Preliminary Comments from Members of the CASAC Ozone Review Panel on EPA's Integrated Science Assessment for Ozone (Second External Review Draft – September 2011)

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Preface, Preamble, Chapters 1 (Executive Summary) and 2 (Integrative Overview)

Please review and comment on the effectiveness of these revisions. Please comment on the extent to which Chapters 1 and 2 comprise a useful and effective approach for presenting this summary information and conclusions. Please recommend any revisions that may improve the scientific accuracy of these summary sections and the conclusions therein.

The CASAC panel expresses appreciation for the major revisions that have been made in this revision of the ISA. Specifically, we applaud the new Preamble, which not only supports this particular ISA, but others as well. Greater uniformity among CASAC documents will be helpful to the agency and to the public. Further scrutiny and review of the Preamble is warranted, not because of substantial problems, but because of the potential future role of this document. I believe that the authors of the Preamble should be identified, and this Preamble should be submitted to an ATS journal or perhaps to Environmental Health Perspectives for peer review and publication. Or perhaps a free-standing Preamble can simply be adopted by the EPA and endorsed by CASAC. Its substance and its future use is too important for it to be overlooked as part of this larger and not widely circulated document.

There is an elephant in the room that CASAC and/or the EPA Ozone Panel should address. Ozone concerns make clear that we need to revisit the Clean Air Act. It is now more than forty years since the Clean Air Act was passed in 1970. It has had an enormous positive influence on health and even has contributed to the economy. However, is it still possible to established air quality standards "allowing an adequate margin of safety…to protect the public health"? We are increasingly aware of susceptible individuals and it is clear that current ozone levels at the current standard have measurable health effects. Is it possible and practical to make further reductions in the standard and in ambient levels?

This problem has become more serious now that the EPA has established a "policy relevant" background level, which appears to range from 0.015 to 0.05 ppm. The ozone standard is approaching background concentrations. I recommend that our committee and CASAC propose that we address this problem. While doing this, we need to be certain that we don't threaten the regulatory process and its historic successes. But if we don't address this challenge, this may also threaten the credibility of the Clean Air Act and the regulatory process and even the role of CASAC.
Of particular importance is clarifying the "framework for causal determination." Continuing clarification of this framework and using it in a consistent way has greatly improved the effectiveness and transparency of ISAs.

I very much appreciated the Preface. It's very helpful to have a historical review of the ozone standard and a discussion of what has happened in the last couple of years. I like having this "story" told in one place. Is Preface the right word? Should it be called "Historic Perspective" or something like that?

We agree with the conclusion in the charge question that Chapters 1 and 2 now "comprise a useful and effective approach for presenting the summary information and conclusions." The executive summary is

now seventeen pages long. That's an appropriate length. Lay people, legislators, and others can conveniently read the key points of the document. I'm not concerned that several parts of this overlap with other parts of the overall ISA. They should. The tables and figures add a lot. The summary is informative and accurate.

I do have one concern. If the reader wants additional information on any particular topic, such as 1.4 Human Exposure or 1.6.4 Populations at Increased Risk or whatever, do we need to guide them to sources and expanded versions of these topics? On the one hand, the current executive summary is easy to read and uncluttered because it lacks citations. On the other hand, how does a reader of the executive summary find an expanded version of the topics addressed here? Perhaps one way is to link the major rubrics with other chapters and components of these chapters. Then one could go there and use other aids, such as HERO to move to an expanded version of the summary as well as the references which support them.

Another solution is to make clear that the integrative summary – to some extent – is an expansion of the executive summary. In similar fashion, how do we move from the integrative summary to the other chapters which support it? It's possible to move from the executive summary to the integrative summary to the remainder of the document, but are there way sin which these paths can be better defined and easier to use?

A major concept of importance is "policy relevant background." I don't see that addressed in the executive summary. Similarly, in Chapter 2, the integrative summary, I also don't find discussion of it.

We applaud adding introductory sections which are specific to this ISA and placing them at the beginning of Chapter 2. We agree that it makes sense to include historical aspects of ozone regulation in the preface. In toto, this makes sense and presents a more logical progression and a more accessible document to readers at multiple levels.

I confess to remaining ambivalent about the length of the ISA. This latest revision is still very long. On the one hand, its length makes it difficult to find key ideas and to focus on what information is most relevant to a review of the current standard. On the other hand, there are so many aspects to understanding ozone toxicology as well as an abundance of new information that leaving things out also seems undesirable. These current revisions and especially the chapter on integrative health and welfare effects (Chapter 2) is a useful and concise overview.

Comments on Other Sections: 1. I draw attention to sections dealing with adaptation. One of the hallmarks of oxidant injury, especially ozone, is the phenomenon of adaptation. There are levels of ozone, or hyperoxia, which produce serious injury or even death in naïve animals. However, in animals chronically exposed to lower levels of ozone or oxygen, there is morphologic and biochemical adaptation. Subsequent exposures to ozone produce a far lower response. This is important in understanding ozone toxicology in humans as well. It also relates importantly to different patterns of ozone exposure. Citizens, who rarely see significant ozone levels and then suddenly have a two to three day episode of high ozone, may be much more affected than those who enjoy steady state ozone exposures all the time.

Minor Comments:

Preamble, Page lv. I'm not sure I am convinced of the first sentence of the second paragraph, lines 15-16. Is it the case that "the most direct evidence of a causal relationship between pollutant exposures and human health effects comes from human exposure studies"? The paragraph then goes on to describe the deficiencies of such studies. An important one not adequately discussed is the fact that the outcomes for such deliberate human exposures must, by definition, be trivial. More severe exacerbations, causing cardiopulmonary disease, aggravating it, or events leading to hospital admissions or mortality – all the things we care about deeply – cannot be addressed in controlled human exposures. It may be true that these studies loom large at the lowest levels of observed ozone effects, but our overall concern about ozone flows more strongly from more serious outcomes detectable by epidemiology and predicted by relevant animal studies.

Dr. David Chock

Comments on the Preface, Preamble, Chapters 1 and 2

This portion of the revised version has greatly enhanced the value of the ISA to its readers. The organization is logical and the presentation is concise and thorough. The authors have done a wonderful job. I have nothing to add except for some minor editorial issues: In Chapter 1, references to tables in the text ought to be by number (e.g., Table 1-1) rather than by location. Presently, Table 1-1 is placed before the text that refers to it as "table below" (Section 1.6). Also, Chapter 1 has no page numbers and line numbers.

Comments on Chapter 3: Atmospheric Chemistry and Ambient Concentrations

This Chapter is very well prepared and represents an excellent summary of our scientific knowledge to date on tropospheric ozone. Section 3.4 points out that any existing monitored ozone concentrations are inadequate to represent North American background (NAB) because any anthropogenic emissions in NA can travel short and long distances to impact all monitors (See page 3-32, lines 16 to 20). Consequently, the NAB needs to be determined by chemistry-transport models (CTMs) such as GEOS-Chem. Section 3.9 is an excellent update of the GEOS-Chem model predictions for different definitions of ozone background. Yet there are issues of model performance that need to be more thoroughly described in the Sections, especially in regard to extreme concentrations relevant to the ozone air quality standard.

Section 3.4.3 discusses ozone background estimations. But there are limited discussions of the extreme value estimates of the ozone background distributions, especially the annual fourth highest values of the daily-maximum 8-hour ozone concentrations. This information is important in the process of setting the NAAQS for ozone when the intended standard is approaching the background concentrations. Figures 3-49 through 3-56 of Section 3.8 show many time series comparisons between measurements and GEOS-Chem model predictions of daily maximum 8-h ozone concentrations for many CASTNET sites in 2006. The comparison for the Trinidad Head site (See Figure 3-55) is particularly interesting because ozone concentrations at this site are quite close to the model-predicted North American background values. While the predicted and observed annual means of the daily-maximum 8-hour ozone concentrations appear quite comparable, it is rather obvious that GEOS-Chem underpredicts the upper extreme values of the concentrations. Assuming that the observed means can be relatively well predicted, it is generally true that chemistry-transport models have difficulties predicting the upper extreme ozone concentrations and, in fact, tend to underpredict them compared to actual observations. (It would be nice if figures similar to Figure 3-11 on page 3-36 of the first draft were included in the current draft.) These underpredictions make it difficult to use CTMs to construct a reliable NA background for regulatory purposes. Note that the distributions for the observed and GEOS-Chem-predicted daily maximum 8-hour ozone concentrations for the high-elevation CASTNET sites during March-August of 2006 (see Figure 3-58 of Section 3.9) look reasonable for the mean and the high end of the distributions. But these are combined distributions of multiple sites, which do not establish the accuracy of model predictions for individual sites. And the air quality standard is supposed to be met by each individual site.
Section 3.7.2 discusses the role of fine-scale modeling. An issue that has almost never been discussed is

the role of subgrid chemistry. All existing CTMs assume uniform chemical reactions within a model grid. But when the time scales of some chemical reactions are short relative to the mixing time scale of the constituents within the grid, spatially non-homogeneous chemistry will occur. Because the range of atmospheric chemical reaction time scales is large, ignoring subgrid chemistry most definitely contributes to modeling errors. Yet the error size is very difficult to ascertain because of the complexity of the nonlinear atmospheric chemical reactions. (Incidentally, contrary to the figure caption, the right panel of Figure 3-56 does not contain a comparison of GEOS-Chem model predictions of different grid resolutions.)

On page 3-3, lines 10 to 13, the description of the role of high-pressure systems in causing high ozone is somewhat confusing. Sinking air associated with a high-pressure system may not necessarily increase stability and decrease vertical mixing because the associated cloudless skies of the high-pressure system, while increasing stability at night, actually promote mixing within the planetary boundary layer in the day time. A more cogent argument, which is also implied in the subsequent description, would be that cloudless skies in the daytime promote photochemical reactions, and sinking air could bring down the high ozone concentrations trapped in the previous night in the lower free troposphere, due to low winds and deep penetrative convection during the day.

Comments on Chapter 4: Exposure to Ambient Ozone

The second external review draft of Chapter 4 is a significant improvement over the first draft, not only in terms of content, but also in terms of organization and scientific accuracy. Tables are now provided that summarize the results of many relevant studies. New information and associated references have been added. In particular, the inclusion of a discussion on averting behavior on high-ozone alert days is helpful. It shows a significant beneficial impact of alert information on asthma hospital admissions for children and the elderly. The Summary and Conclusions section has been shortened and it presents a more concise and accurate description of the Chapter.

There are two issues that the Chapter authors need to pay attention to. First, in Table 4-3 (p. 4-13), most of the results presented appear to be the slopes, rather than the “ratios” as indicated in the table title, for the relations between personal exposure and ambient concentration for a given time duration. We don’t expect the ratio and the slope in a linear regression to be the same unless the intercept and terms like the random-subject effect in the regression model are effectively zero. And there is no indication in the text that this is the case. If the entry for Xue et al. (2005) in the table is any indication, the values for ratio and slope can indeed be quite different.

Second, in Subsection 4.5.3 describing microenvironment-based models, there is a paragraph describing the impact of roll-back adjustment on air quality distributions (p. 4-31, lines 13 to 22). It indicates a vastly different estimated probability of exposure to an 8-h ozone level of at least 70 ppb between children in Boston and children in Los Angeles, when both cities are assumed to meet an alternative 8-h ozone standard of 74 ppb. There is no indication as to whether this is a scientifically reasonable outcome when both cities indeed meet the said standard. In fact, it is most likely not. If the rollback adjustment used is the quadratic rollback for concentrations above the mean of the policy-relevant background (PRB), then obviously the true PRB distribution has been distorted as a result because the portion of the PRB distribution above the PRB mean has been suppressed, more so in the case of Los Angeles than

Boston because of a more drastic reduction requirement. This is unphysical and is an artifact of the selected rollback methodology. The content of this paragraph is more speculative than definitive, and it only reduces the scientific credibility of the Chapter. Its removal is strongly recommended.

Two editorial errors are identified below:

Page 4-18, lines 7-8. The sentence is incomplete.

Page 4-22, line 22. “Figure 3-24” should now be changed to “Figure 3-25” in this version.

Dr. Ana Diez-Roux

Charge Question 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.

Chapter 8 has been revised to incorporate language suggested by CASAC as part of the prior review in order to better define the various terms used. This has helped clarify some of the ambiguity present in the prior version. However, given the importance of the identification of "sensitive" groups for the NAAQS generally, additional clarifications (and accompanying tightening of the language) may be helpful. It is also important to be consistent throughout the ISA in how the various relevant terms are defined and used. Some reframing of how the material on "populations at increased risk" is presented may also be helpful.

Conceptually it is important to distinguish two broad categories:

1. Persons exposed to higher levels of ambient concentrations [for example, because of where they live or because of the activities they engage in (e.g. spend more time outdoors)].

2. Persons who are "more vulnerable" to the adverse health effects of exposure to a given ambient concentration. Persons who are "more vulnerable" are persons who have other characteristics that make it more likely that they will suffer adverse health effects when exposed to a given ambient concentration. In epidemiologic studies this differential vulnerability is manifested through the presence of statistical interaction between ambient levels and other factors or through the equivalent "effect modification" (when the adverse health effects of air pollution are different depending on whether another factor is or is not present). A simpler terminology for this category which avoids the use of the term "greater vulnerability" (which may lead to confusion given past uses) may be "persons with characteristics which magnify the adverse health effects of a given level of exposure."

This "greater vulnerability" (or magnification of the adverse health effects of a given level of exposure) can result from various processes:

(a) characteristics that modify the dose received by the individual for a given ambient concentration. This includes breathing patterns (which modify the internal dose) and air conditioning which modifies the indoor exposure. Note that if "exposure" is defined broadly to encompass the (internal) dose received by the person, both of these factors can be considered to be related to exposure levels (rather than to modifications of the effect of exposure) and could be encompassed under point 1 above. However, practically speaking what is commonly measured in studies is the ambient exposure, so both of these factors operate as effect modifiers in studies of ambient levels (ie the strength of the relation between ambient ozone and adverse health outcomes is modified by breathing patterns and/or by air conditioning because both affect the actual internal dose received).

(b) characteristics that interact synergistically (and ultimately biologically) with exposure such that the adverse health effects of the dose received are greater (or only present) when the characteristic is present. There may be many personal characteristics that interact with air pollution exposures, such that the adverse effects of air pollution are magnified. Some of these may be eminently biological (such as genetic variations or the presence of a pre-existing disease) and others may be more social (such as differences in access to care that result in greater mortality when exposed to a given level of air pollution). Age or lifestage may also modify the adverse health effects of air pollution although at least part of the modifying effects of age may reflect differences in breathing patterns or presence of other diseases.

It is important to note that some population attributes such as low socioeconomic status or certain race/ethnic groups may both (1) be linked to exposure to greater ambient concentrations (through work or residential exposures) and (2) magnify the adverse health effects of exposure through interactions of various factors linked to SES and race/ethnicity with air pollution (e.g. greater prevalence of pre-existing conditions in lower SES groups, interactions of air pollution with other risk factors such as unhealthy diets, or poor access to care). Gender and other factors may also have implications for both sets of processes.

Although all the elements listed above are mentioned in Chapter 8 and throughout the ISA, the framework within which they are presented is not as clear as it could be.

"At-risk population" (which is used to encompass populations variously described as "susceptible, vulnerable, or sensitive") is defined in the preamble as "those populations or life stages that have a greater likelihood of experiencing health effects related to exposure to a pollutant due to a variety of factors. These factors may be intrinsic such as genetic or developmental factors, race, gender, life stage, or the presence of preexisting diseases or they may be extrinsic such as socioeconomic status, activity pattern and exercise level, reduced access to health care, low educational attainment, or increase pollutant exposures (such e.g. near roadways)" (pg xiv).

This is a broad definition that encompasses population features associated with either (1) greater exposure or (2) with factors that magnify the adverse health effects of exposure although this distinction is not made explicit. Chapter 8 restates a similar definition although some of the language in the introduction seems to suggest that the focus of the chapter is not on increased exposure but exclusively on increased vulnerability to a given level of exposure. Section 8.10 on "Heightened exposure" does include a discussion of increased exposure (primarily due to type of work and air conditioning availability) but it seems to be a bit of an afterthought and is not well integrated within the rest of the chapter or sufficiently comprehensive.

If the intent of Chapter 8 (and the term "populations-at-risk) is to allude to populations who have higher levels of exposure and/or who are more vulnerable to a given level of exposure it may be helpful to consider reframing the information presented so that (a) factors related to greater exposure and (b) factors that magnify the adverse heath effects of exposure are clearly distinguished both in the introduction and in the discussion of specific studies.

It could be practical to being with factors related to greater exposure and then discuss factors that modify the adverse health effects of a given level of exposure.

The section on increased exposure could review work showing variations in levels of exposure by key sociodemographic attributes. Work on the modifying effects of breathing patterns or air conditioning could also be reviewed here, as the "effect modifying" effect of these factors is primarily a result of reductions in internal dose or exposure.

The section on factors that magnify the adverse health effects of exposure could be categorized from more proximal to more distal. For example successive categories of effect modifiers discussed could be (1) genetic factors (2) pre-existing disease/conditions (3) other disease risk factors (smoking, diet, BMI, physical conditioning); (4) sociodemographic factors including lifestage, gender, race/ethnicity, SES (which could modify the effects of ozone in part through the more proximal mechanisms).

These suggestions do not imply a radical revision of this chapter which has excellent material but rather a reorganization and reframing. The need to develop this type of general framework was also alluded to in the prior CASAC review.

In general, it would be helpful if each section on an effect modifier had a similar structure (this is done for some sections but is not always consistent): (1) rationale for expecting effect modification of ozone effects specifically with reference to hypothesized mechanisms (this is especially important for more distal factors such as SES and race/ethnicity which could modify ozone health effects through a number of different mechanisms) (2) prevalence of the condition or factor in the population as a way to highlight the population health importance of any effect modification observed (3) synthetic review of key epidemiologic studies clearly distinguishing studies that found effect modification and those that did not (rather than just listing all studies, large and small sequentially) (4) review of any controlled experiments or toxicological studies to highlight mechanisms and biologic plausibility of effect modification (5) general conclusion.

It would be helpful for the reader to get a sense from the literature review of how often this particular factor has been investigated as an effect modifier and whether the studies have been large or small. As is the review simply lists a number of studies with sometimes inconsistent results so it is difficult to get a clear sense of what can be concluded or whether much additional work is needed to clarify the importance of the specific effect modifier. Some statements on the criteria used to select the studies highlighted would be helpful.

In general there are several instances where the language can be sharpened. Often the description of effect modification is not precise (e.g. "factor X increases ozone risk" rather than "factor X magnifies the effect of ozone on risk of disease Y").

In the conclusion section it may also be helpful to acknowledge some of the methodologic challenges inherent in studying modifiers of air pollution effects. Key among these are consistency in the measures of the effect modifiers studied and having sufficient sample size in the various cross-classified categories. These issues may explain some of the inconsistencies observed across studies.

Specific comments

pg. 8-2. The detailed discussion of intrinsic and extrinsic factors may not be necessary since as noted in the chapter, the distinction between both types can be rather arbitrary

pg 8-4 The conclusion of the section in influenza/infections does not appear to match the studies that are reported, most of which appear to report effect modification. The rationale for the conclusion that there is little evidence of effect modification needs to be further developed.

Pg 8-10. A brief summary of all of section 8.1 (linking all pre-existing conditions /diseases to differential ozone effects) would be helpful.

Pg 8-13 The conclusion of section 8.2.1 (Children) does not seem to match the bulk of the evidence presented. A clearer statement of what the evidence shows regarding whether children are or are not more vulnerable to the effects of ozone would be helpful.

Pg 8-14 to 8-16. Sections on older adults and gender. This is clearly a difficult literature to summarize with sometimes inconsistent findings. Sometimes the many studies reviewed are difficult to follow. Greater synthesis (for example first noting all studied reporting effect modification and the noting exceptions) might help give readers a better sense of the bulk of the evidence.

Pg 8-18 to 8-22 The section on genetics is much longer than the others and could be condensed.

Pg 8-30 Consider reframing section on “heightened exposure” along the lines suggested in the general comments so that a broad range of factors related to greater exposure are discussed. Factors that result in greater internal dose (such as breathing patterns) could also be mentioned in this section, although noting that this is manifested as effect modification in epidemiologic studies.

Pg 8-26 lines 20-23. The sentence is unclear. Was there effect modification by census tract household income? What does “regardless of SES” mean?

Pg 8-32 Consider integrating the section on “healthy responders” within a concluding section that highlights difficulties in investigating effect modification and notes that there is additional interindividual variability in responses which are not currently explained by known factors but need further evaluation.

Dr. W. Michael Foster

Charge Question on Chapters 6 and 7: Comment on the extent to which there is sufficient clarity in the presentation of study designs and results; and provide guidance where the interpretation of the scientific evidence may be improved as well as on the soundness of conclusions in these chapters.

Comments on Chapter 6: Integrated Health Effects of Short-term Ozone Exposure

Overall there is a substantial amount of information from a number of scientific disciplines (clinical, epidemiology, toxicology, and pathology) on respiratory effects reviewed in this chapter . In response to suggestions of the Committee for greater clarity and integration of newer studies, chapter 6 has been expanded roughly 30% from the initial ISA version, and now encompasses 233 pages of text, 53 tables, 37 figures, and 31 pages of reference citations. Organization of the chapter is greatly improved and figure legends have been more clearly defined. As requested by the Committee, discussion of animal model studies have been more explicit in the chapter, and an improved and consistent use of the terms “adaptation” and “attenuation” have also now been followed throughout the text. Respiratory structural changes in animal models as a result of exposure to short-term O3 is now more clearly presented.

The older studies are now more explicitly developed with the results from newer investigations and a smoother presentation of the materials is now provided and that encompass both clinical and animal model toxicological, data bases. This is a significant improvement over the prior ISA version, and for which had previously been characterized by the Committee as troublesome, since in large measure the integration of short-term human clinical and epidemiological studies will likely form the predominant bases of the O3 NAAQS review.

The Chapter is divided into sections covering: respiratory effects, cardio-vascular effects, central nervous, and mortality. Sectional summaries have been better developed and provide a clearer interpretation of the data bases, with clear conclusions on possible/potential determinations of causality of a health effect resulting from short-term exposure to O3. For respiratory effects the summation supports a causal relationship between exposure and effect (pulmonary function, pulmonary inflammation). However, mortality as a result of short-term exposure, is suggested in the summation as a likely relationship, but not definitive as causal from O3 exposure. The studies suggesting a causal relationship for mortality are strong (Section 6.6, pgs. 6-193 thru to 6-233), and thus surprising that summation assigns only a likely association between mortality and short-term exposure to O3.

With respect to integrating respiratory effects section, with O3-related mortality section, would be helpful to co-reference within these specific sections the parallel of apparent susceptibilities of nonwhite populations and perhaps link a potential relationship between biologic responses and death (pg. 6-19, li.13-24 and pg. 208. li.3-23).

Additional references for studies that were published after the initial version of the ISA was prepared, and should be considered for integration into the text include the following:

a) to description in the text on pg. 6-109, li. 27-39, and also to Chp. 5, with respect to the TLR4 signaling and pulmonary response to O3, the recent report by Z LI et al, PLos One 2011;6(11):e27137, is helpful as the report clearly defines specifics and translation of the TLR4 pathway leading to ozone-induced lung injury.

b) to description in the text on pg. 6-103, li. 27-34, with respect to identification of a novel subset of lung macrophages (derived from resident intermediate type macrophages) the study by RM Tighe et al, J of Immunol 187:4800-08. 2011, reports upon a new cell-based endogenous protection available to the host from the biological response to ozone.

Several typographic errors in the text that should be corrected include:

a) Pg. 6-141, li. 2.
b) Pg. 6-201, li.1-2.
c) Pg. 6-224, li. 29.

Comments on Chapter 7: Integrated Health Effects of Long-term Ozone Exposure

Overall a number of organ system responses (respiratory, cardio-vascular, reproductive and developmental, CNS, carcinogenic, and mortality) have been reviewed in this chapter. The revised chapter now encompasses 85 pages of text, 13 tables, 5 figures, and 14 pages of reference citations. As requested by the Committee the organization of the chapter has been improved with separate summary and causality determinations for each organ system response that was reviewed.

The determinations in the summaries appear appropriate for the degrees of causality between respiratory (likely), and mortality (suggestive) and long-term exposure to O3.

An additional reference for a study that was published after the initial version of the ISA was prepared, and should be considered for integration into the text includes the following:

a) to description in the text of section 7.4.8, Developmental Respiratory Effects , on pg. 7-59, with respect to post-natal O3 exposure, the recent report by R Auten et al, Amer J Resp Cell Mol Biol 2011 (Nov.3 epub), is helpful as the report clearly defines structural changes to parenchymal lung tissues, and as well demonstrates a persistence of airway functional changes that do not regress following recovery from multi-day O3 exposure throughout postnatal to juvenile stages of lung development.

It would be helpful to provide an explanation in the text for use of the acronym “MSA” on pg. 7-84.

Dr. Judith Graham

General Comments Not Specific to a Charge

1. The ISA is greatly improved. The hard work is obvious. Thank you. There will never be a "perfect" document, but having said that, some significant improvements are still possible.
2. This comment is, unfortunately, identical to the comment I offered on the first ISA draft. The database for O_3 is extremely large and complex, requiring an unusually high degree of insight to describe and interpret well. I am concerned about whether this draft has had adequate external input and peer review. Eight of 27 authors are external; 4 of 14 (previously) 1 of 11 contributors are external; and 10 of 41 (previously 10 of 35) reviewers are external. This should not be interpreted as a criticism of the EPA staff involved. I know many of them and fully recognize that while several of the EPA staff are internationally recognized experts in O3, most do not have scientific expertise in this area. Thus, external experts play a major role for insuring the quality of the ISA. I also know several of the extramural scientists involved and have great respect for them. The CASAC Ozone Review Panel has a collection of experts, but the magnitude of the database is quite large and, at least for myself, I don't claim knowledge of the details of every key toxicology paper. A broader collection of external experts would offer greater assurance that the original papers have been critically interpreted correctly. This is even more important due to the brevity of the descriptions of many of the papers. As a first step, I recommend listing the authors, contributors, and reviewers according to the chapter they addressed, as was done for the 2006 AQCD. It was clear in the 2006 AQCD that the authors, contributors, and reviewers represented an array of world-class experts (EPA and external). As a second step, I recommend using additional external experts to assist in making revisions to the ISA and reviewing the next draft prior to the document being reviewed again by CASAC.
3. Great progress has been made on avoiding the artificial separation of "old" vs. "new" literature. However, it is possible and desirable to go further. To this reviewer, the only important separation is whether the conclusions of causal determination have changed with new information. Otherwise, all separation could be removed and save space at the same time. An excellent example of the problem with the artificial separation is 7-30 L19, the summary and causal determination of chronic respiratory effects. The last sentence of this crucial section says that "The results for the CHS (whatever that is) described in the 2006 O3AQCD remain the definitive line of evidence." If it's definitive, describe it, don't ignore it. Another good example of a problem is on 7-58 L19 ff. This says that only the old information is discussed if new information is not available. This restricts understanding of the weight of evidence. Another way of conceptualizing this is to think about 2 review cycles from now. O3 research is decreasing, so in 10 years there may be no "new data". We all know that there still is risk, but the 2021 ISA will need to describe all the old information *or* have a 1-page ISA referring the reader to the 1986,

1996, 2006, 2011, and 2016 documents. If this separation is an attempt at brevity, it didn't work. The ISA could add all the old relevant studies and still be shorter, or at least not lengthened. The challenge is dealing with relevance and approaches to provide the details to support the conclusions offered.

4. Concentration, exposure, and dose need clarification of definition and then need to be used consistently. For example, consider using Zartarian, Bahadori, and McKone, JESEE, 2005:15, 1-5 http://www.nature.com/jes/journal/v15/n1/abs/7500411a.html . This paper is a summary of a WHO effort that was also adopted by the International Society of Exposure Science to standardize exposure terminology. The first page of Chapter 4 has a good explanation and at some other place, the appropriate sentence from this reference is used for the word exposure. However, later it gets confounded with dose and with concentration x time x ventilation.
5. Several of the figures are very small, making it very difficult to read them.
6. Several of the figures are more than a page removed from the text discussion, making them difficult to follow.
7. Research needs pop up in several places (e.g., 4-32 L2; 6-20 L36) and should be deleted. It is necessary to have an all-or-none approach to research needs.

Chapter 1 Executive Summary (no page or line numbers in text)

General Comment

1. The level of detail and length are reasonable for an executive audience. However, the text is basically a shrinkage of the summaries from each chapter, rather than an executive summary. Consider asking what executives what to know (or even asking them). It is likely that they want to know what are the effects of O3 under ambient exposure conditions, what are the lowest exposures that cause what effects, what populations are most at risk, and what are the health impacts of those effects. Details of atmospheric chemistry, dosimetry, and mode of action are peripheral. Notice I said peripheral to the bottom line; they are important underpinnings. Another approach is to conceive a 5-minute presentation (5 ppt slides) to an executive audience and use that as guidance to revise the executive summary.

Specific Comments

1. Section 1.3 is labeled Atmospheric Chemistry and Ambient Concentrations. However, no concentration information is provided, except for background in the last paragraph. Consider deleting the concentration part and including it under exposure.
2. Section1.4 is labeled Human Exposure.
    a. If a policy maker asked a question about exposures that people (average and "at risk" populations) encountered and the answer had to be non-technical and accomplished in less than a minute, the text would be different.

   b. The first paragraph is misleading. Specifically the text mentions indoor exposure as apparently equivalent weight to outdoor exposures.
   c. Try creating a text that answers the following questions:
      i. What is the non-technical definition of exposure? This is important because I expect that many non-scientists would think more in terms of ambient concentrations.
      ii. What are activity patterns, especially indoors vs. outdoors? This would include exposure durations as well as activity patterns (where is the person and is the person exercising)
      iii. What are daily patterns of exposure (e.g., higher at certain times of day)
      iv. What groups are most "at risk" because of exposure. What puts these groups into a high exposure (or dose) and hence higher risk category?
3. Table 1-1. I have no comments on the table, per se, since it is just a summary of the text. However, please revisit the concept for the table. The current table has a lot of space for "conclusions from previous review", but only the causation class for the 2011 ISA. It is valuable to tell an executive whether the previous conclusions are stable or have changed. If stable, does the new evidence make them even stronger? The most important thing is what the 2011 says. Consider having the longer description under conclusions from 2011 and then under conclusions from previous review just give the class, such as causal. If you keep the text as is, look carefully at the table. Under short-term CNS, it does not give the causal classification for previous review.
4. Section 1.6.4.
   a. The first sentence says that "an examination of populations… allow for the NAAQS to provide an adequate margin of safety…" This is arguable and is a science-policy or policy question and therefore should be deleted as not appropriate to an ISA.
   b. High-end exposure is not even mentioned. It must be added. For example, "outdoor" kids are more at risk than all kids. Same thing for "outdoor" workers. A person with a genetic susceptibility who stays indoors with air conditioning (and perhaps even without air conditioning) is not at risk. A healthy person who does heavy exercise outdoors when O3 is high is at risk.
   c. What is the basis for saying we aren't sure whether COPD puts people at risk. I know that the human clinical database doesn't say they are more responsive. However, since their lungs are already compromised, a small impact may have greater consequences.
   d. As a minor point, naming the genes is far too much detail for an executive summary.
5. Section 1.6.5 This says concentration-response. This is overly simplistic since exposure-dose response is the important metric. Also, this paragraph focuses on epi, but it is equally relevant to controlled studies.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. Text under Figure 1-1, para 1. It says "cumulative" exposures. Fine. However, this would be true for acute exposures as well, which are more relevant to human effects.
2. Section 1.6.3 says emerging evidence, but evidence is emerging everywhere, not just for these categories.

Chapter 2. Integrative Summary

Specific Comments

1. Whole chapter. Use consistent definitions of the terms concentration, dose, and exposure. For example 2-32 L6 refers to exposure-response, whereas 2-33 L11 is concentration-response, although both are referring to epi studies.
2. 2-1 L16-19 Delete "newly" because the chapter summarizes the information available; perhaps it emphasizes the new, but it is not exclusive to the new. Also, delete "policy-relevant...assessment." Insert NAAQS.
3. 2-1 Consider deleting the whole section. It adds nothing and actually is misleading. All the questions say "new scientific information". However, both old and new are used. Some of the old data forms the most important bases of the NAAQS.
4. 2-5 L32 Add "exposure-response" to the list.
5. 2-14 L18. This says low personal: ambient correlations may tend to "obscure the presence of thresholds...". Since thresholds are an important policy-relevant issue, the wording should be more precise. For example, consider adding "if they exist" after "thresholds".
6. 2-17 L9ff. The homology section needs significant expansion. Although the concepts of animal to human extrapolation are there, they are obscured. For example, there is more complete discussion of some of the MOA (including details of lung biochemistry and reactions) than on the underpinnings of extrapolation. The last sentence credits animal tox as a tool in mechanistic and cause-effect. True. However, one of THE most important values is omitted: indication of the range of O3 effects (e.g. lung pathology). Also, add the value of their contribution of MOA; this underpins WOE of causation in human studies.
7. 2-17ff Section 2.6. Indicate the approximate length of exposure for the animal tox studies (e.g., short-term, long-term). It makes a huge difference.
8. Table 2-1. Given its nature, this table will be used a lot. Therefore, precision becomes more important.
    a. I reiterate my concerns about the artificial separation. For example, if the 2006 conclusion is not repeated under 2011, does that mean it is no longer a conclusion?
    b. When concentrations are given, it should be clear whether they were the lowest concentration tested. As examples (I have not listed all of them):

i. 2-18 airway hyperresponsiveness 2011. Says effects at 80 ppb. Were lower concentrations tested and had no effect. Also, typo on "health adults". Also, delete "suggesting a genetic component." This phrase also modifies the humans mentioned in the same sentence. Also, some kind of genetic component is likely to be involved in everything.

ii. 2-18 pulmonary inflammation, 2011 column. It says concentrations less than 73 ppb. This could be 0.

c. When concentrations from human clinical studies are given, please state whether the subjects were exercising or not.

d. 2-19 pulmonary structure and function 2006. Add, ",some of which were irreversible," after "structural alterations"

e. 2-12 bottom right. This says that animal tox shows effects as low as 500ppb. Moffatt et al 1987 showed inflammation in monkeys after prolonged exposure at 400 ppb (see 1996 CD).

9. 2-20 ff. The whole section on respiratory effects has to be revised to clearly indicate whether the human subjects were exercising or not. Sometimes, it is indicated and when not stated the assumption is that no exercise was included. However, this is not the case.
6. 2-31 L2ff. The sentence says that "an examination of populations… allow for the NAAQS to provide an adequate margin of safety…" This is arguable and, in any case, is a science-policy or policy question and therefore should be deleted as not appropriate to an ISA.
10. 2-30 Section 2.6.7.1 This would be a good place to define sensitive, susceptible, and at increased risk, especially since all these terms are used, somewhat interchangeably throughout this section.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. 2-5 L16-17 Delete "or doses" Without doing dosimetric extrapolations, this isn't possible to tell.
2. 2-13 L24 Careful. I define "preliminary" as something in the abstract stage. If this is true, fine. If not, then revision is needed.
3. 2-13 L29. I doubt if dose-response functions were used, using the proper definition of dose.
4. 2-15 L7. Careful about using the word lung. Stick to the accurate terminology and say LRT or RT. L9 also needs clarification. The alveolar region is part of the respiratory tract. Maybe add the word "more" before "into".
5. 2-15 L19 Change "prevent" to "reduce".
6. 2-15 L26ff. Careful, some of these are effects (e.g., modification of immunity, airway remodeling), not MOA.
7. 2-21 L22 I think the "recent toxicological studies" to which you refer are the monkey studies. If so, say monkeys since this automatically adds weight to the findings.
8. 2-22 Figure 2-3 legend. This says that the bottom row have "subclinical". First, delete subclinical and clinical since that refers to a medical interpretation. For example, altered morphology in the bottom row and airways hyperresponsiveness are not subclinical.

9. 2-23 L35. Add "increased" before "susceptibility"
10. 2-33 L15. Add "observable" before "health response".
11. 2-33 L23. Delete "recent". Most of the studies on this figure are "old", going back to 1988.
11. Table 2-1 Editorial: 2-18 bottom, left. Should be phagocytize. 2-19, top right should be healthy.

Chapter 4 Exposure To Ambient Ozone

Specific Comments

1. The focus on exposure as related to epidemiology is appropriate, but excessive. Exposure assessment has great value for interpreting human clinical studies as well as animal tox studies. For example, how many people are likely to be exposed to levels that caused pulmonary function effects in human clinical studies? There is no "exposure misclassification" in clinical studies.
2. 4-2 L5 Delete "may" and insert "do". These specific sources are NOT important to population exposure.
3. 4-10L19-24. Some of these sentences are at odds with each other.
4. 4-12 L1. Add "in this study" after "indicate that." Reason: there are other examples of poor correlations.
5. 4-19 L1ff. This discusses CHAD, saying what it has. Fine, but what is the important information within it that bears on this ISA. The use of Figure 4-3 is very useful. What about giving some figures of outputs like indoor: outdoor activity patterns by age. How about location of kids vs. hour of day by season (e.g., shows that kids are outdoors at high O3 times).
6. 4-19 Section 4.4.2 on Ozone Averting Behavior. This focuses on how people may reduce their exposure, thereby reducing their risk. However, is aversion an "effect"? If a person alters their behavior, could this be considered "adverse?"

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. 4-5 L16. Provide a reference. The table that describes this area says no such thing. For example, L21 is definitive, whereas L23 says it's unclear.
2. 4-12 L7 this says that farm workers spend 100% of their time outdoors. Didn't they sleep indoors?
3. 4-19 L1. Delete "the" and insert "all". Reason, CHAD has all the most important ones. If you doubt that, give Tom McCurdy a call.
4. 4-19 L35. Define these codes relative to concentrations.
5. 4-29 L31. "blood dose" is not relevant for O3 since it doesn't reach the blood.
6. 4-32 L13. I am *highly* supportive of this model development. However, describing models under development is not appropriate for an ISA and it should be deleted.

Chapter 5 Dosimetry and Mode of Action

General Comments

1. This chapter has been greatly improved. In particular, there is more about homology and the MOA section has more MOA than effects. There is still room for improvement. The inclusion of MOA beyond biochemical interactions results in redundancy to subsequent chapters. A decision should be made about cross-referencing Chapter 5 MOA and MOA discussion in Ch. 6 and 7. The only appropriate place for duplication is discussion of causation elements in Ch. 6 and 7.

Specific Comments

1. Throughout, pay more attention to definition of terms, especially dose and RT regions.
2. 5-2 L7 This says "ideally …dose…is ppmxLxh…" This is NOT the ideal. Dose rate is very important since O3 toxicity is not CxT. For example, 10ppm for 1hr is different from 1ppm for 10 hours. Also, several studies in rats and monkeys have shown that intermittent exposure can be more toxic than continuous at the same C. This should be revised to talk about how concentration is different from dose and there can be several ways to express dose.
3. 5-11 Figure 5-4 has males and females. Were there any gender differences?
4. For the most part, the figures are pertinent and useful. A figure of the effect of age would be VERY useful to describe p5-14 discussion of age. Fig 5-5 doesn't add much to a take-home message.
5. 5-13 L12. This says mode of breathing "may not be biologically significant." The rationale for this is not clear and actually is not true as written. NP scrubbing removes a significant amount of O3. Also, the switch to oral-nasal breathing corresponds to exercise level and that redistributes the dose pattern to reach deeper into the lung, with different cell types.
6. 5-16 Summary. Add the concepts of the impact of age, gender, and pre-existing disease.
7. 5-17 L25 is at odds with L32.
8. 5-17 Section 5.2.3. Add a short paragraph that many/most of the studies were conducted in vitro due to the nature of the necessary measurements. Then say that when in vivo studies are described, this will be specified. Some of the language (e.g., chamber concentrations) is true, but misleading.
9. 5-26 whole section 5.3. As acknowledged in many areas of the ISA, concentration, duration, and exercise level are major determinants of effects. However, the MOA section is often deficient in these details. For example, 5-27 L5, was the exposure acute or long-term? 5-27 L14, no concentration or duration were provided. The word exercising is often used—good. However, it should be modified as heavy, very heavy, etc. It is especially important to provide details for low-level human studies. For example, the Peden and Aleis (5-31 L9) had effects at 80ppb. Using a table for the details would provide this information and keep the text simple.

10. 5-26 L30. Earlier, the ISA acknowledged that effects observed at high concentrations may not occur under ambient conditions and therefore unrealistic concentrations would not be used. True. However, the study here was via an endotrach tube (unrealistic already since scrubbing bypassed) to 3ppm. There are several 2-3 ppm animal tox studies. I realize that dosimetrically, they may be within the "order of magnitude" of ambient, BUT. A MOA theoretically precedes an effect and would be more sensitive. For example, why would it take 3 ppm to change a precursor to an effect observed at 0.5ppm? I know there is a detectability issue, and in some cases the so-called MOA study only used one high concentration, but I am suspicious of such studies having any meaning. I would delete them all. However, I know you won't. So, I recommend that you have a discussion of the concentration story, with additional warnings about in vitro not having any homeostasis or any real dose metric. That would at least place these studies in a better context.
11. 5-27 L31. This says that symptoms "led to" spirometric changes. Question- did one cause the other or were they concurrent? This is an important distinction because kids have spirometric changes, but no symptoms.
12. 5-34 Section on barrier function. Add a brief comment about time course since this will be important later to epi and other animal studies. Also, please be very clear about whether the study of permeability was from lung to blood or blood to lung. In many instances it is clear, but not always (e.g. 5-35 L8).
13. 5-34 L2 and L12. Consider deletion of Abraham et al and Foster and Freed studies. They were via an endotracheal tube, so have no C-R interpretation. I realize this is supposed to be MOA, but as discussed in the ISA and above, concentration matters and there are other studies that show similar effects.
14. 5-36 Section 5.3.5 Bronchial muscle sensitization. Bring out the story of the time line since this could have a bearing on epi time lag. The information is there, but "buried."
15. 5-45 L32ff. This is too much of a stretch for a MOA. First of all, the effect of O3 on testicular and sperm function is very uncertain at this time. Then in vitro studies not even using O3 are cited as "one mechanism." This implies there are several mechanisms. This whole paragraph should be deleted as being too speculative.
16. 5-51 L18-19 This implies a non-linear C-R function and hence should be part of the C-R discussion.
17. It is highly likely that dosimetric differences plays a significant role in interindividual variability, it terms of total dose and regional dose. However this is not brought out clearly. The story is primarily in the relationship of effects to dose and dose to anatomy, biochemistry of ELF, and respiratory physiology and then how all these factors have individual differences. For example, you could cite the background range of resting and exercising FEV1 or f and the range of responses in spirometry after O3 exposure.
    a. 5-51 L20 to 5-52 L10 True, but this has no relationship to interindividual variability as written.

   b. 5-52 L11 ff. True, but how is this related to dose.
   c. 5-52 This summary paragraph needs to be revised based on the foregoing comments.

18. 5-57 Section 5.4.2.2 Pre-existing disease. This section of 5 pages slips too far into effects and what will come later in the chapter on susceptibles.
19. 5-65 Section 5.4.2.5 is named Attenuation of Responses.
    a. Please define attenuation here at the beginning. Also, the first paragraph correctly notes that attenuation happens in lung function and symptoms. However, the fact that changes in other endpoints persist in the presence of such attenuation is not mentioned until later. The first few sentence should be introductory and give a "story", which is then explained further below.
    b. A possible unmentioned mechanism for the persistence of some short-term effects and chronic effects is attenuation of PF. For example, rapid shallow breathing reduces O3 dose to the distal RT. With attenuation of this PF response, the distal RT would receive a greater dose. Thus, attenuation is not necessarily a benefit.
20. 5-57 This is 23 lines about co-exposure with particulate matter. However 4 lines are devoted to a VERY unrealistic study with nanotubes (the dose of nanotubes was silly high). This study should be deleted to keep the focus on interaction with ambient PM.
21. 5-65 L4ff. This is an accurate description of attenuation of spirometric responses and symptoms. Later, the ISA explains that concurrent damage occurs. This should be briefly clarified here to avoid the impression that attenuation is a benefit.
22. 5-67ff Summary. This will be read more, making precision of language more important.
    a. 5-67 L27 Insert "some" before "mechanisms"
    b. 5-67 L28 says "may", but figure 5-10 says "contribute". The figure legend should have "some" before "factors" and "likely" before "contribute".

23. 5-68 L1ff. This introduces the section on homology and sensitivity. However, the text is split into dosimetry and homology of response. More importantly, the pieces are not the point of the material to follow. The title should be changed to say something like "Extrapolation from animals to humans", since that appears to be the emphasis of the section. My personal preference would be to expand it to be interspecies and intraspecies extrapolation, but I understand that that could get confounded with other discussions of sensitive subpopulations. The first paragraph should be clarified to indicate the value of extrapolation (mechanism, biological plausibility, cause-effect, and identification of range of effects). Then discuss that the overarching concept is qualitative and quantitative extrapolation, with interspecies dosimetry and interspecies sensitivity being the 2 components. I think of the components as similarities and differences in delivered dose (interspecies dosimetry) and then the similarities and differences in the response to that dose (interspecies sensitivity). Homology is generally analogous to "similar". Thus, there is a homology of lung structure (influencing dosimetry) and a homology of antioxidant capacity and cellular repair mechanisms (influencing sensitivity). Thus, I don't understand how homology is apparently defined here as response, which has a dose component. Then the introductory text can say that there is solid evidence for qualitative extrapolation that if an effect is observed in an animal study, it is likely that such an effect could occur in humans if exposure were sufficient. Quantitative extrapolation (i.e., knowledge of equivalent EFFECTIVE exposures) is currently substantially more uncertain. Then get into the subsections of dosimetry and sensitivity (e.g., homology).
    a. Generally, the tone is on the differences, not the similarities.
    b. In several places (e.g., 5-68. L2) the term "chronic functional responses" is used. "Functional" should be deleted since the most important changes are morphometric.
    c. 5-68 L4. This credits animals with enabling causative determinations. However, equally important is the ability of animal studies to identify the fuller range of potential O3 effects in humans, albeit at an unknown concentration. For example, lung remodeling information is derived from animal morphometry after specified exposures, which can't be done in humans.
24. 5-69 L30ff. This paragraph discusses species differences in antioxidant concentrations and chemical species in ELF. Fine. However, it emphasizes the differences, without saying that net antioxidant activity is likely to be important, but is not fully understood (a lung biochemist like Gary Hatch could provide accurate input to you). Consider deleting Fig 5-11. It adds nothing to understanding.
25. 5-70 L16 Add "Even with these differences…" to the beginning of the sentence. This puts a more positive emphasis on extrapolation.
26. 5-75 L10 to 23. This is important information, but is not homology. It is age-related sensitivity. The next paragraph (L24) is OK but it should be recast as genetic influences on intraspecies sensitivity. This paragraph should be revised to avoid over emphasis on the age component (it's important but not here unless the whole section is expanded to include intraspecies sensitivity.

27. 5-76 L3ff. The word sensitivity is used without definition. The differences in responsiveness could have been due to dosimetry.
28. 5-76 L21-30. Summary. Extrapolation is exceedingly important because it brings animal toxicology into the web of understanding of the effects of O3. So, having 1 paragraph is wholly inadequate. Other sections that have far less importance are longer. The first sentence emphasizes the limitations, rather than the strengths. Balance is needed. Where is the summary of similarities of regional dose patterns? L24-26 should be deleted (not the right place for genetic sensitivity and infant mice).
29. 5-77 L36 Add the importance of animal studies to understanding the full range of potential effects.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. 5-6 Section 5.2.2.1 Consider deleting this section. Earlier sections have tutorials (e.g., RT anatomy) that are useful because they define terminology used throughout. However, these principles are not needed to understand the text.
2. 5-14 L15 Delete "pulmonary physiology" and insert "RT anatomy." Reason, the factors given like TB volume are anatomy not physiology.
3. Be careful to provide all units. Ex: 5-14 L20 and 5-16 L26 O3 absorbed per minute per what? cm2 surface are of LRT?
4. 5-19 Delete Fig 5-7 if you want to save space. It adds nothing.
5. In several chapter the year of the Que is study missing.
6. 5-47 Figure 5-9. Right bottom says epithelial metaplasia and "fibrotic airways". This is too strong. Replace with "fibrotic changes".
7. 5-64 L37 This study is a co-exposure study and should be relocated there.
8. 5-66 L22 Insert "some" before "responses."
9. 5-69 L29 delete "could"

Chapter 6 Integrated Health Effects Of Short-Term Ozone Exposure

General Comments (My focus is exclusively on the human clinical and animal tox studies and may or may not be pertinent to epi)

1. This is greatly improved, but still has a way to go.

2. This chapter is the *most crucial* since it has the information on exposure-response that will be the foundation of the NAAQS. Hence, it deserves the greatest attention to precision and clarity. In my view, five changes are essential to this goal. They are:

    a. Eliminate the distinction between "old" and "new" studies. This problem was reduced in this draft, but is still inadequate. Reasons follow.

i. The NAAQS is based on the scientific evidence, not the recent evidence. There are egregious examples in which an "old" study at 0.12ppm is given short shrift, but a "new" study at .7 or 1ppm gets a lot of space. Thus, to serve the purpose of the ISA, the science needs to be described, independent of date.

ii. The separation causes unnecessary duplication of the explanation of effects. The result is to decrease understanding of the reader.

iii. The fix is relatively easy; it is editorial.

b. Clearly and succinctly explain the lowest effective exposures in human clinical studies. All the information is in the text, but it needs to be drawn together in ONE summary table. This table should answer the question, "What is the lowest exposure that causes changes and what are these changes?" The table would focus exclusively on concentrations from the lowest tested (probably 40ppb) up to 80ppb only. Given the importance of duration and ventilation, possibly have 1 table that only had one duration and ventilation and different ppb's to permit comparisons (e.g., several studies from several labs with similar protocols reached similar conclusions). Another table would have other V's and T's if that helps answer the question. One column would be ppb, spirometric changes, symptom changes, hyperresponsiveness and inflammation. The table would indicate % changes and whether they were statistically significant and, as appropriate, what % of the subjects was most responsive. This would also permit a reader to see the whole of a study in one place and not have to read different sections of the text to see the correlation of spirometry, symptoms, inflammation, etc. I don't want to prescribe a specific table, but one had to wade through a lot of information and a lot of summaries to answer the key question about lowest effective exposure.

c. Describe the severity of the effects observed. This is briefly mentioned for spirometric changes, but not discussed for human clinical inflammatory changes. Such a discussion is needed to scientifically support later decisions on adversity.

d. Describe the relationship of human clinical study protocols to people in the real world. For example, typically only mild asthmatics are subjects, but the real world has a greater range. There are limits to children's studies. What do these exercise levels mean? What population groups are likely to have exercise levels equivalent to those of the human clinical studies observing effects at very low concentrations. How does the duration of exposure, with intermittent exercise, relate to the real world?

2. The discussion of animal tox studies is totally ***inadequate*** and does a disservice to understanding the effects of O3. There are 2 major problems:

a. Lack of tables. It is ***totally unacceptable*** to cross reference tables in the previous CD's (even the 1986 one is mentioned) and then add a description of new studies, some of which are less important than others buried in old CD's. Nobody is going to sit down with 3 documents in front of them. As stated in the text, rat studies may dosimetrically underestimate exposure compared to humans. This makes rat studies at several hundred ppb very relevant. Therefore, morphometric changes or immune-related changes from exposure of rats to 300 or 400 ppb may be very relevant to humans, but impossible to study in humans for ethical reasons. I recognize the desire to keep the ISA short, but it isn't. For example, many pages are devoted to mechanisms of uncertain relevance, while key animal studies showing the range of effects are buried in an old document. Pages are devoted to effects of uncertain relevance (e.g., neuro) while only a few paragraphs are devoted to lung remodeling; probably because of date of the study. To conserve length, the animal tox tables could be truncated to below a specific exposure (e.g., .75ppm).

b. Some text descriptions are quite good if supplemented with tables; others are woefully deficient. For example, lung remodeling is extremely important. The Section 6.2.3.3 (6-79) is under the larger text of pulmonary inflammation, injury and oxidative stress. The forgoing material is primarily on inflammation. The text describes several dozens of studies on lung morphology. The old studies are cross-referenced to old CD's. A quarter of a page is devoted to a listing of dozens of references for "new information on underlying mechanisms." The animal tox literature provides a much broader understanding of the time course of inflammation and structural changes that would be of significant concern if it could be clearly shown that they would occur in humans.

c. Some animal tox sections have tables with the new studies only. This gives a biased picture. It does, however, make fixing it easier since it would be an editing merge with the tables in the old document.

3. The term "tolerance" is used several places, sometimes in the same sentence with "attenuation," (e.g., 6-2 L22, 6-22 L24, 6-102 L27) suggesting that it is being used synonymously. The terms are NOT synonymous. For O3, the term tolerance is traditionally used for animal studies in which a lower concentration is used to protect against a VERY HIGH (e.g. over 10ppm) O3 or some other chemicals. Thus, it has no place in this ISA and should be deleted throughout.

4. There are several examples of duplication to the MOA section of Chapter 5. In some cases, such duplication is useful when summarizing the causation elements. However, in other cases it is superfluous (e.g., 6-60 L1ff). Cross-referencing Chapter 5 should be done more frequently.

Specific Comments

1. Throughout for the human clinical studies, it is essential to better characterize the degree of exercise because it has a major influence on the exposure-response. In some cases the word exercise is not used at all (e.g., 6-4 L31), the word "exercise" is used without modification (e.g., 6-6 L17), the word exercise is modified by an adjective like very heavy, the actual V_E (e.g., 6-5 L23) is given with no indication of what it means. One approach would be to have a table at the beginning of respiratory effects that describes the adjective (e.g., moderate), the corresponding V_E, and the corresponding description (e.g., brisk walking; running a race). Then the text could use the word exercise with the adjective modifier and the tables (to be added!!) could have the V_E.
2. 6-2 L4 ff Add symptoms since they will help define "adversity."
3. 6-2 L34 This says that infection in early life is associated with asthma incidence. True. I thought it was also associated with COPD incidence; please check on the accuracy of this statement.
4. 6-5 L31. This is not "actually a measure of exposure", according to the definition used in chapter 5. It may be typically used as a surrogate of dose. Perhaps, say that the product of CVT has a huge influence and avoid the words exposure and dose.
5. For the 6.6 hr. exposure, add a short discussion about the time course of effects. For example, was the effect observed at less than 6.6 hours? This time course could be important since very few people would be exposed in the real world for 6.6 hours.
6. 6-10 L33ff. this is a very good discussion of the bottom line of an extremely important group of studies. It is only missing a correlation with symptoms, an important element since it was included in these studies and is part of the definition of adversity.
7. 6-56 Section 6.2.1.3 Toxicology section. L22. The Wiester study is the effects of temperature, as well as the time course of functional changes over 5 days. The Tepper et al 1989 (look at Ch. 5 for full ref) is the one with lack of attenuation for structural changes.
8. 6-69 Tox section. The organization of this section should be revisited. Lung lavage studies should be more in one place.
9. 6-79 L24. This is several dozen references, with no explanation other than "new information regarding the underlying mechanisms." Therefore, these lines have no benefit. If the studies are important, they should be in the MOA section of Ch. 5.
10. 6-81 Table 6-17. Good table, but it needs some clarification. Specifically the Harkema et al 1993 needs expansion. There was also an exposure to 0.3ppm with greater impact. The observations need to be expanded to include the interstitial changes and more details of the changes themselves. All the observations need to be consistent in level of detail offered. For the Hyde et al, 1992, what were the morphometric changes?
11. 6-83 L4-9. This is a good example of excessive duplication (significant explanation of gene interactions at 1ppm, with cross reference to chapter 8) that offers no useful information beyond what has been known from the older studies that are ignored.

12. 6-106 L33 This needs to be clarified. Indeed, there is no epi evidence of mortality from infection. However, in the mouse studies, mortality was an *indicator* not an endpoint to be replicated. So, when the text says "little compelling evidence"—evidence for what?
13. 6-140 L34ff. This is the summary section and this area describes the human clinical studies at 60ppb on inflammation. This discussion needs to be expanded to discuss severity interpretation.
14. 6-175 Section 6.3.3 Toxicology. This section is "formally" split into old (6.3.3.1) and new (6.3.3.2). Although other sections often have a separation within the text, they don't formally split it. Such a split results in excess duplication. The split is of more significant concern because some of the old and new show similar effects, increasing the weight of the evidence.
15. 6-176 L3ff. What were exposure durations?
16. 6-176 L11 mentions human studies. Describe them—epi or clinical; exposures.
17. 6-176 L22 to end of section. This is speculation on the mechanism of CVS changes and is located in the old section. First, MOA belongs in Ch. 5. Second, the MOA discussion is longer than the inadequate description of the effects.
18. 6-177 ff Section 6.3.3.2 recent studies. This has a table, which is OK at the end of the section. However, one can't read the text without reference to the tables. For example 7-177 (L19) should give the concentration. There are other examples. The text should stand alone, to a degree, with the details being in the table. The table should have the old studies since in some cases they show the same effect, increasing the weight of the evidence.
19. 6-182 the tox summary has more on MOA than effects. Increase explanation of effects and cross-reference Ch. 5.
20. 6-184 L15. This is a good example of the problem with truncating the description of the old data. The Tepper study was at 0.1ppm, which is considerably lower than many of the new studies described in more detail.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. 6-7 Figure legend Panel A does not have Adams 1998
2. 6-84 L5ff. This section is labeled "Mechanisms of Injury" and is another example of excessive duplication. It should be in the MOA section of Ch. 5; only a cross-reference is needed here.
3. 6-103 L9 this is long-term exposure
4. 6-139 L11 Add the Tepper et al ref
5. 6-141 L2 typo
6. 6-143 L21. Says they "failed to demonstrate". Did they even look?
7. 6-143 L23. Describe the exposure conditions.
8. Animal tox tables throughout. The titles are not consistent. Also, some titles are misleading in that they imply a complete listing of effects when it lists only the new studies.
9. 6-81 Table. Please specify the ages of all the monkeys since a lot of infant studies were conducted.

10. 6-191 Section 6.4.2 This summary includes 3 references out of several dozens. Why were these choses for calling out?

Chapter 7 Integrated Health Effects Of Long-Term Ozone Expousre

General Comments

1. The animal tox tables presented here are better here because, for the most part, they do not artificially separate the old and the new. However, they should be expanded with *all* the relevant studies. Also, in many cases (e.g., 7-19 L14-38), O3 effects are described in the text with no indication of the concentration or duration. This information is sometimes in the tables (but difficult to find); sometimes the study mentioned is not in the table (e.g. 7-19 L31. Basically, the text needs to have minimal info on study protocol (e.g., concentration, adjective or details about duration, species).
2. Several developmental studies of animal pups are described. All of the key papers in this group should be examined for exposure methodology. Very young pups will be close to their mothers and absorption/reaction on maternal fur would reduce the exposure. If they were exposed on bedding, the exposure likewise would be reduced. This methodological issue should be mentioned briefly if there are papers where these variables weren't controlled.

Specific Comments

1. 7-17 L11 This is the beginning of animal tox, including the most important studies of structural changes. I say most important because they cannot be studied in humans, but for many reasons, it is very likely that humans would experience these effects if exposure was sufficient. Thus, the first 2 sentences are a major problem because they essentially dismiss animal tox because of difficulties in extrapolation. Indeed, there are difficulties in quantitative extrapolation, but they are not overwhelming. They are underexplored in this ISA (Ch. 5). It goes on to say (L16) "However, important…nonhuman primates…" This implies that only monkey studies are of relevance. That is just not true. Monkeys, rats, and other species have similar effects in the CAR, although structural details are different (e.g., monkeys have respiratory bronchioles). This whole introductory paragraph needs to be recast.
2. 7-17 Section 7.2.3.1 discusses the non-human primate studies of the Plopper group. The body of work has studies of infant and adults, and in some cases direct comparisons of the influence of age are possible. This needs to be brought out because of the great importance of age being a risk factor.

3. 7-18 L3ff This cites findings that were not statistically significant. This creates a problem since the reader is then uncertain about the statistical significance of the whole document, unless a specific statement is made. Only cite non-statistically significant studies if they are overwhelmingly important and have been examined to determine the reason. For example, if the sample size was very small, there probably was no effect. Maybe the effect was driven by one high responder, in which case, this could be discussed. Another example is on 7-40 L22 in which a statistically insignificant effect (at 1.2 ppm) is discussed.
4. 7-25 L1ff. This paragraph needs to be clarified since some exposures were acute and others subchronic. Also, it is misleading to say "protective adaptation". Indeed, there was an adaptation. But, as described in earlier documents (and to a lesser degree in this ISA), the pattern is that inflammation measures return to normal while measures of cellular remodeling and fibrotic changes increase.
5. 7-30 L9. Similar effects occur in adults so a revision is needed. Also, the first sentence begins with "irreversible morphological changes…, which in turn can influence pulmonary function." True, but it is far more than an impact on pulmonary function. You could say the functioning of the respiratory tract, which is more inclusive.
6. 7-32 L7 Says "cumulative impacts". This suggests a C x T. The tox studies (those presented and not presented) indicate that seasonal exposure can have different and more effects than "continuous" exposure, even though the C X T on seasonal was very much lower. Thus, delete "cumulative" and explain.
7. 7-38 L30. This says that the Rubes study "did not identify specific pollutants and their concentrations." Also, it appears the other 2 studies in this paragraph were similar. So, why cite them if they are essentially useless.
8. 7-39 L10ff. This section describes one O3 study and then cross references the MOA section (5-45) for support. However, 5-45 is very weak and speculative and does not offer support. For example, L 14 says "studies", but only one study is cited.
9. 7-39 L30ff This introduction to effects on reproduction is not supported by the OZONE data (a stretch to include smoker data, intimating that O3 may be similar).
10. 7-59 L4. This study is useless as an interaction study because the dams were exposed to PM intratracheally to 0.48 mg twice weekly for 3 weeks. This is an absurdly high dose. The O3-only group data would be relevant.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. 7-18 L31. This compares the concentration of a chronic animal study to a controlled human exposure study. This is a false comparison.
2. 7-20 L30 Change "effects" to "impacts" because the effects ARE known.
3. 7-25 L8 why are acute studies here
4. 7-69 Table 7-10. The rationale for the order of the studies presented is not apparent. Also, it is labeled "key" studies. Why are studies at 3ppm "key...This is unrealistically high?

5. 7-75 Section on carcinogenic …. Cancer is always a high-attention area, requiring more precision.
    a. The word "dose" should be deleted throughout this section and replaced with "concentration."
    b. 7-76 L10ff discusses the NTP study was very well designed and had adequate power to detect changes of concern. This area has several lines (e.g. L15, L23) with phrases like "marginally significant", "some semblance of a dose-response." Trying to pull out findings that were not supported in the report is not warranted. Also, the study found no effects of 2-year or lifetime exposure of rats, but these negative data are not included. Given the quality of this study and its importance, it should be explained in detail with a table (simply copy the 1996 CD entry).
    c. 7-76 L23. What was marginally significant? Was it a minor difference? Was the power of the study adequate

Chapter 8 Populations Potentially At Increased Risk For Ozone-Related Health Effects

General Comments

1. Generally, the chapter is very good and a significant improvement. However, further improvements would be important.
2. Epidemiology studies from many countries are summarized throughout. Please add a discussion of the strengths and limitations of using such studies from other countries. I wonder to what degree the information is quantitatively (and even qualitatively in some cases) interpretable for the US situation. For example, in some cases pollutant mixtures would be very different, O3 concentrations and patterns could be different, and SES is likely very different (e.g. Low SES in China is likely to be different from low SES in the US).
3. The separation of old and new studies is totally inappropriate. It adds nothing. In some cases it is misleading and is unevenly treated. For example, 8-13 L26ff. It says that previous and current human and tox studies show age effects, but "recent … [epi]…" are inconsistent. What about the old epi. Just describe the studies.
4. Throughout, the level of detail of presentation of concentration, exposure durations, and exercise levels is uneven. Such information should be presented judiciously (e.g., not needed for every study), but evenly.

Specific Comments

1. 8-1 L1 Delete "suggests" and add "indicates". Interindividual variation is real, not a mere suggestion.
2. 8-1. This is a valiant effort at definitions, but falls short. Some comments:
    a. L10 and L11 add to the e.g., with some related directly to O3 (e.g. intrinsic should include preexisting disease).

   b. L 18-20 apparently has 2 "categories", whereas L23 below has 3 categories.
   c. L23-32 (I recall CASAC commenting on a proposed definition, but I can't recall it. Therefore, I'll just comment on what's here.) I support the "at risk" phraseology. I strongly object to there being 3 categories. First, intrinsic and extrinsic are *complete*, and there can be no "third". The term increased dose is indeed a risk factor, but could be a mixture of intrinsic (e.g., anatomy, physiology) and extrinsic (e.g., exercise, contact of greater concentration). It gets even worse by L29 in which a greater exposure is a separate paragraph.
   d. To me, the greatest need is to define how the "sensitivity" term of the CAA is being used in this ISA. This is reasonably well done in the first paragraph in which sensitivity is translated to "at-risk."
   e. Intrinsic and extrinsic are not used in the body of the chapter. Rather, the chapter is organized by risk factors. Perhaps the best approach is to polish the first paragraph and then discuss the complexity of the interactions of many of the factors, bringing in intrinsic (biological) and extrinsic (everything else, including higher exposure, higher dose, SES).
3. 8-2 provides good context.
4. 8-3 around L15. Insert the concept that preexisting disease can present a risk because of less reserve (i.e., the same percent change may have more impact in a person with COPD).
5. 8-10ff Children are a major at risk group, thereby requiring more precision of language. For example, the introduction basically says that the old data show effects in kids and "New evidence, summarized below, further supports…" However, the following has some old details, appropriately so. This illustrates the false dichotomy between old and new.
6. 8-10 L23. Add that kids have less symptoms than young adults.
7. 8-11 L8ff. This is a good example of the misuse of old and new data as an organizational feature. L8 implies that this is the whole of the "old" and L22 begins the new ("recent"). However, later old work is brought in.
8. 8-12 The infant monkey studies are discussed here and later. They are quite important. However, the summary focuses on the infant results ONLY, whereas the introduction to this chapter says the goal is to compare age groups. The entire set of the Plopper studies supports age comparisons, but these comparisons are not brought out here.
9. 8-12 L25 uses the term "protective adaptation", implying it is a beneficial effect. It could be argued that it is a detrimental adaptation, so omit the adjective.
10. 8-14 L1ff In the first paragraph insert the concept that older people may have less reserve, making relatively small changes have more impact.
11. 8-25 Section 8.6 is called BMI and Physical Conditioning. Indeed there is a link. But having them together implies that physical conditioning "may also affect the risk…" (L30). The text doesn't demonstrate that physical conditioning is a risk factor. It could be argued that exercise in the presence of O3 (see later in this chapter) is a risk factor. Please clarify.

12. 8-28 L20 ff. Was there an interaction with SES?
13. 8-29 L29. This says that smokers were at "less risk." They definitely were less responsive, but this is different from "less risk." 8-30 L21 alludes to this by saying "pseudo-protective." The story needs to be consistent.
14. 8-30 Section 8.1 is Heightened Exposure. It should be expanded to be exposure and dose. At present, it focuses on outdoor workers and lack of air conditioning in some households. It does not mention exercise, although that is a major risk factor (e.g., in clinical studies, it takes 500ppb to cause spirometric effects if the subjects are at rest). It does not mention children although they are outdoors in the summer afternoon exercising (a triple risk: kid's developing lungs, encounter high O3 due to time of day, and exercise (i.e., greater dose). The concepts need to be laid out in the first paragraph
    a. Concentration response important
    b. A person has to be exposed; most people spend 90% (get actual estimate from OAQPS) indoors where concentrations are lower, even without air conditioning.
    c. Exercise increases dose
    d. Therefore, people who are outdoors exercising, especially at times of day when O3 high are at greater risk.
    e. Then have the text expand on these concepts. Outdoor worker and air conditioning discussion is generally fine, but other elements are not discussed.
15. 8-31 L34. This says that "increased exposure to outdoor air does appear to confer additional risk…" This needs to be stronger. There is no doubt that increased exposure, etc., really does confer additional risk. The exact concentration and duration can be argued, but this generic statement needs to be stronger.
16. 8-33 L22ff. This is a summary of heightened exposure. IT needs a total revision since the only emphasis is outdoors.
17. 8-33 L27. First, make this a new paragraph since it is a different concept.

Minor Comments for EPA's consideration, but not needing discussion at the meeting

1. I couldn't find a definition for HA in this chapter, but maybe I wasn't looking hard enough. Since it is used frequently, please make sure it's defined here.
2. 8-10 L1 Delete "recent" because it implies that maybe there is an older literature that you aren't describing.
3. 8-11 L18 Insert "lung" before "regional"
4. 8-12 L18 Change "nasal airways" to "respiratory tract".
5. 8-14, L5 and 8-15 L28. Both place say that diminished symptoms allow them to "withstand" increased O3. This language implies this is a benefit. However, it could well be a detriment since they would not seek relief by avoiding exposure.
6. 8-27 L6. Clarify that this effect was with O3 exposure.
7. 8-32 L22. Delete "may". There is no doubt about it.

Dr. David Grantz

Comments on Chapter 9: Environmental Effects on Vegetation and Ecosystems

This Second External Review Draft ISA provides a thorough and scientifically valid presentation of the current state of the science regarding effects of tropospheric ozone on vegetation. I appreciate the reorganization and improved clarity in response to CASAC comments on the First Draft ISA.

The document appropriately captures new information (since the last AQCD) on the molecular and genetic underpinnings of ozone impacts, on available comparisons of chamber-based and more recently published chamberless exposure studies, and the results of several meta-analyses that provide an integration of the previously available information.

The physiological and biochemical mechanisms of ozone impact are presented in sufficient detail to demonstrate plausible mechanisms of injury, without delving unnecessarily into the still poorly characterized signaling cascades that mediate them. However, as noted previously, the mechanism of ozone impact is predicated upon a "sensing" of ozone by the plant which does not describe the process as currently understood. Further revision is suggested. For example, the process described on page 9-10, e.g., lines 18 and 28, and page 9-36, line 29, is much more accurately described on page 9-17, lines 26-30. This latter framework should be used throughout. Also as noted previously, it is important to consider that gene expression is itself a response, contrary to page 9-11, lines 27-28 and page 9-19, lines 20-32. Differences in genomic responses between sensitive and tolerant plants may reflect differences in ozone uptake or detoxification, or they may fundamental differences in gene regulation. It will likely become important to distinguish these. The new information on proteomic differences is an important contribution of this document. To further highlight this contribution, a distinct Conclusions section could be added to Section 9.3.3.2 (page 9-22).

The available knowledge that is most policy-relevant with respect to setting of a Secondary Standard remains the set of yield-loss relationships described in the previous AQCD. These were derived from OTC (i.e. chamber) studies during NCLAN, NHEERL and European OTC studies. The current document appropriately evaluates and ultimately reaffirms previous conclusions based on these studies:

- That ozone impacts on vegetation occur at current and potential future ambient concentrations,
- that exposure-response relationships remain the best available means of quantifying and predicting the impacts,
- that the exact mathematical relationships that best describe these relationships remain unclear although cumulative indices that emphasize high concentrations may outperform means,
- that flux-response relationships including temporal trends in plant susceptibility are promising but not yet sufficiently developed.

An important aspect of Chapter 9 is the inter-comparison of the chamber and chamberless exposure-response data. Using data from the Free Air systems in Urbana IL and Rhinelander WI, the document shows that previous concerns that hypothetical "chamber effects" had skewed and potentially overstated

the effects of ozone on vegetation, are in fact not significant. This is shown clearly by presenting the convergence of yield predictions based on exposure-response relationships from the two types of systems, and on relative yield reductions. A potential improvement to the presentation could be achieved by further consolidating this material along with the meta-analyses, which are now scattered among the description of the exposure technologies (Section 9.2), the results from each type of exposure (including meta-analyses, Sect. 9.4) and the results of the explicit comparisons of the contrasting exposure technologies (Sect. 9.6).

The mathematical definition of the exposure indices (page 9-106, line 14-20) occurs after most of the discussion of them. This could be moved to precede the discussion. Section 9.5.3.1 is poorly focused, and the conclusion that ozone peaks are important is somewhat obscured. The legend for Figure 9-12 is unclear (what is “Mean diurnal.”? and what is “flux cutoff threshold”?). In the legend for Figure 9-18, should define IQR as Inter-Quartile Range. In 9.6.3.5, the reference to Tables 9-18 and 9-19 should be corrected to 9-17 and 9-18.

Effects on photosynthesis are appropriately emphasized. It is recognized that there may be direct as well as indirect impacts of ozone on stomatal conductance. However, there may be other indirect effects in addition to stomatal response to intercellular CO2 concentration, including metabolic communication following ozone attack on the mesophyll (page 9-13, line 7; page 9-37, lines 1-6). Discussion of C4 sensitivity to ozone should include a reference to Grantz and Vu, 2009 (page 9-32, lines 10-19). The mention of nocturnal stomatal conductance is appropriate given its current level of research interest, however this behavior may receive more attention than is warranted based on its prevalence and potential impact on ozone flux. The conclusion that fast growing plants with large stomatal conductance are most sensitive to ozone (page 9-82, line 1) is not consistent with the previous conclusions that the allometric coefficient of slow growing plants is more sensitive (page 9-48, line 31). This inconsistency probably cannot be resolved here, but in this and other cases in this chapter (e.g., stomatal opening vs. closing responses), obvious discrepancies should be acknowledged and whatever differences can be identified (e.g., in endpoints or species) should be noted.

Impacts of ozone on root growth are very important. This has important physiological impacts and may in future be shown to impact carbon sequestration. All available meta-analyses support the conclusion that ozone reduces allocation below ground, as noted in 9.4.3.2. The conclusions in 9.4.3.1 and in 9.4.3.2 are weak and suggest uncertainty where little exists. For example, there are clear population level explanations for the data of Pregitzer et al., 2008 that reduce the level of uncertainty in the conclusion.

The analysis of hydraulic conductance (page 9-70, lines 22-34) is somewhat confused. There is a specific quantitative relationship between stomatal conductance, leaf and soil water potentials, and hydraulic conductance, that is not consistent with the text. The abbreviation for hydraulic conductance (kl?) requires definition at first use. Sap and stem flow are not synonymous (page 9-70, line 38).

Section 9.6.3.3 attempts to demonstrate that the components of an aggregate population do not exhibit the same statistical properties as the population as a whole. While this is true, the cottonwood data in this section more clearly demonstrate that there are outliers in any population.

The Second External Review Draft contains considerable information that will be useful in development of a Secondary Ozone Standard and in providing a summary of the state of the science for multiple applications. The organization is much improved relative to the First Draft and the conclusions are sound. Attention to the above suggestions may help to address the few remaining rough spots.

Dr. Daniel Jacob

Chapter 3 - Atmospheric Chemistry and Ambient 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 03 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 provides a very good overview of the atmospheric chemistry relevant to ozone pollution, the ability of models to describe it, and the ozone concentration patterns over the US. I have a number of suggestions for improving the chapter. The more important ones are given in bold. I would have liked to see some more discussion of long-term temporal trends, and comment more specifically on this below. I would have liked also to see some more discussion of the utility of satellite observations, in particular as top-down constraints on the emissions of ozone precursors. The discussion of background ozone is overall very good but I strongly recommend that supplemental section 3.9 be deleted because it is incorrect and misleading.

Specific comments (page, line):

(3-5, 23) Since off-road mobile sources are so important for NOx it would be useful to comment on what they represent. Agricultural vehicles, ATVs, lawn mowers, …?

(3-7, 37-38) Statement that coniferous forests are the largest source of biogenic VOCs is useless and misleading – deciduous forests are a larger source density of isoprene and this is what matters.

(3-11, 5) Do you mean peroxides rather than epoxides? Epoxides are exotic and low-yield.

(3-11, 8) hydroxycarbonyls do not come to mind as major products of alkane oxidation.

(3-12, 1-3) Isomerization of isoprene RO2s is very tentative and Crounse et al. find it to be unimportant. This whole business of isoprene chemistry and its effect on OH is very uncertain at present and I recommend that the ISA do nothing more than comment on the uncertain state of affairs.

(3-13, 27-30) repeats (3-13, 11-14).

(3-13, section 3.2.3) The discussion of heterogeneous chemistry effects on ozone elaborates on exotic mechanisms of unclear significance while missing two biggies: N2O5 hydrolysis and HO2 uptake. There has been a lot of recent literature on these two processes that have challenged previous literature and increased our knowledge. Some discussion would be in order.

(3-17) The discussion of NOx-limited vs. NOx-saturated regimes is confusing and sometimes seems wrong. The NOx-saturated regime is defined by dominance of NO2+OH as sink for HOx – it doesn't have anything to do with ozone titration. The NOx-limited regime is defined by dominance of peroxide formation as sink for HOx. Ozone production is dependent on "free radicals" (I presume that means HOx, but is awkward because NOx are also radicals) in both regimes. There is no theoretical distinction between the low-NOx and very-low-NOx regimes, except maybe in the upper troposphere but that's irrelevant here.

(3-26, 9-18) I don't understand the point of this paragraph and suggest cutting.

(3-27, 9-12) It is not clear from the figure that the models are doing better in the mountain west than in the southeast.

(3-31, 13) "ozonesonde data" for what altitude?

(3-32, 8-10) the depletion of ozone at Trinidad Head under offshore flow conditions is due to deposition to land, not titration (Goldstein et al., JGR 2004)

(3-36) The text presents as given that wildfires have a large effect on ozone but in my opinion that is uncritical and flies against other evidence. Singh et al. (ACP 2010) found that California fire plumes are not enriched in ozone unless mixed with urban influence. Alvarado et al. (ACP 2011) found no significant ozone production in boreal forest fire plumes during ARCTAS. McKeen et al. found very little fire influence on surface ozone during ICARTT in summer 2004 even though there was a large influence on CO. In my opinion, there is little evidence that US wildfires make a significant contribution to domestic ozone. I understand that opinions may differ but at least the literature arguing against significant ozone from fires should be acknowledged.

(3-37, section 3.4.3) Some mention should be made in that section of the recent McDonald-Buller EST 2011 review article on the ozone background.

(3-38, 2) The GEOS-Chem model bias in the Southeast is for background conditions and is due to excessive ozone in clean air over the Gulf of Mexico, not error in US emissions, cf. Fiore et al. 2003.

(3-40, 5) The GEOS-Chem maximum over the SW in summer is not due to wildfires but to lightning and deep mixing, cf. Zhang et al. 2011.

(3-41, 20) Excessive vertical transport might also be a cause of excessive surface ozone over the subtropical Atlantic.

(3-44, 8-10) This paragraph seems gratuitous. Cut?

(3-47, 24-27) A 20-50 ppb bias would be of considerable concern! Could this be correct? It doesn't seem that it can be stated without discussion.

(3-103, section 3.6.3.1) I would have liked to see more discussion of long-term (multiyear) trends as these are so important for accountability of emission controls, background influences, and effects of climate change. There is a lot of literature on the topic besides EPA reports and Cooper et al. There is the Parrish et al. paper on increasing ozone in western US inflow, the Cohan et al. paper on the accountability of SIPs, the Leibensperger et al. paper on the effect of climate change over the past three decades. Trends have also been very non-uniform across the US, which is acknowledged in the text but I think that a map showing the geographical distribution of trends would be in order.

(3-109, 2) The flat profile appears to reflect the common observation for mountaintop sites due to orographical flow. I don't think that it is characteristic of rural sites. For example, a site like Harvard Forest has large diurnal variability in ozone due to deposition at night.

(3-103, section 3.6.4) This section overlaps with section 3.2.4 where the correlation of ozone with meteorological and chemical variables was much better discussed. I suggest cutting. The section is somewhat misleading, for example strong ozone-CO correlations are routinely observed in rural air in summer.

(3-114, 23) The statement about uncertainty in conversion of NOx to HNO3 and recycling is not helpful without some explanation of the processes involved. It's actually not clear to me what the authors have in mind. N2O5 hydrolysis? Isoprene chemistry?

(3-114, 24-25) The statement that most of the error in ozone modeling is from meteorology and emissions seems unsupported and is in my view misleading because it gets chemistry off the hook. I recommend cutting, here and in the body of the chapter.

(3-117, 3-8) I don't see the utility of saying that satellite instruments do not directly measure atmospheric composition. One could say that about other methods as well. "Stratospheric measurement of the total O3 column" doesn't make sense. I suggest that the authors put a more positive spin on the satellite measurements as these have demonstrated usefulness for ozone in the free troposphere and as top-down constraints on NOx, CO, and VOC emissions.

(3-119, 5) I don't see the point of "However"

(3-119, 8) this factor of two trend in global ozone is since pre-industrial times, not for the past decades. There's a good review paper by Oltmans on trends in background ozone over the past few decades.

(3-119,26-bottom) Again, I think that the section 3.6.4 is not helpful and could be deleted to advantage. That holds for this summary paragraph as well.

(3-125, section 3.9) I strongly recommend that this section be deleted. It used a faulty implementation of GEOS-Chem, with greatly excessive biogenic VOC emissions and ship emissions. The text suggests that the Harvard group stands behind the simulation but in fact it does not. The simulations were done by ICF and were supposed to replicate the Zhang et al. (2011) work, but in fact used a more recent version of GEOS-Chem and did so wrongly. The overall results are strange, beyond what I could attribute to the above errors. I am very familiar with the GEOS-Chem performance for PM, CO,

NOx,etc. which is extensively documented in the literature (no citation to that work is given here). It doesn't look like what is described here. GEOS-Chem is a research model and should not be used without discernment by inexperienced users.

Chapter 10- The Role of Tropospheric Ozone in Climate Change and UV -B Effects

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

Discussion of the importance of ozone as a climate gas is important in view of the need of concerted climate-AQ objectives in future regulations. Discussion of UV-B effects is also appropriate although these appear to be very small. The chapter acceptably delivers on these two topics but it has a number of minor errors. Also, I think that it needs to better inform on the climate effects of ozone precursor emissions, which in my opinion should be the most important item of this chapter because it directly relates to AQ regulation and is not obvious. There should also be some discussion of the new AR5 RFP scenarios as these will guide future climate-AQ studies. Itemized comments are listed below; important ones are in bold.

Itemized comments (page, line)

(10-3,1-26) That whole discussion is not well written and contains some inaccuracies. UV-B scattering does not depend on cloud droplet size distributions since the sizes are in any case much longer than the wavelength. Not clear to me why it would depend on altitude except in subtle ways. The troposphere is not opaque to outgoing IR radiation (atmospheric window). The text fails to mention the most important greenhouse gas (H2O).

(10-3, 27) A greenhouse gas is not defined by its interaction with solar radiation.

(10-3, 30 and 10-17, 25) Factor of 2 increase in tropospheric ozone seems higher than standard estimates, and the same paper is quoted on page 10-12 as reporting a 30-70% increase which is more mainstream.

(10-4, 4-10; 10-6, 1-5; 10-28, 1-6) The SRES scenarios are old history by now. I understand that the published work uses these scenarios but it behooves this report to discuss the new AR5 RFP scenarios, which are radically different in trends of AQ gases and in particular project no increases in the future except for the business-as-usual scenario. One cannot assume anymore that tropospheric ozone will increase in the future.

(10-5, 6) Again the text fails to mention H2O as the principal greenhouse gas.

(10-9, also section 10.3.3) The IPCC bar chart on radiative forcing referenced to emissions would be a very important addition to this report. It would greatly help in conveying the message on the very different sensitivities for the different emissions. Section 10.3.3 discusses older individual studies and gets mired into details (such as the effect of aircraft NOx) but fails to convey the consensus generated in

the IPCC AR4 report including also the effects on aerosol forcing. The numbers in the report should be given here. In particular, an important conclusion of IPCC AR4 is that NOx emissions are climate-neutral within the range of uncertainty.

(10-10, 9) replace “climate” by “global surface temperature”? That would be more defensible.

(10-11, 7) A site in the San Bernardino Mountains is hardly relevant for the global trend in ozone.

(10-13, 6-8) I think that the important point in the Shindell et al. study is that the observed ozone trend since 1950 is much larger than predicted by models.

(10-13, 18-22) An important reason for the large shortwave forcing in the Arctic is the large SZA.

(10-14, 11) A more direct effect is the horizontal transport of heat, which cannot be regarded as a climate feedback.

(10-17, 6-7) Surface air at 30N is not NOx-saturated.

(10-27, 1) 10% for the contribution of the boundary layer to total tropospheric ozone seems low for polluted regions.

(10-28, 12-13) I don’t see the point (also in the text) of citing the older Naik work that “a carefully combined reduction of CO, VOCs and NOx emissions could lead to net cooling”. Having such a “carefully combined reduction” is wishful thinking, both in terms of practical policy and scientific uncertainty.

(10-28, 23) The cooling effects of CH4 controls would in fact be realized immediately.

Dr. Steven Kleeberger

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

The introduction to Chapter 8 has been revised with expanded discussion to better capture the intricacies associated with characterizing populations potentially at greater risk for O_3-related health effects, utilizing the terms identified by the CASAC panel (i.e. intrinsic, extrinsic, increased dose, greater exposure).

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 populations:

Chapter 8 is much improved after revision of the initial draft. The revised chapter has attempted to clarify the terms 'susceptibility' and 'vulnerability' as they pertain to increased risk of detrimental effects following acute or chronic exposures to ozone. As suggested by the CASAC review members, the authors categorized risk of detrimental effects into intrinsic, extrinsic, and increased dose factors. This artificial categorization provides a framework for discussion of the risk factors. The authors also indicate that some of the factors that are included in the three categories are often connected and/or not easily separable for discussion (page 8-2). The categorization is somewhat unwieldy but it does attempt to clarify the difference between susceptibility and vulnerability.

As suggested by the first review, the authors have been more inclusive of animal toxicology literature where the studies support human studies or where human studies have not been performed but biological plausibility suggests importance. This addition enhances the value of the document.

A table that summarized findings of genetic investigations was recommended in the first critique, but was not included in the revised chapter 8. A table should be included.

The summary of Chapter 8 is not particularly useful. While some of the major points are summarized in the text, there was not a comprehensive synthesis of the findings for the reader to consider. This would seem to be especially important for regulatory purposes. A table that summarizes the risk factors and the strength of evidence for their importance in human and animal studies is recommended (see table 6-65, page 6-233 for example).

Other comments:

Page 8-1, line 3; page 8-5, line 23; page 8-6, lines 1 and 17; page 8-8, line 8. Remove 'both'

Page 8-2, line 10. Change sentence to …this chapter is to identify and understand the characteristics…

Page 8-2, line 16. It is not clear what 'different role' means.

Page 8-11, line 11. 'Sycalmptomatic' should be 'Symptomatic'?

Page 8-11, line 22. Change sentence to …epidemiologic studies have examined…

Page 8-18, beginning of section 8.4. While the study by Triche et al is an example of the potential role for genetics in ozone-related health effects, other studies could be cited that provide a better segue to the section including inter-individual (human) and inter-strain (rodents) variation in responses to ozone in otherwise healthy individuals.

Page 8-19, line 7. Change sentence to …low frequency minor alleles and therefore…

Page 8-19, line 19. 'polymorphism' should be plural.

Page 8-19, line 20. 'response' should be plural.

Page 8-20, lines 18-20. The point should be made here that one of the reasons for the inconsistencies could be that different genes may be important for different phenotypes. This point has certainly been demonstrated in rodent studies, and should be included in this section.

Dr. Frederick J. Miller

Chapter 5: Dosimetry and Mode of Action

Pre-Meeting General Comments

The 2nd draft of this chapter has been greatly improved by the addition of material on gas transport principles, the importance of mode of breathing, expansion of the importance of physical activity in determining dose, and the organization of the discussion on the role of the ELF to name just a few areas. The mode of action material has been strengthened by the addition of results from animal studies.

While the chapter is longer than the 1st draft was, it provides a clearer picture of the role of dosimetry in integrating animal and human data for evaluating the potential for O_3 to cause various effects in humans following acute and chronic exposure to this pollutant. The text now does a good job of showing how mathematical dosimetry model results agree with experimental dosimetry results from studies in human subjects.

There is still a need to do a better job of linking some discussions in Chapter 5 to other Chapters and Sections. For example, Section 5.3.7 on Airway Remodeling contains an inadequate discussion of the ability of O_3 to remodel the lower respiratory tract of monkeys as it only discusses changes occurring in adult animals, while ignoring all of the work done by the UC Davis group on infant monkeys. At a minimum, the reader should be referred to Section 7.2.3.1, but more importantly, the reader misses the impact of the implication of these results for children living in areas of higher O_3 levels.

Some of the material that was in the Mode of Action sections in the 1st draft has been moved to Section 5.5.2 on Homology of Response. An example is the discussion of the Dormans et al. (1999) study. However, since the authors have still failed to address a criticism this reviewer raised about this study and other such studies, the comment is repeated here:

> "…the authors need to be careful about making statements that a study shows one species is more sensitive to O_3 than another. A good example of this can be found on page 5-34 starting at line 20. The text states that Dormans et al. (1999) exposed rats, mice, and guinea pigs to O_3 and found guinea pigs to be the most sensitive with respect to alveolar macrophage elicitation and pulmonary cell density in the centriacinar region. And mice were most sensitive to bronchiolar epithelial hypertrophy … and the list goes on. Such statements about sensitivity are simply not valid unless there is normalization to the dose received. One species may remove more O_3 than another in the nasopharyngeal region or one species may receive a greater pulmonary dose."

Concerning the thickness of the ELF in the alveolar region, the authors present the results of Bastacky and colleague (1995) from the laboratory of John Clements for measurements of the thickness of the surfactant layer. Dr. Clements has long been interested in the thickness of the surfactant layer. While they report a "mean" surfactant thickness over "flat alveolar surfaces" of 140 nm, they state that it varies from a few nm to as much as about 900 nm at alveolar wall junctions. Even if only 10% of the alveolar surface has a few nm surfactant layer, this could have a large impact on epithelial injury caused by O_3

and could explain the patchy network of damage that has been observed in animals following O_3 exposure. Thus, it is not appropriate to ascribe all alveolar region changes as being due to ELF reaction products or a cascade of these products; therefore, the text on page 5-18 lines 23 – 25 is a reasonable statement of the likely situation for O_3 absorption in the alveolar region. However, this point is made so subtly in the chapter summary that it is basically lost by the reader.

Pre-Meeting Specific Comments

Page, line	Comment
	There are numerous instances in this chapter where the authors use the word "which" when they should use "that". A Technical Editor should go through the document for grammatical errors.
5-5, 33	There is a confusion imparted by stating that "uptake efficiency" is that same thing as "fractional absorption". They are not the same, and this leads to confusion in Table 5-1 where F_{URT} and F_{LRT} sum to a value > 1. Efficiency refers to the fraction taken up in a region as a function of the total amount of material entering the given region, while fractional absorption is based upon the amount inhaled and represents normalization by region such that their sum cannot exceed 1.
5-7, 23 - 35	This paragraph is confusing because the wording on the 3rd line from the end makes it sound like the model predictions do not agree with the experimental results. However, that is not the case; moreover, the model predictions were made many years before the experimental studies were conducted. Some rewording of this paragraph is in order.
5-8, 4	The text here is an incorrect statement of how Miller et al. (1985) modeled the LRT uptake of O_3. Reactions with the alveolar region ELF (i.e., the surfactant layer) were not excluded – rather the concentration of molecules that can react with O_3 is exceeding small compared to those that are contained in the ELF of the URT and TB regions.
5-8, 12	The text here makes a very important point that should go forward to the summary for this chapter and to the Executive Summary chapter. The variability in path length from the trachea imparts a significant variability in localized acinar dose. Thus, the authors are right on target when they state "This could have implications in regional damage to the LRT, such that even though the average LRT dose may be at a level that would be considered insignificant, local regions of the RT may receive significantly higher than average doses and therefore be at greater risk of effects."
5-8, 19	The authors did not address the point I raised in the 1st draft about the Wiester et al. (1987) paper. The comment is repeated here: "The discussion here does not include Wiester et al. (1987) where only 40% in the total respiratory tract was measured over a concentration range from 0.3 to 1 ppm O_3. Has this study been discredited? If not, then it should be included to reflect that there is not complete agreement in the published literature about how much O_3 is removed in the head in animals."

5-9, 1st full paragraph	The thrust of the results presented in this paragraph is simply a repeat of the findings of Aharonson, who showed that as flow rate increases the localized flux into the tissue increases but the overall uptake decreases due to the shorter residence time of the inhaled air in the given region.
5-9, 34	The authors state that because the O_3 dosimetry model predicts low tissue dose in the trachea, but injury is seen there, that net dose may be a better predictor of local toxic tissue dose. If this were indeed the case, then much more significant effects in the TB region should be seen because the net tissue doses in the trachea and upper portions of the TB region are practically the same as the net dose in the alveolar region.
5-13, 12	The authors state that the difference between nasal and oral uptake is not large and so the difference is probably not biologically significant. However, the most reliable study of those listed in Table 5-1 to address the point of nasal versus oral breathing differences in O_3 uptake is, in the opinion of this reviewer, the study by Nodelman and Ultman (19999). With shallow breathing, these authors showed a reduction from 0.9 to 0.8 for nasal vs. oral breathing. At a flow rate corresponding to moderate to heavy exercise, nasal uptake was 0.4 while oral uptake was 0.25, which is a highly significant difference. Moreover, the reduction in scrubbing efficiency with oral breathing means more O_3 is delivered to the deep lung where the epithelial cells are more sensitive to O_3 exposure. Thus, the text should be modified.
5-13, 15	Shouldn't "of the RT" be "on the RT"?
5-32, 20	This is almost a verbatim restatement of text that appears on the previous page.
5-33, 1-14	Most of the studies discussed here were with very high O_3 exposures and are not likely relevant to human exposure – suggest deleting this paragraph.
5-33, 19	UA as an abbreviation for Uric Acid is not contained in the glossary.
5-35, 26	TLR4 is used both capitalized and not capitalized here and on subsequent pages. Be consistent.
5-38, 26	Instead of "an older study", the authors might consider using "an earlier study".
5-40	The introductory paragraph to Section 5.3.6 is excellent.
5-45, 32	This paragraph should be deleted. The text provides weak evidence at best from in vitro studies and is mostly conjecture.
5-50, 29	The definitive study for understanding the variability in inter-subject changes in FEV1 response to O_3 has not been done because the studies have not controlled for the conducting airway volume, which is likely the driver as modeled by Overton et al. (1996). The authors correctly cite this concern, but the text here should contain a caveat that arises from the results of Overton et al. (1996).
5-57	Section 5.4.2.2 on Pre-existing Diseases and Conditions is very well written. And the text on page 5-58 from line 9 – 12 should go forward to the chapter summary and the Executive Summary.

5-65	The Attenuation of Responses section is difficult to follow because there is a mixture of endpoints that show attenuation and those that do not. The authors might consider a short introductory paragraph where the endpoints that are attenuated with O_3 exposure are listed followed by a listing of those that are not. Then the paragraph could end with a sentence that now these various endpoints will be discussed. In addition, the bottom line does not come through for this section about how attenuation is essentially a "false negative" because other endpoints continue to worsen with more O_3 exposure. Moreover, the animal studies show that various lesions continue to worsen even though endpoints like pulmonary function are attenuated.
5-68	In the introductory paragraph to Section 5.5, the authors state "This will not be a quantitative extrapolation of doses where O_3 effects have been observed". The authors could easily provide an example of how quantitative extrapolation can be done by expanding Figure 5-11 to include a Panel c that is based on Figure 9-6 of Miller et al. (1988). That figure contains data on lavage fluid protein (LFP) levels in rats, guinea pigs, and rabbits as a function of model predicted tissue dose of ozone that has been normalized to take into account differences among the species in body weight and LRT absorption. Moreover, the data from the human studies for this endpoint could be added to Panel c to provide a clear example of how quantitative extrapolation can be done. The importance of such an exercise is not to extrapolate LFP per se. Rather it would illustrate the importance of the animal results underpinning implication for humans. It would also help dispel any "So What" attempt to dismiss the acute changes seen in human studies as being not of concern by laying the groundwork of the importance of effects seen in chronic exposure studies in animals with the knowledge that similar acute effects can be demonstrated in both animals and humans.
5-73, 10	The statement here about animals at rest underestimating risk to humans with exercise is an apple to orange comparison. One needs to normalize to the dose received by each species and convert concentration-response data to dose-response data before such statements should be made.

Dr. Howard Neufeld

Comments on Chapter 9 – ISA for Ozone and Other Photochemical Oxidants

This is a well-written summary of the current state of knowledge concerning the impacts of O_3 on plants and ecosystems. I thought the organization from leaf to ecosystem was excellent, and the discussions were of an appropriate length and depth. I agree with the majority of the conclusions regarding the degree of causality with exposure to O_3. The authors have successfully incorporated most, if not all, of the criticisms of the earlier document and ended up with a very readable and comprehensive account of the impacts of ozone on plants and ecosystems. Most of my comments are minor in nature. At the end, I list those typos that I found.

On page 9-3, the authors state that there have been no methodological advancements since 2006 that have fundamentally altered our understanding of O_3 effects on plants and ecosystems. I think this is too strong a statement. Although it is true that no new "breakthrough" technologies may have been developed in that period, new understanding did arise from using existing technologies in new ways. For example, researchers used the Li-6400 gas exchange system to look intensively at dynamic stomatal responses to O_3, and out of that came the concept of stomatal sluggishness. One researcher (Grulke) did develop a new system that can measure photosynthesis and stomatal conductance while simultaneously applying an O_3 treatment to the leaves. And at the molecular level, advancements in the analysis of arrays have allowed researchers to study how O_3 affects gene upregulation and downregulation. Thus, I would temper this sentence by saying that there were *some* methodological advances.

I was particularly pleased with the review of past literature and the statements confirming that much of the older results are still relevant. I was also happy with the synthesis of the various exposure methodologies and the clear statements and analyses showing the veracity of the results from these earlier technologies. For many years now, the data obtained from either CSTRs (continuously stirred tank reactors) or OTCs (open-top chambers) have been questioned, but these new analyses clearly show that the results obtained from these studies continue to have relevance and are highly correlated with the results obtained from FACE systems. In other words, there is a lot of internal consistency among the various exposure methodologies. This part was very well done.

In Section 9.2.4, which discusses various FACE-type systems, the authors for some reason left out the Finnish FACE system, even though that system is discussed later in this same chapter. I would suggest including a mention of it here (pg 9-6). The same criticism applies with regard to the Kranzberg Forest Exposure system, which is a variant on the more traditional FACE systems.

Bennett and others (2006, Env. Poll., 142:354-366) utilized a gradient in exposure along Indiana Dunes National Park to assess O_3 injury on plants, and I would suggest including that study in addition to the San Bernardino study. Bennett and others clearly showed how pollution from Chicago was affecting plants downwind across Lake Michigan, which is where Indiana Dunes National Park is. The study by Winner et al in Shenandoah National Park, while showing an elevational gradient in injury in plants, should have the conclusions tempered by the fact that there were probably other confounding gradients

involved also, such as higher N deposition and rainfall at higher elevations.

In the discussion comparing results in OTCs and FACE and gradient studies, the authors mention the possibility of chamber effects using OTCs. In many studies, there were indeed chamber effects, but few to no interactions between ozone exposures and chamber, suggesting that using the OTCs for determining relative effects is okay. In studies I did in the Smokies, we directly compared plants in 1X ambient chambers with those growing in non-chambered plots. For most parameters measured, there were no significant chamber effects. See Neufeld et al. (1995, New Phytologist 130:447-459) where there were no chamber effects on black cherry seedlings except for height growth; see also Neufeld et al. (2000, Env. Poll. 108:141-151) where there were no chamber effects for any parameters measured on several conifer species. Perhaps these studies could be included to help show why OTCs are still useful for assessing O_3 impacts on plants.

On page 9-10, the authors simply state that Gregg et al. found "similar" effects as the previous study cited. Given the large magnitude of effects in the Gregg study, I think it prudent to perhaps elaborate just a bit here on the Gregg study to put it into a better context. I know that the authors devoted a separate section later on to Gregg's study, but this one sentence here seems too brief for such a significant piece of work.

On page 9-21 the authors are discussing various proteomic and transcriptomic studies. These topics are well done, and the authors do a nice job of distilling the major patterns that are apparent, even after just a small number of studies have been published. However, perhaps the Biswas et al. (2008) paper (see citations) on wheat genotypes and breeding should be mentioned in either this section (which is discussing genes at the molecular level) or in a later section (i.e., 9.4.4.1, pg 9-61).

On page 9-38 the authors review the causes of decline in photosynthesis due to O_3. One possibility that has not been extensively discussed is whether or not photosynthesis and other physiological processes proceed at near normal rates in those parts of the leaf that do not show O_3-induced stipple: that is, are the green areas that remain uninjured still photosynthetically competent? Most studies of gas exchange simply express rates on a total leaf area basis, without regard for how much of the leaf is showing stipple or injury. When leaves subject to acidic deposition were measured for their gas exchange (Neufeld et al. 1985), the rates were unaffected if expressed on a green leaf area basis, whereas if the necrotic areas were included, this necessarily lowered the rates. If rates are high in visibly unaffected portions of the leaf, then that suggests several things: (1) that effects of O_3 are highly localized within the leaf; (2) that portions of the leaf that are uninjured continue to function at near normal rates, and (3) that declines in photosynthesis due to O_3 may not always result from a general inactivation or destruction of all RUBISCO and associated enzymes, but may simply be due to loss of competent leaf area in those areas exhibiting stipple.

In section 9.4.2, I think the authors could have beefed up their discussion of genetic variation in non-crop species. For example, Somers et al. (1998) and others (Chappelka et al. 2003, Souza et al. 2006) showed extensive genetic variation in symptom expression in the field for both herbaceous and woody plants. Furthermore, this section might benefit from a slight extension of the discussion of the value of genetic diversity among wild plants.

The meta-analyses of Wittig et al. (2009) which purport to show 7% reductions in growth at O_3 as low as 40 ppb are perhaps worthy of further discussion. Although the magnitude of reduction is consistent with previous studies, as pointed out by the authors of this chapter, they are expressed relative to growth in charcoal-filtered controls. There is current discussion as to whether or not a charcoal-filtered control is appropriate if background O_3 is near 40 ppb. Furthermore, none of my tree studies in the Smokies showed any detectable growth reductions in OTCs below ambient levels of O_3. Therefore, I think these conclusions may warrant some rethinking and further analysis.

I was glad to see the discussion of the potential impacts of ozone on lower plants, such as mosses and lichens. These plants cover a substantial portion of the surface of the earth, and while small in stature, have large ecological footprints. If they turn out to be affected by O_3, it could have ramifications for ecosystems around the world.

On page 9-46, the authors refer to the trees in the AspenFACE site as a "forest in Wisconsin". I think this is somewhat misleading. Every one of those trees was planted, so even though it could be considered a forest, it is a highly artificial one. I would augment this by referring to this forest as an "artificial forest", or a "planted forest". And even if it is considered a "forest", it is limited to just three species, or in the other half of the plots, to one species with many genotypes. Neither is typical of a natural forest.

In section 9.4.3.2, Summary, last paragraph, the authors might consider modifying or adding to the conclusionary sentence. I think it is important to stress that many O_3 effects on native vegetation in the field were found at ambient levels of ozone, to distinguish those effects found using elevated O_3 under controlled conditions. It's important to show and explicitly mention that current ambient levels of O_3 are negatively impacting vegetation.

On page 9-54, there is no mention of David Weinstein's analysis of the potential impacts of O_3 on trees in Great Smoky Mountains National Park. This analysis was published in a SAMAB report. I think it is important to include this and to expand the discussion beyond California.

I am surprised that most of the current modeling for O_3 effects on plants is using data that is 20 to 25 years old (see pg 9-56, bottom). Also, these models are all using data obtained from just one research group. Are there no other more current or varied data sets to use to parameterize these models? I find that incredible and somewhat disappointing as it perhaps indicates how the lack of funding has hindered our attempts to learn more about the effects of O_3 on plants.

I was also surprised not to see any papers by Muntifering when discussing the impacts of O_3 on the nutritive quality of crop and range plants. His research group, in conjunction with European researchers, has shown declines in the quality of forage after exposure to ozone (section 9.4.4.2, pg 9-64).

Figure 9.7, pg. 9-69, could possibly be modified to also include a *decrease* in water loss due to the loss of canopy leaf area resulting from O_3 exposure. A loss of canopy would also open up more of the soil surface to direct radiation input, which might enhance evaporation from the soil surface, unless there are feedback loops where vegetation in the lower soil layers becomes denser and shades the soil surface.

When discussing sluggish stomata, the authors should consider that in some cases, stomatal conductance is reduced when stomata begin exhibiting sluggish responses. So, if they fail to respond to environmental stimuli, and remain open, but not to the same degree as in the absence of O_3 exposure, then there may not be *enhanced* water loss relative to a no O_3 condition. It could, in fact, be less.

In section 9.4.9.2, the authors state that after a single 4 hour exposure to O_3 there was reduced pulmonary macrophage phagocytosis in a toad. It would help to specify the actual exposure in ppm*hrs.

I was pleased with the sections dealing with the various exposure indices, and the development of exposure-response functions. The analyses were carefully done and results clearly show detrimental effects of O_3 on the growth of plants, and in particular, the important role for higher O_3 concentrations. The separate discussion of the results from Gregg et al. (2003, Nature) which show an 82% reduction in biomass, presumably due to O_3, compared to less than 20% for other tree species in the same genera in other studies, points out how unusual Gregg's results were. The authors adopt the most parsimonious explanation, which is that these are valid results with no confounding effects. While this is the most logical conclusion to reach at this time, it would seem prudent to wait until someone replicates this experiment before placing too much emphasis on the results.

In conclusion, the chapter is well written, comprehensive, and brings together most of the relevant literature published since the 2006 report.

Typos
Pg and lines
9-12, line 22 – the superoxide chemical structure is missing a minus sign
p-14, line 6 – subscript needed in CO2
9-16, figure 9.4 legend – in section (a), change "reactions" to "reaction and "is" to "are"
9-18 – Arabidopsis is sometimes italicized, and sometimes not. Decide whether to italicize, and then do throughout document.
9-18, line 12 – change MAP kinase to MAPK
9-21, line 14 – insert space before "ethylene"
9-25, line 5, there is an extra period at the end of the line; line 12 – change "was" to "were"
9-28, line 22 – insert "ozone" before "for 60 days…"
Line 37 – insert "ozone" before "conditions"
9-37, line 3 – take out "in" before "have been…"
9-46, line 9 – subscript 3 in "O3"; line 16 – Change "Wittig et al" to "They"; last line – take out "the"
9-47, lines 18-20: rewrite as: "…; however, apparent direct O_3 treatment effects were obscured by high variability in the data."
9-49, line 13 – change "meta-analysis" to plural; change "demonstrates" to "demonstrate"
Line 17 – refer to the forest as a "planted" forest, since it is not natural.
Lines 26-27 – should this conclusion refer specifically to "ambient" O_3?
9-51, Table 9-1 – the first species name is misspelled. Should be "*Apocynum*" with an "m" at end
9-53, Table 9-3 – for Tregro section on carbon uptake, insert "of" at beginning of last line
In TEM section, carbon uptake section, change "vegetations" to singular
9-56, line 23 – take out comma after "damage"

9-57, line 6 – change "provided" to "provide". In fact, rewrite entire paragraph in present tense. Line 15 – insert "the" before "Mid-"; line 19 – change "those" to "these"; line 23 – change "sink" to plural;
9-58, Table 9-3 – use negative exponents in units at bottom of table in line a.
9-59, line 10 – need a space after AQCD
9-61, line 3 – change "phenolics" to singular; line 34 – change "merits" to singular
9-64, line 20 – take out "was" and add "of" at end of line; line 22 – take out "were" and rewrite as: "…considered by these authors.; line 24 – change "demonstrates" to "demonstrated"; line 27 – change "is" to "was"; line 29 – take out period before citation.
9-69, line 3 – insert "widths" after "aperture"
9-70, line 28 – take out "of"
9-74, line 30 – sentence is missing some words. Needs to be fixed.
9-76, line 5 – change "have" to "had"; line 25 – italicize *Quercus ilex*
9-79, line 13 – insert "an" before O3-tolerant; line 26 – change "decreased" to "decrease"; line 31 – change "suggested" to "suggest"; line 32 – change "were" to "are"
9-82, line 25 – insert "the" before "exotic"
9-83, line 34 – change "the" to "that"
9-84, line 4 – insert "showed that" after "community"; line 5 – italics for scientific name; line 6 – take out "most studied" and italicize scientific name again;

9-85, line 26 – insert "the" at end of line; lines 28, 29 – italics for scientific names
9-86, line 12 – insert "the" before "Carpathian"
9-93, line 9 – change "to" to "in" before "plants"
9-94, line 18 – take out comma after "site"; line 20 – take out parentheses around scientific name; line 31 – spell out genus name for Japanese beetle.
9-95, line 11 – change ";" to a comma.
9-97, line 6 – change "were" to "was"; line 7 – insert "litter from" before "trees grown"; line 12 – move scientific name up to line 10 where earthworms are first mentioned; line 30 –change "is" to "are"
9-99, line 18 – take out comma after scientific name
9-100, line 8 – change "that" to "than"
9-101, line 2 – insert "the" before "secondary"; line 12, insert comma before "temperature"
9-102, line 3 – definition of SUM06 is incorrect. It should be "…concentrations at or above 0.060 ppm. Add a comma after "summed". Line 6 – insert "the" before "summed".
9-110, line 7 – change "occurred" to "occur" and "were" to "are"; line 8 – change "were" to "are"
9-111, line 32 – "stomata" should be "stomatal"
9-114, line 25 – insert "the" before "secondary"
9-141, line 10 – add "with" at end of line

Comments on Chapter 10 – ISA for Ozone and Other Photochemical Oxidants

This short chapter is, like the previous one, well written. It clearly summarizes a wide variety of articles on the impacts of tropospheric O_3 on possible climate change and does so in a very readable format. Most importantly, it succinctly summarizes the various states of knowledge concerning tropospheric O_3 impacts on climate change.

I found very little of substance to comment on. Most sections were well done and reached logical conclusions based on the literature and data available. In section 10.3.2.1, pg 10-11, there are no referrals to the EPA Trends reports, which I found puzzling. This section would benefit by their inclusion.

In Section 10.4.4, there are no mentions of Joe Sullivan's, Alan Teramura's or Martin Caldwell's work on effects of enhanced UV-B on plants. Perhaps this is because most of this work was carried out prior to 2006, but even so, if this section is briefly reviewing UV-B effects, their work would be highly relevant here.

On pg 10-6, the authors use the word "deposited" when discussing albedo effects. Albedo doesn't affect the amount of energy deposited to a surface, only the amount that is retained at the surface. Thus, perhaps the authors would consider replacing "deposited" with "retained", since that more clearly reflects the mechanism of action here. On page 10-24, it seems to me that the last two sentences of the aquatic ecosystems section would better fit in the next section on changes in biogeochemical cycles.

The rest of the chapter is very well done, and I have no major comments.

Typos:
10-7, line 2 – I think this statement is too strong. I would insert "some of" before "these processes" and on the next line, replace "is" with "can be"; line 7 – change "are" to "is";
10-13, line 17 – I would again replace "deposited" with "retained"
10-15, line 29 – change "leading" to "lead"
10-16, line 26 – change "at" to "by" before "2030"; line 27 – insert "may" after "precursors" and change "increases" to "increase"
10-25 – insert "the" before "southeastern"

Dr. James Ultman

Comments on Chapter 5: Dosimetry and Mode of Action

There have numerous improvements in the organization and content of this chapter. The addition of an overall chapter introduction now clearly lays out the goals of the chapter. The background information on gross anatomy included in this introduction is also a useful addition. The elimination of the sectional subdivision between research in the previous ISA and newer research has improved the flow and readability of the text. Reversing the order of the sections on ozone uptake (now first) and ozone reaction products (now second) provides a more logical progression to the discussion of MOA. Shifting material on ozone product formation from old section 5.2.2 on to new section 5.2.3 on secondary oxidation was also a logical change in the ordering of the material.

An important aspect of this chapter is associating known ozone reaction products with the biological responses that they might trigger. The chapter competently reviews the literature in this regard, but does not clearly prioritize these alternative pathways. In the chapter summary, it would be useful to venture an educated guess at what substances (including ozone) and its consequent MOA are the most important.

A major criticism in the previous review was that the first part of the chapter on dosimetry was disconnected from the second part of the chapter on MOA. With an improved organization in the revised chapter that separates dosimetric aspects of chemical reactions (Section 5.2) from the effects of reaction products on MOA (Section 5.3), this disconnect has been overcome. Other important criticisms that have also been successfully addressed were: the paucity of information concerning ventilatory changes during exercise (Section 5.2.2.7); and comparative anatomy and dosimetry between humans and laboratory animals (Sections 5.1 and 5.5.1).

Although dosimetric principles have been better explained, there are still some places where further clarification is needed. First, there is still virtually no concrete connection between dosimetry principles and theoretical or experimental observations of dose distribution and tissue damage. Second, specific definitions and consistent use of terminology for the different types of dose has been improved but some further refinements are still necessary. Specific suggests for these changes are included in the detailed comments below.

Detailed Comments:

Page Line

5-1 12 Change “is” to “are”

5-1 16 Change “relevant” to “related”

5-2 fig 5-1 The arrow between inhaled dose and tissue dose suggests that O3 can be absorbed without the need for transport through the ELF. Does this mean that there may be some “dry” spots on the epithelial surface? Some explanation in the figure caption would help.

5-2 fig 5-1 Change caption to read: “Schematic of the O3 exposure and response pathway. O3 transport follows a path from exposure concentration, to inhaled dose, to net dose, to the local tissue dose. Chapter 5 discusses the concepts of dose and modes of action that result in the health effects discussed in Chapters 6 and 7.”

5-2 10 Change “to the concentration of” to “to the quantity of”

5-2 1-13 This paragraph still needs some fine tuning. In particular, it is important to define the different doses that will be used in the chapter. I suggest that definitions of the following terms be incorporated in this paragraph and tied into figure 5.1. It should also be mentioned that the “net dose” represents the O3 that is available for reaction with tissue. The other definitions of dose are more-easily measurec surrogates for the net dose.
1) Exposure concentration.
2) Effective (or inhaled) Dose=concXmin.ventXtime
3) Net Dose=amount or rate of entry of O3 across the gas/ELF interface.
4) Tissue Dose=amount or rate at which O3 or its reaction products reach target tissue sites.

5-5 3 Change “its effective dose” to “its tissue dose”

5-5 7 Change “surfactant.” to “surfactant solution.”

5-5 10-12 It is premature to use the term "uptake" in this paragraph because it is not defined until the next paragraph.

5-5 10-11 Change “Ozone uptake…termed reactive absorption” to “Ozone dose is directly related to the coupled diffusion and chemical reactions occurring in ELF, a process termed reactive absorption.”

5-5 11-12 Change “Thus, the uptake...is related to both” to “Thus, O3 dose depends on both”

5-5 13-15 Delete the first sentence. Change the second sentence from “Ozone uptake is affected by complex interactions between a number of major factors including RT morphology…” to “Ozone dose is affected by complex interactions between a number of other major factors including RT morphology,”

5-5 30 Add the sentence "Measurements of O3 dose have been inferred from simultaneous measurements of airflow and O3 concentration at the airway opening of the nose or mouth (Weister, 1996; Nodelman and Ultman, 1999) as well as at internal sampling catheters (e.g., Gerrity et al., 1988,1995)" between the existing sentences "…O3 dosimetry." and "One method…"

5-5 32 Change "The O3 in the breath that is removed during the breathing period is termed" to "The difference in the amount of O3 inhaled and exhaled relative to the amount inhaled O3 is termed"

5-6 2 Change "fractional uptake of O3" to fractional absorption of O3"

5-6 3 Change "LRT(FLRT) are presented.." to "LRT(FLRT) relative to the amounts of O3 inhaled into the region are presented.."

5-6 5-10 The existing paragraph inadequately dispersion and the relative importance of different transport mechanisms in different airways. I suggest substituting the following paragraph:
"The three-dimensional transport of O3 in the lumen of an airway is governed by bulk flow or convection and diffusion. When modeled as a one-dimensional process in which the radial profiles of axial velocity and O3 concentration profiles are flat, O3 transport along an airway lumen occurs by convection, axial diffusion and a coupled diffusionreaction process called dispersion. Simultaneously, O3 diffuses into the ELF where it undergoes radial diffusion and chemical reaction (Figure 5-3c) (Miller, 1995). The relative importance of these transport mechanisms varies among RT regions for a given level of ventilation in any species. In the URT and major bronchi, bulk airflow tends to be the predominant mechanism for axial transport in the airway lumen, and diffusion dominates chemical reaction in the ELF. However, in the alveolar region of the lung, diffusion is the major gas transport mechanism while reaction dominates in ELF."

5-7 7-11 Similarly, these sentences give an inadequate explanation of dispersion. I suggest changing lines 7 to 11 with the following text:
"profile and diffusion. 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 the O3 that operates in parallel with bulk flow and axial diffusion (a process caused by the ever-present Brownian motion of individual O3 molecules). The 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"

5-7 19-22 Change the last two sentences of this paragraph to:
"Gas molecules close to the alveolar-capillary membrane have almost zero convective velocity with respect to the membrane, and this creates a substantial boundary layer resistance to O3 transfer across the gas-ELF interface. Thus, the transport of O3 through the ELF has a more important role in the peripheral lung than in the TB region."

5-7 6 This paragraph would be a logical place to explain how principles can be used to explain simulation results or data. In particular, how do the expanding summed airway cross-sectional area, increasing surface-to-volume ratio, and decreasing mucous thickness with increasing generation contribute to differences in axial transport and lateral absorption in different lung regions. In combination with changes in mucous thickness, how do these effects explain the tissue dose distribution (predicted by the models and observed in the O3-18 studies) and the net dose (predicted by the models)?

5-8 28 Change "nonlinear reaction kinetics could result" to "non-linear kinetics of O3 uptake fraction"

5-9 3 Change "and 46% between the mouth" to "and 46% during a complete breath in which an O3 bolus penetrated between the mouth"

5-9 6 Replace this line by "resulted because these investigators measurements were based on inhalation alone or was caused by O3 scrubbing by the mouthpiece."

5-9 14-15 Change "(i.e., flow rate…) was" to "(i.e., flow rate×exposure concentration×(1- nasal absorbed fraction)) was"

5-9 25 Change "removes half" to "removes about half"

5-9 32-34 In order to compare the reliability of net dose compared to tissue dose, this paragraph should be expanded. In particular, the O3-18 measurements reveal maximum damage in the CA region and less damage in the more proximal and more distal airways. This is consistent with distribution of O3 tissue dose predicted by single-path models, and suggests that O3 tissue dose is a good predictor of O3 damage. On the other hand, some of the damage might be due to toxic reaction products. The net O3 dose can be an indicator of such products, particularly when the formation of these products is rapid (e.g., in the extreme of an infinitely fast reation, all the O3 that crosses the gas-ELF interface is converted to product before reaching the ELF-tissue interface).

5-10 12 Change "uptake was" to "uptake efficiency was"

5-10 31-33 Replace these lines with:
"reaction rates of O3 are proportional to the O3 concentration. As mentioned above, a weak negative relationship between O3 concentration and uptake efficiency was reported for the nasal cavities by Santiago et al., 2001. Rigas et al. (2000) also found a weak but significant negative dependence of O3 concentration on RT uptake efficiency in exercising"

5-11 4-5 Replace these lines with:
"computational fluid dynamics model was created to investigate O3 transport in a single airway bifurcation (Taylor et"

5-11 13 Replace line 13 with:
"child. This model predicted velocity distributions that were consistent with the earlier work of Schroter and Sudlow (1969), and also reported O3 concentration and wall uptake distributions. The model"

5-12 6-7 Not clear why this particular change was singled out to emphasize. Therefore, either delete the sentence or broaden it to other conditions.

5-13 7 Delete “URT”

5-13 31 Change “the TB” to “the URT and TB”

5-14 32 Change “the upper airways” to “these airways”

5-15 27 Sawyer studied the nasal cavities only. I expect that "1.6-fold higher delivered dose rate to the lungs" was an inference made from Sawyers results. If so, this should be stated.

5-28 31 Change “the product of airway resistance and thoracic gas volume” to “the ratio of airway resistance to thoracic gas volume”

5-14 25 The study of Hu et al.(1994) was not done under exercising conditions. Rather, the inspired and expired flow rates approaches those attained during exercise.

5-68 10 Change “airflow patterns such that major airflow streams are created” to “airflow patterns, particularly the shape of major airflow streams.”