflammation.

Two additional comments regarding modes of action:

- It appears that all (potential) vascular and systemic effects of NO_2 are lumped under "Transduction of extrapulmonary responses" (discussion in Section 3.3.2.8 on pages 3-43 to 3-46, which provides a brief but informative overview). The spectrum of these (potential) effects does not become clear either in the summary of page 3-59 or (even more) in the corresponding entry of Table 3.3 on page 3-57. It is realized that the uncertainties regarding systemic effects (and the MOAs involved in these) are very large; however, the range (and severity) of health effects that have been hypothesized to be related to NO_2 exposures is so wide that a more detailed listing of the biological mechanisms potentially associated with them would be justified.

- It would be informative to identify explicitly MOAs that may be relevant specifically to cases involving co-exposures with other xenobiotics (since inhalation exposures to NO_2 and NO always occur in the context of a complex mixture of atmospheric contaminants as well as for exposures of subjects with health problems (ranging from obesity to asthma and COPD).

References:

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Dr. Jack Harkema

Comments on Chapter 1 - Integrative Summary

General Comments:

The introduction of Chapter 1 provides a good presentation of the ISA's organization and scope, along with definitions of the categories of causality. The evaluation sections on health effects provide an in-depth collective summary of the material presented within the health effects chapters of the ISA. Though each topic area is nicely summarized in a conclusion paragraph that provides the rationale for the determination of causality, the authors do not clearly and consistently identify the body of work that substantially contributed to the selected causality classification. This should be provided clearly both in the text and in the tables.

Furthermore it is not always easy to know if the causality classification was primarily dependent on recent (since the last review) or older studies. This is due in part to a lack of references. There needs to be more consistency in how key studies are referenced throughout this Chapter. Also in this regard, the key health effect findings need to be presented along with their NO_2 exposure data. This too is inconsistent throughout the chapter. In addition, there is too much reliance of terms such a "high quality studies" in the justifications. More specific and robust rationale needs to be presented.

In general there is good integration and summarization of the collective data within a topic area (e.g., Respiratory Effects Associated with Short-term NO_2 Exposure), but more synthesis and critical review needs to be provided between topic areas (e.g., between Respiratory Effects of Short- and Long-term NO_2 Exposures). For example, it is not always clear that the respiratory (or extrapulmonary) health effects being examined in a study are clearly due to short- or long-term NO_2 exposures. A critical assessment of this potential problem of interpretation should be presented, along with the uncertainty it brings to the causality determination. In terms of basic pathology and pathophysiology, one would think that long-term exposures to inhaled pollutants would likely be associated with chronic health effects (e.g., chronic bronchitis, emphysema, atherosclerosis, mortality), while short-term exposures would be associated with acute effects, such as exacerbation of asthma. This is, in part, an issue of biological plausibility that needs critical evaluation. It is especially important now that there is both an annual and 1-hr standard for NOx.

Overall this is a good summary, but more critical synthesis and clarification of the major findings (or lack of findings) since the last review are needed. This will help the Administrator with her policy decisions regarding NAAQS.

Specific Comments:

The Integrative and Executive Summaries are places to identify existing data gaps. This is lacking in this ISA draft, along with suggested areas for future research.
The introductory section on 1-1 provides a paragraph on the major outcomes from the last review. A brief paragraph summarizing the major research findings since the last review would be helpful here as well to set the stage for this Chapter and remainder of the ISA.

1.4.1 The discussion on dosimetry is very limited in its scope. The discussion is focused on general airway fluid, tissue and cellular dosimetric determinants and does not cover important areas such as dosimetry throughout the respiratory tract, impact of exercise and changes in airway dosimetry with age and disease.

1.4.1 Likewise, the potential mode(s) of action for acute and chronic responses to short- and long-term exposures to NO_2 is limited in its scope. There is no acknowledgement of the specific sites of pulmonary injury other important modes of action outside of inflammation, such as sensory nerve responses and airway remodeling.

1-16. More critical evaluation is need on the relationship of long-term NO_2 exposure and respiratory health effects. As written, there does not appear to be enough supporting evidence to increase the level of causality to likely from suggestive in this reviewer's opinion. The associations of respiratory health, incidence of asthma, in new epidemiology studies may still be due to short-term exposures causing exacerbations. More clear and convincing justification is needed in this section to make the case for this change in causality.

1.5. Evaluation of Independent Effects of NO_2. This section provides good documentation with ample references to key studies since the last review and before.

1-40. Indoor NO_2. The influence of outdoor NO_2 on indoor NO_2 is not described in this short section. Neither is there any discussion of the health of effects of indoor NO_2 affecting responses to outdoor NO_2 exposure.

1-50. At-risk populations. Since there is a major concern about the interface of air pollution and obesity, diabetes and the metabolic syndrome, recent studies (or lack of studies) on NO_2 exposure and these newly identified at-risk populations should be addressed.

1-47. Last paragraph does not give support to changing the causality level of the respiratory effects of long-term NO_2 exposure.

1.7. Conclusions. This section would be bolstered by recognizing the recent studies that support changes in causality. The last sentence in this section is rather nebulous and does not clearly state whether there is enough convincing new evidence in regard to concentration-response relationships to warrant a change(s) in current NAAQS.

Comments on the Executive Summary

This is a condensed version of Chapter 1. Many of my comments on the Integrative Summary would also hold for the Executive Summary. In addition, there is a lot of redundancy (too much "cut and paste") in this Summary and Chapter 1 that cheapens the text of both.

The term "Lung function growth" needs to be better explained in both the Executive Summary and Chapter 1 – Integrative Summary.

Dr. Michael Jerrett

Comments on Chapter 2

Not clear when the review begins and ends because some articles prior to 2008 are cited, but some are not, and also there are some key omissions from the review that were published with Chapter 2. Not clear what it means when a study is excluded – please clarify this - based on quality or date or simply an omission?

Not clear from the exposure assessment framework how the EPA will deal with occupational exposures, both indoor and outdoor, within the exposure assessment framework outlined in this chapter.

Also there is likely to be a major on-road exposure of commuters, whether on foot, bicycle, or by vehicle or public transit. More than 90 million Americans are commuters and many millions of children are commuting to school.

The chapter is silent on the issue of physical activity during the point of contact between the NOx and the human receptor; this can have a substantial impact on the intake of the pollutant if we compare for example the intake during sedentary behavior (4.5 L/M) vs. high activity for strenuous exercise (35L/M). Some commentary is needed.

Chapter 6 – Response to Charge Questions

a. In general the at risk categories are useful, but in some cases there were ambiguities and omissions, including:
    - what are the differences between "differences in dose/exposure or differences in exposure to air pollutant concentrations"
    - there are several categories that should be added: persons and families under stress – Shankardass PNAS, and other articles by Cloughty on exposure to violence and on animal studies
    - occupations who are likely to have higher exposure in the occupations (police officers – in vehicle, on foot or bike; postal workers; courier drivers and bicycle couriers; others working outside)
    - commuters to work and school (in vehicle and in active commute by walking or biking)
    - children attending schools with high NO2 exposure, which may contribute to their overall exposure
    - there was no mention of potential climate effects, and it would be useful to examine whether climate variables modify the effects of NO2

b. With the exception of omissions noted above, the literature review accurately reflects the epidemiologic, human exposure and toxicological studies. Summary tables at the end of each section, similar to the table at the end of the genetics section would help to distill the reasons for the causal decisions.
c. On asthma, there are older articles (Sahsuvaroglou et al. 2008 shows effects in children without hayfever, particularly in older girls; Steib et al. 2014 in contrast finds effects in children with

allergies). It would also useful to compare the Children's Health Study results for the older and the younger cohorts in terms of effect sizes, etc.

d. With asthma, document seems to stretch draw such strong conclusions after nearly two pages of caveats about the results.

There is substantial evidence that NO2 exposures are not equally distributed among the population, but instead follow an inverse social gradient such that the socially disadvantaged groups face generally higher exposures. Since these groups are also potentially more susceptible, this has been referred to as double jeopardy. Some recognition of this literature and it's potential for generating great health effects is needed (IOM 1999 "Toward Environmental Justice: Research, Education, and Health Policy Needs", Jerrett et al. 2001, O'Neil et al. 2003, Morello-Frosch et al. 2012 and several others have made this point in general). The main issue here is that there are cumulative exposures and vulnerabilities that cluster in the same places and individuals. The main issue raised by public commenters is valid; that there multiple co-exposures that affect individuals and populations with numerous vulnerabilities (obesity, diabetes, high occupational exposures, smoking). Even if you cannot quantify or identify studies that have dealt with this issue.

Other General Comments on Various Chapters and General Organization

It would be useful to have a summary table showing the causal determinations from the last review vs. those in this review, with an emphasis on highlighting the changes from the last review

Example Table with Several Elements Key to the Issues of Confounding and Effect Modification of NO2 Effects

Health Outcome Associated with NO2	Co-Pollutant Confounders with likely direction of modification	Co-Pollutant Modifiers where NO2 is an adjuvent	Other Confounders or Modifiers
Asthma exacerbation	UFP (-), BC (-), Metals (-), Other Particle Species (-), VOCs (-), Ozone (+/-), others	Allergy-inducing pollens, molds, other time varying allergens or pollutants where NO2 could act as an adjuvant, etc	Noise (M or C), Weather, Season, other time varying factors
Asthma hospital admissions			

It would be useful to have some summary of the effects observed from the particle species caused by NOx rather than just referring to the PM ISA, which is now quite dated.

There is a growing literature on metabolic effects of air pollution and several studies have found associations between NO2 and diabetes (Coogan et al., Chen et al. 2013, Brook et al. 2009, Brook et al. 2013). There should be a separate section dealing with metabolic outcomes.

Given the high level of spatial variability in NOx, it seems that some priority should be given to studies that use within-city exposure estimates, rather than those using central site monitors, for the long-term studies. It was not always clear from reading Chapt 1 if the adequate weighting was being given when studies using central site vs. within city estimates of NO2 were being compared (e.g. ACS vs. Harvard Six Cities) – both are central monitor studies and should not be held up as that relevant for NOx. There is likely to be a much higher level of measurement error when the central sites are being used for exposure assessment when compared to the within city studies. If these comparisons treated the exposure assessments equally and were used as a factor in determining causality, there should be a reweighing than de-emphasizes the studies using central monitors and to emphasize those studies that have used modeled estimates or monitored estimates that match the scale of variation in NOx (10-100s of m).
The spatial distribution of sources in relation to receptor population will have a large impact on the intake fraction of NOx. Because much of NOx has local sources from traffic, the intake fraction of NOx is likely quite large compared to other pollutants. Could the EPA include some mention of this in their review.

The reference to the annual average exposures based on the monitoring locations is likely an under-estimate of exposure because very few of the monitoring sites are located in areas of high traffic density, but a large portion of the population does live in these areas. A caveat is needed in reporting the levels in Chapter 1 and elsewhere.

There is not enough detail on noise as a potential confounder or effect modifier. Traffic noise has been associated with several outcomes that are similar to those examined in the ISA, and it is one of the confounders could be important. More European studies estimate this exposure and in this instance they should be consulted.

More emphasis should be given to understanding the micro-environment concentrations as was done in the HEI Health Effects of Traffic Report. In that report all concentrations even recorded in a given micro-environment were reported. If the EPA cannot undertake this, then please include the HEI pot.

Along similar lines, there are likely many gradient studies that have not been identified (Paulson's studies in LA for example).

Dr. Joel D. Kaufman

This is a large and impressive compilation of information and overall it is reasonably well organized. Some sections are well-written while a few are not as well-written, reflecting the multi-author nature of the document. I focused my attention and comments here on the chapters primarily describing the integrated health effects of short-term and long-term exposure to oxides of nitrogen (Chapters 4 and 5, respectively).

Overall, these two chapters appeared to represent a reasonably complete review of the literature since 2008, with salient earlier references, collected up to a time-point a bit more than a year ago. I understand that additional literature will be incorporated that is published between that time and a few months from now.

An overarching issue with the ISA is regarding the degree to which NO_2 and NOx exposure assessment in epidemiological studies (especially in studies of long-term exposure) are fundamentally studying near-source combustion-derived pollutants (especially but not exclusively traffic-related air pollutants) or are they specifically studying effects of oxides of nitrogen exposures. This distinction would become less important if regulatory efforts proceed to address sources of pollution in a multi-pollutant context. However, from discussion at the March 2014 meeting, it appears clear that the agency plans to move forward with this ISA focused explicitly on NO_2 (and NOx), as an exposure separable from the suite of pollutants with which it travels. This decision is reasonable given the constraints which exist, but requires a bit more consistency, for example, with attention to how studies are described in the ISA. In this context it is not helpful to describe health effect studies as being about traffic-related air pollution. It would be more helpful to delete descriptions of individual studies regarding whether they are traffic studies and instead to be consistent in describing for each study: the observed associations with NO_2 (or NOx) and the ability to be confident that the exposures and health effects assessed can be attributed to oxides of nitrogen.

Since there is an increase in the ISA authors' confidence in levels of causation between NOx and most categories of health outcomes, this requires: 1) that the reader understand the criteria and processes for determinations of causality; AND 2) that the reader understand the body of evidence underlying each potential determination. Regarding the first point: While some committee members felt that the framework for causal determinations was not clear and well road-marked in the document, I consider that the ISA authors have done a good job with this and that while some table improvements could be made, for the most part the process is clear.

On the other hand, the document could use additional organization efforts to demonstrate the evidence underlying causal determinations. To some extent this is a matter of re-organizing the description of study types in a way that will better relate to health outcomes. For some outcomes, it is reasonable to re-think the importance of some lines of evidence with regard to important health endpoints.

There are four major health effect categories for which important increases in casual determinations have been made and which are reviewed in the ISA. For short-term NO_2 exposures this includes respiratory effects, cardiovascular effects, and total mortality. For long-term NO_2 exposures this represents respiratory effects. For each of these outcomes, I believe that the reporting of outcomes in the ISA can be structured in a way that better informs our understanding of causal relationships.

For short-term NO_2/NOx exposures, the respiratory effects are driven primarily by studies regarding exacerbations of asthma or airway hyper-responsiveness among those with asthma, and secondarily by other respiratory effects such as COPD exacerbations and undifferentiated respiratory disease outcomes. While it is reasonable to separate studies into experimental designs and observational designs as has been done, it would be much easier to review the evidence regarding the causal relationship and coherence of evidence from observational studies of asthma exacerbations (which by definition only occur in those with asthma), if the studies were described together, rather than being separated by artificial study design distinctions. Observational study evidence regarding asthma exacerbations is found in studies of respiratory symptoms, studies of asthma medication use, studies of spirometric outcomes, studies of fractional exhaled nitric oxide concentrations, studies of hospital admissions, and studies of emergency department visits, and are strongly supported by the experimental evidence in airway responsiveness controlled exposure studies. The observational studies from all studies of asthmatics, without regard to study design, should be reviewed as a collective whole and not lumped with studies of non-asthmatics in this regard. This criticism holds for other health outcomes and for both short-term and long-term exposure studies: artificial distinctions derived from study design differences obscure the effort to determine if there is a health effect causally related to oxides of nitrogen exposure.

For short-term NO_2 exposures with regard to cardiovascular effects, the findings as reported further obscure the important distinctions between outcomes of primary importance and those which should be of secondary importance in determining health effects of potential regulatory significance. Outcomes of primary importance should be actual clinical events, or changes in validated subclinical measures which are strongly associated with the clinical events observed in populations. Outcomes of secondary importance are those which assess a measurable physiological or biochemical alteration for which a within-individual change has not been clearly found to predict (or be associated with) the clinical events observed in populations. These outcomes of secondary importance can still play a role in causal determinations not as outcomes in their own right, but rather to inform issues of biological plausibility (modes of action) and to potentially inform issues of concentration-response relationships--but only to the extent that the outcome is associated with the clinical events of interest.

As an example, it is presumed that the underlying driver of short term health effects of concern for NO_2/NOx on cardiovascular effects are the *triggering of* myocardial infarction, or stroke, or lethal arrhythmia, or possibly decompensation of pre-existing congestive heart failure. While many lethal arrhythmias are associated with myocardial infarction, some derive from separate causes, as a result it would be useful for the ISA review to divide the evidence into these four sets of data (triggering of MI, lethal arrhythmia, stroke, CHF worsening), regardless of study design. The epidemiological studies which will be most informative are studies of confirmed acute myocardial infarction or other ischemic heart disease (IHD) outcomes, confirmed arrhythmia, confirmed stroke, and studies of cardiovascular admissions and mortality, for which we can anticipate that mortality effects will be dominated by IHD and stroke. Studies of sudden cardiac death, and studies of lethal arrhythmias noted in implantable-cardioverter-defibrillators (AICDs) will be most informative for the effect on lethal arrhythmia as distinct from IHD. Studies of congestive heart failure would be limited primarily to medical records or hospitalization studies. Additional health outcomes of primary importance which are described in the ISA would be studies of blood pressure (a valid health outcome in its own right) and ST segment depression (a validated marker of subclinical IHD). In my opinion, all of the other noted health outcomes (heart rate variability, QT-interval duration, and blood biomarkers of cardiovascular effects) would be considered of secondary importance, since in most cases a within-individual change in these

measures has not been clearly associated with the clinical events observed to be associated with NO_2 or NOx in populations. As noted above, these outcomes of secondary importance do serve to inform issues of biological plausibility (modes of action) and to potentially inform issues of concentration-response relationships, but only to the extent that changes in the measure is clearly associated with the clinical events of interest—which is hazy for many of these.

I have similar concerns regarding the description of the evidence regarding long-term exposures and respiratory effects. An organization of the review which focused on all studies regarding incidence of asthma (separately in children and in adults) and not separated by study type, would make for a more coherent understanding of the strength of the evidence.

In addition to these organization points, I have comments on two additional major areas:

Exposure assessment in epidemiological studies of long-term exposure

The ISA does not meaningfully distinguish between modern studies which have can determine fine scale intra-area gradients for oxides of nitrogen (as via land-use regression or other hybrid fine-scale approaches) as compared with studies using nearest monitor or coarse gridded dispersion models. This distinction is critically important in interpreting the long-term exposure studies and is given short-shrift here.

Meta-analysis of airway provocation studies

The ISA section 4.2.2 discusses the effect of short-term NO_2 exposure on airways responsiveness. The limited original analysis described in this section of the ISA was reasonable and appropriate. This "meta-analysis" did not include pooling of individual level data beyond that which was available in the published studies. It would have been helpful if the hypothesis to be addressed in the meta-analysis was explicitly stated at the beginning of the section. There were many sources of heterogeneity between the study protocols, and the authors of the ISA separated individual subjects/studies according to whether the subjects were asthmatic and whether the experimental protocol involved exercise. I infer that the hypothesis (a reasonable one) was that responses to NO_2 would be most notable in asthmatics, and responses would be attenuated with exercise. A more comprehensive analysis should discuss the role of asthmatic status and asthmatic sub-phenotype (atopic or non-atopic, childhood- or adult-onset, exercise-induced bronchospasm or not, if known), exercise, provocative agent, the temporal aspects of response, as well as definition and/or extent of adversity, but this can be deferred to a supplement or a free-standing peer-reviewed publication.

Specific minor comments:

On page I-17 line 5, I don't believe that the sentence describes what is meant to be implied. Rather than there being limited biological plausibility, I believe this statement should be that that there is limited experimental evidence to directly inform an assessment of biological plausibility. There is plenty of biological plausibility that the same processes that happen acutely could extend to a long-term effect, and little reason to believe it would be otherwise.

Table 4-1 does not describe HDM in the legend.

Page 4-194 line 28: true but the vast majority of these is believed to be primarily due to MI.

Page 4-196 lines 1-2: overstates what can be inferred from the studies cited.

Section 4.3.4. The importance of ST-segment changes is quite different from the importance of QT-interval studies. The ST-segment study is a study of cardiac ischemia and needs to be characterized as such—it is highly relevant to understanding ischemic heart disease. It shouldn't be lumped with studies of QT changes which are studies of entirely different electrophysiological changes and are more related to mode of action and are important primarily for the arrhythmia outcome.

Page 4-2110 lines 28-30: This is not a study about blood pressure and I'm not sure what it's doing here.

Page 4-214 lines 25-33: This is not a study about blood pressure. If you want to put it with the FMD study previously, you could make these a section in modes of action section or something.

Section 4.3.7. This section stands out in my comment regarding organizational structure. It would make more sense to categorize in the types of cardiovascular disease events first, and then into whether data is from hospitalization, other clinical event ascertainment, or mortality data. Doing it the way you've done it separates out the similar outcomes and makes it harder to see consistent message on strength of evidence.

Section 4.3.7.3. Again this stroke evidence should be described with the other stroke evidence.

Page 4-248, lines 17-8: Again: in synthesis section, issue isn't whether NO_2 is associated with HOSPITAL ADMISSIONS for IHD, but whether all sources of research (mortality, hospital admissions, clinical epi studies) provide consistent evidence of association between NO_2 and ischemia and IHD events. Studies of ST segment changes even belong here, but calling out of study type does not belong here.

Page 4-249, lines 1-3. What is evidence for this very strong statement? I would argue entire HRV section belongs elsewhere.

Section 5.2.3.1. This section is not well organized or clear. What other kind of studies are there here other than epidemiological studies?

Page 5-34, lines 26-27. While this whole section is not particularly well-written, this particular sentence doesn't make sense at all to me.

Section 5.2.3.2. I'm not sure why this whole section doesn't simply end after line 8 "No recent studies were available."

Section 5.2.4. Why are hospitalizations a section rather than have the outcomes of the studies used to categorize the hospitalizations used to put them in with the outcome of interest? It doesn't really make sense. These are also epidemiological studies. Also, the descriptions of the studies don't provide enough idea of how the NO_2 exposure was assessed.

Section 5.2.5. Symptoms in children with asthma diagnosis belong in the section on asthma as a study of exacerbations of asthma. Other respiratory symptoms can be separated out as some kind of nonspecific respiratory symptom studies.

Page 5-46, line 29: It's true that nasal eosinophils participate in allergic disease, but they are not allergic disease in their own right. This study would belong in mode of action if anywhere.

Page 5-47, lines 16-37: both of these studies are of asthma, so it's not clear why they are here rather than with asthma studies.

Dr. Michael T. Kleinman

Comments on the Executive Summary

The summary adequately presents the purpose of the ISA, the scope and methods that were used. The summary of source and exposure-related information is given in great detail and could possibly be shortened by limiting the discussion to what is different from what was reported in the 2008 ISA. On the other hand, the discussion of the basis for strengthening the causal determination for the evaluated health effect categories does relate to new information and perhaps could be expanded since this is the going to greatly influence discussion of any proposed changes to the current NAAQS.

Comments on Chapter 3: Dosimetry and Modes of Action

a. The discussion of the unlikelihood of NO2 penetrating through lung lining fluid does not address the heterogeneous nature of the chemical composition and thickness of the lining fluid as a function of location in the respiratory tract. The lining fluid in conducting airways is thicker and of different composition from that in alveolar spaces. The lining fluid in the alveolar region is thinner (on the order of 0.2 μm)[1], is rich in surfactants and plays a role in the innate defenses of the lung. The models estimate that NO2 can penetrate 0.6 μm so NO2 might be able to penetrate to cell surfaces. The information in Table 3-1 might be expanded to separately discuss the chemistry of airway and alveolar lining fluids in the context of what fraction of inhaled NO2 penetrates to those regions.
b. To the extent that NO2 dosimetry models predict penetration of NO2 to the alveolar region given the relatively small volume of alveolar lining fluid there might some utility to examining potential cross species effects on innate immunity functions mediated by the constituents of alveolar lining fluid.
c. The discussion of endogenous NO and NO2 should mention the possibility that endogenous production may be great enough in small selected spatial regions of the respiratory tract that the local anti-oxidant capacity is exhausted and thus exogenous oxidant insults could overbalance the system and increase the likelihood of an adverse effect.
d. There are some specific issues that could be mentioned with regard to populations such as individuals with acute respiratory distress syndrome that could be more sensitive to NO2 reactions with lung lining surfactants.

1. Ng AW, Bidani A, Heming TA: **Innate host defense of the lung: effects of lung-lining fluid pH**. *Lung* 2004, **182**(5):297-317.

Dr. Timothy V. Larson

1. The Executive Summary is intended to provide a concise synopsis of the key findings and conclusions of the ISA for a broad range of audiences. Please comment on the clarity with which the Executive Summary communicates the key information from the ISA. Please provide recommendation on information that should be added or information that should be left for discussion in the subsequent chapters of the ISA.

- The summary contains a lot of jargon, e.g. 'average daily 1-hour maximum', 'microscale' or that is potentially confusing to most readers.
- Need to better describe the relevance of panel studies to the standard setting process. This is more clearly laid out in the Integrated Summary. A clear statement is needed on how this information will be used to arrive at the key findings, including the issue of co-pollutant confounding.
- Table 1-1 implies that epi studies that adjust for confounding by other pollutants is the main reason for going from 'likely causal' to 'causal'. Although this is an important factor, it was not the only reason for this change. As such the wording in this important summary table needs to emphasize all lines of evidence, not just the epi studies. This is stated clearly in the conclusions section, but not in this summary table.

2a. Please comment on the usefulness and effectiveness of the summary presentation. Please provide recommendations on approaches that may improve the communication of key ISA findings to varied audiences and the synthesis of available information across subject areas.

- For the general air pollution community, a shorter (~5-7 page) summary would be useful perhaps organized around Table ES-1 or Table 1-1 with a brief rationale that focuses on what evidence was necessary to go from suggestive to causal (e.g. epi results robust to confounders, epi results consistent across cities and across different NO_2 exposure metrics, human clinical results consistent with epi outcomes, and animal tox mechanisms consistent with both human clinical and epi metrics.).

2b. What are the Panel's thoughts on the application of the Health and Environmental Research Online (HERO) system to support a more transparent assessment process?

It is very useful.

2c. To what extent does Chapter 1 communicate the key scientific information on sources, atmospheric chemistry, ambient concentrations, exposure, and health effects of oxides of nitrogen as well as at-risk lifestages and populations? What information should be added or is more appropriate to leave for discussion in the subsequent detailed chapters?

Chapter 1 provides an excellent summary of the ISA. Section 1.5 should be kept here in its entirety. The wording in Table 1-1 needs to be revised to balance the importance of human clinical, epidemiology, and panel studies of total personal exposure.

2d. What are the Panel's thoughts on the rationale presented for forming causal determinations for NO_2 *exposure only and considering epidemiologic results for associations between NOX and health effects in causal determinations for* NO_2 *(Sections 1.4.1 and 1.4.3)?*

The biological rationale supporting the idea that NO per se is not the toxic agent is reasonable. However, there is also an air quality rationale for not using NOx as a surrogate for NO_2, namely the variation in the NO_2/NOx ratio as a function of distance from major roadways. This also needs to be emphasized.

2e Section 1.5 discusses available information that is not necessarily included in the health effect chapters on potential confounding by copollutants and other factors as well as the potential for NO_2 *to serve primarily as an indicator of traffic-related pollutants and traffic proximity. This discussion is in Chapter 1 because it integrates information across Chapters 2, 4, and 5. Please comment on the extent to which this discussion is informative in describing how the evidence of independent effects of* NO_2 *is evaluated in this ISA. Does the discussion accurately reflect the available evidence? If this discussion is informative, what information could be added or removed to improve the discussion. Should the discussion remain in Chapter 1 or should it be moved to another part of the ISA?*

I think this section is very informative and a more complete discussion of these issues than is currently in the Executive Summary. The rationale for assessing confounding in the epi studies needs more emphasis.

The discussion about the differences in near-road gradients in NO_2 versus UFPs or BC needs to be given further thought given that the upwind values vary by pollutant (gradients are not normalized to on-road values prior to comparison) and that epidemiological studies have relied on monitors placed away from the road where these gradient differences are not very pronounced. The panel studies with personal monitoring do not appear to have strong copollutant confounding, an important point made here. These latter studies should also be pointed out in Table 1-1 as additional supportive causal evidence.

2f. Please comment on the extent to which the discussion of various policy-relevant considerations is clearly described and integrates relevant information (Section 1.6). Please identify any other relevant information that would be useful to include.

This is an excellent discussion. However, I am puzzled by the statement on page 1-52, lines 7-11, that refers to 'suggestive evidence'. This seems to downplay the human clinical studies relative to epidemiology and, to the extent that it implies that epidemiological evidence is most important, violate the rules of evidence set out at the beginning of the document.

General Comments on other sections of the document

plxx, line 27 Not just error in near road exposures, but error in estimated exposures at locations distant from measured values used to develop exposure surfaces.

p lxx, line 24 NOx is also an indicator of other correlated pollutants such as BC and UFP.

p lxxii, line 6 This conclusion of an independent effect is not necessarily true for all traffic related pollutants

p lxxv, line 25 not as clear for BC and UFP as for CO. These are coming primarily from different classes of mobile sources, the former from heavy duty vehicles and the latter from all vehicles under heavy load.

p lxxvii, line19 This conclusion contradicts earlier statements about the absence of cofounding by copollutants

p lxxx, line 33 Earlier in this section the relevant distance was cited as 15 m. Maybe include some earlier statement about the magnitude of concentration elevation within 500 m to support this conclusion.

p 2-41, line 13 Is this the source of the 15m statement in the executive summary?

p2-43, line 25 See also Wang et al Atm Environ. 45 (2011) 43-52.

p 2-46, line 2 see also Jensen et al Atm Environ 2009, 53(1), 23-39.

p2-47, line 12 see also Wania et al J. Env. Management 94 (2012) 91-101; Salmond et al STOTEN 443 (2013) 287-298.

p2-59, line 15 also might want to refer to models that include building wake effects such as OSPM (www.au.dk/ospm) or Austal2000 (www.austal2000.de/en/home.html).

p2-61, line 31 See also Yuval et al Atm Env 79 261-270 2013 (non linear optimization model); Wilton et al STOTEN 408, 1120-1130, 2010 (hybrid dispersion, LUR model for NOx); Lindstrom et al (2013) Environmental and Ecological Statistics doi:10:1007/s10651-013-0261-4 (NOx spatio-temporal model with disperson-based covariate)

p2-82 fig 2-2- needs distance labels

p2-83, line 11 This is true for classical errors like exposures to indoor NO_2 that are not accounted for in traditional air pollution epi studies with outdoor exposure surrogates. It is not necessarily true for exposure misspecification if the predictor variables vary in quality between locations.

p2-70, line 12 All studies in Table 2-4 are for at least a 24 hour average value. Any data on correlations of one hour averages?

p4-188 Fig4-11 results shown for vonKlot et al for beta-agonist is not obviously consistent with those reported in the original paper

Dr. Jeremy Sarnat

General Comment

Generally, I believe that the draft ISA presents a comprehensive collection of the science regarding NO_2. The interpretation of this body of work is largely coherent and I support many of the recommended changes that may affect future policy decisions aimed at regulating this pollutant. My main comments on the draft ISA center primarily on the weight given to results from two-pollutant epidemiologic models (co-pollutant models) in decisions related to causal determination status. Although my comments may be broadly applicable to determination decisions across the ranges of exposures and effects, I believe the implications are most pronounced for the science and uncertainties related to short-term NO_2 exposures and respiratory effects, which are the focus of my observations below.

Chapter 4

The evidence from the 2008 NO_2 ISA and findings published since, continue to implicate NO_2 as a likely *independent* causal factor of acute adverse respiratory response. However, I find the justification to change the status to 'causal' based largely on the use and application of epidemiologic results from co-pollutant models to be unjustified, with results that do not 'rule out…confounding, and other biases' as stipulated in the causal framework guidelines. Specifically, I don't believe the co-pollutant results presented in this draft ISA sufficiently preclude the possibility that either: a) NO_2 is serving as a surrogate of traffic pollution mixtures or traffic components more causally associated with short-term respiratory response; or that b) NO_2 may play some role in independently eliciting short-term respiratory response within a complex mixture, but that this effect is minor relative to the effect attributable to its other correlated co-pollutants.

There are several related aspects to the discussion of confounding, correct model specification and co-pollutant modeling.

a) *Confounding of* NO_2 *by other 'criteria' pollutants*. The 2008 ISA results, as well as more recent findings, provide strong evidence that the NO_2-related health risk estimates are unlikely confounded by other, ubiquitous urban air pollutants (e.g., O3, SO2, PM, CO). The population-based epidemiologic modeling examining short-term respiratory and, especially the mortality results are numerous and convincing. Despite this, very few co-pollutant analyses have examined confounding from other traffic-related pollutants, including VOCs, particulate organic, and transition metal species. The results presented in Chp 4 examining short term NO_2 exposures and corresponding changes in lung function, serve as an example. Of the 53 short-term NO_2 and acute respiratory studies cited in Table 4-7, including numerous panel and small cohort designs with excellent exposure and health characterizations, only 9 studies (17%) specifically measured non-criteria pollutant components we typically associate with traffic emissions (i.e., UFP, BC/EC, BTEX, particulate organic species). Of these, only a couple included comprehensive chemical speciation of the exposure measurements. With the exception of a very small number of these findings (Delfino et al., 2008, for example), it was not clear whether NO_2 was independent driver of lung function response. While these outcomes deal with lung function exclusively, similar trends exist for other acute endpoints, including AHR and pulmonary

inflammation. The relative dearth of NO_2 and traffic related co-pollutant results is also noted in several sections of the ISA (Page 1-14, for example).

Finally, I feel the results from the few measurement studies including specific traffic trace components (Brook et al., 2007, for example), highlight the potential that strong collinearity exists between NO_2 and other traffic species. Since, we hypothesize that these traffic species elicit respiratory responses (as well as responses in other organ systems) via similar biological pathways as NO_2, this further raises the concern that they may serve as confounders.

b) *Model specification*. Specification for most of the co-pollutant models examining acute respiratory outcomes primarily focuses on the issue of confounding solely (i.e., what is the effect estimate of NO_2, while controlling for another pollutant), rather than the potential for joint effects or effect modification. These latter scenarios appear to me to be equally plausible in characterizing NO_2 short-term health respiratory effects, and that NO_2 along with a complex suite of particles and gases, may elicit response via inflammation-mediated pathways. A key area of uncertainty is whether epidemiologic models more properly designed to assess the effects of pollutant **mixtures**, either in a more properly specified joint effects or effect modification setting, that may include interaction terms among the pollutants, are more efficient and provide better fits to the C-R relationship than model with two, independent pollutant terms. Currently, there are a very limited number of studies who have attempted to model NO_2 a part of a mixture. In revisions to the final ISA draft, I would recommend a greater discussion of alternative approaches for characterizing NO_2 within a mixture (i.e., Bayesian modeling as done with the mortality results or various factor analytical and source apportionment approaches). Of particular interest are the APHENA findings (Katsouyanni et al., 2003), where greater PM risks were observed in cities with high NO_2 concentrations, and whether similar patterns exist for short-term NO_2 and acute respiratory response.

A related source of uncertainty regarding specification of the co-pollutant models is the potential non-linearity of associations between NO_2 and its co-pollutants. The use of linear expressions, within a co-pollutant setting, to control for confounding of non-linearly correlated co-pollutants could lead to imprecision and/or bias; an appearance of effects associated with NO_2, where they do not exist. Modeling NO_2 with higher order pollutant terms could be a more appropriate means of addressing confounding in these circumstances. NO_2 formation and NOx chemistry differs between low and high O3 regimes (as noted on Page 2-7). It makes sense, therefore, that epidemiologic models with both terms may also want to consider non-linear terms when formally assessing confounding.

c) *Limits of assessing confounding through co-pollutant models*. There is acknowledgement in various parts of the ISA that co-pollutant models may have limitations in assessing potential confounding (Page 4-2, for example), and there is some very limited discussion of unspecified or residual confounding. I believe this discussion deserves greater attention. What specifically are the implications for the observed epidemiologic results from improper modeling of confounding? Is bias likely to occur, or a lack of precision? Which pollutants may be more susceptible to

potential bias and errors resulting from this modeling approach? A number of investigators have approached this from a biostatistical modeling framework (e.g., L. Sheppard and her group, for example) and could offer insight into framing this source of uncertainty. At the very least, greater attention to the shortcomings of co-pollutant models would enhance transparency.

Taken together, I cannot support the following statement from Section 1xxv of the Preamble, as well as similar statements throughout the draft ISA: '*In the current ISA, the causal determination is strengthened from likely to be a causal relationship to causal relationship because the recent epidemiologic evidence reduces the previously identified uncertainty regarding confounding by other traffic-related pollutants.*'

Correlations between NO_2 *and other pollutants*. There is a useful discussion about the potential for confounding from correlated co-pollutants in the NO_2 exposure assessment sections of the ISA (Pages 2-69 through 2-83). Along with the epidemiologic results and the controlled exposures and toxicology, these exposure and measurement findings can inform the question of whether NO_2 is a potential confounder or indicator of specific sources. Despite this, there is limited integration of these results as they relate to potential confounding, as addressed throughout Chapters 4 and 5.

Section 2.6.4.1 (Page 2-70) is vague about the role of averaging time on observed strengths of association between NO_2 and its co-pollutants. The results generally describe correlations over 24h integrated periods, with some daily 1h max correlations as well. Are there any studies who have examined more temporally resolved associations? I suspect that we will see stronger correlations between NO_2 and especially the traffic components. If acute health effects are also occurring on these scales, then these associations will be useful to study.

Dr. Richard Schlesinger

Comments on Chapter 3

1. Section 3.2.1. This is more of a summary rather than an introduction to the scope of the Chapter.

2. p 3-6, lines 14-15. What is the reference for the statement about basal nitrite levels remaining unchanged?

3. p 3-10, line 31. Sentence should read "…and other factors."

4. p. 3-14, lines 4-17. This paragraph is redundant of material previously discussed

5. p 3-17, lines 3-4. What is the source for the comment about sensitivity to endogenously produced oxidants?

6. p 3-17, lines 21-26. This is aimed at indicating why endogenous NO_2 levels will not be affected by inhaled NO_2. However, while endogenous NO_2 may not be systemically distributed per the discussion, there could potentially be an increase in reaction products in the tissues due to changes in levels of endogenous NO_2.

7. p 3-18, lines 16-25. This part of the paragraph should be in Section 3.2.3. On page 3-17, it is noted that NO_2 reacts with some antioxidants resulting in production of nitrite, yet there is no indication of whether this would affect toxicity of inhaled NO_2. However, on p 3-18, it seems to be inferred that there may be toxicity of nitrite from NO or NO_2. In addition, the last sentences which indicate uncertainty about the relative contribution of endogenous NO_2 with low level inhalation exposure seem to contradict the comment noted in # 5 above that endogenous oxidants will likely not affect toxicity of inhaled oxidants.

8. p 3-17, lines 7-9. There are more recent references for the role of nitrite on muscle

9. p 3-18, lines 1-19. It is not clear why effects of such high levels are discussed.

10. p 3-29, lines 5-16. It is not clear why the discussion of gas partial pressures are in the section on neural reflexes.

11. p 3-13, lines 9-10. Where have these cells been demonstrated?

12. p 3-19, Endogenous NO_2. The discussion seems to be about NO rather than NO_2.

13. p 3-41. Section 3.3.2.6.3. This section should be part of the prior section, 3.3.2.6.2 and not a separate section.
14. p 3-43, line 14. Is it correct to say that the NO_2 exposure enhanced "..preexisting emphysema in animal models" or would it be better to say "preexisting emphysema-like conditions…."?

15. p 3-46, line 23-25. Here again it seems to contradict statements about the relative roles of endogenous and exogenous NO_2.

16. p 3-54, line 28-29. Sentence should read, "….may lead to development and exacerbation of…."

17. p 3-57. Summary. The last sentence noted that inhaled NO_2 may contribute to the endogenous body burden of NO_2 species, yet in many places earlier it is stated or inferred that this does not occur. There needs to be some consistency about this issue.

Comments on Chapter 4

1. p.4-21, line 18-20. The surface dose is likely related to airway caliber.

2. p. 4-65. After line 26 there needs to be a better statement of conclusion related to lung function that integrates all of the findings in the disciplines rather than just summarizing various points.

3. p. 4-108. As above, there needs to be a statement of conclusion related to this section.

4. P. 4-183, line 22-25. There seems to be somewhat of a disconnect between this statement and prior statements in Section 4.2.9. For example, here it indicates that there are associations between NO_2 and hospital admissions for all respiratory causes, but on page 4-181 line 13-14 it is noted that evidence suggests a causal relationship between NO_2 and respiratory effects primarily evidenced only by asthma morbidity. Then, on page 4-185 lines 27-38, again the main evidence is noted as referring to asthma exacerbation. Thus, it is not clear whether causality is being proposed for just asthma or for all respiratory causes.

5. p.4-194, line 34-38. It is not clear why focusing on ventricular arrhythmias has resulted in inconsistent evidence.

6. p. 4-242, line 10-13. The first paragraph on page 4-241 indicates that there was little evidence for CV effects based upon studies in the 2008 ISA. However, here it states that epi data continues to support an association between NO_2 and CV effects. Continues from what?

7. p. 4-249, line 16-19. Here it is noted that inconsistencies across studies and limited evidence does not support effects observed in hospital admissions and CV mortality. However, on p. 4-247 line 30 it is noted that epi studies consistently demonstrate NO_2 associated hospital visits for CV effects and mortality. The two statements seem contradictory.

8. p. 4-282 line 12-18. There seems to be a contradiction here. In the first sentence, it is noted that the NO_2 mortality association is robust in copollutant models, but this is followed by the statement that it is hard to disentangle independent effects of NO_2 from those of other measured or unmeasured pollutants, adding to uncertainty. So, what exactly is robust and what is not.

Dr. Elizabeth A. (Lianne) Sheppard

These comments address some overall impressions of the document as well as my detailed review of Section 2.6, Chapter 5 and parts of Chapter 4 (specifically the meta-analysis).

Organization and clarity

Overall the organization of the document is very good and much better than the 2008 NOx documents.

Some key elements that I have appreciated are:

- Inclusion of the Preamble to clearly put the objectives of the ISA and the review process into context.
- Division of summaries into the executive summary, longer chapter 1 and results-specific summaries is helpful (though a bit repetitive for anyone reading multiple summaries in one sitting – I think this is unavoidable and the inclusion of multiple types and levels of summary is needed). With both the Executive Summary and overview Chapter 1 readers get a good overall perspective of the evidence and conclusions.
- Table 1-1 is a good overview of results for inference
- Integration of evidence from animal and human studies as a function of endpoint.
- Good discussions of the evidence in the context of the causal conclusions that are drawn.
- Well-designed tables that focus on the information needed for causal conclusions.
- Great cross-referencing of the document facilitating navigation.
- Excellent and easily accessible supporting information by integrating the HERO database

Exposure modeling and exposure measurement error

One of my major suggestions is that better/different attention be paid to exposure modeling and the concept of exposure measurement error, particularly in the context of epidemiological studies of long-term exposures where the focus is on spatial exposure variation. I believe that scientific understanding of the role of exposure in epidemiological inference to be at the cusp of reaching a deeper level of insight and I suggest that recognition of the potential of the emerging insights be incorporated into this document. I think such a discussion is even more important for NOx than for PM because NOx is a much more spatially heterogeneous pollutant and thus has more potential for epidemiological study findings to be impacted by the details of the exposure modeling and monitoring network. In the list that follows I give some specific suggestions based on my reading of Chapter 5. Many should be incorporated into the wholesale revision of section 2.6 that is needed, particularly Section 2.6.5.

1. I suggest incorporating better summarization of the exposures used in the long-term epidemiological studies into the document. Results tables in Chapter 5 should incorporate more than just the type of exposure model used.
2. There should be some perspective included on the epidemiological inferences that can be drawn from the diverse set of exposure modeling strategies used in the cited papers (from e.g. nearest monitor, land use regression, dispersion modeling). There aren't yet any definitive statements that can be pulled from the existing literature, but I think the discussion can be broadened to reflect the dynamics of the exposures used in many studies and the aspects of them that may affect inference.

Here are some: type of exposure model (most notably contrasting those that rely on measurements vs. physical and/or chemical models alone), spatial extent of the study and monitoring network, source of the monitoring data (e.g. regulatory network only or study-specific measurements), simplifying assumptions inherent in the work (e.g. are 2-4 weeks of data assumed to represent an annual average?), approach to smoothing/modeling over space (focusing on whether the model is "up" to capturing the sources of spatial heterogeneity in the pollutant), alignment of the monitoring and subject locations, size of the monitoring network (i.e. number and density of monitors used to develop the exposure model) and monitor siting criteria (e.g. are specific locations systematically omitted due to regulations?, how well does the monitoring network represent the study subjects?).

3. There should be some direct statements about the importance of the relatively high spatial variability of NOx in the evaluation of exposure assessment for epidemiological study inference. Unlike PM, which is spatially a much more homogeneous pollutant, the approach to exposure modeling of NOx and the set of monitors used in a given study, with respect to their numbers and locations, could have a major impact on the inferences drawn. Some of these ideas are included in Chapter 2; we should consider whether the points can be made more clearly.

4. I suggest some discussion could be added about specific judgments about specific exposure models that are then applied to inference about NO_2/NOx effects, most likely emphasizing the studies used to judge causality. I suggest that it would be appropriate to give higher weight to studies that do a better job taking into account the street network in the inference (note that in some applications there may be technical reasons why obvious choices, such as LUR models, aren't always better; see Szpiro et al 2011 Epidemiology) and less weight to those that will miss it completely. This may be particularly important for NO_2/NOx (vs. e.g. PM). Here are some suggestions:
    a. Models that rely only on the existing regulatory network (at least prior to the near-road monitoring network) may not adequately capture the increased exposure near roads due to too few monitors in the network that are sited near roads.
    b. Nearest monitor exposures (e.g. Miller et al 2007) may not reflect NOx exposures for many individuals (again depending on how the monitors are sited), thus potentially strongly affecting the ability of such studies to detect health effects if they indeed exist. It could be interesting to contrast the relative merits of nearest monitor exposure estimates for spatially heterogeneous NOx vs. the much more spatially smooth PM2.5.
    c. IDW exposure estimates (e.g. Lipsett 2011) may smooth over road networks too much, unless there is an extremely spatially dense monitoring network used. Again the ability to detect NOx effects may be extremely poor in such a situation.
    d. Dispersion models may only capture some sources of NOx. There could also be important systematic errors in dispersion models due to how key assumptions are made and implemented. This would increase the uncertainty of the findings from studies that rely on dispersion models as the estimates could be better or worse than one might anticipate if the true exposures were known. Because some of the errors are likely to be systematic with dispersion models, it may be more difficult to characterize the direction of the impact on health effect estimates.

5. I suggest that the document be revised to expand and update the measurement error perspective for inference about health effects. The discussion in Chapter 2 is not complete or up to date. Among the changes that are needed, the revision should include a review of a recently published discussion paper (Szpiro & Paciorek, 2013 Environmetrics with discussion by Spiegelman, Thomas, Hodges,

Peng). That paper focuses on cohort studies where the key source of exposure variation is spatial; this perspective needs to clearly be stated as part of the discussion. Of particular importance are the following concepts:

a. Exposure predictions have measurement error that can be decomposed into Berkson-like and classical-like components. The Berkson-like component comes from the prediction not capturing all the variation of the true exposure. The classical-like component comes from the uncertainty in the estimates in the exposure model. Neither component is true Berkson or classical (thus the "-like" terminiology) because the information used to derive the predictions is shared across all subjects. (There is mention of Berkson- and classical-like errors in Chapter 2, but I did not see these terms defined in the document. My review of the concepts here is intended to make sure the understanding of these concepts comes across clearly.)
b. The monitor and subject locations should be compatible, i.e. come from the same underlying location distribution.
c. Spatially structured adjustment variables in the health model should be included in the exposure model.

6. In Chapter 2 I think the target exposure for inference should be defined in the context of the exposure measurement error discussion. Is it and should it always be total personal exposure? Or should it be personal exposure to ambient-source pollutants? When is it appropriate to consider ambient concentration as the target exposure for inference? In measurement error research, there are a whole host of issues in understanding the role of measurement error when the target exposure is ambient concentration. It will be important to consider those, and to address them distinctly from the issues that arise when the target exposure for inference is total personal exposure.

In Chapter 5 there seems to be an artificial distinction in the document between "measured" NO_2 and modeled NO_2. I would dispute that an estimate of NO_2 based on IDW or nearest monitor is any more "measured" than an estimate based on LUR.

Exposure assessment and measurement error comments based on Chapter 2 review

Overall I think considerable reworking of the exposure assessment and measurement error section (2.6, particularly 2.6.5) is needed. Some overview of exposure assessment can be included for its own inherent value but this should be reduced/rebalanced. Notably, much of the discussion of exposure assessment should be done in the context of epidemiological study inference. My suggestions for key aspects of the revamped discussion include:

- Directly consider study design: exposure questions are fundamentally different for panel studies, time series studies, and cohort studies (both cross-sectional and longitudinal)
- Address whether total or ambient personal exposure is (and whether it should always be) the relevant exposure of scientific interest. For many studies ambient concentration is used as the exposure metric and there should be some consideration of its direct performance from a measurement error perspective (even if one could argue it is not the relevant exposure of scientific interest).
- Distinguish the measurement error discussion to separately focus on the target parameter of interest from an epi study (they are different when one uses personal exposure or ambient concentration) and properties of measurement error due to how exposure is measured and/or

modeled. Discussion of bias in the current document conflates the two features and leads to confusion.

- Make sure simplifying assumptions are clearly stated as they can become extremely important in the evaluation of work. One example is simplification of the total personal exposure model into a partitioning of ambient and non-ambient sources without distinction to where these occur. (I.e. the document moves from the richest framing of total personal and non-ambient source exposure (eq 2-1 and 2-7) to some strong simplifying assumptions (eq 2-8 and 2-9)) We need to be careful to not be misled by such simplifications. For NOx, near and on-road exposures may dominate, so work that ignores these sources could reach misleading conclusions. But if the simplified exposure model is treated as "correct", then this challenge could be missed.
- Make sure the discussions of properties of measurement error clearly separate developments in the context of time series designs (where temporal variation in pollution is paramount and aggregation has some important impacts) and cohort study designs (where spatial variation is crucial and prediction models are used to obtain exposure estimates for individuals).
- Make sure the discussion of the various modeling approaches is balanced with respect to understanding the target for epidemiology: estimation of the health effect parameter. Also make sure there is insightful use of results. For instance, in reporting R2 from LUR models, it is important to understand whether these are out of sample assessments and if so, whether or not they are optimized for the data (i.e. evaluated around the best fit line) or not (i.e. evaluated around the 1:1 line).
- I don't think the conclusion that health effect estimates tend to be biased towards the null is always correct or sufficiently nuanced. It also ignores the uncertainties in the estimates, i.e., estimation of their standard errors; these are critical for inference.
- Make sure the temporal and spatial scales of the data are always understood.

Additional specific comments: I have many comments in the text I have appended below. A few of them are summarized here.

- Dispersion models section: It would be good to include some discussion of what aspects of the space-time NOx field dispersion models miss and what they might get wrong (e.g. over-/under-estimate).
- P 226: The personal-ambient relationships section focuses on time series studies; this should be clear up front. I suggest subdividing the section into time series and cohort studies (and possibly also panel studies)
- The use of "statistical significance" needs work. E.g. see some examples on p. 226

Short-term controlled exposure meta-analysis

See response to charge question 5.f. below.

Additional comments about health studies, effect estimates, and causality

I am struggling with how one determines a "high quality" study and how one weights the myriad features that could influence study findings. In addition to the exposure measurement error issues I have discussed at length, I note a few examples:

- Crouse et al 2007 is a hospital-based case-control study focusing on breast cancer. Controls were any of 32 cancers that led to hospitalization, with exclusion of certain cancers thought to be occupationally related (liver and intrahepatic bile duct, pancreas, lung, bronchus and trachea, brain and central nervous system, leukemias, and lymphomas). The approach to control selection as well as other factors could be impacting the effect estimate in this study.
- Gruzieva et al 2013 is a longitudinal cohort study in Stockholm. Most of the findings are consistent with a wide range of effects on asthma and wheeze, from protective to harmful. I am concerned that there could be a number of reasons why the findings could be less than robust: there was decreasing participation over time and analysis was based on GEE (meaning the analysis makes an implicit assumption that the data are missing completely at random; this is often not true when there is dropout as occurred in this study); exposure is predicted from dispersion models with time-varying emissions inventory input datasets; there is a stong age-related trend in the NOx distribution in the study; and the main findings, while limited, relate to exposure in the first year of life. Many of the above features could be impacting the health effect estimates and their uncertainties in this study. (There are some related issues with Gehring et al 2010)

In reviewing the causality determination for long-term exposure and respiratory outcomes, I am concerned that the effort to be comprehensive is leading to effective over-interpretation of the literature or under-appreciation of the factors that will contribute to null study findings even if there is a true effect. For instance, both Gruzieva et al 2013 and Gehring et al 2010 are included in Table 5-9 as supporting the consistent evidence of increases in asthma incidence, but I would not characterize the full set of findings in those studies as consistent evidence for an asthma incidence effect. Both provide some evidence, and Gehring more than Gruzieva, but it is not as strong as the table reference implies.

In conclusion, I suggest clear definitions of "high quality" be added and that some studies be given less weight based upon how informative they are likely to be towards determining the causal relationship between NOx and health effects. Reasons to downweight studies should include exposure assessment in cohort studies when it does not adequately capture fine scale variation, and features of the study design or analysis that may affect the validity of the inference.

General comments

I wonder if the HERO database could be leveraged to create and store study-specific summaries that are longer than what one can include in the text or tables. These summaries could address a whole host of study-specific issues that may be better tuned to a particular study. Mostly these would be aligned with papers, but occasionally several papers from the same study could be combined. This may provide an opportunity to include additional judgments that are fundamentally important but not formulaic.

I think industry-funded studies should be flagged. In the future, this feature should be incorporated into the assessment of the weight of evidence. There has been a major move in the area of responsible conduct of research to recognize financial conflicts of interest and acknowledge the role of funding source in publications.

As an organizational suggestion, since many folks are working from a pdf file now, could the page numbers that appear in Adobe Reader also be printed on each page? This will help with cross-referencing during discussions and in using the comments.

Responses to charge questions

2.e. Please comment on the accuracy, level of detail, and completeness of the discussion regarding exposure assessment and the influence of exposure error on effect estimates in epidemiologic studies of the health effects of NO_2.

See my extended comments above for details. The discussion is currently incomplete and isn't always properly framed. It needs to be completely reframed and reworked.

Chapters 4 & 5

5.a. To what extent do the discussions in this chapter accurately reflect the body of evidence from epidemiologic, controlled human exposure and toxicological studies?
It is important to get a complete sense of the literature but at the same time to not put too much weight on studies that don't need it. The most weight should be put on the highest quality studies. These should be identified where possible and appropriate. Studies that may be misleading for one reason or another (e.g. due to analysis approach, exposure metric used or data that goes into the exposure assessment, confounding control, funding source) should be discounted in summarizing the body of evidence.

Summarizing the whole set of studies in a table tends to give them equal weight implicitly. Is this always appropriate?

It will be important for the discussions of the long-term exposure epi studies to fully capture whether they properly capture fine-scale variability of NOx.

5.b. Please comment on the balance of discussion of evidence from previous and recent studies in informing the causal determinations.

See Dr. Sarnat's comments

5.c. Please comment on the adequacy of the discussion of the strengths and limitations of the evidence in the text and tables within Chapters 4 & 5 and in the evaluation of the evidence in the causal determinations.

There is a concerted effort to be thorough and thoughtfully address the strengths and limitations of the evidence. Tabular compilations are helpful. One concern that I have, but that is difficult to address, is that there are a number of aspects of epidemiological studies that suggest that their evidence could be misleading – yielding effect estimates either stronger or weaker than one would expect. To the degree possible, this should be incorporated into the discussion of strengths and limitations. If more back-up documentation is needed, perhaps HERO could be leveraged.

5. d. What are the views of the panel on the integration of epidemiologic, controlled human exposure, and toxicological evidence, in particular, on the balance of emphasis placed on each source of evidence? Please comment on the adequacy with which issues related to exposure assessment and mode of action are integrated in the health effects discussion. Please provide recommendations on information in other chapters of the ISA that would be useful to integrate with the health effects discussions in these chapters.

I like the integration of all different types of studies in a single chapter. The challenge is that the material becomes unwieldy and difficult to digest. This is a challenge for the review and new approaches to how to give review assignments may be one solution to this problem.

See my comments above for the need to bring in better perspective about exposure assessment and its impact on epidemiological inference. The ability to capture fine scale spatial variability in long-term exposure epi studies is fundamental to their utility for inference about NO_2.

5. e. Please comment on the appropriateness of using experimental and epidemiologic evidence for morbidity effects to inform the biological plausibility of total mortality associated with short-term (Section 4.4) NO_2 *exposure and in turn, to inform causal determinations*.

Yes…

5. f. Section 4.2.2 discusses the effect of short-term NO_2 *exposure on airways responsiveness. This section focuses primarily on an EPA meta-analysis developed for this ISA of airway responsiveness data for individuals with asthma and secondarily on the potential of various factors to affect airways hyper-responsiveness independently or in conjunction with* NO_2 *exposure in controlled human exposure studies. This material presently is unpublished and we ask the Panel to provide the peer review for the analysis, in particular, to comment on the appropriateness of the methodology utilized for the meta-analysis, the conclusions reached based this analysis, and its use in the draft ISA. With regard to factors potentially affecting airways responsiveness, please comment on the adequacy of this discussion. Are there other modifying factors that should be considered?*

The data and results are summarized in Tables 4-1 to 4-5. It was not clear to me from reviewing Tables 4-1 and 4-2 which studies or parts of them are included in the analyses in Tables 4-3 to 4-5. Based on the meeting discussion this information is documented in the tables, so it may be just incorporating a few clarifications in the text to make it easier for readers to pick up the information quickly.

The use of the sign test is OK, but it has low power. However, while this is a meta-analysis there is no consideration of between-study heterogeneity. Some consideration of whether (or not) it should be done should be included in the document. Accounting for study heterogeneity would give different relative weighting to the information from each subject.

The amount of AHR and the importance of the sign as an indication of an effect needs to be clearly documented.

I agree this analysis should be included in the document and I don't see any strong reason to question it. A clear statement of the scientific objective(s) of the analysis should be included. More information (as in the form of a paper that could ultimately be published and in the meantime included as an appendix) would be helpful for allowing CASAC to do a more thorough peer review.

5. g. The 2008 ISA for Oxides of Nitrogen stated that one of the largest uncertainties was the potential for health effects observed in association with NO_2 *exposure to be confounded by correlated*

copollutants. To what extent has evidence that informs independent effects of NO_2 *been adequately discussed in Chapters 4 and 5 and appropriately interpreted as reducing uncertainty (for example, evaluation of copollutant model results)? Has the current draft ISA appropriately considered recent epidemiologic findings regarding potential copollutant confounding in causal determinations? Please provide comments specifically for respiratory effects, cardiovascular effects, and total mortality of short-term* NO_2 *exposure*.

There is still considerable challenge in sorting out co-pollutant effects in epi studies. How can we separate NO_2 exposure alone from traffic? Many epi studies use NO_2 as a marker for traffic-related pollution.

5. h. To what extent is the causal framework transparently applied to evidence for each of the health effect categories evaluated to form causal determinations? How consistently was the causal framework applied across the health effect categories? Do the text and tables in the summaries and causal determinations clearly communicate how the evidence was considered to form causal determinations?
There is some unevenness across endpoints.

5. i. What are the views of the panel regarding the clarity and effectiveness of figures and tables in conveying information about the consistency of evidence for a given health endpoint? In particular, was the use of the tables and figures in both the text and online in the HERO database effective in providing additional information on the studies evaluated? Are there tables and figures in the ISA that would be more appropriate to include as a resource in the HERO database?

The tables and figures do an excellent job of condensing lots of information. This is very helpful. My only concern is that this summarization implicitly weights all the studies the same (particularly in the tables where the CI's aren't as easy to perceive) and I'm not sure this is always appropriate.

The HERO database access is an outstanding resource. It tremendously facilitated my ability to review specific points made in the document. (The bigger limitation is the amount of time needed to actually carry out such reviews. However, the barrier of accessing the original papers has been completely removed and this is an awesome step forward.)

Specific comments by document page (pdf page numbers used)

1. P. 74 (1xxiv) 26-7: The reason may be more related to design, feasibility, and data rather than cause.
2. P 80 3-5: Exposure measurement error doesn't always attenuate health effect estimates
3. P 91 3-6: This sentence reflects a mismatch of two different measurement-related concepts: that the target parameter of interest is different when exposure vs. ambient concentration is used, and that the uncertainty of the exposure quantity used in the model can have measurement error consequences. In general it would be worthwhile being extremely clear when talking about measurement error what the target exposure should be. Is it always personal exposure? Total or only to ambient source? When do we think the target exposure is acceptable to be ambient concentration? There are a whole host of measurement error issues even when focus is on ambient concentration at a person's representative location.

4. P 91 8-10: It is important to distinguish short-term studies that focus on temporal variation from long-term studies that focus on spatial variation.
5. P 91 35: Presumably this interference is a source of systematic error that may vary spatially? If so, this may have implications for epidemiology.
6. P 104 8 and 10: These ranges are the same. Is one an error?
7. P 106 l 14-18: The NO_2 means are quite different for NO_2 and NOx. Correct?
8. P 117 24-6: Is it worth mentioning this point in the summary?
9. P 118 13+: Mention time averaging in this summary
10. P 118 17-8: .25 is not higher. 18-9: Work on wording, since .41 is moderate, not poor or inverse.
11. P 118 23-4: Mention epidemiological study design as another reason confounding will vary.
12. P 123 30-1: Statement needs more support.
13. P 135 4-6: Not always. More important may be the impact on the CI. See my extended comments on exposure and exposure measurement error.
14. P 196: There is an implicit assumption in this conceptual model that personal exposure is the relevant exposure of scientific interest. I think this should be stated outright (probably in a different section) along with the reality that most epi studies use ambient concentration as a surrogate of exposure. When we talk about measurement error we need to identify whether we are focusing primarily on the role of ambient concentration as a surrogate for personal exposure, or the difficulty of accurately capturing an individual's ambient concentration. Both are important issues but they should be addressed differently from a scientific point of view. The section on the conceptual model could follow a section that talks about choice of the target exposure of interest, retitled to focus on the conceptual model for total personal exposure.
15. P 199 heading: I suggest retitling to insert "of Ambient Concentration"
16. P 199 14-16: This statement is fine alone, but not all exposure estimates are necessarily appropriate when the focus is on estimating a health effect parameter in an epidemiological study. More clarity on this point needs to be added.
17. P 201 new section: It would be good to include some discussion of what aspects of the space-time NOx field dispersion models capture vs. miss, and what they might get right vs. wrong (e.g. over-/under-estimate).
18. P 205 7-8: This is a good point. It is also very important to mention is that not all R2 estimates are the same. It depends on whether the evaluation is "in- sample" or "out of sample". For out-of-sample estimates it also depends on whether the R2 is evaluated around the 1:1 line or around the best fit line. R2 estimates that are centered on the best fit line won't pick up systematic bias. This can be an important feature when evaluating a model in a new area.
19. P 206 section: These stochastic population exposure models are not appropriate to use as predictors for inference about epidemiological health effects. They are very useful for risk assessment.
20. P 226 section 2.6.5: The study design is a very important feature here since for epidemiological study inference, the way one "gets the exposure estimate wrong" matters. This will strongly depend on the study design.
21. P 226 10-11: I don't think this conclusion is always correct or sufficiently nuanced. Revise.
22. P 226 16: It is fine to focus on time series studies here, but I think that should be clear up front. I would suggest subdividing this section into time series studies and cohort studies (and possibly also including cross-sectional, i.e. kinds of studies that rely on spatial exposure variation). It may also be appropriate to add panel studies as a separate consideration since they can capture both temporal and spatial variation and don't also aggregate like time series studies.
23. P 226 19: I agree with this statement but I think it also reflects one of the problems with discussing exposure measurement error in air pollution studies. There are two kinds of bias that can lead to

attenuation: 1) As in this sentence, using concentration instead of exposure so the alpha gets absorbed into the health effect parameter estimate. The issue here is that the target parameter of inference has changed when concentration is substituted for total personal or ambient source exposure. 2) Attenuation bias due to presence of classical measurement error. The two kinds of biases are often conflated but their implications are different.

24. P 226 20: This sentence, starting with "personal", marks the transition in this paragraph from talking about an aggregated population exposure to individual exposures. I suggest splitting these apart for greater clarity.
25. P 226 22-3: I don't understand this statement. Why would there be any "computation" in a total personal exposure measurement?
26. P 226 26-7: Why is statistical significance the determining feature for the literature being mixed? Studies can give reasonably consistent even when only some of them produce statistically significant findings. Line 27-30: This following statement suggests to me that there is much more than statistical significance going on.
27. 227 1-2: I don't know how meaningful this statement is without knowing the temporal scale of the data and also the defining characteristics of the study populations. That is too much detail for the goals here, but consider if there is a different perspective to be included in the discussion. Just the same, if a summary statistic (average, median) is to be mentioned, I think what one is summarizing should be indicated.
28. 227 8-9: While this statement is fine, it reminds me that the implications depend upon the epidemiological study design.
29. 227 14-6: This can be correct but it doesn't necessarily mean that the central site measurement doesn't provide some incredibly useful exposure information for inference about health impacts. I think this is particularly true for time series studies because of their enormous power and the advantages of aggregation in facilitating understanding the health impacts of a shared exposure.
30. 227 18-20: I agree with this statement. I suggest it be used to help us understand how to interpret epi studies of different designs, rather than to merely focus on downward bias of epi effect estimates. However, the “by nonambient sources” part of the sentence is confusing to me. Perhaps it is the wording? Does the mention of nonambient sources connect to the "not well detected by" or the "were influenced by"?
31. 235 3: Classical error gives you a noisy estimate of the true exposure, not bias in the exposure itself. (At least using the most basic definition of pure classical error.) It induces bias in the health effect parameter (often called beta) in an epi study, not the exposure itself. It is also important to note that classical error also gives incorrect standard errors of the beta parameter estimate; these can be too big or too small.
32. 235 5-10: I agree with this statement but the work was all done in the context of time series study designs. I don't believe similar work has been done for cohort studies so I don't think the statement can be made as broadly as it is written here.
33. 235 11-2: The use of "-like" here is a very important idea. These terms have not been defined yet in this document and they should be defined before they are used. They were introduced by Szpiro et al 2011 Biostatistics. The independence condition in the definitions of pure Berkson and classical errors is not required for the "-like" errors.
34. 235 15: Once again, this is discussed in the context of time series studies. This needs to be made clear since the results may not be the same for other study designs.
35. 235 21-3: These are broadly understood properties of Berkson and classical measurement error in the context of linear disease models. Also mention that the standard errors of the health effect estimates are typically incorrect in the classical error setting. Indeed, in both settings, the observed SE when

plugging in exposure with measurement error can be biased, leading to incorrect coverage of 95% confidence intervals.

36. 235 27-9: I need to know the time scale of the data in order to make sense out of this summary. This suggestion is particularly important for people who haven't read the paper.
37. 235 30: Why is the statistical significance so important? What does it tell us about factors that influence measurement error?
38. 235 33: 24-hour average?
39. 236 1: What does the statistical significance tell us?
40. 236 2-4: Again, make sure the statement is in the context of the questions being addressed. Here we need to understand at least the time scale of the data being considered. We also need to focus on the factors we need to know for the intended epidemiological inference.
41. 236 section and line 7: Are these all the same study design? It is important to distinguish the issues in time series studies from those in cohort studies. Also it appears that the target exposure in this section is no longer personal, but is now ambient concentration that reflects a subject's spatial location.
42. 236 8-9: So this implies that the monitors don't reflect the spatial characteristics of the people. But the population in a time series study is widely dispersed spatially. Was that taken into account?
43. 236 15: Insert “in a time series study design”
44. 236 15-6: I think that 95% CIs are much more informative than p-values.
45. 236 17-8: The reference RR in a simulation study is the true value which is known. (This is OK as is, but it shows that we can detect effects in the absence of measurement error, and it does not show anything about the ability to estimate the true value in the base case.)
46. 236 18-9: These results are trivially different. Drop?
47. 237 20: Szpiro et al focuses exclusively on cohort studies. The "true" exposure in that paper is ambient concentration. So the issues are about inference when ambient concentration are predicted. That paper doesn't also address personal exposure.
48. 237 21-3: This is garbled. The assessment of the prediction accuracy was for the exposure. The assessment of bias was for the health effect parameter beta. That evaluation also focused on the uncertainty of the beta estimate as quantified by root mean square error.
49. 237 27-9: This is an incomplete and somewhat misleading summarization. The scenario being described is when there was not very much variation in a predictor in the monitoring dataset (but not the subject data) for the third covariate in the exposure model. Poor estimation of the regression parameter for that covariate led to classical-like measurement error that affected the health effect inference. Also it is important to recognize that the R2 was pure out of sample assessment in the study population. (Such an out of sample assessment is straightforward in a simulation study, but often impossible in practice since subject exposures are unknown.)
50. 237 32: The paper was about predicting exposure for inference about health effects. The added value of the third covariate in the prediction model was small in the monitoring data, even though it was an important determinant of the true exposure. This paper pointed out the impact of including that covariate (which did belong in the model) on health effect inference.
51. 237 33-4: This is really garbled. The paper makes it clear that there are both Berkson-like and classical-like errors operating in this setting. In the scenario quoted the classical-like error is dominating. Classical-like error does not always lead to attenuation bias.
52. 237 35: The target study design has switched again? Also it is important to acknowledge what was assumed to be true in the simulations. Even if the CTM doesn't reflect population exposures in reality, the simulations would still show it to have added value because it is assumed to be truth here.
53. 238 10-2: Unclear to me. I’d need to read this paper carefully to understand what is intended here.

54. 239 Table: Exposure measurement error typically is quantified and addressed in terms of its impact on the health effect estimate, not on the exposure measurement itself.
55. 239 section: A discussion of CHAD is useful but I don't think it pertains to a discussion about exposure measurement error for epidemiological study inference. Simulated exposures should not be used in epidemiological studies.
56. 241 1: While I don't disagree with this statement, very few epi studies have time-activity data and very few use personal exposure as the exposure metric, so I'm not sure what the point is here.
57. 241 section: I'm not sure how much of this section should be kept. Regardless, whatever material is retained should be revised to focus on its importance w.r.t. exposure measurement error.
58. 241 7-8: This comment doesn't really pertain to this section: In the document we should address whether it is ambient NO_2 that is the focus or any NO_2. NO_2 is a molecule, so why does its source matter? Do we care more about ambient NO_2 because of what else comes with it? Or because of regulation?
59. 241 8: Insert "daily average" before "NO_2 data" or the correct time scale.
60. 242 8: How does one get an association with prediction error?
61. 242 20-1: Effect on what? I would probably agree with this but again it depends on what one is quantifying.
62. 242 21-3: How?
63. 242 33-4: Does this refer to the bias? What was the significance test?
64. 243 1: I suggest this is "a" model, not "the" model. It would be applicable for a cohort or cross-sectional study that is focusing on continuous outcomes. It is important to recognize that there are additional issues in understanding the role of measurement error in disease models that have nonlinear link functions (such as log or logit).
65. 243 equation after 6: How are the two equations for Y equal?
66. 243 14-6: I don't think the conceptualization of exposure using alpha was ever meant to capture all of these factors. I think it is misleading to think that from a scientific perspective the alpha parameter captures spatial variation (other than what amounts to spatial structure in time-activity and infiltration).
67. 243 17-9: I don't think that this statement is correct for cohort studies.
68. 243 19-20: How many locations are of interest in air pollution epidemiology where there are few NO_2 sources, e.g. that don't have trafficked roads crisscrossing them?
69. 243 20-3: I think clearer conclusions can be drawn.
70. 244 3-4: I don't understand this logic.
71. 244 5-6: Meaning that alpha is constant and between 0 and 1?
72. 244 7-8: a) Honestly we only measure concentration so how could we use a different exposure metric in time series studies? b) Work discussed above, such as Goldman, assesses whether or not it is OK to use measurements at a central site monitor in time series studies. Why not say that? I'm not sure the points have come across clearly.
73. 244 13: It would be useful to review Setton 2011 to find out what is happening with LUR vs. spatial smoothing and impact on inference about health. It must have been a panel study, correct?
74. 244 15: Wording. I think the epidemiologic model is of a health outcome and our interest is the effect of NO_2 on it.
75. P 244 17: Bias in what?
76. 244 18-21: The details of this work should be assessed carefully to understand why there was more bias from an exposure based on LUR rather than a "monitor-based approach for mapping" (what is that?) Putting these results together with those of Szpiro et al discussed above, one might be able to get some more revealing insight into what is happening in this study.

77. 244 28-31: I don't agree with this statement. These studies were panel studies and because of the aggregation in time series studies the impact of space could be fairly different in the two designs. Also be careful about what is measurement error and what is the impact of a different target parameter of interest.
78. 244 33-4: Is this a helpful perspective?
79. 244 36: Insert "air exchange rate, as previously" before "defined"
80. 245 8-10: Does this paper inform our understanding of exposure measurement error and its role in inference in epidemiological studies?
81. 245 conclusions: The measurement error conclusions need to be revamped after section 2.6 is revised.
82. 245 27-8: I think this statement with the follow-up sentence is a bit strong and also misleading to imply that e.g. a dispersion model estimates personal exposure.
83. 245 31-3: I think this statement is on track, but could be clearer. First it depends on what exposure is being estimated. Second, the errors will be related to features of the underlying NO_2 space-time field (where space includes how an individual moves through it), measurements that are used to develop the estimates (which is where instrumentation error comes in), and the models that link the two to produce exposure estimates.
84. 245 34: See my previous comments for suggestions of how to reframe this argument. Bias is not the only important feature of exposure measurement error. The effect on the SEs is in practice often much more important.
85. 246: Is the bias towards the null because of the difference in the target parameter when concentration is used or because of error in estimating concentration in a particular study?
86. 661 9-12: To the degree that contrasts are over time, the kinds of confounders that are important will be different than for studies that rely only on contrasts over space.
87. 664: 21-22: I suggest this result also supports the idea that no residual confounding is operating at either level and that exposure measurement error is not more problematic at one level than the other. (Where levels are between and within community)
88. 664 22-7: I don't understand the importance of this discussion. Of course the HR varies as a function of the increment used in the reporting. For comparing estimates I suggest using the same increment between and within communities.
89. 665 36-7: This suggests (to the degree that TRP is an adequate proxy for NO_2) that there is no contextual effect of NO_2 beyond that captured by TRP. This does not mean that the effect of NO_2 went away, but rather that it is all captured in the TRP exposure. To really make sense out of these findings it would be good to understand how correlated are the community-average TRP measures with the central site NO_2.
90. 665 4-6: Presumably this exposure is combining between and within community variation? Say so. It will be important to make sure that the within- community estimate is consistent with this. There can be between-community confounding that is difficult to control in these studies, so addressing whether it is likely there is important. (This is addressed below so is OK.)
91. 666 3: It appears that most of the exposure variation in this study is temporal. The ability to predict spatial variation from 13 sites is extremely limited.
92. 666 18-20: Since this immediately follows discussion of Islam, I suggest putting in the reference to Lee again here.
93. 667 9-11: I think a little more detail is needed in this discussion. Was this a survival analysis? How was the timing of incidence addressed in the analysis?

94. 696 25: How does this belong in the long-term exposure section? Does it even make sense to do a time series study using monthly exposure? This is completely in the timeframe where we expect confounding to be operating.
95. 736 Gruzieva paper: How much do we trust this estimate? It is based on emissions inventory data not measurements
96. 743 7-9: I don't recommend IDW interpolation for NO_2. It could miss all local sources, depending on how the monitors are sited.
97. 751 1: I suggest more skepticism/perspective w.r.t. exposure quantification should be added.
98. 751 23: wording
99. 754 8-10: The long-term/short-term exposure period discussion seems counter-productive here. Isn't the key point the duration w.r.t. the pregnancy?
100. 754 14-18: I would think it would be best to characterize all of the exposures w.r.t. pregnancy duration and timing of development.
101. 755 2: This is a picky point, but the goal is not finding associations but understanding the evidence. This statement implies that studies that lack statistical significance don't provide any evidence. Consider rephrasing to say something like the evidence from the limited number of studies available was consistent with no associations.
102. 755 9: Should seasonality be included in the list?
103. 748 26: Throughout gestation is helpful framing.
104. 761 12: I don't understand what "measured" means here. LUR also uses measured NO_2, just after predicting it from a model. IDW is just a different model -- it doesn't use "measured" NO_2 any more or less than LUR.
105. 761 14-5: Here is a place where understanding the monitoring design may help us understand these results. In general for NOx I would trust LUR results more than IDW, unless the monitoring network were quite rich and well placed.
106. 761 25-7: This description doesn't really give good perspective on what is happening here. Was this just a power problem? Were these results consistent with the ones where "associations were found", but just no longer statistically significant? Or did more than that change?
107. 776 table:
    a. If possible, it would be helpful to also include a measure of spread in this. Can we report the range across subjects?
    b. I'm glad to see the exposure assessment approach. Please add more details, e.g. # monitors and other aspects as can be reasonably summarized.
    c. It is notable that there are many different exposure models used. We don't know how much impact they have on inference but we should be aware that the results could be inducing both false positives and false negatives driven in part by the exposure modeling approach.
108. 777 Hansen exposure: So where is the contrast coming from if it is only one city? Time? Then what about seasonality and other secular trends? Were they appropriately adjusted for?
109. 781 Volk
    a. CALINE discussion: Unclear. CALINE should be able to predict at homes. Does this refer to the model considering all roads within 5 km?
    b. IDW discussion: When there are two exposure models described, how is the reader supposed to understand which one contributes to the reported results?
110. 781 Becerra:
    a. Same comment as above: how do we know which predictions apply to which estimates?
    b. Both models described here are monitor-based
111. 783 26-7: wording implies all are statistically significant

112. 788 3: Both of these studies rely on city-average monitor estimates.
113. 814 7-8: Meaning some are inverse or that they are not statistically significant?

Dr. Helen Suh

Charge Question 2

Chapter 2 provided a solid starting point for the discussion of exposures to nitrogen oxides, containing many of the key pieces needed to understand exposures to NO_2 and their connection to epidemiological and other health studies. The section on exposure in Chapter 2 would be improved, however, through a re-organization of the section. This reorganization could follow several possible structures. One such possible structure may be (in order):

a. A brief subsection that discusses exposure-related issues relevant to epidemiological studies and a statement of what the ISA considers to be the exposure or exposures most relevant to determination of NO_2 health impacts (e.g., personal exposures to NO_2, or personal exposures to NO_2 of ambient origin, or ambient NO_2 concentrations). In so doing, this subsection would serve as an introduction and would provide a framework for later subsections.

b. Exposure distribution summaries (general levels and distributions of ambient concentrations, personal exposures, etc.), with specific focus on the exposures most relevant to epidemiological and other studies. These distributions should include a discussion of how exposures vary by space (within a city and across cities) and time (hourly, daily, and yearly). Since exposure data on spatial and temporal variability at each of the above spatial or temporal scales may not be available, the discussion on certain aspects of the distributions may be brief – perhaps limited to what is known and identification of the knowledge gaps.

c. Discussion of exposure-related issues relevant to epidemiological studies

    - *Exposure error*: include subsections regarding (1) personal-ambient concentration relationships, (2) factors contributing to exposure error (e.g., spatial variability, differential infiltration, time/activity patterns, home ventilation, and personal behavior), and (3) statistical issues discussing impact of exposure error on risk estimates from short-term and long-term health effect studies. This section would incorporate the exposure related discussion currently in Chapter 1 Executive Summary, with Chapter 1 revised to be more a synthesis of exposure error and epidemiological study findings.

    - *Confounding*: include subsections regarding (1) relationships among personal NO_2 and co-pollutant exposures, between indoor and personal NO_2 and co-pollutant exposures, between ambient co-pollutant concentrations and personal NO_2 exposures, and between personal NO_2 exposures and ambient co-pollutant concentrations and (2) implications of these co-pollutant associations on short-term and long-term epidemiological study findings

As a note, the above sections should take care to discuss the issues relative to specific epidemiological study designs – including time-series studies, cohort studies of short-term impacts, and cohort studies of long-term impacts. Should this section become too lengthy, it may be advisable to separate the exposure sections into a separate and new Chapter.

Chapter 4/5

The basis of causality determinations for each outcome should be defined in more detail, especially with regards to the potential for confounding of NO_2-attributed health impacts. For example, the quality of the study with regard to control for confounding should be defined at least in large part based on the co-pollutants relevant to the health outcome of interest. Of note, for short-term cardiovascular and total mortality effects, most studies did not control for traffic related pollutants, such as black carbon (BC), which have been linked to short-term cardiovascular effects in other studies. Given this, it is unlikely that the potential for confounding is ruled out with sufficient confidence or deemed minimal in short-term cardiovascular or mortality (for which majority of causes are cardiovascular in nature) health studies that do not control for BC and other traffic related pollutants. As a result, the "likely causal" determinations should be reconsidered or further justified.

Charge Question 6

Chapter 6 evaluates scientific information and presents conclusions on factors that may modify exposure to NO_2*, physiological responses to* NO_2 *exposure, or risk of health effects associated with* NO_2 *exposure. Consistent with the ISAs for ozone and lead, conclusions on these at-risk factors inform at-risk lifestages and populations.*

a. How effective are the categories of at-risk factors in providing information on potential at-risk lifestages and populations? Is there information available on other key at-risk factors that is not included in the first draft ISA and should be added?

b. To what extent do the discussions in this chapter accurately reflect the body of available evidence from epidemiologic, controlled human exposure, and toxicological studies, including the extent to which evidence indicates that the effects of NO_2 *exposure are independent of other traffic-related copollutants?*

c. Please comment on the consistency and transparency with which the framework for drawing conclusions about at-risk factors has been applied in this ISA.

d. To what extent is available scientific evidence on factors that modify exposure to NO_2 *discussed in the chapter and adequately considered in conclusions for at-risk lifestages or populations?*

Response

The Chapter does a thorough job summarizing information in the previous chapters regarding factors that may increase health risks from nitrogen oxide exposures. The Chapter sections were generally well organized. I particularly liked how each section began with a discussion of the overall import of the at-risk category. The Chapter would be improved significantly if it focused on a synthesis of the findings by risk factor, rather than repetition of study findings. Further, the Chapter would also be improved through greater organization, as it was hard to separate and navigate among the large number of health endpoints and the diversity of study populations and designs that were often discussed for each at-risk factor.

a. The at-risk factors are categorized rather broadly, including genetic factors, pre-existing conditions, socio-demographic factors, and behavioral factors. These categories are appropriate, encompassing each of the identified at-risk factors. However, the list of specific at-risk factors should be expanded to include housing factors (other than residential location), such as presence of indoor gas stoves and/or home ventilation. While there is limited data with regard to their impact on the NO_2-health relationship, there is some data on their impact on NO_2 exposures.

   It would be helpful to discuss how the identified at-risk measures are related to one another, in order to provide information about whether certain at-risk measures may be acting as surrogates for another at-risk factor. For example, obesity rates may be higher in individuals of lower SES; as a result, it is possible that SES may be acting as proxy for obesity (or another correlated at-risk measure) in effect modification studies of SES.

   Correspondingly, the beginning of the Chapter mentions the possibility that multiple at-risk factors may impact the health impacts of NO_2; however, the discussion that follows does not discuss this possibility further. To address this issue, discussions of at-risk factors should be expanded to include, for example, discussions of effect modification of asthma by lifestage or obesity by lifestage. In both examples, it is possible that any differential impacts of asthma or obesity may differ for children, adults, and older adults.

b. Table 6-2 provided a nice summary of the studies used to make determinations of effect modification by genetic variation. Sections for other at-risk factors would benefit from inclusion of a similar table. Further, the section would be improved substantially if the results from the various studies were presented for each at-risk factor as a synthesis rather than as individual study findings, especially since the individual study findings were presented in Chapters 4 and 5. In addition, the Chapter would be improved with the addition of (1) evidence indicating that the effects of NO_2 exposure by at-risk factor are independent of other traffic-related co-pollutants and (2) a discussion of the strengths and weaknesses of the relevant studies.

c. As before, the relative strengths and limitations of the studies were not discussed or otherwise indicated, even though as discussed in earlier chapters, some studies were found to carry more weight than others. As a result, it was difficult to weigh the evidence, other than to simply count the number of affirmative or null studies. As was done in Chapters 4 and 5, each section would benefit from a table that summarizes the studies that contribute to the causal determination. In addition to the relevant studies, this table should describe what indicator of the at-risk factor was used in the study, the study population, the results, and other relevant information. By including such a table, it would be possible in the text to discuss only the "high quality" and/or relevant studies, which may help to support the causal determination.

Dr. Ronald E. Wyzga

Charge Questions for Chapters 4 and 5:

a. *To what extent do the discussions in this chapter accurately reflect the body of evidence from epidemiologic, controlled human exposure and toxicological studies?*

The information provided is mixed. In some cases it is extensive and helpful in reaching a conclusion. In other cases, the information provided needs to be augmented. It is not always clear when and which co-pollutants were considered in analyses. Some portions of the description do not differentiate among co-pollutants. The statistical significance of results is often not indicated, and summary statements such as "positive but imprecise" are not helpful. See specific comments below.

c. *Please comment on the adequacy of the discussion of the strengths and limitations of the evidence in the text and tables within Chapters 4 and 5 and in the evaluation of the evidence in the causal determinations.*

It varies thoughout the chapters. In some cases the input for the evidence is comprehensive and allows one to make a reasonable judgment; in other cases it is not. See specific comments below.

d. *What are the views of the panel on the integration of epidemiologic, controlled human exposure, and toxicological evidence, in particular, on the balance of emphasis placed on each source of evidence? Please comment on the adequacy with which issues related to exposure assessment and mode of action are integrated in the health effects discussion. Please provide recommendations on information in other chapters of the ISA that would be useful to integrate with the health effects discussions in these chapters.*

Again the integration differs according to the health endpoint considered. See specific comments below. In general, there is limited discussion of the relationship between personal and ambient exposures and how these differences could impact the results.

e. *Please comment on the appropriateness of using experimental and epidemiologic evidence for morbidity effects to inform the biological plausibility of total mortality associated with short-term (Section 4.4) and long-term (Section 5.5)* NO_2 *exposure and in turn, to inform causal determinations.*

This is clearly appropriate.

f. *Section 4.2.2 discusses the effect of short-term* NO_2 *exposure on airways responsiveness. This section focuses primarily on an EPA meta-analysis developed for this ISA of airway responsiveness data for individuals with asthma and secondarily on the potential of various factors to affect airways hyperresponsiveness independently or in conjunction with* NO_2 *exposure in controlled human exposure studies. This material presently is unpublished and we ask the Panel to provide the peer review for the analysis, in particular, to comment on the appropriateness of the methodology utilized for the meta-analysis, the conclusions reached based this analysis, and its use in the draft ISA. With regard to factors potentially affecting airways responsiveness, please*

comment on the adequacy of this discussion. Are there other modifying factors that should be considered?

I would like to see this information presented in a paper format before making any judgments about suitability for publication. There also needs to be some discussion of what is the appropriate cutoff response to define adversity. Is a one per cent change adverse? It could be useful to consider a sensitivity analysis to indicate how robustness of the meta-analysis conclusions.

g. *The 2008 ISA for Oxides of Nitrogen stated that one of the largest uncertainties was the potential for health effects observed in association with* NO_2 *exposure to be confounded by correlated copollutants. To what extent has evidence that informs independent effects of* NO_2 *been adequately discussed in Chapters 4 and 5 and appropriately interpreted as reducing uncertainty (for example, evaluation of copollutant model results)? Has the current draft ISA appropriately considered recent epidemiologic findings regarding potential copollutant confounding in causal determinations? Please provide comments specifically for respiratory effects, cardiovascular effects, and total mortality of short-term* NO_2 *exposure.*

The consideration of co-pollutants varies considerably throughout the document. See specific comments below. It is clear that some co-pollutants are more relevant than others in that their concentrations in ambient air are correlated with those of NO_2 and there is some evidence suggesting that these co-pollutants are also associated with the health effect under consideration. Ideally one would have the resources to examine all competing co-pollutants, not only in each study, but also in terms of evaluating their roles in impacting the health effects studied. For example, is there greater evidence associating some cardiovascular endpoint with EC than NO_2? In addition it is important to note that the concerns of covariates in the short-term and long-term studies are different. In one case we are concerned with the spatial correlations among various pollutants; in the other we are concerned with temporal correlations. This draft appears to focus on the latter. The role of NO_2 in a complex air pollution mixture is also ignored, but the existing framework for considering NAAQS precludes or greatly limits this consideration.

h. *To what extent is the causal framework transparently applied to evidence for each of the health effect categories evaluated to form causal determinations? How consistently was the causal framework applied across the health effect categories? Do the text and tables in the summaries and causal determinations clearly communicate how the evidence was considered to form causal determinations?*
I do not believe that it is consistent. I was particularly troubled with its application to reproductive effects. Perhaps better guidance from the Agency on the extent of evidence required to make a causal inference could help here.

i. *What are the views of the panel regarding the clarity and effectiveness of figures and tables in conveying information about the consistency of evidence for a given health endpoint? In particular, was the use of the tables and figures in both the text and online in the HERO database effective in providing additional information on the studies evaluated? Are there tables and figures in the ISA that would be more appropriate to include as a resource in the HERO database?*

The value of the information in Tables and Figures varied considerably. I felt that more attention should be given to the influence of co-pollutants on analytical results. I personally did not access the HERO database. I now know how to access it and look forward to using it.

Specific comments:

Executive Summary: I assume that changes in the document will be reflected in any revised Executive summary.

Chapter 1:

I assume this Chapter will be rewritten when the document is revised; I nevertheless provide comments on this Chapter as well as on the material in subsequent chapters.

p. 1-13, l. 24: From what we know from existing studies, there may be some indication of the co-pollutants of particular concern in teasing out the influence of NOx as opposed to co-pollutants. I would like the document to acknowledge the co-pollutants of greatest concern and to indicate where they have or have not been considered. There are parts of the document that appear to accept that consideration of co-pollutants is adequate if the issue is partially addressed.

p. 1-16, ll. 13-20: Given the potential role for co-pollutants, it might be useful to provide a brief understanding of the biological plausibility for the co-pollutants of greatest concern.
ll. 23-26: to what extent were co-pollutants addressed in this study?

p. 1-17, ll. 19-33: to what extent were co-pollutants addressed in these studies?

p. 1-19, ll. 32-35: Can we say anything about the biological plausibility of the relevant co-pollutants of concern?

p. 1-20, ll. 14-18: I am concerned about the roles of EC and OC as well.

p. 1-21, ll. 9-10: See above comment.

p. 1-23, l. 13: See above comment.

p. 1-24, ll. 14-16: or that traffic was not appropriately characterized. I don't find this to be a strong argument.
p. 1-25, ll. 1-3: Were these results independent of relevant co-pollutants?

p. 1-27, section 1.4.7: There could be some discussion of nitro-PAHs and known carcinogens that form when NOx is present on the atmosphere. Also, the issue of latency or of the historical levels of NOx should be discussed.

p. 1-29, Table 1: OC should also be mentioned as co-pollutant of interest. The biological plausibility argument ignores the mixed results seen in experimental studies. Recent studies provide some additional evidence but do not resolve the issue of whether NOx effects are independent of co-pollutants. There is remaining uncertainty that need be mentioned.

p. 1-30: I am also concerned about the limited studies that also examined co-pollutants, particularly EC and OC, which have been shown to be associated with cardiovascular effects in other studies.

p. 1-31: See above comment.

p. 1-32: See above comment.

p. 1-33: See above comment.

p. 1-27, l. 2: add OC as well.
ll. 11-12: This does not mean that NOx is a poorer surrogate than other pollutants; it does suggest that the correlations between NOx and other pollutant s are not constant over the gradient from roadways. The value of a pollutant characterizing traffic is dependent on how one defines that gradient. Unfortunately, we generally only have data from one monitoring station in an area..
ll. 15-26: Given the higher correlations between NOx and CO and EC (I would also add OC.), more attention should be given to these pollutants in the document.
ll. 32-33: the key co-pollutants are in line 33, except possibly for PM in line 32. There are also some findings to the contrary. This summary ignores the many cases where co-pollutants did change the results for NOx.

p. 1-41, ll. 23-28: there are also studies where the contrary is true: a traffic effect persists and the NOx association goes away with adjustment for traffic; hence there are two sides to this argument and the document only discusses one side.

p. 1-43, ll. 3-17: This discussion should also discuss differences in measurement error.

p. 1-49, ll. 5-11: Indoor exposures could also play a role here.
p. 1-54, l. 24: I would delete the word “compelling”.

p. 1-55, l.4: There are also people who travel on roads.

p. 2-70, l. 8, l. 19: define “moderately”.
This section also needs to consider EC and OC in more detail and to differentiate between spatial and temporal correlations.

Table 2-4: Add a column EC (and possibly OC).

p. 2-77, Figure 2-19: Add rows for EC, OC.

p. 2-84: ll. 4-6: This may explain why there are seasonal differences in results as presented in Chapter 5.
ll. 10-20: This result troubles me and its implications for the study results in Chapters 5 and 6 need be discussed.

p. 2-85, Table 2-9: What is the difference between “ambient” and “outdoor”?

p. 2-90, Table 2-10: It would be interesting to see what the correlations are between personal NO_2 and ambient levels of relevant co-pollutants, both spatially and temporally.

p. 2-93, ll. 8-20: Good discussion.

p. 4-3, l. 19: for ozone, PM, and CO. But can we say anything about EC, OC, UFP, or organics?
l. 25: are these concentrations relevant?

p. 4-4, ll. 6-8: and the low correlations between personal exposures and ambient levels of NO_2.

p. 4-13, l. 15: Is there a clear and accepted definition of "adverse"?

p. 4-31, Figure 4-1: Can this be redrawn with results when co-pollutants were considered?

p. 4-33: Why is there a discrepancy in the Holguin results presented in Table 4-7 and in Figure 4-1?

p. 4-34: The Spira-Cohen et al. results suggest that another pollutant (EC) is more important. This indicates the difficulty of making inferences when the focus is on only one pollutant.

p. 4-35: Why is there a discrepancy in the Dales et al. results presented in Table 4-7 and in Figure 4-1?

p. 4-53, ll. 26-28: Can we have a Table or Figure which clearly shows the influence of co-pollutants on the estimated NO_2 effects. I also have problems with lumping all co-pollutants together; some are clearly more correlated with NO_2 and/or biologically relevant than others. It is the more highly correlated and biologically relevant pollutants that need be addressed.

p. 4-55, l. 13: Do not lump all co-pollutants together.

p. 4-85, ll. 8011: Why is this result not presented in Chapter 3.

p. 4-86, Figure 4-2: Can results with co-pollutants be added to this Figure? Why is the all subject personal exposure result of Delfino not represented in this figure?

p. 4-88: Why is there a discrepancy in the Greenwald et al. results presented in Table 4-14 and in Figure 4-2?

p. 4-100, ll. 4-6: It should be noted that co-pollutants were not considered in these results. I also think the differences between indoor and outdoor exposures in Greenwald et al. are relatively ambiguous.

p. 4-101, 1l. 3-30: I would urge the authors to consider each co-pollutant separately.

p. 4-102, ll. 23-30: Ozone and SO2 are less relevant co-pollutants as others, such as CO, EC, OC.

p. 4-108, l. 18: But there are counter examples as well: Greeenwald et al., Lin et al., and Timonen et al.

p. 4-108, ll. 30-32: But there are also the cases where there is little correlation between personal and ambient exposures. See p. 2-84. To be fair these results should also be discussed here.

p. 4-113, l. 13: are these exposures relevant?

p. 4-124: Can we include results with co-pollutants in Figure 4-3? Why are the results of Schildcrout et al., Gillespie-Bennett et al., and Zora et al. not included as well as the wheeze results of Spira-Cohen et al.?

p. 4-136, l. 5: What does "imprecisely associated" mean?
l. 34: can the authors provide a range of multidays.

p. 4-137, ll. 9-12: Although the estimates are positive they are not statistically significant. Positive results are noteworthy, but statistical significance also plays a role, and given the numerous tests in a given study, the multiple comparisons issue should also be raised.
l. 14: I don't think one can fairly support the "independent association" assertion. The only co-pollutants considered are not the most relevant ones: CO, EC, OC. Several studies found effects of the other pollutants as well. Anderson et al. reported significantly diminished results when NO_2 was considered jointly with PM10.
l. 36: and in some cases lost statistical significance.

p. 4-144, l6: Robust in what way? Across cities, robust to consideration of co-pollutants?

p. 4-145: l. 19 but lost statistical significance.

p. 4-146, l. 1: "Robust" in what sense?

P. 4-153, ll.. 1-4: Do you mean to imply that Cakmak et al. did not consider single pollutant models?

p. 4-154: Many of the associations presented on this page were not statistically significant; although statistical significance is not the "end-all", it is noteworthy and it should be clearly indicated whether a result is or is not statistically significant. I also note that there are often many statistical tests are performed within the context of a specific study or paper; hence there is also a multiple comparisons problem which is rarely addressed. This could impact results that are barely statistically significant, such as the result of Son presented on p. 4-153.
ll. 7-8: were these associations statistically significant; it would useful to present the estimates and confidence intervals for the shorter lag results.

p. 4-155, ll. 31-33: what is meant by "remained robust"; remained positive but not statistically significant?

p. 4-158, ll. 1-7: This portrays one of the conundrums we face with NO_2 results. Associations tend to be stronger in the warmer months when NO_2 levels are lower. Some discussion of this issue should be included; it could be that individuals spend more time outdoors in warmer months; hence personal exposures may be higher. Do we have any data to address this possibility?

p. 4-160, ll. 17-18: it should be noted that although this result is positive, it is not statistically significant.

p. 4-167, Figure 4-5: I find the results of Darrow et al. curious. Why is the association between day and night exposures so different? I would expect daytime exposures to be more highly associated with personal exposures. Some discussion of this issue could be of value.

p. 4-167, l. 20: "positive", but not statistically significant.

p. 4-171, ll. 11-12: Can we generalize to all central monitors? I suspect the results are dependent upon monitor location with respect to sources and terrain.

p. 4-173, ll. 9-12: Results were positive but not statistically significant.

p. 4-176, ll8-13: It would be better to consider the possible co-pollutants individually rather than lumping them all together.

p. 4-177, Figure 4-9: can results with co-pollutants be included here as well?
p. 4-179, ll. 20-22: were results statistically significant?

p. 4-184, ll. 17-19: It should be noted that BC, EC, UFP, PNC appear to influence the results of NO_2 associations more than other pollutants.

p. 4-185, ll. 12-14: I find this result troubling. If NO_2 per se were responsible for effects, we would expect stronger results for personal exposures.
ll. 27- : There nevertheless remain uncertainties; to be comprehensive, this paragraph should mention these as well.

p. 4-186, l. 7: I would delete the word "compelling".

p. 4-197, l. 32: which other pollutants?

p. 4-198, ll. 1-15: did these studies consider co-pollutants?
ll. 18-24: the results using personal or indoor exposures should also be presented here as well as the results for co-pollutants.

p. 4-199, ll. 5-9: Can estimates and confidence intervals be presented here? Were the results statistically significant?

p. 4-200: Table 4-25 should also present results for co-pollutants.

p. 4-209, l. 3: present numbers. What is "borderline"?

p. 4-232, ll. 12-14: can numbers be presented; to what extent were they attenuated or less precise. Which results were statistically significant?
ll.26-28: Given the limited consideration of the co-pollutants that are most relevant, this statement is an overstatement.

p. 4-236, l. 1: EC was not considered.

ll. 28-38: can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 4-237, ll. 8-9: But was EC considered in any co-pollutant analyses?

p. 4-246, ll. 4-5: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 4-247, ll. 9-11: but only a limited number of co-pollutants were considered; given this, the conclusion is too strong.

p. 4-248, l. 7: insert "limited" before "copollutant models".

P. 4-254, Figure 4-16: It is important to identify which co-pollutants were considered in each case.

p. 4-255, Table 4-35: See above comment.

p. 4-256, ll. 7-8: Given the uncertainties and limited examination of results from co-pollutants, is this conclusion justified. I believe it tis too strong.
Table 4-36: Some of the key co-pollutants (e.g., EC,OC) were not considered. In some cases the effects of EC were greater than NO_2.

p. 4-267, ll. 19-21: what about other important co-pollutants?
ll. 24-27: which co-pollutants were considered?

p. 4-269, ll. 20-30: Was there any explicit consideration of NO_2 per se?

p. 4-285, Table 4-41. It is important to articulate those copollutants considered. Grouping them is not helpful.

p. 5-5, l. 37: This result is not statistically significant.

p. 5-4-18: Section 5.2.2: This section should indicate whether any co-pollutants were considered? Also it is important to indicate which results were statistically significant and which were not.

p. 5-19-24: Section 5.2.2.2: The above comment applies here as well.

p. 5-24-34: Section 5.2.3.1: Same comment as above.

p. 5-36, ll. 3-26: Were any co-pollutants considered?

P. 5-37,l 4. : Can you provide numbers? What is meant by attenuated? Does significance change?
ll. 7-38: Were any co-pollutants considered?

p. 5-38, ll. 1-19: Were any co-pollutants considered?

p. 5-39, ll 16-27: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-41, Table 5-3: I don't understand the first paragraph under Comments. Please clarify.

p. 5-42: Are there any co-pollutant model results for Gehring et al.

p. 5-45, l.4: Can numerical results and confidence intervals be presented? Were the results statistically significant?
l. 21-22: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-46, l. 8: what does" positive but imprecise" mean? Can numerical results and confidence intervals be presented? Were the results statistically significant?
ll. 17-28: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-47, l. 8: what were the other measures? Co-pollutants?

p. 5-48, ll. 1-7: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-49, ll.20-21: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-60, l. 29: does "fully adjusted" include adjustments for co-pollutants?

p. 5-71 ll. 1-2: what about EC and OC?

p. 5-72, ll. 4-5: I have problems with looking at the statistical significance of correlation co-efficients; given enough observations, any non-zero correlation will be significant; I don't know what this really means other than one rejects a correlation of zero. I would place more weight on the R^2 estimates.

p. 5-84,ll. 17-20: This suggests the importance of considering co-pollutants in order to understand the role of NO_2 in observed health effects.
i. 31: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-85, ll. 1-17: Are there any results from analyses with co-pollutants?

p. 5-93: Table 5-12: Please indicate which studies demonstrated statistically significant associations, with and without consideration of co-pollutants

p. 5-97, ll. 28-31: what about other co-pollutants EC, OC, PM?

p. 5-117, Table 5-13: Do any of these studies consider co-pollutants? Which ones?

p. 5-124, ll. 13-16: Do we really have sufficient evidence to make this assertion? To what extent were co-pollutants ruled out? How much of the limited evidence is statistically significant?
ll. 28-31: Do we really have sufficient evidence to make this assertion? To what extent were co-pollutants ruled out? How much of the limited evidence is statistically significant?

p. 5-25, ll. 6-9: Do we really have sufficient evidence to make this assertion? To what extent were co-pollutants ruled out? How much of the limited evidence is statistically significant?

p. 5-126, Table 5-15: Please indicate which results are statistically significant? And which have considered co-pollutants?

p. 5-132, ll. 9-13: Can numerical results and confidence intervals be presented? Were the results statistically significant?
ll. 23-32: Can numerical results and confidence intervals be presented? Were the results statistically significant?
l. 24: What is meant by "less precise"?

P. 5-133, 1-5: Can numerical results and confidence intervals be presented? Were the results statistically significant?

p. 5-143, Table 5-19: Please clarify the differences between Krewski et al. (2000) and Krewski et al. (2009). They appear to give conflicting results.

p. 5-156, Table 5-21: If there is an association between NO_2 and cancer, there is clearly a latency period, and concentrations for the epidemiological studies in this table should reflect this latency.

Dr. Junfeng (Jim) Zhang

Overall, this is an impressive first draft of ISA for NO_2-Health Criteria. The document reflects thorough and systematic review of the literature. The overall structure of the document is well thought out. Below are my specific comments.

1. Executive Summary: At its current format, this Summary is not very useful, because it reads like a condensed version of Chapter 1. I think it is necessary to have an Executive Summary, but it should concisely describe the overall objectives of ISA, review approaches, major findings from the review, and conclusions/recommendations. It does not necessarily follow the structure of Chapter 1. Rather, it should reflect that this comes from an integrated review/thought process.

2. Chapter 1: In general, I like the way this chapter is written in linking the major points stated in this chapter to more detailed descriptions and discussions in subsequent chapters. However, I also feel it is difficult to get a clear overall picture, as the chapter attempts to cover all but loosely connected points raised in subsequent chapters. I think a more effective approach is to describe the major findings in each subsection and to provide cohesive connections among the subsections, naturally leading to the Conclusions from an integrated (rather than fragmented) analysis. For example, on page 1-11, the last sentence of the 2nd paragraph, "however, the contribution of near-road exposure to … is not well characterized" as a concluding sentence of a concluding paragraph of this section is awkward. Such statements make the chapter reads fragmented.

3. Page 1-14: Line 9-11: "These studies are considered… thus minimizing the potential for publication bias". It is very hard to understand such a statement without context. Then when I read the subsequent chapter, I realize this is perhaps referring to confounding rather than publication bias.

4. I think one way to help integrate the evidence on NO_2 health effects, observed from epidemiological and toxicological studies (including controlled human studies), is to present a diagram showing possible biological pathways linking NO_2 exposure and various endpoints reviewed in the entire report (see example for $PM_{2.5}$ – Brook et al, in Circulation). This will help the discussions about the causal determination.

5. Table 2-9: It would be useful to provide Indoor-to-outdoor concentration ratios when data are available to derive I/O ratios.

6. Page 3-46, line 33: NO_2 and NO are not free radicals.

7. Page 3-47, Line 1: delete "it' between "As a result" and "there may be…"

8. Table 3-3: The information on biological pathways presented here may be organized into a chart and placed in Chapter 1 (see Comment 4 above).

9. In Chapter 4 tables 4-25 and 4-27, etc (Rich et al 2012), this is a study conducted during the 2008 Beijing Olympics. Please see Health Effects Institute Report 174, where more detailed

quantitative analyses of biomarker-pollutant relationships (including two-pollutant models) are presented.

10. Figure 4-17: figure caption needs to indicate % increase in mortality per how much increase in NO_2 concentration.

11. Table 4-38: same comment as above, what is the unit change in NO_2?

12. In Chapters 4 and 5, limitations using two-pollutant models to control for confounding effects should be toned up. Two-pollutant models help to assess whether the effects from NO_2 are independent from a second co-pollutant, but in many cases (especially when co-pollutants are highly correlated), these models still cannot sort out the confounding effects.