UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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November 14, 2012

EPA-CASAC-13-001

The Honorable Lisa P. Jackson
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 Ozone and Related Photochemical Oxidants (Third External Review Draft – June 2012)*

Dear Administrator Jackson:

The Clean Air Scientific Advisory Committee (CASAC) Ozone Review Panel discussed its review of the EPA's *Integrated Science Assessment for Ozone and Related Photochemical Oxidants (Third External Review Draft – June 2012)*, hereafter referred to as the Third Draft ISA, during a public meeting on September 11, 2012 and a public teleconference on November 5, 2012. The CASAC's consensus responses to the agency's charge questions and the individual review comments from the CASAC Ozone Review Panel are enclosed. The CASAC's key points are highlighted below.

The CASAC commends the EPA for substantial revisions to the Second Draft ISA based upon its prior advice (March 2012). The Integrative Summary now clearly captures the critical components of the lengthy ISA document. The EPA has expanded the discussion of ambient concentrations of ozone, which now is comprehensive, clear, and up to date. The discussion of exposure to ambient ozone is considerably improved over earlier versions and effectively presents current perspectives on ambient ozone exposure.

The CASAC has additional comments and recommendations on improving the document and believes that with the completion of the recommended revisions outlined below and in the consensus responses, the ISA will serve as a scientifically sound foundation for the risk and exposure assessment and policy assessment documents for the ozone National Ambient Air Quality Standards (NAAQS) review.

Dosimetry and Mode of Action

In general, the EPA has adequately addressed the major CASAC comments on this topic from the prior review. The chapter on dosimetry and mode of action could be further improved by revising the title to include species homology and giving greater attention to dosimetry and the toxic effects of ozone in the

upper respiratory tract. Additionally, the section on systemic inflammation should include a discussion of the findings from a recent controlled human exposure study.

Integrated Health Effects of Short- and Long-Term Ozone Exposure

The CASAC appreciates the EPA's clear articulation of the rationale for retaining the "suggestive to be causal" designation of the relationship between short-term ozone exposure and cardiovascular effects. However, the CASAC's scientific judgment is that the designation should be "likely to be causal" based on consideration of the totality of the evidence (toxicologic, epidemiologic and human clinical studies) related to cardiovascular effects. Moreover, the EPA's designation of the relationship between short-term ozone exposure and mortality is "likely to be causal." This short-term association is driven by cardiovascular mortality, the leading cause of death. For consistency in interpreting evidence, the relationship between short-term ozone exposure and cardiovascular effects also should be designated as "likely to be causal." The EPA should consider incorporating findings from the recent controlled human exposure study that substantially strengthen the human clinical data.

Populations Potentially at Increased Risk for Ozone-Related Health Effects

The new classification scheme for synthesizing the evidence and categorizing the strength of evidence regarding factors that place populations "at risk" is a welcome addition to the ISA. Using this framework, it is important to explicitly specify the basis for making determinations for each factor considered. The conceptual clarity of the chapter has been improved by distinguishing factors associated with increased exposure to ozone from those that modify the effect of ozone on health. However, there is still some conflation of these factors in the discussion of the evidence. The EPA should continue to develop precise and consistent terminology to describe "populations at risk." In general, as recommended previously, it will be useful to clearly distinguish three types of factors that may result in populations being at "increased risk": (1) increased exposure; (2) increased dose; and (3) increased sensitivity to a given exposure or dose.

Environmental Effects: Ozone Effects on Vegetation and Ecosystems

Overall, the CASAC commends the EPA for a useful summary of welfare effects on vegetation. The CASAC strongly supports maintaining and strengthening the conclusion that ozone at current ambient concentrations impacts crop yield and development, and recommends that this conclusion be carried forward to the risk and exposure assessment and policy assessment documents. The EPA should include a more in-depth discussion of the implications of competitive interactions among species that differ in ozone sensitivity and provide a better explanation of the apparent discrepancy between empirical and modeling studies in this area.

The CASAC appreciates the opportunity to provide advice on the ISA. There is no need for further review of the ISA by the CASAC. The CASAC looks forward to the EPA response to the advice provided here.

Sincerely,

/signed/

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

/signed/

Dr. Jonathan M. Samet, Immediate Past Chair
Clean Air Scientific Advisory Committee

Enclosures

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Consensus Responses to Charge Questions on
EPA's Integrated Science Assessment for Ozone and Related Photochemical Oxidants
(Third External Review Draft – June 2012)

Preamble; Legislative and Historical Background (formally Preface)

The CASAC Panel recommended flow diagrams be included in the Preamble for clarity of presentation. In the Preface, the Panel noted several omissions in the recent history and recommended updating the history to include recent decisions. The Panel also recommended renaming the Preface to reflect its historical content. Several diagrams were added to the Preamble to more effectively and clearly communicate the process of ISA development and the NAAQS review process. The Preface was renamed to reflect its content and revised to include a more complete and up-to-date history of activities.

Please review and comment on the effectiveness of these revisions. Please comment on the extent to which these sections of the ISA provide a useful and effective format for presenting introductory materials for this and future ISAs. Please recommend any revisions that may further improve the clarity of discussion.

Preamble

The Preamble is generally well-written and has an appropriate scope.

Figure I does not correctly depict the relationships among the *Integrated Science Assessment for Ozone and Related Photochemical Oxidants* (ISA), *Risk and Exposure Assessment for Ozone* (REA), and *Policy Assessment for the Review of the Ozone National Ambient Air Quality Standards* (PA); for example, linkages should be shown from the ISA to both the REA and the PA.

The term "integration" is used (Page lix, lines 5-6), but it is not clearly defined and explained. The intended meaning appears to be that evidence regarding causality for health effects was integrated across different types of information, including epidemiologic, toxicologic, and controlled human exposure studies. Furthermore, integration is considered with respect to interactions among effects. An explanation of the intended meaning of "integration" merits at least a paragraph, since this is such an important theme not only for the Preamble, but also for the Executive Summary and the Integrative Summary. Integration should be highlighted later in the document when its applications occur.

Although the categories of epidemiologic studies (Page lxiv, lines 9-12) are well-known to some experts, they may not be well-known to all readers. It would be helpful to include text explaining each of these categories.

The term "objective" is used (Page lxix, line 5) with regard to weight-of-evidence inferences, however, the classification relies on the judgment of scientists, typically from consensus of a group that has reviewed the evidence. The review method is qualitative and should be acknowledged as such. Transparency of the approach is critical, but, given the reliance on expert judgment, the classification of evidence has some inherent subjectivity and conclusions may not be fully consistent from group to

group. The description of making weight-of-evidence inferences should better reflect these characteristics of the process.

The Preamble is very useful and should be included in the Integrated Science Assessments for each of the criteria air pollutants, with adaptation as appropriate. The EPA should consider developing the discussion of the causality framework into a manuscript for submission to a journal. However, the framework should be a dynamic and useable document that is subject to iterative improvement.

Legislative and Historical Background

The Legislative and Historical Background is well written and the scope is appropriate, but it tells only part of the story. The historical review omits some of the key history, such as the difference between the level set in the 2008 rulemaking and the CASAC's range of recommended levels. Some of the history is better summarized in the other ozone documents, particularly the PA. Although different authors prepared the ISA versus the REA and PA, the historical information should be consistent across the various ozone documents.

Chapters 1 (Executive Summary) and 2 (Integrative Summary)

In response to CASAC comments, the language in Chapter 1 was simplified and figures were removed to improve the readability for a non-technical audience. Call-outs were added to Chapters 1 and 2 for ease of accessing more detailed discussions in the rest of the ISA. Both chapters were updated to reflect revisions in subsequent chapters. In Chapter 2, relative to the last draft of the chapter, there is increased integration of health effects evidence across scientific disciplines and health endpoints. Discussion of heterogeneity in risk estimates was expanded and synthesized.

Please comment on the adequacy of these and other changes to the chapters and recommend any revisions to improve the discussion of key information. Please recommend any revisions that may further improve the clarity of discussion.

Chapter 1

The Executive Summary is much better written than in the previous draft and the EPA is to be commended for the improvements.

The Executive Summary should include a brief summary of current ozone concentrations in the United States because the general reader may not be familiar with such information. Furthermore, there should be a brief introduction of the health concerns associated with exposure to ozone.

Greater clarity is needed on the definitions of the health outcomes and whether the outcome categories overlap (Page 1-5, Table 1-1). In particular, health outcomes are listed for respiratory effects, cardiovascular effects, and central nervous system effects, followed by "total mortality." Are the first three limited solely to morbidity within the effect category, or do they also include mortality? Does the category of "total mortality" include mortality from "respiratory effects"? The table should be clearly documented to answers these questions.

With regard to climate effects (Page 1-12, lines 9-17), there seem to be two relevant questions that should be addressed:

1. Is there a causal relationship between changes in tropospheric ozone concentration and effects on climate?
2. Can the magnitude of the effects be characterized with confidence?

An answer of "no" to the second question does not preclude a finding of causality for the first.

There is no mention of the best exposure index for welfare effects. The Executive Summary fails to make clear the scientific basis for later consideration of the form of the secondary standard, which can and should be different than that of the primary standard. Although this conclusion was reached in the previous NAAQS review, there is no mention of this determination in the current Executive Summary. This gap needs to be addressed.

Chapter 2

The revised Integrative Summary has captured the critical elements of the ISA. It is well written and provides appropriate linkages to places in the documents that address the topics that are summarized.

The explanation of "integration" on Page 2-1 seems clearer than in the Preamble. The EPA is encouraged to develop one working definition of integration and apply it consistently throughout the document.

The figure summarizing ozone chemistry (Page 2-6) emphasizes the stratosphere, which is not a focus of this document, while giving inadequate attention to the topics most relevant to the ISA. The figure should be redrawn to put greater emphasis on the most relevant aspects of ozone photochemistry.

The summary on Page 2-11, lines 29-31, is confusing in that the reported three-year averages across the United States appear to be at or below the background levels indicated in Table 2-1. This could be, in part, an artifact of reporting median values here compared to the mean values given on page 2-8. Some discussion of the numbers here compared to the North American (NA) background estimates is needed. Alternatively, it may be better to report mean values and ranges (e.g., 95% frequency range) rather than just median values.

It is unclear whether there was any quantification of the correlation between the metrics on Page 2-33, lines 10 to 15. It is also unclear what factors should be considered when comparing metrics and whether only epidemiologic studies should be considered. This section should be expanded so that the reader is not left with the impression that it does not matter which metric is used.

Figure 2-3 states that ozone can affect leaf "production." There did not appear to be any literature cited in Chapter 9 that specifically looked at production. Thus, the issue of production should be looked at more carefully, and there should be consistency between Chapter 9 and this chapter.

Chapter 3 – Atmospheric Chemistry and Ambient Concentrations

In revising Chapter 3, particular attention was given to estimates of background ozone concentrations. At the request of CASAC, new studies published after completion of the prior draft were evaluated and added to the discussion in Section 3.4. There is also increased focus on background estimates relevant to the fourth-highest maximum daily 8-hour average ozone concentrations.

Please comment on the adequacy of these and other changes to the chapter and recommend any revisions to improve the discussion of key information. In relation to ambient and background ozone concentrations, is material clearly, succinctly, and accurately provided? Where appropriate, please provide guidance that may refine the scientific interpretation and/or improve the representation of the science.

The changes to Chapter 3 are responsive to prior CASAC comments and substantially improve this chapter. The addition of metrics for background estimates relevant to the fourth-highest maximum daily 8-hour average (MDA8) ozone concentrations are very useful. This new draft is up to date, comprehensive, and clear. The remaining issues should be handled in the final round of revision.

1. Figure 3-1 is too complicated for the non-specialist, a comment also made on the Second Draft ISA. Showing the stratosphere in such detail gives the misleading impression that the stratosphere is a large source of ozone for the troposphere.

2. The text should note that the ozone coming down to the troposphere in stratospheric intrusions is not necessarily natural, i.e., it could have originated from production in the troposphere followed by transport to the lowermost stratosphere. Although Lin et al. (2012) considers any ozone that has crossed the chemical tropopause surface as stratospheric, Zhang et al. (2011) consider ozone to be stratospheric only if it was produced in the stratosphere. The latter definition is recommended.

3. The text gives the impression that high ozone background concentrations at higher elevation sites are due to stratospheric influence and intercontinental transport. In fact, these concentrations reflect the general increase of background ozone concentrations with altitude in the troposphere due to increasing ozone lifetime.

4. The ozone background is a topic of active discussion in the atmospheric chemistry community. The chapter should make more ample reference to McDonald-Buller et al. (2011) as the closest thing to a consensus statement from the atmospheric chemistry community on the magnitudes and sources of background ozone.

5. The presentation of satellite data should mention their utility for constraining estimates of the emissions of ozone precursors (nitrogen dioxide and formaldehyde) and the long-range transport of pollution (carbon monoxide). The role of satellite data in helping to estimate or constrain estimates of background ozone concentrations in the free troposphere should be further emphasized, including in the concluding section. On the other hand, two important limitations of satellite observations of tropospheric ozone should be noted: (1) they require monthly averaging to reduce noise, and thus are of little value for synoptic-scale variability; and (2) they are insensitive to surface concentrations.

6. In considering the natural ozone background concentrations, the ISA should include a study by Knapp and Soule (2007) that shows: (1) a declining trend in the mid-latitude cyclone frequency and a delaying trend in the time of first onset of the cyclone in a year during the period of 1900-2004, and (2) a significant association between these trends and an increasing trend in areas burned by wild fires in the Northern Rockies.

7. As discussed further in the individual member comments, two new manuscripts have relevance to this chapter. Huang et al. (2012) discusses western U.S. background ozone and Turner et al. (2012) describes a simulation of the impact of summertime cyclone frequency on ozone contrary to what is described in the chapter.

Chapter 4 – Exposure to Ambient Ozone

Revisions made to Chapter 4 in response to CASAC comments included: adding maps that integrate population density, placement of ozone monitors, and concentrations at the monitors; a discussion of long-term concentration averages typically used as exposure metrics in epidemiologic studies; and adding time activity information. These revisions more closely link the information in Chapter 4 with subsequent health information in Chapters 5-8.

Please comment on the adequacy of these and other changes in responding to the Panel's comments. Please provide comment on revisions that may further improve the utility of this chapter for interpretation of epidemiologic results in subsequent chapters.

The revised Chapter 4 is a considerable improvement over earlier versions. The additional maps and discussions add clarity to the material covered. Additional refinements are suggested in the individual member comments, but the chapter effectively presents current perspectives on ambient ozone exposure.

Chapter 5 – Dosimetry and Mode of Action

Chapter 5 was updated considerably in response to CASAC comments with revisions including expanded characterization of the potential for ozone reaction products versus ozone itself to elicit observed health effects, attention to specific exercise levels utilized in the human studies, and enhanced discussion of species homology and interspecies sensitivity.

Please comment on the extent to which these revisions help to provide the underlying mechanistic and dosimetric information for interpretation of health effects evidence in later chapters and recommend any revisions to improve the discussion of key information.

This chapter has been greatly improved by reorganization and by the additional text added to various sections. Overall, the chapter is well organized and well written. The authors have done an excellent job of addressing the major comments of the CASAC on the Second Draft ISA. However, the chapter still does not address a few relevant topics (e.g., poor reliability of predicted ozone penetration distance in the extracellular lining fluid (ELF) due to the large uncertainty in substrate concentrations and reaction rate constants) and lacks clarity in some areas (e.g., the difference between "bulk flow" and convection

and the role of axial diffusion compared to radial diffusion). Individual comments by Panel members identify these areas and offer suggestions for improving the text associated with them.

Although the authors state that there are two main purposes to the chapter (i.e., to describe the principles that underlie the dosimetry of ozone and to discuss factors that influence it), species homology is an important component of the chapter and the title of Chapter 5 should be changed to reflect this.

The chapter needs to be strengthened in its treatment of the upper respiratory tract (URT) with regard to both dosimetry and ozone toxicity. The chapter text gives the impression that ozone uptake is homogeneous throughout the nasopharyngeal airways, but estimates made with computational fluid dynamics show site specificity of uptake that correlates well with the nasal pathology observed in various studies. Individual Panel member comments provide suggestions on specific points that need to be addressed and strengthened.

The section on systemic inflammation should include a discussion of the recent controlled human exposure study by Devlin et al. (2012) showing that ozone can cause an increase in vascular markers of inflammation, changes in markers of fibrinolysis, as well markers that affect autonomic control of heart rate and repolarization. Because these findings provide biologic plausibility for the epidemiology studies that associate ozone exposure with mortality, a reference to the appropriate section in Chapter 6 should be included. The results of Devlin et al. (2012) are important additions to the overall database of controlled human exposure studies. The EPA should highlight the novel findings of this research and the results that support previous findings by others, as well as discuss the strengths and limitations of the study relative to the elucidation of the mode of action of ozone.

Chapters 6-7 – Integrated Health Effects of Short- and Long-Term Ozone Exposure

Revisions made to Chapters 6 and 7 included increased integration of recent evidence with key findings from previous reviews, and further integration across chapters, particularly with information from Chapters 4 and 8. One key example of further integration across chapters is the expanded discussion of exposure assessment methods and measurement error issues with linkages to Chapter 4 and discussion of their potential influence on heterogeneity of results among studies.

Careful consideration was given to a CASAC recommendation that the causal determination for cardiovascular effects from short-term ozone exposure be increased to "likely to be a causal relationship." There was strong toxicological evidence and consistent, positive associations between short-term ozone exposure and cardiovascular mortality in epidemiologic studies. However, controlled human exposure studies were limited in number and provided inconsistent results. Likewise, epidemiologic studies showed inconsistent findings for cardiovascular morbidity (e.g., heart rhythm, physiological biomarkers, and hospital admissions or emergency department visits). Based on extensive review and discussion of the evidence the decision was made to retain the "suggestive of a causal relationship" conclusion for cardiovascular effects from short-term ozone exposure.

Please comment on the extent to which there is sufficient clarity in the revised presentation of study designs and results. Please provide recommendations where the interpretation of the scientific evidence may be improved as well as comments on the soundness of conclusions in these chapters.

The CASAC finds that chapters 6 and 7 have been substantially improved in the Third Draft ISA. Further suggestions for improvements to the clarity, interpretation of scientific evidence, and soundness of conclusions include:

1. There has been improvement in separating the discussion of short-term and long-term exposure effects. However, there are still instances where the discussion intersperses short-term and long-term exposure effects. If this organization is retained, a brief explanation should be provided to avoid confusion and improve clarity.

2. The approach to distinguishing effect modification from interaction runs counter to common usage in the epidemiology community. It is not clear that the very specific definition of interaction that is presented in order to allow discussion of "interaction" as distinct from effect modification is useful. It would be less confusing if the discussions of topics included in the effect modification subsection and the interaction subsection were combined.

3. The CASAC appreciates the clear argument put forward to retain the causal designation of the relationship between short-term ozone exposure and cardiovascular effects as suggestive rather than likely. The disagreement between the CASAC and the EPA on this issue is due to the relative weighting of the available evidence. The CASAC weights the toxicological findings, the findings on (total) mortality (and the EPA designation of this relationship as likely to be causal, which logically implicates cardiovascular mortality given that this association is driven by cardiovascular mortality), and the limited human clinical data, more heavily than the inconsistency in the findings on the morbidity endpoint. The EPA should consider incorporating findings from Devlin et al. (2012) that substantially strengthen the previously meager human clinical data. Although these recent findings are based on a relatively small number of subjects and only one, relatively high, exposure concentration, they are from a controlled laboratory setting. Additional references (detailed in individual comments from Dr. Michael Foster) also provide scientific support for associated cardiac results established by Devlin et al. (2012).

4. The presentation of susceptibility in these chapters should be aligned more closely with that in Chapter 8.

5. The new-onset asthma studies are described as providing "the strongest epidemiologic evidence for a relationship between long-term ozone exposure and respiratory effects." This conclusion rests on two studies from the California Children's Health Study (CHS). McConnell et al. (2002) can be characterized as initial findings in a full cohort that have not yet been replicated in other studies. Islam et al. (2008) is a gene-environment interaction study in which no ozone main effect was observed. The characterization of the new-onset asthma studies should be reconsidered.

6. Care should be taken in attributing effects to long-term ozone exposure for certain endpoints such as hospitalizations and emergency department visits, where effects can also be due to short-term exposure. Support for long-term effects could be obtained with observing persistent long-term effects after control for short-term effects in these studies, or by estimating impacts of long-term exposure that are substantially larger than would be estimated from short-term effects alone.

Chapter 8 – Populations Potentially at Increased Risk for Ozone-Related Health Effects

The CASAC encouraged the development of standard terminology and concepts for assessing populations at risk that could be applied broadly across the NAAQS pollutants. To help synthesize the evidence, a new classification system was created for considering risk factors. Similar to the approach used to determine causality, each factor was evaluated and classified based on the weight of evidence within and across disciplines. Throughout the chapter, effort was also made to distinguish between greater ambient exposure and/or greater internal dose versus greater adverse health effects at a specific dose when describing the evidence that could potentially result in a population being at increased risk of an ozone-related health effect.

Please comment on the adequacy of these revisions to clarify the consideration of potential at-risk populations and recommend any revisions to improve the characterization of key findings and scientific conclusions.

The chapter is much improved by the new classification scheme for synthesizing evidence and categorizing strength of evidence regarding factors that place populations "at risk." This classification scheme may be elaborated on further in the future but it is an excellent addition. It is important to be explicit in specifying why a particular determination was made for each of the factors.

The chapter is also improved by language that begins to distinguish factors associated with increased exposure to ozone from those that modify the effect of ozone on health. However, there is still some conflation of these factors in the discussion of the evidence. The CASAC encourages the EPA to continue to develop precise and consistent terminology to describe "populations at risk." In general, it may be useful to clearly distinguish three types of factors that may result in populations being at "increased risk":

1. Increased exposure: for example, outdoor workers will have greater exposure (e.g., concentration x time) than indoor workers;

2. Increased dose (e.g., concentration x minute ventilation x time), given a certain exposure: for example, persons who exercise will receive a greater dose for a given ambient exposure, children may have breathing patterns that result in increased dose, given a certain ambient exposure (NOTE that this can be manifested as effect modification of the ambient exposure in epidemiologic studies but is not true effect modification in the sense of point 3 below).

3. Increased sensitivity to a given exposure or dose: for example, persons with certain genetic characteristics or certain diets that experience abnormally strong adverse health effects.

Most of the evidence reviewed and summarized using the evidence criteria focuses on factors affecting increased sensitivity to a given exposure or dose (although there is some discussion of factors affecting increased exposure and factors affecting increased dose). Given that the definition of population at risk is broad and appears to encompass all three types of factors, it may be useful to either clearly define the focus of the chapter (as focused primarily in points 2 and 3) or broaden the discussion to encompass point 1 more broadly.

Chapter 9 – Environmental Effects: Ozone Effects on Vegetation and Ecosystems;
Chapter 10 – The Role of Tropospheric Ozone in Climate Change and UV-B Effects

The CASAC Panel provided a number of important comments that led to focused revisions of these chapters. In Chapter 9, clarifying statements were added related the effect of ozone on root growth. In Chapter 10, a description of the RCP scenarios from the IPCC Fifth Assessment Report was added. Additionally, the discussion of radiative forcing from ozone precursor emissions was expanded. The discussion in Chapter 10 of tropospheric ozone health and welfare effects related to UV-B shielding was revised to be more concise with clear conclusions. At the end of both Chapter 9 and 10, a table of causal determinations was added at the request of CASAC.

Please comment on the revisions to these chapters and the adequacy, scientific soundness, and usefulness of the material presented and recommend any revisions to improve the discussion of key information.

Chapter 9

Overall, the CASAC commends EPA for an important summary of a large body of complex research in Chapter 9. The CASAC is impressed with the depth of the review of welfare effects on vegetation in the revised chapter and with the clear effort by the EPA to respond to the CASAC's suggestions. The extended analysis of the molecular basis of plant responses to ozone is very appropriate, reflecting the recent maturation of these lines of research. The molecular scale should be added to the Introduction, as a level at which ozone impacts on plants may be identified. The chapter summary refers to "very different mechanisms" that may mediate crop damage from acute versus chronic exposures to ozone. However, the supporting arguments are not clearly presented and might be added in final revisions, given the considerable literature regarding this potential source of uncertainty. In considering the signaling pathways related to ozone response, there is little evidence that a plant has a specific ability to sense or detect ozone, in the sense that jasmonate vapor activates specific pathways. The response to oxidant challenge appears to be non-specific.

The chapter appropriately determines that recent proteomics results support the earlier conclusions from gene expression studies. The chapter has a lengthy discussion about potential inconsistencies that hypothetically could occur, but that did not occur. The proteomics results should be stated more positively without detailed consideration of these hypothetical potential inconsistencies. Ozone impacts the protein content and activity of ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO). Protein content responds to reduced synthesis and enhanced degradation, while activity of existing protein responds to modification of the active site and to activity or content of RUBISCO activase. These elements are mentioned throughout the text but a clear synthesis is lacking, and the analysis could be brought forward to a single location early in the chapter.

The CASAC supports the conclusion in the Third Draft ISA that the results of newer chamberless designs, including Free Air and Ambient Gradient protocols, provide qualitative and quantitative support for relationships between yields or biomass productivity and concentration-based exposure metrics obtained using earlier open-top chamber (OTC) techniques. The EPA has clearly demonstrated that uncertainties regarding artifacts due to exposure technology have been largely resolved, validating much earlier research, and broadening the database of species that can be used in risk assessment. The depth of science supporting ozone impacts on crop yield and development is very strong and is effectively

demonstrated in the chapter. Use of relationships obtained with crop species in risk assessment appears to be well justified, and potentially powerful given the abundant data sets available. During the final revisions of the document, these conclusions should be maintained, and where possible, strengthened, due to their critical importance.

The CASAC concurs with the EPA conclusions about the use of flux-based metrics. It is clear that flux is the biologically-relevant ozone parameter, but as the chapter notes, the complexities of application remain too high for its current use in a regulatory context. In one study (Gonzalez-Fernandez et al., 2010), flux performed no better than exposure in predicting the yield of wheat. The role of effective flux is well considered in this draft chapter, with appropriate literature citations, although it is not yet clear what may determine effectiveness. Publications by Sandermann (2008), Booker et al. (2012), and others have raised questions regarding the assumed dominant role of apoplastic antioxidants, including ascorbate, in determining resistance. The strong case made for apoplastic ascorbate as the first line of defense in this draft may be somewhat overstated.

The section on stomatal responses is much improved. There remains some confusion in the text in addressing possible bidirectional responses of stomata to ozone and the unexpected failure of stand transpiration to decline in response to ozone under some conditions. The issue of sluggish stomata is well considered, though its ability to account for data such as that of McLaughlin et al. (2007) on stream flows may be limited. Recent evidence suggests that ozone may, under some conditions, even cause stomatal opening. This observation is briefly considered in the context of prolonged exposures, but in final revisions of the document the EPA should carry these potential impacts throughout the full discussion of stomatal responses to ozone.

The CASAC recognizes that the treatment of hydraulic conductance in this chapter is not carried forward into risk assessment, and thus may be of secondary priority. However, a few factual inaccuracies in the analysis of leaf area specific hydraulic conductance, and its relationships with stand transpiration and stomatal conductance, should be addressed for clarity. A more careful discussion based on Uddling et al. (2008, 2009, 2010) would be useful.

Figure 9-1 is important, but should be improved. A better integration and labeling of Figures 9-1 and 9-2 could provide greater clarity. There should be explicit consideration of boundary layer and stomatal resistances to ozone uptake. Figure 9-2 might be more useful if it appeared closer to 9-1, and were renamed “anatomy” instead of “microarchitecture”.

The modeling study on tulip poplar (Weinstein et al., 2001) is appropriately considered, including the conclusion of moderate impacts on basal area. However, the text should discuss, in more depth, the implications of competitive interactions among species that differ in ozone sensitivity. The model suggests that competitive release would predict a short-term increase in abundance of red maple and black cherry, followed by a larger decrease in total stand productivity. In OTC studies on seedlings of these same species, black cherry was ranked the most sensitive to ozone and red maple the least sensitive. This apparent discrepancy between empirical and modeling studies should be better explained to avoid confusion. Similarly, the gradient-plus-chamber design with cottonwood of the Gregg et al. (2003) study provides coherence to the data but the results remain somewhat unusual, which should be acknowledged, particularly given the emphasis to be given to this study in the risk assessment.

Additional higher order interactions with ozone should be considered. These include changes in litter decomposition due to ozone-induced defoliation, increased radiation penetration, soil surface drying and temperature, emission of volatile organic compounds (VOCs) that may enhance ozone formation, effects on biodiversity, and effects on albedo. Ozone-disease interactions have been demonstrated and ozone-nitrogen interactions are a major problem in many areas of the United States. Excess nitrogen is known to directly affect plant responses to ozone and pathogens, although the interactions may be complex. An additional study to consider including in the final draft of the chapter would be Von Tiedemann (1996).

Chapter 10

Chapter 10 is well written and adequately summarizes a body of complex and not well-characterized relationships. As a stand-alone chapter it is quite informative, though additional brevity could be achieved by greater reference to Chapter 3. The EPA should consider the possible expansion of section 10.4.5 to attempt attribution of some fraction of ultraviolet B (UV-B) impacts on vegetation to changes in ozone, parallel to studies cited in the preceding two paragraphs and the ecosystem studies in 10.4.4. The CASAC is supportive of the final paragraph of the current draft as an indication of future research needs. UV-B effects on plants should receive greater emphasis.

The CASAC supports the discussion (10.3.3.5) of a potential feed-forward mechanism involving the biological carbon cycle, with its reference to Chapter 9. Other changes caused by tropospheric ozone and mediated by altered radiative forcing also could be considered, both here and in Chapter 9, including the effect of increasing temperature due to tropospheric ozone on biodiversity, plant species migration, consequent impacts on albedo, and associated feed-forward responses.

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Appendix A

Compendium of Individual Comments by CASAC Ozone Review Panel Members on EPA's Integrated Science Assessment for Ozone and Related Photochemical Oxidants (Third External Review Draft – June 2012)

Mr. George A. Allen

The markup version made review of 3rd ERD much more efficient - this is greatly appreciated.

The changes to Ch. 3 are very responsive to panel comments and substantially improve this chapter of the ISA over the 2nd ERD; the staff are to be acknowledged for their efforts in this revision. The addition of metrics for background estimates relevant to the fourth-highest maximum daily 8-hour average O3 concentrations are very useful.

The issue of the contribution of "stratospheric O3" is still not well resolved. High elevation sites may have elevated O3 relative to other sites in the region simply because of the lack of O3 sinks at elevation. The consistently high O3 at Mount Washington, NH is an example (see my comments on the first draft ISA document for details).

Minor comments:

3.4.1.1 , pg 3-34, line 11-12: The Thompson estimate that "roughly 20-25% of tropospheric O3 over northeastern North America during July-August 2004 was of stratospheric origin" could be on the high side. 2004 was one of the cleanest surface O3 summers in the New England for the entire decade; see: http://www.epa.gov/region1/airquality/standard.html

3.4.1.2: Importance of wildfire as a source of O3. A possible confounder here is interference from elemental mercury in woodsmoke with the UV FEM method. There is Hg in WS. Figures 3-15 and 3-16 show a hot-spot for modeled O3 in central Idaho. There are no data to support these modeled results according to staff at ID-DEQ, but there was a large wildfire event there in 2007. Per this source, in 2007 wildfire emissions were often computed using the total fire acreage from the incident reports, as if all the acres were all burning every day. This could lead to a large overestimate of wildfire emissions. Currently, satellite-corrected daily fire acreages are used via the SMARTFIRE system to simulate only the fire acreage burning each day. In 2008, Desert Research Institute released a report for ID-DEQ on wildfire's influence on O3 during summer 2007 in the Treasure Valley (near Boise, ~ 130 km SW from the "red spot"):
http://www.deq.idaho.gov/media/352767-ozone_treasure_valley_report.pdf
While not in the open literature, there may be useful analysis in this report for this issue.

3.5.6.1, Pg 3-80, line 29: Class-I airshed (not -i)

3.6.3.1, Pg 3-133, line 29: (Leibensperger, 2008)

A paper that is currently under open review and is relevant to western US background O3 at Mt. Bachelor and Trinidad Head is listed below. If it is finalized soon, it may be of use to this ISA.

ACP Discussions:
Impacts of transported background pollutants on summertime western US air quality: model evaluation, sensitivity analysis and data assimilation by M. Huang, G. R. Carmichael, T. Chai, R. B. Pierce, S. J. Oltmans, D. A. Jaffe, K. W. Bowman, A. Kaduwela, C. Cai, A. J. Weinheimer, L. G. Huey, and G. S. Diskin
http://www.atmos-chem-phys-discuss.net/12/15227/2012/

Mr. Ed Avol

General Comments:

In my opinion, this document has been carefully and consistently improved over earlier versions and represents a useful and objective assessment of the current evidence (at the time of document publication). At this time, I have no substantive over-arching comments to contribute.

Specific Comments:

1. Chapter 1 - Executive Summary, pg1-3, lines 3-14: Could/should there be another sentence or quantified detail on EPA's approach to the background (formerly PRB)? Because effects have been observed at ever-lower concentrations, and the background levels are creeping upward, being explicitly aware of the absolute difference between the current NAAQS, the current level of observed effects, and the current estimates of background seem to be important anchor points. Moreover, the use of multiple background values (US, North American, world-wide, etc) will make it difficult for many readers to understand the issue. It would be much better to clearly define what each of the background values represent, select one for use, and explain why that choice was made.

2. Chapter 2 - Integrated Summary: pg 2-30, lines12-15: "This is the first time…to suggest that long-term exposure to O3 may play a role in the development of the disease…" is incorrect. What about McConnell et al (Lancet, 2002: Feb 2:359(9304):386-91), which showed increased incidence of asthma in schoolchildren who played on 3 or more sports, and argued this was evidence of a causal link between O3 exposure and asthma?

3. Chapter 4 - Exposure to Ambient Ozone: pg4-24, line 3 to 4: "…the most important human activity databases…" seems unnecessarily judgmental. Suggest that one of the following should be used instead: numerous, many of the important, several.

4. Pg4-25, section title 4.4.2 should be "Ozone-Averting Behavior"

5. Pg4-25, line 4: how does "…being active outdoors when air quality is better…" *reduce* exposure to O3? Do you mean exercising at times of lower pollution?

6. Pg 4-32, line 22: should be corrected to read "…reflect lack of ***NOx*** sources…"

7. Pg4-39, line 4: Author's name should be spelled McConnell, not Mcconnell.

Dr. Joseph D. Brain

Comments on Preamble; Legislative and Historical Background (formally Preface)

The CASAC Panel recommended flow diagrams be included in the Preamble for clarity of presentation. In the Preface, the Panel noted several omissions in the recent history and recommended updating the history to include recent decisions. The Panel also recommended renaming the Preface to reflect its historical content. Several diagrams were added to the Preamble to more effectively and clearly communicate the process of ISA development and the NAAQS review process. The Preface was renamed to reflect its content and revised to include a more complete and up-to-date history of activities.

Please review and comment on the effectiveness of these revisions. Please comment on the extent to which these sections of the ISA provide a useful and effective format for presenting introductory materials for this and future ISAs. Please recommend any revisions that may further improve the clarity of discussion.

In the charge questions, it suggests that "Legislative and Historical Background" be substituted for the term "Preamble." I agree with this. The proposed title better describes the contents. For some reason, the document itself still has the rubric "preamble." Let's change it as suggested.

I am deeply impressed with this accomplishment. It certainly meets, perhaps exceeds, my expectations. I find the ISA an extremely valuable tutorial in terms of the entire process leading up to the ISA. It also explainins how it will be used in the REA and policy assessment. The diagrams, while necessarily complex, are understandable. I like Figure 2 and the use of the ovals and finally the circle showing how the vast amount of literature is selected and summarized and finally focused on ISA conclusions.

The document is far more than a roadmap leading to decisions by the administrator. Especially important is the articulation of the EPA framework for causal determination. We applaud the discussion of different sources of data and how such diverse areas as exposure assessment, epidemiologic studies, chamber studies, and especially animal studies are evaluated and integrated into a "framework for causal determination."

I applaud and support the idea that this initial chapter on "Legislative and Historical Background" be part of all future CASAC ISAs. This current draft is generic and not focused exclusively on ozone. It’s appropriate. Having an accepted and common strategy articulated at the outset will simplify work on other future pollutant focused ISAs.

Having expressed my enthusiasms, there is a penalty/consequence. Because this part is so important and will be used repeatedly, it deserves further scrutiny. I suggest that a subcommittee be established to further refine this document. Perhaps this subcommittee should include individuals from both the EPA and CASAC who would work together. Furthermore, I propose that this document be peer reviewed and published in a journal, such as one of the ATS journals. I suspect that the new vision for the Proceedings of the American Thoracic Society (PATS) would be delighted to publish this. Even after this, this should be a living document. While replicated for future pollutants, it should be possible to improve it.

Comments on Chapters 1 (Executive Summary) and 2 (Integrative Overview)

In response to CASAC comments, the language in Chapter 1 was simplified and figures were removed to improve the readability for a non-technical audience. Call-outs were added to Chapters 1 and 2 for ease of accessing more detailed discussions in the rest of the ISA. Both chapters were updated to reflect revisions in subsequent chapters. In Chapter 2, relative to the last draft of the chapter, there is increased integration of health effects evidence across scientific disciplines and health endpoints. Discussion of heterogeneity in risk estimates was expanded and synthesized.

Please comment on the adequacy of these and other changes to the chapters and recommend any revisions to improve the discussion of key information. Please recommend any revisions that may further improve the clarity of discussion.

The EPA staff are to be commended for their responsiveness to CASAC recommendations. They have done an excellent job in pulling together and presenting in a very readable format the summary of the evidence and conclusions contained in the ISA. That this is accomplished in 13.5 pages is commendable. In previous meetings, CASAC members suggested that an Executive Summary should be approximately this length. It would be the place to start for policy makers, legislators, lay people, or members of the scientific community.

Having dealt with the generic aspects of the ISA as presented in the "Legislative and Historical Background," it now moves to the specific topic of ozone. The rubrics are clear and the order of the rubrics make sense. I applaud the use of bolded text to reflect summary statements about causal relationships for different outcomes or concerns. The chapter is comprehensive and covers the nature of ambient ozone, human exposures and health outcomes, as well as the effects of ozone on ecosystems and agriculture.

I do have one criticism, although I recognize the difficulty of responding to it. In the very first eleven lines of text, the executive summary reminds the reader of the current NAAQS for ozone. However, after that, when discussing all these multiple aspects of ozone, there is a distinct lack of any information regarding ozone concentration. If I were the casual reader, I would want to know not only what environmental and health outcomes might be affected, but also link that to ranges of ozone concentrations where such changes might be expected to occur. Again, all the statements of causal or likely causal relationships, immediately raise the question: at what ozone concentrations do these relationships occur. Perhaps those ozone levels cannot be usefully introduced in every section of the document, but somewhere, perhaps in the conclusion, or in a table, it might be useful to include the range of ozone concentrations which both occur and might elicit the outcomes being described.

Naturally, the Integrative Summary needs to be longer than the Executive Summary. The current length of 53 pages is appropriate. This section represents the next level of summary for readers who want more information. I welcome the links that connect the sections of this document to corresponding topics in subsequent chapters. That one can access supporting tables and figures by clicking on links makes the document more user friendly.

This summary goes much farther in linking concerns to ozone levels. For example, Table 2-2 explicitly mentions ozone levels where the observed effects were measured. (Perhaps one solution to the paucity of ozone quantitation in Chapter 1 could simply be a statement that for readers who want more

information about specific ozone levels, they should turn to Chapter 2, the integrative summary. The chapter is well organized and represents responsiveness to previous CASAC concerns as well as a cogent summary of the rest of the document.

Dr. David Chock

Comments on Chapter 3: Atmospheric Chemistry And Ambient Concentrations

The current version of Chapter 3 is yet another improvement over the previous version. The most noteworthy is the expansion of the discussion on background O3 concentrations and the comparison of CASTNET-observed regional means and model-predicted fourth-highest values of MDA8 O_3 concentrations for 2006. Recently published relevant studies have also been included. Except for the few points described below, this new draft can be considered up to date, comprehensive, and clear in presenting the atmospheric chemistry and ambient concentrations of ozone.

The discussion by Leibensperger, et al. (Atmos. Chem. & Phys. 8, 7075-7086, 2008. Note: This reference is missing in the Chapter's reference list.) (See page 3-132, lines 20-32.) on the reduction in frequency of summertime mid-latitude cyclones since 1980 and its potential consequences in offsetting almost half of the air quality gains in the Northeastern US during the summers of 1980-2006 is highly significant and needs further verifications, as global warming becomes progressively more serious. The paper assumed a linear additive relation to describe the impacts of the rates of ozone-precursor emission changes and cyclone frequency variations on the rate of change of high-ozone days. One concern is that, because of the nonlinearity of ozone chemistry, the resulting slope of the detrended number of high-ozone days vs. the detrended number of cyclones may not be fully independent of the systematic variation of ozone-precursor emissions. Use of climate-chemistry models could help verify the validity of this assumption, and further establish the slope of high-ozone days vs. cyclone frequency with ozone-precursor emissions held fixed. A recent paper by Turner et al. submitted for publication in Atmos. Chem. & Phys (A.J. Turner, A.M. Fiore, L.W. Horowitz, V. Naik and M. Bauer, "Summertime cyclones over the Great Lakes Storm Track from 1860-2100: Variability, trends, and association with ozone pollution." http://www.ldeo.columbia.edu/~amfiore/publications/Turner2012_submitted.pdf) actually addresses this very issue using the Geophysical Fluid Dynamics Laboratory's CM3 Climate Model that also incorporates tropospheric and stratospheric chemistry. The authors found a similar decrease in the summertime mid-latitude cyclone (tracked by the Great Lakes Storm Track) frequency over the 21st century. But when they held the emissions of ozone precursors and aerosols and stratospheric ozone-depleting agents constant at the 2005 levels while allowing all other greenhouse-gas emissions to follow a moderate global warming scenario (with a representative concentration pathway leading to stabilization of radiative forcing at 4.5 W/m^2 by 2100), they found a small negative slope of -0.18 for the detrended number of summertime high-ozone events (defined as days with MDA8 greater than 102 ppb) per detrended number of summertime cyclones in the Northeastern US, and that as a result, the cyclone frequency can only explain no more than 10% of the high-ozone-day variability. The same conclusion was reached for other ozone concentration thresholds as well, including the 84 ppb used by Leibensperger, et al. This information is highly pertinent and ought to be included in the ISA provided that the paper is accepted for publication at the time of the final publication of the ISA.

In regard to the natural background emissions as global warming progresses, the ISA should include the information from a study on the potential impact of the frequency trend and the time trend of the first mid-latitude cyclone onset on the trend of areas burned by wild fires in the Northern Rockies. (See Knapp, P.A. and Soule, P.T.: "Trends in midlatitude cyclone frequency and occurrence during fire season in the Northern Rockies: 1900-2004." Geophys. Res. Lett. 34, L20707, doi:10.1029/2007GL031216, 2007) This study shows a significant decline in the frequency and a

progressively delayed first onset of mid-latitude cyclones in the 20th century especially since the mid-1980s, and that both conditions are significantly linked to an increase in the forest areas burned by wild fires in the Northern Rockies between August and October.

The Chapter does not address the rollback methodology. This is alright given that the methodology will be described in the REA. Also, the rollback methodology is not really part of the science but a tool that attempts to mimic realistic ozone concentration distributions as the ozone concentrations move toward attainment.

Two minor points:

(1) It would be helpful if the type of ozone concentrations plotted (8-h daily max ozone concentrations or MDA8) are indicated in the figure captions of all the correlation and COD figures. (Figs. 3-35 through 3-40 and Figs. 3-116 through 3-155)

(2) The new additions in the multiyear trends section are useful, especially with the trends for different regions of the country. The metric used for Figures 3-50 and 3-51 need to be indicated. Is it the median, or the mean, or what, of the observed annual 4th highest MDA8 concentrations over all the monitors of each of the regions for each year?

Comments on Chapter 4: Exposure to Ambient Ozone

In my view, this third revised version of Chapter 4 has addressed all the major comments provided by CASAC and is definitely an improvement over the second revised version. First, the authors have expanded the discussion on the spatial and temporal variability of ozone exposure. A new section titled "Exposure Duration" has been added to discuss how short-term exposure and long-term exposure can be linked to ambient ozone concentrations of corresponding durations. There was no discussion on the potential for confounding in epidemiological studies by co-pollutants in the long-term exposure. This may well be an area where further studies are needed.

The third revision no longer contains the paragraph that reveals a spurious impact of quadratic rollback that tends to squeeze the ozone concentration distributions excessively for areas with high ozone concentrations as the areas approach attainment of the ozone air quality standard.

Another improvement of the current version is the fact that maps showing monitor locations, observed ozone concentration levels and population distributions for Boston, Atlanta and Los Angeles are now included. Cross-referencing with other chapters of the ISA has also been improved.

In addition, Tables 4-1 through 4-3 have been revamped and improved upon. In Table 4-3, ratios and slopes are now distinguished and separated (Note: on line 5 of p. 4-15, "identified" should be changed to "identical."). Two new tables (Tables 4-4 and 4-5) have been added. Table 4-5 is especially relevant to dosimetry determination and is referred to in Chapter 5 (See line 34 of p. 5-16).

In my view, the Chapter is in excellent shape and there is no need for further improvement.

Dr. Ana Diez-Roux

Comments on Chapters 6-7 – Integrated Health Effects of Short- and Long-Term Ozone Exposure

Please comment on the extent to which there is sufficient clarity in the revised presentation of study designs and results. Please provide recommendations where the interpretation of the scientific evidence may be improved as well as comments on the soundness of conclusions in these chapters.

The chapters have been revised to address many of the concerns in the prior review. In general the evidence is comprehensively presented and conclusions are sound. As requested, information on effect modification has been incorporated in various sections although it may not always be entirely consistent with what is presented in chapter 8. (A relatively minor technical detail is that it is not clear why there are separate sections on effect modification and interaction in chapter 6 since they refer to the same concept.)

Based on the material presented, I understand why the decision was made to retain "suggestive of a causal relationship" for short term effects of ozone on cardiovascular risk. However, as noted in the Preamble, controlled human exposure studies demonstrating an effect is not a requirement for determining that the relation is causal. Toxicological evidence and epidemiologic mortality evidence support a likely causal relation. It thus appears that in this case the determination hinges on the importance that is given to mortality evidence (which is quite consistent) vs. morbidity evidence (which is somewhat inconsistent).

Comments on Chapter 8 – Populations Potentially at Increased Risk for Ozone-Related Health Effects

Please comment on the adequacy of these revisions to clarify the consideration of potential at-risk populations and recommend any revisions to improve the characterization of key findings and scientific conclusions.

The chapter is much improved by the new classification of factors and the implementation of a systematic way to synthesize the evidence regarding what factors place populations "at risk". The classification scheme for evidence is a major improvement.

However, the chapter continues to conflate factors that result in increased exposure with factors that modify the impact of exposure on the risk of an adverse health outcome (this is quite apparent in the introduction). Most of the discussion under the various categories focuses on effect modification. Perhaps noting any specific instances where the factor in question increases risk simply through increased exposure (rather than through interacting with exposure) might help resolve the issue.

Dr. William Michael Foster

Comments on Chapters 6-7 – Integrated Health Effects of Short- and Long-Term Ozone Exposure

Revisions made to Chapters 6 and 7 included increased integration of recent evidence with key findings from previous reviews, and further integration across chapters, particularly with information from Chapters 4 and 8. One key example of further integration across chapters is the expanded discussion of exposure assessment methods and measurement error issues with linkages to Chapter 4 and discussion of their potential influence on heterogeneity of results among studies.

Careful consideration was given to a CASAC recommendation that the causal determination for cardiovascular effects from short-term O_3 exposure be increased to "likely to be a causal relationship." There was strong toxicological evidence and consistent, positive associations between short-term O_3 exposure and cardiovascular mortality in epidemiologic studies. However, controlled human exposure studies were limited in number and provided inconsistent results. Likewise, epidemiologic studies showed inconsistent findings for cardiovascular morbidity (e.g., heart rhythm, physiological biomarkers, and hospital admissions or emergency department visits). Based on extensive review and discussion of the evidence the decision was made to retain the "suggestive of a causal relationship" conclusion for cardiovascular effects from short-term O_3 exposure.

Please comment on the extent to which there is sufficient clarity in the revised presentation of study designs and results. Please provide recommendations where the interpretation of the scientific evidence may be improved as well as comments on the soundness of conclusions in these chapters.

As in my perception of the initial and 2nd draft versions of external review draft for Chapter 6, this 3rd iteration of Chapter 6 is completely encompassing on many areas of controlled laboratory exposure studies to ozone. Chapter 6 has now been expanded and with more than sufficient clarity to now include 274 pages of text with a total of 54 Tables and 36 Figures, and 30 pages of listed references (approximately n=540 references). Enough is enough, and the integration and descriptions of the early literature with the more recent, is excellent.

Based upon the information presented in Chapter 6, I am satisfied with the description that induction of cardiovascular effects from short-term ozone exposure are "suggestive of a causal relationship" as is listed/proposed in the Overall Summary of Table 6-54 in the text of Chapter 6 (pg. 6-274).

For Chapter 7, focusing upon integrated health effects of long-term ozone exposure, the information is as well encompassing with respect to the toxicology data and the human epidemiologic finding to date on this topic. As more data become available, sections that emphasize epi-genetic and central neural effects will likely be developed and/or become more influential to policy on acceptable levels of risk from ozone exposure to individuals (pregnant woman, infants and young children).

Comment on Preamble

Suggested edit to text from Preamble Section, pg. lxiii, li 24 – 37; pg. lxiv, li 1-4:

"… However, controlled human exposure studies by experimental design are structured to address specific questions. For example, characterization of bio-molecular and physiologic outcomes in response to a uniform concentration range of a specific ambient air pollutant and/or combination of air pollutants and with strict adherence to exposure conditions and montoring of host outcomes. The high precision necessary for exposure conditions and effect/response characterizations cannot be accomplished using an epidemiologioc-based experimental design. Although epidemiologic studies are of the highest relevance for associating human health risk to an environmental exposure, these studies lack the precision of lab-based controlled human exposure studies. Although some controlled human exposure studies have included health-compromised individuals such as those with respiratory or cardiovascular disease, these individuals may also be relatively healthy and may not represent the most sensitive individuals in the population. Thus, observing an effect in controlled human exposure studies does not necessarily mean that a direct causal relationship is present; however essential dose-response profiles and ranges of response severity can be established with these studies.…"

Additional References on Cardiovascular Effects

Additional publication references (recent) that should receive consideration on the issue of ozone being causal to cardiovascular adverse effects. These references in addition to the RB Devlin and co-authors pub (Circulation 2012; 126:104-11) for which many of the CASAC panel are now familiar, also have some bearing on the topic of causality of ozone and CV effects. These publications relate to investigations or human based studies within controlled lab settings or epidemiologic based approaches and one of the publications gives consideration to at risk individuals with co-morbidities and genetic susceptibility.

Hampel, R., Breitner, S., Zareba, W., Kraus, U., Pitz, M., Geruschkat, U., Belcredi, P., Peters, A., Schneider, A., and for the Cooperative Health Research in the Region of Augsburg KORA Study Group (2011). Immediate ozone effects on heart rate and repolarisation parameters in potentially susceptible individuals. *Occupational and Environmental Medicine*, 69: 428-36.

Hampel, R., Breitner, S., Schneider, A., Zareba, W., Kraus, U., Cyrys, J., Geruschkat, U., Belcredi, P., Müller, M., Wichmann, and Peters, A. (2012). Acute air pollution effects on heart rate variability are modified by SNPs involved in cardiac rhythm in individuals with diabetes or impaired glucose tolerance. *Environmental Research*, 112:177-185.

Sivagangabalan, G., Spears, D., Masse, S., Urch, B., Brook, R. D., Silverman, F., Gold, D. R., Lukic, K. Z., Speck, M., Kusha, M., Farid, T., Poku, K., Shi, E., Floras, J., and Nanthakumar, K. (2011). The effect of air pollution on spatial dispersion of myocardial repolarization in healthy human volunteers. *Journal of the American College of Cardiology*, 57(2):198-206.

The Sivagangabalan et al. (2011) study utilized a controlled laboratory setting and healthy subjects (sample size of 25) and a combination of exposures (including ambient particulate, ozone, combination of ambient particulate and ozone, and filtered air) and found the combination of ozone with ambient particulate altered dispersion of ventricular repolarization. The Hampel et al. (2011) study utilized an

ambient air pollution exposoure design with electrocardiogram (ECG) data collected on an hourly basis in patients (metabolic disorders) and healthy subjects (sample sizes of 64 and 46, respectively) found immediate ozone effects on heart rate and repolarization.

Dr. H. Christopher Frey

Comments on Preamble, Legislative and Historical Background

Please review and comment on the effectiveness of these revisions. Please comment on the extent to which these sections of the ISA provide a useful and effective format for presenting introductory materials for this and future ISAs. Please recommend any revisions that may further improve the clarity of discussion.

Preamble

The Preamble is generally well-written and has an appropriate scope.

Figure I is not entirely accurate with respect to the interaction between the ISA and the REA. The ISA is meant to inform the REA. However, there is no connection or linkage shown between the ISA and REA. Instead, they are depicted in the diagram as if they are independent and parallel steps in the review process. The diagram is also not internally consistent in how it depicts decision nodes. For example, 'agency decision making and draft proposed notice' is a decision making step, but a decision node is not used. The diagram has two boxes for CASAC but there is only one CASAC. For these reasons, the diagram should be redrawn.

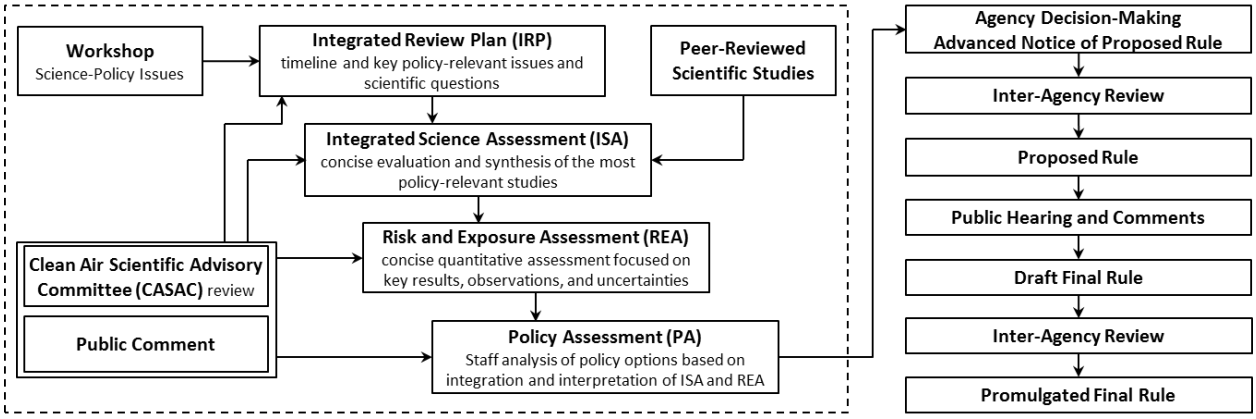

Figure. An Alternative Depiction of the Key Steps in the Review of National Ambient Air Quality Standards.

An example is given here that takes into account the following considerations:

- The IRP, ISA, REA, and PA are sequential. Although there is some overlap in the timing of drafts the latter three, they are ultimately meant to build upon precursor documents. This order is important and should be explicitly depicted.
- CASAC review and Public Comment can occur concurrently, but they are not exactly the same thing. CASAC review is required under statute by an advisory body. Public Comment is separately required under statute. Thus, they merit at least their own boxes. However, the concurrency of these activities can also be depicted, as illustrated in this example.
- For simplicity, decision nodes are not used here, although the diagram could be modified to include them. Administrator decisions would occur at least four times, however: at preparation of

the advanced notice of proposed rulemaking, at the time of the proposed rule, at the time of the draft final rule, and at the time of the final rule.

- Inter-agency review is shown as per the original Figure I. However, for clarity, the diagram could also indicate at what point(s) do OMB review take place? Also, is a Regulatory Impact Assessment (RIA) required as input to OMB review? This should also be shown.
- The parts of the planning process and the sequence of the IRP, ISA, REA, and PA do not involve the EPA Administrator and rather are based on activities of EPA staff and interactions with CASAC and the public. Thus, these activities are qualitatively different from the decision actions that involve the Administrator. For this reason, the former are grouped within a large box on the left and center of the draft diagram. The sequence of boxes on the right side involve decisions of the Administrator. The latter could be made more explicit with notations in the diagram.

Page lv, lines 12-13: are references within HERO classified as implied here (screened, considered, included)? Does HERO enable searching for references based on the NAAQS pollutants for which they were used?

Page lviii, line 37: introduce the figure at the beginning of this paragraph, and make sure that the text and figure are consistent.

Page lix, lines 5-6: the term 'integration' is used, but it is not clearly defined or explained. Here, "as an example," a possible type of integration is given. However, are there other types of integration that are a goal of this document? It is not clear that integration goes beyond the 'example' given here. The intended meaning appears to be that evidence regarding causality for health effects was integrated across different types of information, including epidemiologic, toxicologic, and controlled human exposure studies. Furthermore, integration is considered with respect to interactions among effects. The notion of integration may merit at least a few sentences if not a paragraph. This also applies to the executive summary and especially the integrative chapter 2.

Page lx: Figure III has the term "Evergreen Literature Search". This term is not defined in the text, and it is not clear what it means. Suggest using another term and defining/explaining it in the text, so that the text and the figure are consistent. Also, the Figure uses the term "Peer Input." Please explain in the text what this means. Who are the Peers? Are they internal or external to EPA? How are they selected and by whom?

Page lxi, section on "EPA Framework for Causal Determination." Is "integration a key or inherent part of this framework? It seems to be. It would be useful to make a statement about this here.

Page lxii, lines 11-12: delete "In estimating the … it is recognized that" – this will not result in any loss of content and will make the point more clear – i.e. it is simply the case that "Scientific findings incorporate uncertainty."

Page lxii, line 27… quantitative analysis approaches go beyond meta-analysis. Such approaches could be broadly described as being based upon 'statistical inference' or 'expert elicitation.' These two categories could be added to the example of meta-analysis.

Page lxii, lines 30-33: minor comment – break the long sentence into separate sentences at "Publication bias can [delete 'also'] result in …" substantive comment: some explanation would be helpful – e.g.,

"because of unaccounted for factors that lead to inter-city variability, such as housing type or activity patterns" at end of last sentence. Otherwise, the text leaves the reader with the impression that effects estimates for single cities are larger than for multiple cities as a general rule, which really isn't the case.

Page lxiii, line 4: this is another location where the notion of 'integration' seems to apply and should be mentioned.

Page lxiv, line 3: insert "for some effects endpoints" after "in epidemiologic studies"

Page lxiv, lines 9-12: although the categories of epidemiologic studies are well-known to some experts, they may not be well-known to all readers. It would help to include a sentence or two explaining each of these categories.

Page lxv, lines 4-6: this statement is not very clear. Part of the lack of clarity is that it is not clear as to what is meant by 'independently observed associations for multiple pollutants." Suggest having more than one sentence, with each sentence being clearer. For example:

- One pollutant might be associated with or causal for more than one type of adverse effect. Some studies might evaluate a pollutant without considering other pollutants: in such cases, multiple studies conducted independently of each other might attribute different health effects to the same pollutant, without identifying the full range of health effects associated with that pollutant.
- Two or more pollutants might be associated with or causal for a particular type of adverse effect. Thus, different studies conducted independently of each other might identify only a subset of the relevant pollutants as being associated with a particular affect.

Page lxv, lines 20-25: this was difficult to read because it wasn't clear from the formatting that this quote would go on for five lines. Having a long quote in a technical document is generally awkward. Either paraphrase, or format the long quote with a line return, italics, and reduction of side margins by ½ inch.

Page lxvi, line 12: 'exposure misclassification' should be defined or explained. The text should be clear as to whether this is the same as exposure error, which is used earlier in the same paragraph, or is it a subset of exposure error.

Page lxvii, line 4: seems to be missing "may be" after 'smaller differences'

Page lxvii, line 30: what is an 'exposure dose'? Exposure and dose are not really the same thing.

Page lxviii: the aspects of the Aspects that involve integration should be noted. For example, assessment of "coherence" requires integration, as does 'analogy'. As a minor comment, there should be column headers in this table, such as "Aspect" and "Description"

Page lxix, line 5: "objective" – my working definition of 'objective' is that different people using the same methods should get the same answer (i.e., it is reproducible). It is not clear to me that different scientists who use the Aspects in Table I would get the same answer for causality determination. The method is very qualitative and thus seems to be inherently subjective. On the other hand, even if not all scientists or teams of scientists would arrive at the same answer, it is possible to document the information used and inferences made sufficiently so that the rationale for a particular finding is

explained… this relates to the notion of 'transparency.' Thus, the causality framework can be subjective and transparent, which seems to be a more accurate description than to claim it is objective.

Page lxix, lines 27-28: integration is mentioned here- referring to integration across disciplines (presuming that this refers to epidemiology, toxicology, and clinical disciplines?) and endpoints. This and other examples of integration should be mentioned upfront when integration is first defined.

Table II: some have commented before, perhaps in other reviews of other pollutants, that the categories seem a bit unbalanced: i.e. there is a not a category for 'not causal.' An argument could be made that such a category is not useful nor possible – e.g., one cannot prove a negative in scientific hypothesis testing, but only show through repeated experiments that no effect was observed. It would be useful to have a brief discussion of this, to preventively fend off (unfounded) accusations of bias in the categories.

Page lxxii, line 18 "use various methods" is vague. Earlier, multi-variable statistical analysis is mentioned as the typical method… does this text mean to imply some other methods? Or does this have to do with design of the epi. study?

Page lxxii, line 30: this is a sufficiently important point that a reference should be cited here, for linearization of the C-R function and inability to identify a threshold.

Page lxxiv, line 5: "estimated" seems to be better than "available" here. Seems like a goal rather than an all encompassing accomplishment. Also, is there a key reference that can be cited for this paragraph?

Page lxxiv, line 15, 16: should be questions, not sentences.

Page lxxvi – would it be worth stating something about lack of sustainability of ecosystem services as an adverse effect?

Legislative and Historical Background

The Legislative and Historical Background is well written and the scope is appropriate, but tells only part of the story. The historical review seems to be carefully written to state some facts about each review process, when EPA promulgated a new standard, and the outcome of any relevant court decisions along the way. As such, it does not make statements regarding the claims of the lawsuits mentioned, only the outcomes of court decisions. It also does not indicate the role of CASAC except that as part of the reconsideration of the 2008 rule, CASAC was consulted. The review does not describe the extent to which CASAC advice was not fully considered in setting the 2008 standard, nor the results of the CASAC advice regarding reconsideration of the rule. This is pointed out as an observation. The results of the CASAC review are a matter of public record: in this regard, it doesn't matter whether they are described here. However, a reader not familiar with this topic would not get much from this review.

Comments on Chapters 1 and 2 (Executive Summary)

Please comment on the adequacy of these and other changes to the chapters and recommend any revisions to improve the discussion of key information. Please recommend any revisions that may further improve the clarity of discussion.

Chapter 1 (Executive Summary)

The executive summary is much better written than in the 2nd draft of the ISA. The comments here are relatively minor.

Page 1-1, lines 3-4: The relationship between the ISA and REA is not adequately depicted in Figure I, as noted in earlier comments.

Page 1-3, lines 1-2: something is wrong with this sentence – it needs to be proofread and rewritten. Probably 'resulting in' and 'that' need to be deleted. Is it really the case that concentrations in rural areas are 'often' higher than in urban areas? Is this with respect to geographic area or exposed population?

Page 1-5, Table 1-1: it is essential to have more clarity as to the definitions of the health outcomes and whether they overlap. In particular, health outcomes are listed for respiratory effects, cardiovascular effects, and central nervous system effects, followed by 'total mortality.' Are these first three limited solely to morbidity within the effect category, or do they include mortality? Does "total mortality" include mortality from 'respiratory effects"? The table should be documented to make extremely clear as whether mortality is included in all effects categories, or only the total mortality categories. If the former, then the conclusions for the first three effects categories should clearly address both morbidity and mortality. If the latter, than the conclusions for total mortality should address the contributions from each of the first three categories, plus any other relevant categories of health outcomes.

Specific comments on Table 1-1:

Short-term

- respiratory effects: Mortality is mentioned on pages 2-21 and 2-24. Thus, does the causal relationship here include that for mortality, or it just for morbidity?
- For cardiovascular effects, the conclusions seem clear that this is only for morbidity in the previous review. Same for the current review?
- For CNS effects, the text is not entirely clear – is this related only to morbidity for the previous review? Current review?
- For Total Mortality, the text mentions 'non-accidental' mortality – but this could be anything other than an accident. Does this include respiratory, CVD, and CNS related causes? 'cardiopulmonary-related mortality' is mentioned – what about respiratory-related mortality, as mentioned later in the ISA?
- If there is a 'causal relationship' for respiratory effects, and if these include mortality, then why is the conclusion for total mortality only 'likely to be a causal relationship'?

Page 1-6, line 34: 'indicators' seems better than 'measures' here.

Page 1-8, Table 1-2: the last review was in 2008, or 1996? Explain why conclusions from 1996 are given here, and why there are no conclusions from the 2008 review.

Page 1-11, line 11: "the W126 metric" is written as if the reader should already know what this is. What is it? - e.g., "W126 is a weighting scheme for combining ambient O3 concentrations…"

Page 1-11, 18 "It is important to note that…" delete this phrase.

Page 1-12, lines 9-17. There seem to be two relevant questions here, and it would help to address each separately in the text:

1. Is there a causal relationship between changes in tropospheric O_3 concentration and effects on climate?
2. Can the magnitude of the effects be characterized with confidence?

An answer of no to the second question does not preclude a finding of causality. As noted in Table I on page lxviii, 'a small magnitude of an effect estimate may represent a substantial effect in a population."

As written, the discussion in this paragraph implies that there is uncertainty as to the extent of the effects, but it does not clearly state that there is uncertainty as to whether an effect will occur – e.g., is there uncertainty regarding the sign of the change in surface temperature? If, however, the issue is that surface temperature is not a sufficient indicator of adverse effect, that should also be stated.

Page 1-12, lines 28-30: the finding should be in bold.

Page 1-13: Table 1-3, for clarity, give a reference to the previous review (2008 review?).

<u>Chapter 2: Integrative Summary</u>

Page 2-1: the explanation of 'integration' here seems a bit more clear than in the preamble.

Page 2-3, lines 16-36: This paragraph goes into far more detail than the other text in this section. It seems out of place here, and might be moved elsewhere in this chapter.

Page 2-3, lines 26-27: run-on sentence.

Page 2-5, lines 19-20: "processing on cloud and aerosol particles." The term 'processing' as used here is jargon with a particular community, but seems out of place here. The intended meaning seems to be something like 'physical transformations of'…

Page 2-6, Figure 2-1: personally, I don't think this figure is very effective. Visually, it puts a lot of reader focus on the stratosphere (not really a focus of this document) and very little focus on the things that matter the most to this document, which are cramped into the middle/lower right side. The key message to convey to the reader is the following:

$NO_2 + hv \rightarrow NO + O$
$O + O_2 \rightarrow O_3$
VOCs degrade to produce free radicals such as HO_2*

$NO + HO_2^* \rightarrow NO_2 + OH^*$
(most NO_x is emitted as NO)
The OH* can attack VOCs, leading to more VOC degradation, producing HO_2^* which oxidized NO to NO2 which leads to photodisocciation of NO_2 and formation of O_3. VOC's can also undergo photo-dissociation in some cases, also producing HO2* as a byproduct.
Also,
$CO + HO_2^* \rightarrow CO_2 + OH^*$
The OH* from oxidation of CO can attack VOCs, thereby producing more HO_2^* and leading to more O_3 formation.

Page 2-6, line 2-3: "Similar basic process…" similar to what? (each other?). Is CO oxidized to CO2 by photochemistry? Not clear that this is really 'similar.'? (e.g., CO + HO2 → CO2 + OH is not a photochemical reaction). CO is mentioned in the text but not shown in the diagram.

Page 2-6, line 7 replace 'for' with 'precursors to'

Page 2-7: it would help to have the brief description of monitor networks moved from section 2.2.3 to 2.2.2. This would provide essential background for the reader when CASTNET is mentioned in the section on atmospheric modeling. In the current version, CASTNET is mentioned in section 2.2.2 but is not explained until section 2.2.3.
Page 2-8, lines 10-21: for clarity, I think the intended meaning is (~50km x ~50 km)… i.e. the distances are variable in both directions. "The R2 for both models are…" is not very clear. R2 compared to what? Is this a comparison to CASTNET data? Table 3-1 is references, but is exactly the same as Table 2-1 given on this same page, and neither has any documentation of R2.

Page 2-8, Table 2-1:

- MDA8 is undefined in the table header. Please spell it out or define in a footnote.
- CASTNET is not explained. What is this? (explain in a footnote)
- There is no explanation in the column headers for the model results as to why two sets of values are given in each table cell. There is a footnote that attempts to explain this, but the footnote is not cited in the table. Tables should be documented so that they are clear to the reader. E.g, there could be a 'super' header over all data columns that clearly states "Ozone Concentration (ppb) ± One Standard Deviation (ppb)"
- What exactly is the meaning of the standard deviation? i.e. what is the sample from which it is calculated? Does it represent spatial variability, temporal variability, or both?
- The units should be clearly indicated in the table header(s), not relegated to a footnote.
- Number of monitor sites could be a column rather than a parenthesis in the region name.
- The table caption and the content don't match. For each site, there should be one row for model predictions that include North American anthropogenic precursors. There should be a second row for the estimated North American background concentration. To which category does the CASTNET data belong?
- It would help to have a summary in the text of the main quantitatively findings. i.e. the estimated NA background concentration ranges from 24 to 42 ppb based on GEOS-Chem, and 27 to 42 ppb based on GEOS-Chem/CAMx, depending on region and season. The implication (which is important later when reviewing various epidemiologic studies) is that it is rare to have ambient concentrations less than about 40 ppb, and rarer still that they are down to 20 ppb.

Page 2-11, lines 29-31: the summary here is confusing in that the reported three year averages across the U.S. appear to be at or below background levels indicated in Table 2-1. This could be in part an artifact of reporting median values here compared to mean values on page 2-8. Some U.S. sites may have low O3 concentrations – are these summaries weighted by population? If not, then perhaps too much weight is given to sites representing few people that happen to have low O3 concentrations. Some discussion of the numbers here compared to the NA background estimates is needed. Alternatively, it may be better to report mean values and ranges (e.g., 95% frequency range) than just median values.

Page 2-12, lines 7-9: what "uncertainties" should be "considered"? This is vague.

Page 2-12, line 18: "are processed" is shop jargon among air quality modelers, but the intended meaning here seems to be 'transformed' or 'that react and transform'…

Page 2-12, lines 22-23: 'chemical scavenging' may not be understood by all readers. On p. 2-6, this could be explained.

Page 2-13, lines 8-14: the text here is awkward and does not seem internally consistent. "correlations… exhibit negative correlations…" is awkward. If correlations are 'modest at best,' why is there a definite 'need to take' them into consideration? In any case, is this statement really needed in the integrative overview?

Page 2-5, lines 2-3: this point could be explained with the following ideas: there is definitely a bias in the concentration-response relationship compared to the true but unknown exposure-response relationship, since exposure concentrations are lower than ambient concentrations, on average. However, if the ratio of exposure to concentration is approximately constant with respect to time, then epidemiologic models can appropriately determine if there is covariation in the health effect response compare to variation in ambient concentration. The strength of the statistical covariation in R and C is the same as that for E and C if there is a constant ration between E and C.

Page 2-15, lines 13-14: since there is not a very clear delineation of exposure methods earlier in this section, it is not clear as to the basis of this concluding statement. The impression is that only ambient concentration is used as a surrogate of exposure in epi studies. What other 'methods of exposure assessment' were used?

Page 2-20, lines 12-16: does this causality determination apply only to morbidity or does it also include mortality? See also page 2-20, lines 37-39; page 2-12, lines 15-16

Page 2-21, lines 34-35: based on the prior sentence, this conclusion appears to include respiratory mortality. Not clear, however, if the conclusion for respiratory mortality on previous page was 'likely to be causal.' How could the weight of evidence for total mortality be less than that for constituent causes of mortality?

Page 2-31: someone should proofread this – the first full paragraph here seems repetitive of previous material.

Page 2-32, - it would help to have an intro paragraph that explains why the topics that follow are deemed to be policy relevant.

Page 2-32, line 5: “reviewed” rather than “conducted”

Page 2-33, lines 10.5 to 15: Section 2.5.4.2 appears to have implications for the form of the standard. This could be mentioned. “Some [short-term?] epidemiologic…”? “conducted [comparative?] analysis…”? This first paragraph is not very clear. Why not start by listing the exposure metrics, then state that multiple metrics were compared in some short term studies, and then state that in long-term studies, only one metric was used per study and thus comparisons are not available. This would be more clear.

Related to this section, is there any quantification of the correlation between the metrics? What factors should be considered when comparing metrics? Is it only epidemiologic studies that should be considered? Because this section ends here, the reader is left with the impression that it doesn’t matter what metric issued. This seems incomplete.

Page 2-35, section 2.5.4.4. Given that NA background is estimated at approximately 25-40 ppb, it is unlikely that any epi study would have data from which to evaluate C-R below this range. This should be explained. Several times statements are made about ‘low density of data’ or ‘sparse data’ at the low end of the concentration range, but such situations cannot be found (or very rarely) based on ambient air.

Page 2-36, lines 10-19: delete ‘it should be noted that’. The discussion here could be better or more clear. There are differences in the average ratio of exposure to concentration when comparing cities because of factors such as… why not just take these factors into account when comparing cities? “A national or combined analysis may not be appropriate…” if these factors are not taken into account.

Page 2-37, line 1…. Such as activity patterns, housing type and age distribution, prevalence and use of air conditioning, [etc.].

Page 2-37, lines 14-16: given the 2008 economic downturn, the issue of unemployment is in the news every day. The finding here, however, does not mean that someone who just lost their job is suddenly subject to higher risk from exposure to ozone. Some discussion of the basis and limitations of these relationships would be helpful.

Page 2-37, lines 18-20: A/C usage is not the only influential factor.

Page 2-38, lines 1-13: this paragraph was very nice.

Page 2-50, lines 15-16: see earlier comment on this topic.

Page 2-51: section 2-8. This section doesn’t really add much to the chapter. Table 2-4 is a shorter version of Table 2-2, which is useful, but it is not essential. Table 2-5 seems to be the same as Table 2-3. Table 2-6 could be in the previous section.

Dr. David A. Grantz

Comments on Chapter 9

The revised chapter provides a very thorough review of the many fields of investigation that go into welfare effects on vegetation. The Agency has addressed many of CASAC's suggestions in a very helpful way.

It is important that the chapter reaffirms the validity of the Open Top Chamber studies using newer chamberless designs. It is important that the exposure response relationships, between yields or biomass productivity and concentration-based exposure metrics, are reaffirmed by comparison with these newer studies, and used in the chapter to quantify ozone impacts on vegetation.

The chapter does a nice job of introducing and dismissing for the moment the use of flux-based metrics. It is clear that flux is the biologically relevant ozone parameter, but as the chapter notes, the complexities of application remain too high for its current use in a regulatory context.

The revised discussion of ozone impacts on root growth and carbon allocation below ground is balanced and useful.

There remain only a few weak spots that might be considered:

Major concerns:

The Agency has stated the intention to address some weaknesses in the treatment of water relations, but these remain incomplete in this version of the chapter. For example, on page 9-47, it is stated that ozone may "disrupt whole-tree water use", which to me implies a reduction. But the next sentence assumes that tree water use is increased. This is an example of a larger problem in fully addressing the bidirectional responses of stomata to ozone.

The revisions have emphasized sluggish stomatal responses to dynamic environments, and that these may result in reduced closure (i.e. increased conductance relative to controls) at night. This may explain the often cited data of McLaughlin on stream flows (page 9-75), along with similar effects in daylight. However, more recently there are indications that stomata may actually open in response to some ozone exposures that include relatively brief pulse exposures (e.g. Paoletti and Grulke, 2010, already cited). This observation is briefly considered in the context of prolonged exposures on page 9-96, but should inform the stomatal discussion throughout the chapter:

page 9-13 last paragraph;
page 9-15 top;
page 9-30 at beginning of section 9.3.5.1;
page 9-37 middle paragraph;
Figure 9-7 on page 9-73;
page 9-74 end of first paragraph;
page 9-75 as an alternative to sluggishness in explaining McLaughlin's data.

The treatment of hydraulic conductance (page 9-74, bottom) is still not adequate as written. Leaf area specific hydraulic conductance must increase if leaf area declines after sap wood is laid down. This is algebraically mandated. Increased hydraulic conductance may lead to increased stomatal conductance which leads to increased tree and stand water use; but hydraulic conductance itself cannot increase or maintain stand water use. Sluggish stomatal conductance cannot have any bearing on hydraulic conductance. The suggestion that the proportion of leaves in the sun could maintain or increase water use is not supportable. Total sunlit leaf area would not likely change, although the stomatal conductance of these remaining leaves is likely to increase (again with increased leaf area specific hydraulic conductance), thereby potentially maintaining stand water use. The Uddling papers that are cited in this section do not appear to support any of these arguments, except that water use in the stand was not diminished by ozone.

Other concerns:

Figure 9-1 remains fairly weak but important. The top section, O_3 Uptake and Physiology, does not really address ozone uptake, despite the label. It should show a schematic of boundary layer and stomatal resistances. All of the elements under this heading more properly belong under the heading below, Effects on Leaves. The reference to Figure 9-2 in the top section is not necessary and somewhat misleading as 9-2 shows anatomy rather than process. The figure caption might expand upon the meaning of the cryptic blue bar Differential Sensitivity, to explain that mechanisms in each of the component pathways might factor into resistance.

Figure 9-2 might be more useful if it appeared closer to 9-1. It might be renamed "anatomy" instead of microarchitecture which implies something to do with membranes and organelles. The meaning of the double arrow coming off O_3 at the bottom (with the question mark) is unclear.

In a few places the significance of a section of text is masked by reference to its further consideration elsewhere. For example, a conclusion might be added to the next to last paragraph and last paragraph of section 9.2.6 (why is Morgan's 8% important, what did the comparison of FACE and OTC show) rather than simply reference sections 9.2.5 and 9.6.3.

As mentioned in reviews of both previous drafts of this chapter, there is little evidence that a plant has any ability to sense or detect the presence of ozone (9.3.1 and elsewhere), at least not in the sense that an insect detects a pheromone or even that a plant can detect jasmonate vapor. The response to oxidant challenge is quite non-specific.

The chapter makes the strong point that recent proteomics support very strongly the earlier conclusions from transcription (top of page 9-12). It may not be necessary to explain all the possible problems that might have, but did not, confound this interpretation. The conclusion could be simply, and more powerfully, stated in a single sentence.
Among the possible controls on ozone uptake (fifth line, 9-13), is destruction of ozone by reaction with volatile organics in the gas phase outside the leaf (e.g. Fares et al., 2010; Journal of Experimental Botany, 61, 629-633; not yet cited). The importance of this is not yet demonstrated, but it is potentially important.

Figure 9-5: the legend might be expanded to define some of the acronyms in the figure (TF, PR etc.).

The summary in 9.4.4.3 (page 9-67) refers to “very different mechanisms” for acute and chronic exposures. However, it is not clear that this summarizes previous discussion of this subject.

Comments on Chapter 10

This chapter does a nice job of laying out complex material that is often at an early stage of understanding. If necessary some condensation could be achieved by reference to Chapter 3, but as a stand-alone chapter it is quite informative.

A possible area of expansion (10.4.5) would be to make a first attempt to attribute impacts on vegetation due to UVB to changes in tropospheric ozone. The existing final paragraph of this section is adequate to call for further research, but a semi-quantitative concluding analysis could be attempted, along the lines of studies cited in the preceding two paragraphs and using ecosystem studies cited in 10.4.4. Although a comprehensive review of UVB effects on plants is outside the scope of the Ozone ISA, these effects might receive a bit more attention in this section, to lay the ground work for the later 10.4.5 which should be a central piece of the chapter.

The discussion (10.3.3.5) of a potential feedforward mechanism involving the biological carbon cycle, with reference to Chapter 9, is useful. However, other changes caused by tropospheric ozone and mediated by altered radiative forcing may occur. For example, a subject that is not discussed in Chapter9 or Chapter 10 is the effect of increasing temperature due to tropospheric ozone on biodiversity, plant species migration, and consequent impacts on albedo.

Overall, the chapter is very nicely constructed.

Dr. Jack Harkema

Comments on Chapter 5

Please comment on the extent to which these revisions help to provide the underlying mechanistic and dosimetric information for interpretation of health effects evidence in later chapters and recommend any revisions to improve the discussion of key information.

The author(s) has done an excellent job revising this chapter and responding to the panelists' suggestions from the last review. Overall the chapter is well organized and well written. The revisions markedly improve this chapter and the revised chapter now provides a clear and detailed overview of the dosimetric and mechanistic information that is pertinant to the current understanding of ozone-induced health effects discussed in subsequent chapters. This chapter, now nicely sets the stage for the Health Effects chapters that follow. I have only a few additional minor comments and suggestions.

Page 5-2. Introduction and Fig. 5-1 are well done.

Page 5-3. Fig. 5-2. The *extrathoracic region* should be relabeled as the *Nasopharyngeal and Laryngeal Region*, to better represent the RT anatomy, and this change should be appropriately reflected in the text. See if this figure has been revised in a more current ICRP.

Page 5-9-10 5.2.2.2. The nasal passages are also a target site for both dosimetry and toxicity in both humans and laboratory animals (rodents and nonhuman primates). This should be mentioned somewhere in this section, to set the stage for the next section 5.2.2.3 URT ozone removal and dose.

Page 5-10. 5.2.2.3. This section is well written but some mention of the direct toxic effect on the URT (e.g., nasal toxicity) should be made to set the stage for the adverse nasal responses (e.g., rhinitis, epithelial remodeling, decrease in mucociliary fuction) described later in this chapter and elsewhere in the health effects portions of the ISA. In addition, the discussion on the uptake of ozone in the URT gives the impression that uptake is homogeneous throughout the nasopharyngeal passages. Laboratory animal studies using computational fluid dynamic estimates indicate that uptake is site specific in the URT, like it is in the LRT, and this correlates well with the location of nasal pathology caused by ozone exposures in laboratory animals and human subjects living in high ambient ozone regions (e.g., Mexico City). See papers by Carey SA et al. 1) Persistent rhinitis and epithelial remodeling induced by cyclic ozone exposure in the nasal airways of infant monkeys. Am J Physiol Lung Cell Mol Physiol. 2011 Feb;300(2):L242-54 and 2) Three-dimensional mapping of ozone-induced injury in the nasal airways of monkeys using magnetic resonance imaging and morphometric techniques. Toxicol Pathol.2007 Jan;35(1):27-40.

Page 5-14-18. 5.2.2.5. The mode of breathing in light of physical activity (5.2.2.7) is an important factor of ozone uptake in all regions of the RT – URT and LRT. The nasal contribution to breathing with exercise is influenced by race and gender (5.2.2.6) – See paper by Bennett et al. J. Appl. Physiol. 95: 497-503, 2003. Effect of exercise on nasal uptake of ozone in healthy human adults is documented in the paper by Sawyer et al. J. Appl. Physiol. 102:1380-1386, 2007.

Page 5-19. 5.2.2.8. Some summary of URT dosimetry should be made in this concluding section.

5.2.2.30. Good review of ELF and its importance in RT exposure to ozone.

Fig. 5-7. Very good figure.

Page 5-34. Line 24. Ozone-induced rhinitis characterized by an influx of neutrophils in the nasal mucosa has been demonstrated in several laboratory animal studies (e.g., rodents and nonhuman primates) as well as in humans subjected to controlled ozone exposures.

Page 5-34-39. 5.3.3. Since several older studies (e.g. 1990's) are being referenced in this section on the initiation of inflammation, some mention should be made of the results of acute and subchronic studies of nonhuman primates conducted at UCDavis where investigators morphometrically determined the severity of the neutrophilic inflammatory response in the URT and LRT at much lower airborne concentrations (e.g., 0.15) than that commonly used in mice or rats (see Harkema JR, Plopper CG, Hyde DM, et al., Am J Pathol. 1993 Sep;143(3):857-66 and Am J Pathol. 1993 Sep;143(3):857-66). These studies are cited later in the chapter but under a different context (see 5.4.2.5. *Attenuation of Responses*).

In addition, the persistence of URT inflammation associated with epithelial injury and changes in antioxidants have been recently reported in infant monkeys exposed to ozone. This should be cited here or elsewhere in the ISA (see Carey SA et al. Am J Physiol Lung Cell Mol Physiol. 2011 Feb;300(2):L242-54.).

Pages 5-49-50 5.3.7. In this section on airway remodeling, the epithelial hyperplasia and metaplasia in URT and LRT of monkeys exposed to 0.15 and 0.30 ppm ozone should be cited in this section as well (see Harkema JR, Plopper CG, Hyde DM, et al., Am J Pathol. 1993 Sep;143(3):857-66 and Am J Pathol. 1993 Sep;143(3):857-66). These studies are cited later in the chapter but under a different context (see 5.4.2.5. *Attenuation of Responses*).

Page 5-50-52. 5.3.8. In this section on systemic inflammation, some mention of the recent controlled exposure study by Devlin RB et al. Circulation. 2012 Jul 3;126(1):104-11 should be made here or elsewhere in the ISA.

Page 5-53. Fig. 5-8. Good summary figure for this section.

Page 5-56-57. Well written revisions in *Inter-individual Variability in Response*.

Page 5-75. Fig.5-9. Nice summary figure.

Page 5-84-85. Nice overall summary for his chapter, but again URT dosimetry and toxicity is not mentioned. A brief summary statement in this regard is needed.

Comments on Chapter 8

Please comment on the adequacy of these revisions to clarify the consideration of potential at risk populations and recommend any revisions to improve the characterization of key findings and scientific conclusions.

The authors have done an excellent job revising this chapter and responding to the panelists' suggestions from the last review. They have developed standard terminology and a new classification system for the evidence for potential at-risk factors. This provides a clear and useful method of evaluation for determining the weight of evidence for increased risk of ozone-related health effects for each potential at-risk factor or subpopulation. This has markedly improved the chapter. The new or revised tables also nicely summarize and clarify the text. Using the term "at-risk" as the all-encompassing term used for groups with specific factors that increase the risk of ozone-related health effects seems appropriate and is well explained in the introduction. Below are a few minor comments and suggestions.

Page 8-3. Introduction and Table 8-1 is well done.

Page 8-3-9. In general, studies cited in 8.1 (Genetic Factors) are approriate and are clearly and concisely described. There is, however, a lack of consistency in documenting ozone exposure metrics in both the epidemiological and controlled human/animal exposure studies. Such details for each key study could be handled in the tables (see comment below).

Table 8-2 This is a clear and concise summary of the key findings of recent studies examining a variety of gene variants as risk factors for enhanced ozone-induced health effects. The authors should consider adding a column for the exposure metric(s) used in each study. For example, the ozone concentrations and exposure regimens used for each controlled human exposure study could be stated here, rather than in the text. A similar table for the laboratory animal studies could also be developed.

Page 8-9. 8.2 Pre-existing disease/conditions. The selected disease/conditions are all appropriate. Some studies on air pollution and cystic fibrosis, however, are not included. I would suggest that the authors consider citing the following studies in the appropriate subsections.

Cystic Fibrosis:

1. Jassal MS, Yu AM, Bhatia R, Keens TG, Davidson Ward SL. Effect of residential proximity to major roadways on cystic fibrosis exacerbations. Int J Environ Health Res. 2012 Jul 27. [Epub ahead of print] PubMed PMID: 22838501.

2. Kamdar O, Le W, Zhang J, Ghio AJ, Rosen GD, Upadhyay D. Air pollution induces enhanced mitochondrial oxidative stress in cystic fibrosis airway epithelium. FEBS Lett. 2008 Oct 29;582(25-26):3601-6. Epub 2008 Sep 24. PubMed PMID: 18817777; PubMed Central PMCID: PMC2603298.

3. Goss CH, Newsom SA, Schildcrout JS, Sheppard L, Kaufman JD. Effect of ambient air pollution on pulmonary exacerbations and lung function in cystic fibrosis. Am J Respir Crit Care Med. 2004 Apr 1;169(7):816-21. Epub 2004 Jan 12. PubMed PMID: 14718248.

Diabetes:

1. Hoffmann B, Luttmann-Gibson H, Cohen A, Zanobetti A, de Souza C, Foley C, Suh HH, Coull BA, Schwartz J, Mittleman M, Stone P, Horton E, Gold DR. Opposing effects of particle pollution, ozone, and ambient temperature on arterial blood pressure. Environ Health Perspect. 2012 Feb;120(2):241-6. Epub 2011 Oct 20.

PubMed PMID: 22020729; PubMed Central PMCID: PMC3279434.

Pages 8-32-34. 8.4.2. A reference or two of similar studies on PM exposure and obesity would be helpful to place the ozone/obesity studies in context with other air pollutants.

Page 8-33. Line 26. References are needed for studies in obese mice that shown enhanced pulmonary effects with ozone exposure.

Page 8-36. 8.5 Summary. This concluding section is well done. Table 8-5 is a good addition.

Dr. Daniel Jacob

Comments on Chapter 3 – Atmospheric Chemistry and Ambient Concentrations

In revising Chapter 3, particular attention was given to estimates of background O3 concentrations. At the request of CASAC, new studies published after completion of the prior draft were evaluated and added to the discussion in Section 3.4. There is also increased focus on background estimates relevant to the fourth-highest maximum daily 8-hour average O3 concentrations.

Please comment on the adequacy of these and other changes to the chapter and recommend any revisions to improve the discussion of key information. In relation to ambient and background O3 concentrations, is material clearly, succinctly, and accurately provided? Where appropriate, please provide guidance that may refine the scientific interpretation and/or improve the representation of the science.

This chapter is much improved from the 2nd draft ISA. I have a number of comments but they can all be handled in a final round of revision.

1. 3-3: I believe that CASAC previously found Figure 3-1 to be too complicated and unclear for the non-specialist and I agree. There is no need to show the stratosphere in such detail.
2. 3-16, line 9: the significant role of Br chemistry as a global sink for tropospheric ozone should be mentioned with references to von Glasow et al. (JGR 2005), Yang et al. (JGR 2005), Parrella et al. (ACP 2012)
3. 3-14, line 23: somewhere in this section (not necessarily here) it should be mentioned that the lifetime of ozone increases with altitude, from days in the boundary layer to months in the upper troposphere, as this explains why background ozone increases with altitude.
4. 3-19, lines 23-34: I don't think that the theoretical explanation of the low-NOx and high-NOx regimes is correct. There are several errors. Ozone production is limited by the supply of HOx radicals in the same way in the low-NOx and high-NOx regimes. In the low-NOx regime, VOCs do act as a sink of OH. Also, in the high-NOx regime ozone production decreases with increasing NOx. See discussion in the Jacob [1999] book. In the low-NOx regime, P(O3) ~ [NO]*sqrt (P(HOx)); in the high-NOx regime, P(O3) ~ ([VOC]/[NO2])sqrt(P(HOx)).
5. 3-19, lines 9-21: Again I don't think that the theoretical distinction between the low-NOx and very-low-NOx regimes is correct. I see no theoretical distinction between the two. In both cases, loss of HOx radicals is by conversion to peroxides and this is what defines NOx limitation.
6. 3-22, lines 7-8: I disagree that the use of indicator species has been restricted to urban areas. Use has been all over, including at many rural locations.
7. 3-22, line 21: It would be appropriate to cite Martin et al. [GRL L06120, 2004] as the originator of the method of using HCHO/NO2 from space to diagnose the chemical regime for ozone production.
8. 3-26, lines 3-5: I don't see the point of the discussion. All weather models assimilate data of all kinds.
9. 3-32, lines 24-28: singling out anthropogenic methane is confusing because NAB calculations generally use present-day methane without trying to remove CNA sources. Not clear what is the point of "contributing to global concentrations of O3" which seems to confuse the issue more than anything.

10. 3-32, line 32: Figure 3-7 seems more confusing than helpful.
11. 3-39: I think the point should be made that screened ozone concentrations at TH might be representative of Pacific air arriving over the US but cannot be viewed as US background because of loss over land.
12. 3-41, lines 24-25: somewhere it should be pointed out that the ozone coming down to the troposphere in stratospheric intrusions is not necessarily natural, i.e., it could have originated from production in the troposphere followed by transport to the lowermost stratosphere. Lin et al. 2012 label as stratospheric any ozone that has crossed their chemical tropopause surface; this is a very different definition from that used by the GEOS-Chem group (e.g., Zhang et al. 2011) in which ozone is stratospheric only if it was produced in the stratosphere.
13. The ozone background is a topic of active discussion in the atmospheric chemistry community, which generates considerable confusion outside that community. We recommend that the chapter make more ample reference to McDonald-Buller et al. (EST 2011) as the closest thing to a consensus statement from the atmospheric chemistry community on the magnitudes and sources of the ozone background.
14. 3-43, lines 21-22: it should be pointed out that the GEOS-Chem version used by Zhang et al. 2011 included the Linoz mechanistic treatment of stratospheric ozone, avoiding the previous problems in the simulation of STE identified by Fusco and Logan.
15. 3-44, lines 5-9: in the body of the text Zhang et al. 2011 point out that the effect of increasing grid resolution is to increase the background ozone by 1-2 ppb and that this is due to better resolution of vertical eddy motions. This might be worth pointing out as it would be of interest to the readership.
16. 3-45, lines 23-26: the higher background at high-elevation sites simply reflects the general increase of background ozone with altitude in the troposphere, which is due to increasing lifetime with altitude (drier air) and deposition at the surface, and has little to do in the mean with STE or intercontinental pollution.
17. 3-45, lines 6-11: the ambiguity of "contributions" applies to all sensitivity simulations for ozone. This is why it's better to use "enhancement". I don't mind to much the use of "contributions" if it facilitates communication, but then the caveat about nonlinearity should be general.
18. 3-54, lines 33-34: I don't know about this, GEOS-Chem and CAMx have completely different heritages and would be different in chemical mechanism, natural emissions, deposition…
19. 3-55, lines 24-33: Indeed I think that model resolution is largely a red herring with regard to modeling stratospheric intrusions. As shown by Rastigeyev et al. [JGR 2009], which would be worth citing, numerical diffusion is almost grid-independent.
20. 3-57, lines 4-6: again, one has to be very careful of how the stratospheric contribution is defined. Not all of this 70 ppb was likely produced in the stratosphere (see previous comment). The distinction is very important in assessing the stratospheric contribution to the background.
21. 3-64, line 10: I would say that this second question is ill-posed. Some have tried to address it by tagging odd oxygen in their model according to source but this is very ambiguous because of chemical interactions between ozone having different tags. This document would do a service by pointing out that the question of how much a source "contributes" to ozone is just ill-posed and the first question is the good one to use.
22. 3-76, section 3.5.5.5: This section could mention the value of satellites for constraining emissions of ozone precursors (NO2, HCHO) and the long-range transport of pollution (CO). Also, it seems that the value of satellites to constrain background ozone originating from the free troposphere should be played up more, also in the concluding section. On the other hand, it should also be pointed out that tropospheric ozone observations from space generally require

monthly averaging to reduce noise, and thus would be of little use for observing synoptic-scale variability. Also point out lack of sensitivity to surface concentrations, although this could change with the use of Chappuis bands for the retrieval (Natraj et al., Atmos. Environ. 2011).

23. 3-143, lines 15-16: I don't think that the difficulty of separating natural from anthropogenic sources rises to the importance of being in the conclusions section. It's a fine point and open to misinterpretation.
24. 3-143, lines 29-31: it should be pointed out that ozone production is NOx-limited except in urban cores.
25. 3-145, lines 1-11: a nod to uncertainty from halogen chemistry would be appropriate.
26. 3-147, lines 27-36: see earlier comment about the second question being ill-posed.
27. 3-148, lines 33-35: there are in fact a number of mature tropospheric ozone products from space, see for example the TES/OMI intercomparison by Zhang et al. [JGR 2010]. The utility of satellites for monitoring ozone precursors (NO2, HCHO, CO) would also be useful to mention.
28. 3-150, line 21: I wouldn't say "In contrast". Surface ozone over the US has also increased greatly since pre-industrial times!

Dr. Steven Kleeberger

Comments on Chapter 8 – Populations Potentially at Increased Risk for Ozone-Related Health Effects

The CASAC encouraged the development of standard terminology and concepts for assessing populations at risk that could be applied broadly across the NAAQS pollutants. To help synthesize the evidence, a new classification system was created for considering risk factors. Similar to the approach used to determine causality, each factor was evaluated and classified based on the weight of evidence within and across disciplines. Throughout the chapter, effort was also made to distinguish between greater ambient exposure and/or greater internal dose versus greater adverse health effects at a specific dose when describing the evidence that could potentially result in a population being at increased risk of an O3-related health effect.

Please comment on the adequacy of these revisions to clarify the consideration of potential at-risk populations and recommend any revisions to improve the characterization of key findings and scientific conclusions.

Adequacy of the revisions to clarify the consideration of potential at-risk population:

The third draft of the ISA chapter 8 is a considerable improvement of the previous draft. The new classification system requested by the CASAC is adequate and should be able to be applied to the other NAAQS pollutant, as requested. Tables 8-1 and 8-5 are very useful. As recommended by CASAC, the staff has included summarizations of classification recommendations for each of the effect modification factors throughout the chapter, and they are also helpful.

Other comments:

Page 8-8, line 9. I would rephrase the sentence that begins with "Therefore it is unclear…". The study by Kenyon et al used a very different exposure protocol (1 ppm/8 hr/day for 3 days) compared to the previous investigations of the role of Nos2 in response to O3 (0.3 ppm for 3 days). Likely, the different outcomes are dependent on the exposure regimen, and not unusual for these kinds of studies. I would state that the role of inducible nitric oxide in mediating response to O3 is likely dependent on the exposure concentration and duration.

Page 8-9, Table 8-5. I largely agree with the evidence classifications for the potential at risk factors included in table 8-5. They are consistent with the summary statements that follow the discussion in the text. My only disagreement is that staff has concluded that the evidence is "suggestive" for genetic background as a potential risk factor. I would suggest that the evidence is adequate rather than suggestive. In those studies that were adequately powered to investigate the role of functional variants in human populations, an effect of genotype (GSTs, NQO1, etc) was found. The Vagaggini (2010) investigation was very small and underpowered to investigate the contribution of a genetic polymorphism, and the Kim (2011) study was also small and the authors indicated that increasing the number of subjects would likely have indicated a significant effect modification of GSTM1. The potentially different effects are likely a function of differences between exposure protocols (concentration and duration) and phenotypes measured. It is clear we don't have a good understanding

of the role of specific genes for the specific phenotypes and/or for different exposure conditions, but it I think it is reasonable to say that there is adequate evidence that genetic background is a potential at risk factor. Furthermore, the overwhelming evidence from animal studies suggests not only is genetic background important, but specific genes have been identified that modify specific phenotypes.

Page 8-14, line 24. I recommend reiterating that the controlled human exposure studies investigated effects of O3 exposures in mild asthmatics only. It may be hypothesized that more severe asthmatics may be at even greater risk than mild asthmatics, but those studies will not be performed because of ethical considerations.

Page 8-29, line 34. Staff indicates "an inverse association was also present…but was not statistically significant". If it the association was not statistically significant, then I would not suggest an inverse association exists. I would remove this sentence.

Minor comments:

Page 8-10, line 3. Change sentence to read "…may represent potentially large at-risk populations."

Page 8-12, line 37. considerable should be considerably.

Page 8-13, line 38. Oyarzun reference should be at the end of the previous sentence.

Page 8-19, line 6. asthma-eD should be asthma-ED

Page 8-19, line 33. resemble should be resembles

Page 8-27. Insert "a" between "for" and "family".

Page 8-29, line 3. Citations are confusing in context of the sentence.

Page 8-33, lines 24-26. It is not clear what "responses…appear to differ" means. Presumably, this refers to the findings by Shore et al that the responses differ as a function of the exposure regimen. It may help to include the concentration and duration of exposure for the "acute O3 exposure" and the "lower concentration".

Dr. Frederick J. Miller

Comments on Chapter 1 - Executive Summary

General Comments

Overall, the Executive Summary is well done and is at the appropriate level and detail. The authors have captured most of the salient points that rise to the "30,000 foot level" of a summary of key findings and conclusions. However, there is one area that the Executive Summary is deficient in as well as in Chapter 9. There is no mention of the best exposure index for welfare effects. The Executive Summary fails to make it clear that the secondary standard should take on a different form than the primary standard. While this conclusion was reached in the previous review cycle, there is no mention of this aspect in the current Executive Summary. This deficiency needs to be corrected.

Specific Comments

Page, line	Comment
Scope & Methods	"factors affecting inhaled dose" should be added to the list of items in this section. A logical place for inclusion is after "mode(s) of action". Dosimetry and Mode of Action is a section later on in the Executive Summary. The title of this section will need to be changed if the authors accept the suggestion of this reviewer to modify the title of Chapter 5.
1-3, 3	The 3 should be a subscript. Suggest a global "Search and Replace" for O3 to change to "O_3".
1-4, 29	Insert "in" between "provided subsequent".
1-5, 4	"See" does not need to be capitalized.

Comments on Chapter 2 - Integrative Summary

General Comments

The Integrative Summary chapter in the 3rd draft of the ISA has captured the essence of what such a chapter should contain and how it should be organized. The authors should be congratulated for a job well done. The presentation of the material is well written and provides appropriate linkages to other parts of the document that expand upon the points being made in the integrative summary.

Specific Comments

Page, line	Comment
2-23, 1-3	A single epidemiology study is cited as the basis for the statement "is suggestive of a causal relationship between O_3 exposure and CNS effects". This is a weak argument, particularly given there is a much greater body of evidence for O_3 and cardiovascular effects yet the authors only conclude "suggestive of causal relationship". The recent results of Devlin et al. (2012) published in *Circulation* should bolster the case for O_3 and cardiovascular effects being "likely causative".

Table 2-2	The category "Pulmonary Structure and Function" makes no mention of the neonatal primate studies in the right-hand column of the table. This is not consistent with the discussion in Section 2.5.3 on page 2-26.
2-33, 3	Strongly suggest that "possible" be deleted. There is no doubt that the outdoor workers have greater internal doses because of the amount of time spent outdoors and the increased minute ventilation levels required by the work they do.
2-34, 13	The recent paper by McDonnell et al. (2012) published in *Inhalation Toxicology* clearly establishes a threshold for FEV_1 changes and will need to be included here and in the appropriate section in Chapter 6. The paper has major implications for the risk analyses for this endpoint.

Comments on Chapter 5 - Dosimetry and Mode of Action

General Comments

The 3rd draft of this chapter has been greatly improved by the reorganization of the chapter and by the additional text added to various sections. The authors have done an excellent job of addressing the major comments on the 2nd draft provided by CASAC.

The authors start Section 5.1 of the chapter by stating that there are two main purposes to the chapter -- to describe the principles that underlie the dosimetry of O_3 and to discuss factors that influence it. However, this reviewer believes there are three main. In addition to the two already stated, species homology is discussed in the chapter and is a major component of the thrust of the chapter. In addition to adding this aspect to the introductory paragraph of the chapter, this reviewer suggests that Chapter 5 be renamed as "DOSIMETRY, MODE OF ACTION, AND SPECIES HOMOLOGY". Some of the specific comments and points addressed below should also be carried forth to the Integrative Summary chapter.

Specific Comments

Page, line	**Comment**
Fig. 5-1	Suggest ozone exposure box be on lines above with a downward arrow going to "Inhaled O_3 Dose". This would allow the remaining boxes in the figure to all be on the same line.
5-2, 11	Minute ventilation has not been defined nor has C or t. They should be defined so the reader does not have to go back and forth to the glossary.
5-3, 8	"to larynx" should be replaced by either "to the end of the larynx" or by "to the beginning of the trachea".
Fig. 5-3	Suggest the title of the figure be modified by replacing "alveolus" with "alveolar region".
5-9, 21	Recommend deleting "Conversely," and beginning the sentence with "The".
5-20, 2	This is not a sentence – rewording is needed.
5-26, 17-29	Analogous to the calculations and assumptions presented here, similar computations would likely show that O3 can penetrate the alveolar region surfactant layer in most locations to a much greater extent than what Pryor estimates. This is particularly the case if one takes note of the comments provided by Dr. Jim Ultman regarding the

	assumptions used and the sensitivity of the results to the value of the reaction rate constant.
5-28	5.2.3.1 Summary The summary fails to capture some of the important facts discussed in Section 5.2.3. For example, there is no mention of the ELF decreasing as one proceeds distally in the LRT nor that the gas exchange region is lined by surfactant and that the thickness of the surfactant layer is more than an order of magnitude less than the thickness of the ELF in the distal portions of the TB region. Moreover, the reference to and depiction of Figure 5-7 leads the reader to think only about RT regions with a mucous layer. Figure 5-7 should be redrawn to clearly identify that a surfactant layer is present in the alveolar region.
Sec. 5.4.6	This section on Airways Remodeling may be an appropriate place to convey the potential that the alveolar level injury may be due, in part, to the direct reaction of O_3 with the Type I and Type II epithelial cells in this region.
Sec. 5.4.9	The authors should convey that the key events discussed in the summary are not in any order of sequence of occurrence.
5-59, 32	The Kim et al. (2011) study involved 6.6 hr exposures and not 2 hr exposures.
5-76, 11	There is also a transitional epithelium in the nose. Add this type to the list.
5-77, 16	Insert a comma after “differences”.
5-84, 13	The authors should make a more positive statement here. This reviewer suggest replacing the last sentence of the paragraph with “Nonetheless, if experimental animals show pathophysiological consequences of exposure, the overall weight of the toxicological evidence supports the likelihood that qualitatively similar effects occur in humans given appropriate exposure scenarios.” Such a sentence is more in agreement with the kind of statement the authors make in the Section 5.5.3 Summary.
5-84, 25	This reviewer suggests a more positive “the cup is half full” sentence is appropriate. The last sentence of the 5.5.3 Summary should be replaced with a sentence such as “Thus, these considerations can impact quantitative comparisons between species.”
5-85, 32	The thrust of the previous comment carries over to the chapter summary. Suggest replacing “limit” with “can complicate” so the sentence would read “Even though interspecies differences can complicate quantitative comparisons between species, ……”. This type of change should be carried forward to line 2 on page 2-18 in the Integrative Synthesis chapter

Dr. Howard Neufeld

Version 3 of the ISA for Ozone and Photochemical Oxidants is much improved over the previous two, and as a result, I do not find any major problems with this document. I have divided each of my chapter comments into two parts: comments, questions and suggestions for the text, and then a list of typos at the end. I did read several chapters outside of those assigned to me (Chapters 2 and 3) and I have included those few comments below. I commend EPA personnel and their authors and contributors for the hard work that went into preparing this assessment.

Comments on Chapter 2

The paragraph beginning on line 24 seems contradictory to me. The first sentence states that some animal studies provide evidence of cardiovascular effects, despite some conflicting studies on humans, and then the paragraph goes further into a discussion about the lack of coherence into controlled human studies, ending with what seems an unusually strong statement that there is evidence suggestive of a causal relationship between O_3 and cardiovascular effects. Perhaps rewording this would alleviate what seem to me internal contradictions here.

Page 2-36, lines 1 and 2: There is a statement here concerning the great uncertainty about the nature of the relationship of the C-R curve below 20 ppb. I would think that this is always going to be the case, since there is almost nowhere in the world now where ambient O_3 is ever that low for any prolonged period of time. As a consequence, the importance of finding out the shape of this relationship at such low concentrations seems low to me.

Page 2-37: The document states that there is evidence that people living in cities with low O_3 experience greater effects of O_3 exposure, but there is no follow-up or speculation as to why this might be so. Perhaps a sentence could be added here for explanatory purposes.

Figure 2-3 states that O_3 can affect leaf "production". Leaf production to me means either the rate or number of leaves that a plant produces in a certain time period. I really didn't see any literature cited in Chapter 9 that specifically looked at production, yet there are several studies purporting to show such effects (Talhelm et al. 2012, Ecosystems; Ranford and Reiling 2007, Environmental Pollution; Prozherina et al. 2003, New Phytologist). Most studies have focused on the well-known fact that O_3 can hasten leaf senescence, but only a few have looked at effects on production. In one study (Yamaji et al. 2003, Global Change Biology) O_3 even increased leaf production rates. I think the subject of "production" per se, should be looked at further, since rates of production can translate in large effects on growth and yield later on. Both direct (toxicity) and indirect (re-allocation of C, altered environment due to leaf loss) O_3 effects should be evaluated.

Typos in Chapter 2

2-38, line 12: insert "by which" after "endpoints"
2-39, legend of Fig. 2-3: change "pathway" to "pathways"
2-44, line 24: change "increase" to "increased"

Comments on Chapter 3

3-106: Are the subscripts Equation 3.1 correct? As I read the equation, the numerator and denominator within the parentheses are identical, which would make that part of the equation equal to 1 all the time.

Typos in Chapter 3

3-4, line 15: take out "is" before "driven"
Figure 3-2: poorly reproduced and hard to read – perhaps that can be improved
Figure 3-45: In the title, "Smokey" should be "Smoky"
3-132, line 29: citation after Leibensperger is incorrect and needs to be fixed

Comments on Chapter 9

9-1, line 12: This is the first assessment that has an extended section on the molecular responses of plants to ozone. Therefore, in the introduction where the authors are summarizing the various scales at which O_3 exerts its effects, I found it curious that the molecular scale was left out. I suggest inserting "molecular" before organ.

9-4: I think that the authors have done a good job showing that the results of the earlier OTC studies corroborate the findings from chamberless systems, and this result, which is extremely important, because it validates much earlier research, and broadens the database of species that can be assessed, should if anything, be made even more explicit in this document.

9-15, Figure 9-2: I recognize that this is a simplified diagram, but it should be noted that some crops have equal numbers of stomata on the upper and lower surfaces, so ozone and other gases don't always enter just through the lower surface. I don't think the distribution of stomata (amphistomatous – equal on both surfaces; hypostomatous – greater number on one surface) has ever been related to susceptibility to ozone injury.

9-28: A recent paper by Fitzgerald Booker and others questions the role of apoplastic antioxidants in determining resistance to ozone in plants. That citation is: Booker, F.L., K.O. Burkey and A.M. Jones. Re-evaluating the role of ascorbic acid and phenolic glycosides in ozone scavenging in the leaf apoplast of *Arabidopsis thaliana* L. Plant, Cell and Environment 35:1456-1466.

9-31: Most of the substance of this page concerns reductions in the quantity of RUBISCO due to O_3, but O_3 also causes significant declines in RUBISCO "activity". Activity is the rate at which the enzyme works, which is a function, in part, of how many sites are activated and fixing CO_2. Activity is mentioned later in the document, but I think there should be a separate and fuller discussion of how O_3 alters activity, along with a brief explanation of the main type of activity measured (K_{cat}: mol CO_2 mol^{-1} of RUBISCO active sites sec^{-1}) and how that is affected by O_3. Ozone can affect activity via O_3 impacts on RUBISCO activase, or by direct alterations of the structure of the RUBISCO enzyme itself which causes an active site to become non-functional. While O_3 impacts on RUBISCO activase are mentioned later on page 9-38, I think this should be brought up earlier.

Relative to the discussion on biomonitoring (9.4.2.1) Gretchen Smith just published an article updating 16 years of the Forest Health Monitoring program (Smith, G. 2012. Ambient O_3 injury to forest plants in Northeast and North Central USA: 16 years of biomonitoring. Environ. Monitor. Assess. 184:4049-4065). I think it is an important enough paper to include in this ISA, despite its late publication date.

9-56, lines 3-27: This section summarizes the results of a modeling study by Weinstein et al. (2001). This study concentrated on tulip poplar and concluded that there would be moderate impacts from ozone on basal area. It also noted that competitive release would cause an eventual short-term increase in abundance of red maple and black cherry, but that later on, large decreases would occur. In OTC studies on seedlings of these same species, black cherry was ranked most sensitive to O_3 and red maple least. I think the discrepancies between the empirical and modeling studies should be more fully explained. Otherwise, this is a somewhat confusing section.

9-63, lines 12-19: The ISA notes that there does not appear to have been any selection for more O_3 resistant genotypes in the past few decades. However, it does not state whether or not farmers tend to plant the more resistant varieties, which would bias against yield losses. Is there any information on this?

9-67: In the summary, perhaps the authors can consider adding in the fact that nutritional value, yield and/or growth losses can occur without the appearance of visible symptoms on the leaves of plants.

9-74: The section on stomatal responses is very much improved. I want to commend the authors for reworking this section. With regard to using flux vs exposure to construct concentration-response functions, please note the paper in Atmospheric Environment that shows for wheat varieties, flux was no better (maybe even worse) than exposure when relating to yield. That citation is: Gonzalez-Fernandez, I., A. Kaminska, M. Dodmani, E. Goumenaki, S. Quarrie and J.D. Barnes. 2010. Establishing ozone flux-response relationships for winter wheat: Analysis of uncertainties based on data for UK and Polish genotypes. Atmospheric Environment 44:621-630.

9-80, lines 7-29: What about indirect changes in litter decomposition due to O_3 that result from alterations in canopy structure that then change the radiation and water balance of the soil beneath the trees? Might not O_3 affect decomposition via these indirect methods given that it can cause leaf loss from the canopy trees and greater penetration of radiation to the forest floor, which would cause it to dry out and slow decomposition?

9-104: I didn't recall seeing any mention of O_3-disease interactions. For example, if a plant is stressed by O_3, does it become more or less susceptible to bacterial or fungal infections, and vice-versa, if a plant is diseased, is it more or less susceptible to O_3? Following up on this, it appears that O_3-N interactions, and those with disease might need some discussion, unless this was taken care of in the 2006 document. But multi-factor studies need to be emphasized, and given that N deposition is a major problem in the U.S., and that excess N is known to directly affect plant responses to O_3 as well as their ability to resist diseases, I think this should be given more attention. See this citation: Tiedemann, A. 1996. Single and combined effects of nitrogen fertilization and ozone on fungal diseases on wheat. Journal of Plant Diseases and Protection 103:409-419.

9-145: This section discussing Gregg et al.'s work (2003) is much improved and puts this study into better perspective, given its unusual results.

Typos in Chapter 9

9-20, line 19: put a space between "were" and "upregulated"
9-46, line 9: put a comma after "species"
9-49, line 9: a word seems to be missing after "fine-root". Should it be biomass, production or growth?
9-51, line 8: put first parenthesis in front of author instead of date.
Line 16: take out the semi-colon and replace with a comma.
9-53, line 22: the term "plant functional groups" is defined, and then the acronym given is PFT. Shouldn't the acronym logically be PFG?
9-54, Table 9-2: in the TEM row, under the ozone effect column, I think you need to insert the word "derived" after "empirically".
9-65, line 30: there is a line break here that should be removed.
9-75, line 16: just use integer values for O_3 ppb.
9-137, line 33: unscheduled line break.
9-157-9-190: All of the references do not have scientific names italicized, nor did they retain any of the super- or subscripts.

Comments on Chapter 10

Like the preceding chapter, this one is well written and I have no substantive comments to make that would improve it. It reads very well and nicely summarizes the literature in this area.

The same comments regarding the formatting of the references in Chapter 9 apply to this chapter as well.

Dr. Armistead (Ted) Russell

Comments on Chapter 3

EPA staff have done a commendable job in addressing CASAC comments. There are a few things that should be kept in mind by those who will use the ISA and for staff to address during the next revision (some from before).

1. While the discussion on page 3-84 is a very good start, it would be good to provide more quantitative relationships between the various metrics (e.g., 1-hr max, 8-hr max and 24 hr avg) beyond just the correlation coefficient. This is important to better interpreting the health results. I would like to see quantitative relationships between the metrics on a city-by-city basis, if possible, showing the range of relationships (i.e., slopes, intercepts). This section can better reference itself to interpreting the epidemiologic study results later on. Much of this information can be referencing the appendices.
2. Please provide an objective approach to deal with uncertainty and bias in the model (or whatever approach you recommend for use) estimates of background. This is a science issue.
3. Provide more information on multi-year trends, not just of the higher end of the distributions, but also the daily 8-hr maximums at the lower percentiles for various cities, e.g., an extension of the information in Table 3-6, though for a longer period and some specific cities. This information can be used to assess how current controls are impacting not only the peaks, but also the lower ozone levels, which is important for assessing health benefits.
4. I would still have liked to see more of a scientific assessment of the rollback model that will be used in the REA. BenMAP and APEX will use some modeling results (or a fusion of data and various models). The ISA should assess the inputs to that modeling.

Dr. Helen Suh

Comments on Chapter 4 - Exposure to Ambient Ozone

The revised chapter is well-done and successfully incorporates the suggested comments and improvements from the previous review. The chapter is thorough, with its findings incorporated more fully into the other chapters of the ISA. The discussion of monitor siting, surrounding populations, and ozone concentrations was particularly improved and noteworthy.

Additional comments:

- Tables 4-2, 4-3: Add column indicating mean or range of observed ambient ozone concentrations for stated sample durations. This will help assess whether correlations vary with concentration.

- Page 4-18: The section beginning "However, some insight may be provided by an analysis of correlations between O3 and other criteria pollutants, such as is provided in Section 3.6.4…." It is not clear how this analysis provides evidence for co-pollutant correlations over periods corresponding to long-term exposures, given its focus on relationships between 24-h mean and 8-hr daily maximum concentrations. It would preferable to examine correlations across the month, warm season, and year, as this would correspond best to chronic epidemiological study findings.

- A paragraph should be added to Section 4.6.1 ("Non-Ambient Ozone Exposure") discussing the relation between ambient ozone concentrations and corresponding personal exposures, as it is a key aspect of exposure error and is discussed in preceding sections.

- Page 4-50, line 16: I would include the word figure to the sentence to read "This figure provides…" for clarification.

- Page 4-54, lines 8-12: I think that clarification of the following statement is needed: "The use of fixed-site concentrations results in minimal exposure error when: (1) O3 concentrations are uniform across the region; (2) personal activity patterns are similar across the population; and (3) housing characteristics, such as air exchange rate and indoor reaction rate, are constant over the study area." It is not true that exposure error will necessarily be minimal in those cases; perhaps it is more accurate to say that the inter-individual variability in exposure error across a population will be low when all of the following conditions are met …"

- A definition of the terms "short-term" and especially "long-term" would be helpful. This definition should be consistent with how the terms are used in subsequent chapters, especially Chapters 6 and 7.

Dr. James Ultman

Comments on Chapter 5 - Dosimetry and Mode of Action

Please comment on the extent to which these revisions help to provide the underlying mechanistic and dosimetric information for interpretation of health effects evidence in later chapters and recommend any revisions to improve the discussion of key information.

The panel's suggested revisions to the 2nd draft of this chapter included: strengthening of the discussion on dosimetry principles; addition of discussion on extrapolation modeling; clearer presentation of the direct contributions of O_3 versus its reaction products to adverse responses; and inclusion of the relationship between physical activity levels and breathing conditions.

In this third draft of the ISA, there is a more careful definition of the various quantities found in the literature to quantify dose. In addition, dosimetry principles have been described in more detail. However, there still remains some lack of clarity with respect to dosimetry principles (*e.g.* the difference between "bulk flow" and "convection," and the roles of axial diffusion compared to radial diffusion). The SPECIFIC SUGGESTIONS below provide some guidance in improving the final draft.

With respect to extrapolation modeling/species homology and the breathing pattern during exercise, the new draft ISA has been substantially improved. Also included is an increased discussion of the ability of O_3 to penetrate the ELF and cause toxicity. An important point that is missed is the poor reliability of predicted penetration distance because of the large uncertainty in substrate concentrations and reaction rate constants that determine the half-life of O_3 in ELF. See the SPECIFIC SUGGESTIONS given below

SPECIFIC SUGGESTIONS

Page	**Line**	**Comment**

5-6 **13** The word "function" is misleading. Perhaps, the authors mean to say that "efficiency refers to the O3 absorbed in a region expressed as a fraction of the total amount of O3 entering the region."

5-6 **18-20** It would be clearer to say that "For studies that reported fractional absorption of O3 boluses, the equivalent fractional absorption of a continuous inhalation of O3 was estimated as the sum..."

5-8 **In entire section 5.2.2.1**, there is a tendency to confuse longitudinal transport in respired gas from the URT to the lung periphery with radial transport between the series resistances of the gas boundary layer at the airway wall and the reactive absorption occurring in the ELF. I suggest the following rewording of this section:

“5.2.2.1 Gas Transport Principles

The three-dimensional transport of O3 in the lumen of an airway is governed by diffusion associated with the Brownian motion of gas molecules and convection that depends on local velocity patterns. Simultaneously, O3 is absorbed from the gas stream into the ELF where it undergoes simultaneous radial diffusion and chemical reaction.

When air flows through an airway, O3 located near the tube center moves faster than O3 near the tube wall where frictional forces retard the flow. This non-uniformity in the radial profile of velocity gives rise to an axial spreading or dispersion of O3 that operates in parallel with bulk flow and axial diffusion. The detailed shape of the velocity profile is affected by the flow direction through bifurcating airway branches (Schroter and Sudlow, 1969). The velocity profile is nearly parabolic during inhalation but quite flat during exhalation. Thus, there tends to be greater axial dispersion during inhalation than during exhalation. Dispersion also depends on the nature of the flow, that is, whether it is laminar (i.e., streamlined) or turbulent (i.e., possessing random velocity fluctuations). Because turbulent flow flattens velocity profiles, it may actually diminish dispersion. In humans, turbulent flow persists only a few generations into the RT. The persistence of turbulence into the RT also varies by species and flow rates. For example, airflow is nonturbulent in the rat nose at any physiologic flow rate but may be highly turbulent in the human nose during exercise (Miller, 1995).

The relative importance of axial convection, diffusion and dispersion varies among RT regions for a given level of ventilation. In the URT and major bronchi, axial convection and dispersion tend to be the predominant mechanisms. Moving into more distal areas of the RT, the summed cross-sectional area of the airways rapidly increases and linear velocities decrease, leading to a greater role for molecular diffusion. The principal mechanism of gas mixing in the lung periphery is molecular diffusion (Engel, 1985).

Absorption of O3 at the airway wall depends on a concentration boundary layer on the gas side of the airway wall as well as simultaneous radial diffusion and chemical reaction within the ELF (Figure 5-3c). (Miller, 1995). The boundary layer caused by slowly moving gas near the airway wall can be an important component of the radial diffusion resistance to O3 absorption. This diffusive resistance increases with distal penetration into the RT with one study reporting that the gas boundary layer contributes 53% of the overall 18 diffusive resistance in the URT, 78% in the proximal LRT, and 87% in the distal LRT 19 (Hu et al., 1994). The geometry of airway surfaces also affects local O3 absorption. For example, nasal and lung regions receive different O3 exposures or doses (Miller and Kimbell, 1995); and larger surface-to-volume ratio with the smaller airways in women enhances local O3 uptake and reduces the distal penetration volume of O3 into the RT of women relative to men (Ultman et al.,2004).”

5-10 7 Remove " total absorption,"

5-11 8 Uptake efficiencies were previous defined as fractions, not percentages. Consistent definitions should be used.

5-11 9 Change "(totaling..." to "totally a cumulative efficiency from the mouth of..."

5-11 12 "cumulative uptake efficiencies" instead of simply “uptake efficiencies”

5-11 27-30 I don't understand this sentence. Do you mean that even though that "The decrease in efficiency caused by a higher flow rate is more than compensated by the greater O3 concentration that is delivered to the airway."

5-14 25 Add "into the upper airways" after “uptake fraction”

5-14 26 Delete "at each Vp"

5-16 19 Change "Normalized to" to "Even after adjusting for differences in surface area…”

5-17 20-21 Reverse order: put high flow rate first and low flow rate result second)

5-18 6 You mean "net dose" instead of "mucus layer dose?"

Three paragraphs beginning on pg 5-26, line 3 and ending on page 5-27, lines 3. This material is confusing and does not make the important point that estimating the penetration of O_3 through the ELF is confounded by the uncertainty in our knowledge of reaction parameters. I suggest replacing all of that material with the following text.

> “Taking into account the high reactivity and low water solubility of O3, Pryor (1992) estimated the distance that O_3 can penetrate into an ELF layer before it reacts with endogenous substrates to form other more long-lived reactive species, thus initiating a reaction cascade. These calculations utilized the Einstein-Smoluchowski equation to compare the time t_{diff} for O3 to diffuse a distance d to the half-life t_{rx} of O_3 in its simultaneous reaction with substrates (Equation 5-1).
>
> $t_{diff}=d^2/2D_{O3}$ and $t_{rx}=\ln 2/k_sC_s$ (Equation 5-1)
>
> where D_{O3} is the O_3 diffusion coefficient in ELF, k_s is the bimolecular reaction rate constant of O_3 with reactive substrate s in ELF, and C_s is the molar substrate concentration. Importantly, it is assumed in the derivation of t_{rx} that the substrate is far in excess of O_3 so that C_s is spatially uniform in the ELF. To within some proportionality constant, the distance that O_3 pentrates can be estimated by equating t_{diff} to t_{rx} such that
>
> $d\propto(D_{O3}/k_sC_s)^{1/2}$ (Equation 5-2)
>
> We can be reasonably certain that the O_3 diffusion coefficient anywhere in the ELF is in the range $D_{O3}\sim10^{-5}$-10^{-6} cm^2/sec, but values of the k_sC_s product for the reaction of O_3 with specific substrates are much less reliable. Moreover, we do not actually know which substrates make the most important contributions to k_sC_s and how these contributions vary from airway region to airway region. By asserting that polyunsaturated fatty acids are the primary reactive substrate, Miller and coworkers (1985) estimated that $k_sC_s=1198$ sec^{-1} in mucous and $k_sC_s=21.4$ sec^{-1} in alveolar lining fluid. Pryor and colleagues (1992) estimated value of $k_sC_s=10^6$ sec^{-1}, by assuming reduced glutathione is the primary substrate in mucous. A value of $k_sC_s=2.5\times10^5 sec^{-1}$ was extracted from *in vivo* measurements of O_3 uptake into the mucous layer of the nasal cavities

(Santiago *et al.*, 2001). These studies suggest that there is an uncertainty in the magnitude of k_sC_s within mucous by a factor of 1000, and that k_sC_s may be more than 100 times greater in mucous than in alveolar lining fluid.

With their estimates of $k_sC_s=10^6$ sec^{-1} and $D_{O3}=10^{-6}cm^2/sec$, Pryor (1992) concluded that O_3 could not penetrate a mucous layer even as thin as 0.1 μm. Comparable computations with the $k_sC_s=1198$ sec^{-1} value of Miller would indicate that O_3 penetrates a mucous layer as thick as 3μm. Since mucous layer thickness is on the order of 10μm in large airways and 0.1μm in small airways, these results have entirely different implications regarding the direct role of O_3 in damage to underlying epithelium versus the role of toxic reaction products.

In the nasal passages, in particular, a diffusion analysis of *in vivo* O_3 uptake measurements made at different air flows indicated that the O3 penetration distance (0.5 μm) is considerably less than the thickness of the nasal lining layer (10 μm) (Santiago et al., 2001). A computational fluid dynamics model was able to predict experimentally measured O_3 uptake only when the presence of a nasal mucus layer thickness was considered (Cohen-Hubal et al., 1996), further indicating the need to properly account for the reaction-diffusion processes in the mucous layer."

Comments on Chapter 8 - Populations Potentially at Increased Risk for Ozone-Related Health Effects

Please comment on the adequacy of these revisions to clarify the consideration of potential at-risk populations and recommend any revisions to improve the characterization of key findings and scientific conclusions.

The panel's suggested revisions to the 2nd draft of this chapter included: specifying two broad processes that can place populations at risk, either greater ambient exposure/internal dose or greater innate biological sensitivity; providing additional synthesis of key conclusions; and enhanced discussion of the methodological challenges inherent in studying modifiers of ozone effects.

The revisions to this chapter are well done. This is particularly true of synthesizing the results of the many diverse laboratory and epidemiology studies. The chapter introduction now defines specific classifications for potentially at-risk populations. These classifications in Table 8-1 parallel the causality classification in table II in the PREAMBLE. The main difference is the combination of "causal relationship" and "likely to be a causal relationship" catagories from table II into one category called "adequate evidence" in table 8-1 (What was the rationale for doing this?). The use of this at-risk classification system in the section summaries and in the overall chapter summary provides a much improved basis of identifying and prioritizing potential at-risk subpopulations.

The chapter introduction now provides a clear distinction between exposure/dose and biological sensitivity as separate factors in creating an at-risk group. Discussion of dosimetric versus biological effects specific to particular groups has also been added to various subsections of the chapter. It is not apparent to me, however, that discussion of methodological challenges such as inconsistent measures of effect modifiers and inadequate sample sizes have been improved.

Dr. Sverre Vedal

Comments on Chapter 6 - Short-term Exposure

1. Ozone and cardiovascular disease.
   EPA charge: *Careful consideration was given to a CASAC recommendation that the causal determination for cardiovascular effects from short-term O_3 exposure be increased to "likely to be a causal relationship." There was strong toxicological evidence and consistent, positive associations between short-term O_3 exposure and cardiovascular mortality in epidemiologic studies. However, controlled human exposure studies were limited in number and provided inconsistent results. Likewise, epidemiologic studies showed inconsistent findings for cardiovascular morbidity (e.g., heart rhythm, physiological biomarkers, and hospital admissions or emergency department visits). Based on extensive review and discussion of the evidence the decision was made to retain the "suggestive of a causal relationship" conclusion for cardiovascular effects from short-term O_3 exposure.*

   p. 6-218 & Table 6-54 (p. 6-274). The arguments in favor of retaining "suggestive of a causal relationship between short-term exposures and CV effects" are well reasoned. However, note that cardiovascular, CNS, reproductive and developmental effects are all assigned a causal grade of "suggestive", although the evidence for CV effects is far more substantial than that for either of the other two. Although published after the cutoff for publications to consider in the ISA, the recent Devlin publication (Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 2012 Jul 3;126(1):104-11. Epub 2012 Jun 25) removes one of the arguments the resulted in not taking CASAC's advice, ie, lack of human clinical evidence. It is a publication that one would consider for which making a time-cutoff exception should be considered. The only remaining roadblock to assigning the ozone (short-term)-CVD relationship a "likely to be causal" grade is the lack of consistent epidemiology morbidity findings. I therefore still favor advancing the causal grade to "likely."

2. PFT responses asthma vs. non-asthma. On balance, there is really little evidence that asthmatics in controlled human studies exhibit more marked PFT responses than non-asthmatics (pp. 6-19 thru 20). This is not necessarily the case for other endpoints such as airways inflammation. This needs to be kept in mind as the process moves to the REA and policy assessment stages. Other measures of airway function than FEV1 (eg, airways resistance) do show that asthmatics may show an effect of ozone on bronchoconstriction vs. just reduced inspiratory capacity (and reduced FEV_1 as a result), while in non-asthmatics, this bronchoconstrictive effect is a trivial effect (ie, bronchodilators do not prevent or reverse reduction in FEV_1 in non-asthmatics).

3. Long-term and short-term exposures. Long-term vs. short-term mixed together in the same section is confusing and gives a skewed picture of the evidence. For example, CNS (p. 6-219, line 29, and top of p. 6.220 for rationale): respiratory effects (P. 7-3, line5).
4. Effect modification (section 6.6.2.2) vs. interaction (section 6.6.2.3). These are typically synonymous. So, while the use of "interaction" is defined here to have an unusual specific meaning, it might be helpful to add a few sentences motivating the distinction.

5. Miscellaneous
    a. In asthmatic children, lung function decrements occur together with symptoms? - . 6-166 (line 3-5). How addressed?
    b. Newer multi-city studies in asthmatic children (Schildcrout 2006; O'Connor 2008), which should arguably carry the most weight, are not convincing or show no effects on respiratory symptoms or medication use (Figure 6-11, p. 6-88 and Figure 6-11, p. 6-92). Findings from these newer studies should either be criticized and given less weight, allowing the current conclusions to stand, or else given appropriate weight and allowed to influence the conclusions.

Comments on Chapter 7 - Long-term Exposure

1. New-onset asthma. Regarding new-onset asthma, this is described as providing "the strongest epidemiologic evidence for a relationship between long-term ozone exposure and respiratory effects" (p. 7-39, line 12). The "strong" evidence referred to comes largely from the California Children's Health Study (CHS) cohort. The earlier finding from this cohort was that number of outdoor sports a child participated in was associated with new-onset asthma, but only in high ozone communities. This finding was only moderately compelling, being hampered by the relatively small number of cases, and by the observation that participation in tennis drove most of the association. The new findings on new-onset asthma take a gene-environment interaction approach to a larger number of cases (n=160). The emphasis is on gene main effects, somewhat unfortunately, rather than on the ozone exposure main effect, which ignores the fact that modification of ozone effects by genetic polymorphisms does not require a gene main effect. That is, genes that influence new-onset asthma may have nothing to do with how ozone might cause new-onset asthma. Having said that, the genes assessed (reported) in the CHS study (HMOX-1 and GSTP1/GSTM1) might well be of interest in influencing ozone effects. The primary finding was that the protective gene main effect was lost in the higher ozone communities (p. 7-7, line 7, Figure 7-1 [p. 7-8]), a finding that was replicated in another part of the cohort; ie, protection was only present in low ozone communities. The more important finding in our context would have been a demonstration of an ozone main effect on new-onset asthma, with secondary modification of the ozone effect by genetic polymorphisms. Effect modification, it can be argued, should only be explored after a main effect is observed, although in the gene-environment setting, strong modifying effects of a relatively unusual polymorphism, it could be argued, might not be reflected in an exposure main effect. In the full cohort, there was no association between ozone exposure and new onset asthma, ie, there is no main effect on new-onset asthma (p. 7-4 thru 5, lines 39-4). Also, in the study of traffic effects on new-onset asthma, no effects of ozone were observed. In light of the above, ie, dependence on interaction effects to argue for an effect and the small pilot study on outdoor sports, it is difficult to argue that the evidence on new-onset asthma provides "the strongest epidemiologic evidence for a relationship between long-term ozone exposure and respiratory effects," unless the remaining evidence is weak. The association between respiratory mortality and long-term ozone exposure in the ACS cohort provides arguably stronger evidence and to my mind is a more justifiable basis for moving the relationship between respiratory effects and long-term ozone exposure from suggestive to likely to be causal.

2. Long-term exposure metrics for respiratory hospitalizations and ED visits (section 7.2.2). One needs to be careful in ascribing associations to long-term exposures when short-term exposures could potentially cause the associations. When it is claimed that long-term exposure to ozone is associated with increased ED visits or hospitalization, and only long-term exposure metrics are employed, it is not known whether short-term or long-term exposures are responsible. Control (adjustment) for short-term exposure effects would potentially allow one to isolate an effect of long-term exposure, but this is not done. Also, as noted (p.7-17, lines 13-34), effects from long-term exposure that are substantially larger than those that can be attributed to short-term exposure effects can also be used to argue for effects of long-term exposure.

3. University studies. As opposed to what is stated (p. 7-19, line 17), there was no effect of ozone on level of lung function in the Tager 2005 study, only an interaction between baseline FEF25-75/FVC on the relationship between ozone and FEF25-75.