Preliminary Comments from Members of the CASAC Oxides of Nitrogen Review Panel on EPA's Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (First External Review Draft – November 2013)

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

General Comments

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

Charge Questions

a. To what extent is the information presented regarding characteristics of sources, chemistry, monitoring concentrations, and human exposure accurate, complete, and relevant to the review of the NO2 NAAQS?

Source characterization, NOx chemistry, and summaries of monitored concentrations are appropriately covered. Both NOx chemistry and human exposures to NOx are complex topics covered in this chapter; both are covered in sufficient detail. The issue of exposure mis-classification and the errors it introduces in analysis of NO2 health effects is clearly explained. The spatial gradients and non-ambient sources of urban NO2 can lead to substantial uncertainties in personal exposures; this is discussed in great detail.

b. To what extent are the analyses of air quality presented clearly conveyed, appropriately characterized, and relevant to the review of the NO2 NAAQS?

The air quality analysis presented in this chapter is clearly presented and characterized in sufficient detail in ways that support the NO2 NAAQS review. I would suggest that the 1-hour maximum NO2 concentrations in Table 2-1 be reviewed; a 1-hour value of 360 ppb NO2 is inherently suspect and may be due to instrument calibrations or potential exceptional events that were not removed from the data set. It might be helpful to include the related (same hour) 1-h max NO concentrations to this table (just one additional column).

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

The source category groupings and related emission data and discussion clearly show the relative contributions to NOx across different source types. Spatial scales are important for NO2 given the very wide dynamic range of concentrations from elevated near-source urban concentrations to far rural locations where nearly all NOx has been either converted into other oxidized nitrogen species or removed from the atmosphere.

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

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

e. Please comment on the accuracy, level of detail, and completeness of the discussion regarding exposure assessment and the influence of exposure error on effect estimates in epidemiologic studies of the health effects of NO2.

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

Specific Comments

There are many discussions of the literature in this chapter that present results for NO, NO2, or NOx in an inconsistent manner. In the same paragraph, for the same specific topic, study results are sometimes cited for NO, another study for NO2, and a third for NOx, making it difficult to compare results across related studies. An example of this is pg. 2-40, lines 4-27. It may be that some studies only reported results for only one of these pollutants, but I suspect in many cases both NO and NO2 data were reported. When only one pollutant was reported, it would be helpful if that was noted if the discussion includes references to the other pollutants.

NO2 and NOx play very different roles in exposure assessment. The ISA does make it clear that NO2 is the component of NOx shown to be of concern for health effects, and that NOx is preferred to NO2 as a marker of exposure to a wide range of near-road pollutants that could be expected to have health effects, since it is mostly conserved at the neighborhood to small urban spatial scale. Thus both play important but very different roles in health effect assessments. This distinction gets lost in some of the discussion in this chapter.

Pg 2-4 lines 102: this discussion of HNO3 deposition reads like wet deposition dominates, but dry deposition is also a major sink.

Pg 2-10 lines 9-11: it would be helpful to add the fraction of NO2 in NOx for non-catalyzed diesel emissions for comparison. It could be noted here that CDPFs have not been allowed for several years now because of these increased NO2 emissions.

Pg 2-11, Highway Vehicles. The recent final Tier 3 rule for gasoline engine emissions and lower S gasoline will provide a substantial reduction in NOx. Reductions of ~ 25% will rapidly be

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

Pg 2-12 lines 26-28: the HEI ACES phase 2 results were published in early December and thus should be included in the revised ISA. These results are summarized in the press release at:
http://www.healtheffects.org/Pubs/ACES-Phase2-Final-Press-Release-120413.pdf
The full report is at:
http://crcao.org/reports/recentstudies2013/ACES%20Ph2/03-17124_CRC%20ACES%20Phase2-%20FINAL%20Report_Khalek-R6-SwRI.pdf
The report's results indicate that emission reductions substantially exceeded those required by the 2010 HDD engine rule.

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

Pg 2-21 and -22, section 2.4.2, Other Methods for Measuring NO2. This discussion mentions the cavity attenuated phase shift (CAPS) method, which is sensitive and specific to NO2. It is worth noting that one commercial CAPS NO2 monitor now has FEM approval and a second commercial CAPS monitor is in the final stages of FEM approval at ORD. These methods are expected to be a practical alternative (in terms of cost and operational effort) to the traditional CL-moly converter FRM monitor. One consideration in wide routine network deployment of CAPS or any other method that only measures NO2 (e.g., does not measure NO) is the potential loss of NOx data; NOx is often the only widely available exposure surrogate for on-road pollutants.

Pg 2-29 lines 10-12: the revised ISA should include specifics on the number of operational near-road NO2 sites, and if at all possible, summaries of data from those sites.

Pg 2-40 lines 28-38 and next page: this discussion of the EPA NO2 near-road pilot study should note that these were passive integrated samples of at least one-week duration and thus do not reflect short-term (e.g. hourly) concentration patterns.

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

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

Dr. Matthew Campen

Comments on Chapter 3

a) *Given the ubiquity of reactive substrates and reaction rate of NO2 with these substrates, it appears unlikely NO_2 itself will penetrate through the lung lining fluid to the epithelium (see Table 3-1). Please comment of the adequacy of the discussion of NO_2 uptake and reactivity in the respiratory tract.*

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

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

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

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

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

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

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

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

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

3.3.2.4 Epithelial Barrier Function

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

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

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

While exceedingly high exposures are often detailed, many times in discussions of human studies these facts are omitted. Channell et al and Huang used 500 ppm for 2 h and saw significant effects – this seems important information, in light of the studies where neural effects were not observed until mice were exposed to >10,000ppm. Moreover, by limiting the outcomes of Channell et al to "changes in blood lipids and increased levels of plasma soluble lectin-like receptor for oxidized low density lipoprotein", the upshot of observing inflammatory signaling resulting from the whole plasma is lost. These functional outcomes require some further consideration, given the low concentrations of NO2 and that similar outcomes were seen with diesel emissions (which contain a comparable amount of NOx).

3.3.4 Perhaps examples in the literature could be used to show that NOx exposure leads to upregulation of NO2/3, S-NOs and nitrated lipids? This section just seems a bit too academic.

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

Comments on Chapter 5

Fig 5.1 could use a more descriptive caption.

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

Dr. Ronald C. Cohen

Comments on Chapter 2

The chapter provides a useful overview.

Detailed comments follow:

Section 2.2

Figure 2.1 could be more clear:

isoprene nitrates and Alkyl nitrates are subcategories of RONO2; nitroaromatics and nitroPAHs are closely related and they are not directly related to RONO2. They have direct C-N bonds.

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

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

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

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

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

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

Section 2.3

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

see for example:
A.R. Russell, L.C. Valin, and R.C. Cohen, *Trends in OMI NO2observations over the United States: Effects of emission control technology and the economic recession*, Atmos. Chem. Phys. 12, 12197-12209, 2012. Note that many of the figures used in the report are also in this paper--but were peer reviewed unlike the ones in the report. There is not a significant difference in the point made by the images though.

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

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

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

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

Section 2.4

pg 2-19 lines 16-27

The best reference on the MoO convertors is Winer et al. 1974. After that paper it was widely accepted in the scientific community that the FRM for NO2 should be interpreted as NOy. There is absolutely nothing new about the more recent papers. If you ask the authors of the 2007 papers why they wrote them (and I did)--the answer you get is that regulatory agencies in the US and Europe couldn't be made to pay attention to the Winer et al. result without new measurements. I believe there was new attention because some people recognized a commercial opportunity for patentable technology.

I think the claim of variable sensitivity to positive interferences is too general. There is variable sensitivity to HNO3 based on inlet designs that fail to transmit HNO3 to the convertor and occasional materials issues prevent reduction of HNO3 to NO, however there is no variability in the sensitivity to RO2NO2 (e.g. PAN) or RONO2 (e.g. isoprene nitrate) molecules.

pg 2-19, line 28 The statement is not correct. There are numerous measurements prior to those referenced that make the same point--they just didn't label themselves as such because the scientific

community had moved on to calling the FRM NO2 method an NOy detector. For example there is an extensive literature attempted to close the NOy budget-comparing FRM measurements to the sum of distinct measurements of individual nitrogen species.

See for example:

Fahey, D. W., G. Hubler, D. D. Parrish, E. J. Williams, R. B. Norton, B. A. Ridley, H. B. Singh, S. C. Liu, and F. C. Fehsenfeld, Reactive nitrogen species in the troposphere: Measurements of NO, NO2, HNO3, particulate nitrate, peroxyacetyl nitrate (PAN), O3, and total reactive odd nitrogen (NOy) at Niwot Ridge, Colorado, J. Geophys. Res., 91(D9), 9781 – 9793, 1986.

and a review of those issues in:

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

pg 2-21 line 4 should read: "products, including HNO3, PAN and its analogues and total RONO2.

pg 2-21 lines 5 and 6 should be deleted. A quite accurate estimate (+/-30% or better) of true NO2 can be arrived at from NO and O3 measurements thus provided a good measure of the size of the interference to any FRM "NO2" measurement.

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

Section 2.4.2

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

Section 2.4.3

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

pg 2-26 line 9 delete " from ...and since NO2 is mainly a near surface pollutant ..." to the end of the sentence. The mixing heights are not directly related to the point being made. They only come in very indirectly as the NO2 lifetime is longer at higher NO2.

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

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

Section 2.4.4

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

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

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

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

Section 2.5.1

page 2-37 line 1: define short; I think the answer is ~4hrs. also should read "to PANs, RONO2 and HNO3" define highly variable; I think it would be correct to say concentrations of NOx decay on e-folding length scales of approximately 50km in summer and 200 km in winter. There is direct evidence for that in the satellite observations including the figures already in this report and also in L.C. Valin, A.R. Russell and R.C. Cohen, Variations of OH radical in an urban plume inferred from NO2 column measurements, Geophys. Res. Lett. 40, 1856-1860, 2013. and references therein. Also in numerous other papers using the NOAA aircraft to fly downwind of urban and powerplant plumes and measurements along a transect of urban plumes such as the Sacramento one.

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

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

Sections 2.5.2 and 2.5.3

These sections would be easier to read if the intro section had a separate discussion of NO/NO2/O3 chemistry and how titration works. Specifically how the ratio of NOx to O3 affects the behavior.

pg 2-40 lines 26 and 27 the conclusion that NO2 is freshly emitted is likely incorrect and is not substantiated. Simple analysis of the rate of conversion of NO to NO2 indicates NO2 would be 5 ppb 10 seconds after mixing out of the exhaust plane.

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

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

pg 2-43 The analysis presented on this page is somewhat confusing and convoluted. It would be more straightforward to present NOx first and then discuss partitioning of that NOx into NO and NO2.

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

pg 2-43 lines 9-12 Absolute NO gradients are not evidence for the stated effect. The sentence should be deleted. The proper evidence would be NO/NO2 ratios.

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

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

pg 2-47 lines 21-22 satellite observations are not concentrations, they are columns. It would be correct to say satellite observations converted to concentration using a model of the vertical distribution of NO2.

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

pg 2-49 The discussion of O3-NOx relationships in this chapter is not well connected to the long standing understanding of those relationships. It will help if the chapter has a better introduction to the NO/NO2/O3 chemistry as that chemistry explains a lot of the correlations discussed. Also, the larger spatial scale relationships between NOx and O3 are better understood that indicated in this document, see for examples S.E. Pusede and R.C. Cohen, On the observed response of O3 to NOx and VOC reductions in San Joaquin Valley California 1995-present, Atmos. Chem. Phys. 12, 8323-8339, 2012 and the references therein.

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

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

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

pg 2-65 lines 7-27 Since it has already been noted that the FRM has a positive bias due to sensitivity to PAN, RONO2 and HNO3, it should be noted here that the agreement between the FRM and this other sensor implies similar biases in the other sensor.

pg 2-68 lines 8-9

NO2 doesn't react with organic radicals to produce RONO2--or at least such reactions are too slow to matter. The reactions that produce RONO2 are NO3 and NO reactions.

pg 2-68 lines 33-36 Note NO2 reacts with O3 to form NO3 (as discussed later in the text) I'm not sure how that fits into the analysis presented in the referenced papers, but it is an important consideration for interpreting the experiments described.

pg 2-70 The figure referenced should separately identify near roadway and other studies as we expect different correlations in the two regimes. In both NOx would be correlated with other primary pollutants but in the near field of emissions the reaction of NO with O3 results in increases in NO2 while decreases in other primary pollutants are decreasing. As presented the figure suggests there is unexplained variability.

pg 2-71 lines 11-27 There are many, many studies describing why the relationships of ozone and NO2 are expected to be nonlinear. One reason there are few studies describing a linear correlation is that attempts to do so are unlikely to survive peer review as they are presenting a model of the relationship that is known to be flawed. The Pusede and Cohen paper listed above include many relevant references to the issue--but it is by no means comprehensive or complete.

pg 2-20-2-71 and figure 2-19 also pg 2-78 line 5
The role of near road titration on observed correlations should be explicitly discussed. We expect in the near field that ozone and NO2 will be anti-correlated. This issue should not be referred to as "complex chemistry." Then in the far field of a single plume, the two will be positively correlated. However, comparing two different plumes (or one plume at two different initial NOx) the increase in ozone will not be a linear function of NOx.

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

pg 2-80 line 30 NO2 is not prevalent in vehicle exhaust. NO is.

pg 2-82 and Fig 2-20.
I think the figure is misleading because the physically relevant parameter is not the increase in a pollutant divided by its background concentration but the absolute enhancement over the background. There are many analyses of plumes in atmospheric science that show that enhancement ratios defined in this way (e.g. Delta CO enhanced : Delta NOx enhanced) remain conserved during mixing with a background while the ratios to the background vary. On the relevant times scales there are no known losses of NOx or CO, so an analysis that indicates the two behave differently is odd and should be treated with caution.

pg 2-102 lines 12-13 I do not think the diesel statement is relevant. If NOx is less than O3, then on time scales of 100 sec (e.g. 300m at 3m/s winds) NO/NO2 and O3 approach a photostationary state independent of whether emission is as NO or NO2.

pg 2-102 I think the observation that should be highlighted here is the dramatic drop on weekends in cities in the US (~50%) and the long term trend (~30% 2005-2012). Those large changes provide a significant opportunity for new epidemiological studies of the short term health response (weekdays vs weekends) and of the benefits of long term reductions (2005-2012). These issues are much more important to understanding the health effects of NO2 than whether NO or NO2 is emitted from tailpipes.

Dr. Douglas Dockery

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

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

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

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

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

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

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

In this ISA, there is a clear enunciation of "weight of the evidence criteria causal determination" (Table 11, page l). Five levels of evidence are defined – **Causal** relationship, **Likely** to be a causal relationship, **Suggestive** of a causal relationship, **Inadequate** to infer causal relationship, and **Not Likely** to be a

causal relationship. The ISA finds that the evidence has grown stronger for a causal relationship with ambient NO_2 compared to the 2008 ISA for all health end points considered.

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

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

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

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

Dr. Philip M. Fine

Comments on Chapter 2

Page 2-47, second paragraph in Chapter 2.5.4
The text states that while mean concentrations are highest in the first and fourth quarters, maximum concentrations are highest in the second and third quarters. Table 2-1 is cited for support of these seasonal trends, but the Table does not include seasonal data. Furthermore, much of the discussion in this chapter describes higher peak NO2 concentrations in winter, as one would expect from meteorological considerations. The statement that higher maximums are seen in the spring/summer months should be corrected or supported with data.

Page 2-50, Figure 2-16
The blue shaded range in the figure is not explained. Is it the full range across all sites, 5/95 %iles, or standard deviations? It needs some explanation in the caption.

Dr. Panos Georgopoulos

Comments on Chapter 3

Chapter 3 characterizes scientific evidence on the dosimetry and modes of action for NO2 and nitric oxide (NO). Dosimetry and modes of action are bridged by reactions of NO2 with components of the extracellular lining fluid and by reactions of NO with heme proteins, processes that play roles in both uptake and biological responses.

a. Given the ubiquity of reactive substrates and reaction rate of NO2 with these substrates, it appears unlikely NO2 itself will penetrate through the lung lining fluid to the epithelium (see Table 3-1). Please comment of the adequacy of the discussion of NO2 uptake and reactivity in the respiratory tract.

The assumption that it is unlikely for NO_2 itself to penetrate through the lung liing fluid to the epithelium appears generally reasonable. However, describing the interaction of NOx with the extracellular lining fluid (ECLF) in terms of classical (Fickian) diffusion processes and homogeneous chemical reactions would be an oversimplification that may be insufficient with respect to describing actual *in vivo* ECLF/NOx system dynamics. There is a need to understand and describe mechanistically the spatiotemporal dynamics of NO_2 transport and reaction within the various microenvironments of the respiratory system, taking into account that the ECLF is far from homogeneous, both across the respiratory system and within particular microenvironments (such as the alveolar microenvironment). These observations should also apply to NO, which in fact is known to enter alveolar epithelial cells, but potentially through processes that are not diffusion-dependent (e.g. Brahmajothi et al., 2010).

References:

Brahmajothi, M.V., Mason, S.N., Whorton, A.R., McMahon, T.J., and Auten, R.L. 2010. Transport rather than diffusion-dependent route for nitric oxide gas activity in alveolar epithelium. *Free Radic Biol Med* 49 (2):294-300. DOI:10.1016/j.freeradbiomed.2010.04.020

b. Since existing dosimetric models for NO2 do not consider the probability of oxidants/cytotoxic products reaching target sites, it was concluded that these models are inadequate for within or cross species comparisons. Please comment on the validity of this conclusion and identify and comment on the validity of any alternative conclusions.

The conclusion that existing dosimetric models for NO_2 are inadequate is in fact valid. Development of a detailed mechanistic conceptual comprehensive NO_2 dosimetry model, followed by subsequent computational implementation, is critically needed, along the lines of similar efforts that have taken place in recent years (e.g. Asgharian et al., 2011). Such a model should explicitly account for different life-stages and altered health states (development, obesity, aging, etc.), in a framework that takes into account existing hypotheses for NO_2/NO transport and transformation in the respiratory system. Even

during its development, this model would provide a useful tool for hypothesis generation and rational design of future laboratory studies. Of course, pursuing development of this model cannot take place as part of the current review process but it would be important for specific dosimetry modeling needs to be identified. It would also be important at the present time to summarize explicitly the major deficiencies and uncertainties associated with the lack of valid NO_2 dosimetry model; it is recommended to consider including such a summary in the form of a brief table in Section 3.2, where these issues are discussed.

References:

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

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

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

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

References:

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

Fukuto, J.M., Carrington, S.J., Tantillo, D.J., Harrison, J.G., Ignarro, L.J., Freeman, B.A., Chen, A., and Wink, D.A. 2012. Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species. Chem Res Toxicol 25 (4):769-93. DOI:10.1021/tx2005234

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

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

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

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

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

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

Two additional comments regarding modes of action:

- It appears that all (potential) vascular and systemic effects of NO_2 are lumped under "Transduction of extrapulmonary responses" (discussion in Section 3.3.2.8 on pages 3-43 to 3-46, which provides a brief but informative overview). The spectrum of these (potential) effects does not become clear either in the summary of page 3-59 or (even more) in the corresponding entry of Table 3.3 on page 3-57. It is realized that the uncertainties regarding systemic effects (and the MOAs involved in these) are very large; however, the range (and severity) of health effects that have been hypothesized to be related to NO_2 exposures is so wide that a more detailed listing of the biological mechanisms potentially associated with them would be justified.
- It would be informative to identify explicitly MOAs that may be relevant specifically to cases involving co-exposures with other xenobiotics (since inhalation exposures to NO_2 and NO always occur in the context of a complex mixture of atmospheric contaminants as well as for exposures of subjects with health problems (ranging from obesity to asthma and COPD).

References:

Atochina-Vasserman, E.N., Beers, M.F., and Gow, A.J. 2010. Review: Chemical and structural modifications of pulmonary collectins and their functional consequences. Innate Immun 16 (3):175-82. DOI:10.1177/1753425910368871

Matalon, S., Shrestha, K., Kirk, M., Waldheuser, S., McDonald, B., Smith, K., Gao, Z., Belaaouaj, A., and Crouch, E.C. 2009. Modification of surfactant protein D by reactive oxygen-nitrogen intermediates is accompanied by loss of aggregating activity, in vitro and in vivo. FASEB J 23 (5):1415-30. DOI:10.1096/fj.08-120568

Supplementary References for Consideration by the USEPA

Akella, A., and Deshpande, S.B. 2013. Pulmonary surfactants and their role in pathophysiology of lung disorders. Indian Journal of Experimental Biology 51 (1):5-22

Ather, J.L. 2013. Inflammasome Activity in Non-Microbial Lung Inflammation. Journal of Enviromental Immunology and Toxicology 1 (3):108. DOI:10.7178/jeit.20

Ather, J.L., Ckless, K., Martin, R., Foley, K.L., Suratt, B.T., Boyson, J.E., Fitzgerald, K.A., Flavell, R.A., Eisenbarth, S.C., and Poynter, M.E. 2011. Serum amyloid A activates the NLRP3 inflammasome and promotes Th17 allergic asthma in mice. Journal of Immunology 187 (1):64-73. DOI:10.4049/jimmunol.1100500

Ather, J.L., Hodgkins, S.R., Janssen-Heininger, Y.M., and Poynter, M.E. 2011. Airway epithelial NF-kappaB activation promotes allergic sensitization to an innocuous inhaled antigen. American Journal of Respiratory Cell and Molecular Biology 44 (5):631-8. DOI:10.1165/rcmb.2010-0106OC

Atochina-Vasserman, E.N., Winkler, C., Abramova, H., Schaumann, F., Krug, N., Gow, A.J., Beers, M.F., and Hohlfeld, J.M. 2011. Segmental allergen challenge alters multimeric structure and function of surfactant protein D in humans. American Journal of Respiratory and Critical Care Medicine 183 (7):856-64. DOI:10.1164/rccm.201004-0654OC

Baja, E.S., Schwartz, J.D., Coull, B.A., Wellenuis, G.A., Vokonas, P.S., and Suh, H.H. 2013. Structural equation modeling of the inflammatory response to traffic air pollution. J Expo Sci Environ Epidemiol 23 (3):268-74. DOI:10.1038/jes.2012.106

Cirino, G., Distrutti, E., and Wallace, J.L. 2006. Nitric Oxide and Inflammation. Inflammation & Allergy - Drug Targets 5 (2):115-119. DOI:10.2174/187152806776383143

Dadvand, P., Basagana, X., Figueras, F., Martinez, D., Beelen, R., Cirach, M., de Nazelle, A., Hoek, G., Ostro, B., and Nieuwenhuijsen, M.J. 2014. Air pollution and preterm premature rupture of membranes: a spatiotemporal analysis. American Journal of Epidemiology 179 (2):200-7. DOI:10.1093/aje/kwt240

Dadvand, P., Nieuwenhuijsen, M.J., Agusti, A., de Batlle, J., Benet, M., Beelen, R., Cirach, M., Martinez, D., Hoek, G., Basagana, X., Ferrer, A., Ferrer, J., Rodriguez-Roisin, R., Sauleda, J., Guerra, S., Anto, J.M., and Garcia-Aymerich, J. 2014. Air pollution and biomarkers of systemic inflammation and tissue repair in COPD patients. European Respiratory Journal. DOI:10.1183/09031936.00168813

Delamater, P.L., Finley, A.O., and Banerjee, S. 2012. An analysis of asthma hospitalizations, air pollution, and weather conditions in Los Angeles County, California. Science of the Total Environment 425:110-8. DOI:10.1016/j.scitotenv.2012.02.015

Faustini, A., Rapp, R., and Forastiere, F. 2014. Nitrogen dioxide and mortality: review and meta-analysis of long-term studies. European Respiratory Journal. DOI:10.1183/09031936.00114713

Figueira, T.R., Barros, M.H., Camargo, A.A., Castilho, R.F., Ferreira, J.C., Kowaltowski, A.J., Sluse, F.E., Souza-Pinto, N.C., and Vercesi, A.E. 2013. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. Antioxid Redox Signal 18 (16):2029-74. DOI:10.1089/ars.2012.4729

Gerlofs-Nijland, M.E. 2013. Influence of NO_2 on pulmonary toxicity in mice sub-chronically exposed to diluted diesel engine exhaust. Bilthoven, Netherlands: National Institute for Public Health and the Environment.

Ghosh, J.K., Heck, J.E., Cockburn, M., Su, J., Jerrett, M., and Ritz, B. 2013. Prenatal exposure to traffic-related air pollution and risk of early childhood cancers. American Journal of Epidemiology 178 (8):1233-9. DOI:10.1093/aje/kwt129

Gold, D.R., and Mittleman, M.A. 2013. New insights into pollution and the cardiovascular system: 2010 to 2012. Circulation 127 (18):1903-13. DOI:10.1161/CIRCULATIONAHA.111.064337

Gurley, L.R., London, J.E., Dethloff, L.A., DStavert, D.M., and Lehnert, B.E. 1989. Analysis of proteins in bronchoalveolar lavage fluids during pulmonary edema resulting from nitrogen dioxide and cadmium exposure. In Techniques in Protein Chemistry, edited by T. Hugli. San Diego, CA: Academic Press Inc.

Hiraiwa, K., and van Eeden, S.F. 2013. Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants. Mediators of Inflammation 2013:619523. DOI:10.1155/2013/619523

Janssen-Heininger, Y.M., Persinger, R.L., Korn, S.H., Pantano, C., McElhinney, B., Reynaert, N.L., Langen, R.C., Ckless, K., Shrivastava, P., and Poynter, M.E. 2002. Reactive nitrogen species and cell signaling: implications for death or survival of lung epithelium. American Journal of Respiratory and Critical Care Medicine 166 (12 Pt 2):S9-S16. DOI:10.1164/rccm.2206008

Johannesson, S., Andersson, E.M., Stockfelt, L., Barregard, L., and Sallsten, G. 2014. Urban air pollution and effects on biomarkers of systemic inflammation and coagulation: a panel study in healthy adults. Inhalation Toxicology 26 (2):84-94. DOI:10.3109/08958378.2013.856968

Johnson, J.Y., Rowe, B.H., Allen, R.W., Peters, P.A., and Villeneuve, P.J. 2013. A case-control study of medium-term exposure to ambient nitrogen dioxide pollution and hospitalization for stroke. BMC Public Health 13:368. DOI:10.1186/1471-2458-13-368

Karthikeyan, S., Thomson, E.M., Kumarathasan, P., Guenette, J., Rosenblatt, D., Chan, T., Rideout, G., and Vincent, R. 2013. Nitrogen dioxide and ultrafine particles dominate the biological effects of inhaled diesel exhaust treated by a catalyzed diesel particulate filter. Toxicological Sciences 135 (2):437-50. DOI:10.1093/toxsci/kft162

Kastner, P.E., Le Calve, S., Zheng, W., Casset, A., and Pons, F. 2013. A dynamic system for single and repeated exposure of airway epithelial cells to gaseous pollutants. Toxicology in Vitro 27 (2):632-40. DOI:10.1016/j.tiv.2012.11.011

Khafaie, M.A., Salvi, S.S., Ojha, A., Khafaie, B., Gore, S.S., and Yajnik, C.S. 2013. Systemic inflammation (C-reactive protein) in type 2 diabetic patients is associated with ambient air pollution in Pune City, India. Diabetes Care 36 (3):625-30. DOI:10.2337/dc12-0388

Kim, K.H., Jahan, S.A., and Kabir, E. 2013. A review on human health perspective of air pollution with respect to allergies and asthma. Environment International 59:41-52. DOI:10.1016/j.envint.2013.05.007

Layachi, S., Rogerieux, F., Robidel, F., Lacroix, G., and Bayat, S. 2012. Effect of combined nitrogen dioxide and carbon nanoparticle exposure on lung function during ovalbumin sensitization in Brown Norway rat. PLoS One 7 (9):e45687. DOI:10.1371/journal.pone.0045687

Levy, I., Mihele, C., Lu, G., Narayan, J., and Brook, J.R. 2014. Evaluating multipollutant exposure and urban air quality: pollutant interrelationships, neighborhood variability, and nitrogen dioxide as a proxy pollutant. Environmental Health Perspectives 122 (1):65-72. DOI:10.1289/ehp.1306518

Lin, W., Brunekreef, B., and Gehring, U. 2013. Meta-analysis of the effects of indoor nitrogen dioxide and gas cooking on asthma and wheeze in children. International Journal of Epidemiology 42 (6):1724-37. DOI:10.1093/ije/dyt150

Lundberg, J.O., and Weitzberg, E. 2013. Biology of nitrogen oxides in the gastrointestinal tract. Gut 62 (4):616-29. DOI:10.1136/gutjnl-2011-301649

Malaviya, R., Laskin, J.D., and Laskin, D.L. 2014. Oxidative stress-induced autophagy: Role in pulmonary toxicity. Toxicology and Applied Pharmacology 275 (2):145-151. DOI:10.1016/j.taap.2013.12.022

Matsui, E.C., Hansel, N.N., Aloe, C., Schiltz, A.M., Peng, R.D., Rabinovitch, N., Ong, M.J., Williams, D.L., Breysse, P.N., Diette, G.B., and Liu, A.H. 2013. Indoor pollutant exposures modify the effect of airborne endotoxin on asthma in urban children. American Journal of Respiratory and Critical Care Medicine 188 (10):1210-5. DOI:10.1164/rccm.201305-0889OC

Mölter, A., Agius, R., de Vocht, F., Lindley, S., Gerrard, W., Custovic, A., and Simpson, A. 2014. Effects of long-term exposure to PM10 and NO_2 on asthma and wheeze in a prospective birth cohort. Journal of Epidemiology and Community Health 68 (1):21-28. DOI:10.1136/jech-2013-202681

Montagne, D., Hoek, G., Nieuwenhuijsen, M., Lanki, T., Pennanen, A., Portella, M., Meliefste, K., Eeftens, M., Yli-Tuomi, T., Cirach, M., and Brunekreef, B. 2013. Agreement of land use regression models with personal exposure measurements of particulate matter and nitrogen oxides air pollution. Environmental Science and Technology 47 (15):8523-31. DOI:10.1021/es400920a

Paulin, L.M., Diette, G.B., Scott, M., McCormack, M.C., Matsui, E.C., Curtin-Brosnan, J., Williams, D.L., Kidd-Taylor, A., Shea, M., Breysse, P.N., and Hansel, N.N. 2013. Home interventions are effective at decreasing indoor nitrogen dioxide concentrations. Indoor Air. DOI:10.1111/ina.12085

Persinger, R.L., Blay, W.M., Heintz, N.H., Hemenway, D.R., and Janssen-Heininger, Y.M. 2001. Nitrogen dioxide induces death in lung epithelial cells in a density-dependent manner. American Journal of Respiratory Cell and Molecular Biology 24 (5):583-90. DOI:10.1165/ajrcmb.24.5.4340

Persinger, R.L., Poynter, M.E., Ckless, K., and Janssen-Heininger, Y.M. 2002. Molecular mechanisms of nitrogen dioxide induced epithelial injury in the lung. Molecular and Cellular Biochemistry 234-235 (1-2):71-80

Proietti, E., Roosli, M., Frey, U., and Latzin, P. 2013. Air pollution during pregnancy and neonatal outcome: a review. J Aerosol Med Pulm Drug Deliv 26 (1):9-23. DOI:10.1089/jamp.2011.0932

Rider, C.V., Boekelheide, K., Catlin, N., Gordon, C.J., Morata, T., Selgrade, M.K., Sexton, K., and Simmons, J.E. 2014. Cumulative risk: toxicity and interactions of physical and chemical stressors. Toxicological Sciences 137 (1):3-11. DOI:10.1093/toxsci/kft228

Savitz, D.A., Bobb, J.F., Carr, J.L., Clougherty, J.E., Dominici, F., Elston, B., Ito, K., Ross, Z., Yee, M., and Matte, T.D. 2014. Ambient fine particulate matter, nitrogen dioxide, and term birth weight in new york, new york. American Journal of Epidemiology 179 (4):457-66. DOI:10.1093/aje/kwt268

Signorelli, S., Moller, M.N., Coitino, E.L., and Denicola, A. 2011. Nitrogen dioxide solubility and permeation in lipid membranes. Archives of Biochemistry and Biophysics 512 (2):190-6. DOI:10.1016/j.abb.2011.06.003

Silkstone, R.S., Mason, M.G., Nicholls, P., and Cooper, C.E. 2012. Nitrogen dioxide oxidizes mitochondrial cytochrome c. Free Radical Biology and Medicine 52 (1):80-7. DOI:10.1016/j.freeradbiomed.2011.09.024

Silveyra, P., and Floros, J. 2012. Air pollution and epigenetics: effects on SP-A and innate host defence in the lung. Swiss Med Wkly 142:w13579. DOI:10.4414/smw.2012.13579

Steenhof, M., Janssen, N.A., Strak, M., Hoek, G., Gosens, I., Mudway, I.S., Kelly, F.J., Harrison, R.M., Pieters, R.H., Cassee, F.R., and Brunekreef, B. 2014. Air pollution exposure affects circulating white blood cell counts in healthy subjects: the role of particle composition, oxidative potential and gaseous pollutants - the RAPTES project. Inhalation Toxicology 26 (3):141-65. DOI:10.3109/08958378.2013.861884

Sunyer, J., Basagana, X., Belmonte, J., and Anto, J.M. 2002. Effect of nitrogen dioxide and ozone on the risk of dying in patients with severe asthma. Thorax 57 (8):687-93

Teichert, T., Vossoughi, M., Vierkotter, A., Sugiri, D., Schikowski, T., Schulte, T., Roden, M., Luckhaus, C., Herder, C., and Kramer, U. 2013. Association between traffic-related air pollution, subclinical inflammation and impaired glucose metabolism: results from the SALIA study. PLoS One 8 (12):e83042. DOI:10.1371/journal.pone.0083042

Tillett, T. 2013. When blood meets nitrogen oxides: pregnancy complications and air pollution exposure. Environmental Health Perspectives 121 (4):A136. DOI:10.1289/ehp.121-a136

USEPA. 2009. Status Report: Advances in Inhalation Dosimetry of Gases and Vapors with Portal of Entry Effects in the Upper Respiratory Tract. U.S. Environmental Protection Agency. Washington, DC. EPA/600/R-09/072. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=212131

USEPA. 2011. Status Report: Advances in Inhalation Dosimetry for Gases with Lower Respiratory Tract and Systemic Effects. U.S. Environmental Protection Agency. Washington, DC. EPA/600/R-11/067. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=238343

USEPA. 2012. Advances in Inhalation Gas Dosimetry for Derivation of a Reference Concentration (RfC) and Use in Risk Assessment. U.S. Environmental Protection Agency. Washington, DC. EPA/600/R-12/044A. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=244650

Vadillo-Ortega, F., Osornio-Vargas, A., Buxton, M.A., Sanchez, B.N., Rojas-Bracho, L., Viveros-Alcaraz, M., Castillo-Castrejon, M., Beltran-Montoya, J., Brown, D.G., and O'Neill, M.S. 2014. Air pollution, inflammation and preterm birth: a potential mechanistic link. Medical Hypotheses 82 (2):219-24. DOI:10.1016/j.mehy.2013.11.042

Vawda, S., Mansour, R., Takeda, A., Funnell, P., Kerry, S., Mudway, I., Jamaludin, J., Shaheen, S., Griffiths, C., and Walton, R. 2014. Associations Between Inflammatory and Immune Response Genes and Adverse Respiratory Outcomes Following Exposure to Outdoor Air Pollution: A HuGE Systematic Review. American Journal of Epidemiology 179 (4):432-42. DOI:10.1093/aje/kwt269

Wang, K. 2012. Application, Evaluation, and Process Analysis of the US EPA's 2002 Multiple-Pollutant Air Quality Modeling Platform. Atmospheric and Climate Sciences 02 (03):254-289. DOI:10.4236/acs.2012.23025

WHO. 2013. Review of evidence on health aspects of air pollution – REVIHAAP Project. World Health Organization. Copenhagen, Denmark. http://www.euro.who.int/__data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report-final-version.pdf

Wilson, G.S., and George, J. 2014. Physical and chemical insults induce inflammation and gastrointestinal cancers. Cancer Letters 345 (2):190-5. DOI:10.1016/j.canlet.2013.07.011

Wittkopp, S., Staimer, N., Tjoa, T., Gillen, D., Daher, N., Shafer, M., Schauer, J.J., Sioutas, C., and Delfino, R.J. 2013. Mitochondrial genetic background modifies the relationship between traffic-related air pollution exposure and systemic biomarkers of inflammation. PLoS One 8 (5):e64444. DOI:10.1371/journal.pone.0064444

Dr. Jack Harkema

Comments on Chapter 1 - Integrative Summary

General Comments:

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

Furthermore it is not always easy to know if the causality classification was primarily dependent on recent (since the last review) or older studies. This is due in part to a lack of references. There needs to be more consistency in how key studies are referenced throughout this Chapter. Also in this regard, the key health effect findings need to be presented along with their NO2 exposure data. This too is inconsistent throughout the chapter. In addition, there is too much reliance of terms such a "high quality studies" in the justifications. More specific and robust rationale needs to be presented.

In general there is good integration and summarization of the collective data within a topic area (e.g., Respiratory Effects Associated with Short-term NO2 Exposure), but more synthesis and critical review needs to be provided between topic areas (e.g., between Respiratory Effects of Short- and Long-term NO2 Exposures). For example, it is not always clear that the respiratory (or extrapulmonary) health effects being examined in a study are clearly due to short- or long-term NO2 exposures. A critical assessment of this potential problem of interpretation should be presented, along with the uncertainty it brings to the causality determination. In terms of basic pathology and pathophysiology, one would think that long-term exposures to inhaled pollutants would likely be associated with chronic health effects (e.g., chronic bronchitis, emphysema, atherosclerosis, mortality), while short-term exposures would be associated with acute effects, such as exacerbation of asthma. This is, in part, an issue of biological plausibility that needs critical evaluation. It is especially important now that there is both an annual and 1-hr standard for NOx.

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

Specific Comments:

The Integrative and Executive Summaries are places to identify existing data gaps. This is lacking in this ISA draft, along with suggested areas for future research.

The introductory section on 1-1 provides a paragraph on the major outcomes from the last review. A brief paragraph summarizing the major research findings since the last review would be helpful here as well to set the stage for this Chapter and remainder of the ISA.

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

1.4.1 Likewise, the potential mode(s) of action for acute and chronic responses to short- and long-term exposures to NO2 is limited in its scope. There is no acknowledgement of the specific sites of pulmonary injury other important modes of action outside of inflammation, such as sensory nerve responses and airway remodeling.

1-16. More critical evaluation is need on the relationship of long-term NO2 exposure and respiratory health effects. As written, there does not appear to be enough supporting evidence to increase the level of causality to likely from suggestive in this reviewer's opinion. The associations of respiratory health, incidence of asthma, in new epidemiology studies may still be due to short-term exposures causing exacerbations. More clear and convincing justification is needed in this section to make the case for this change in causality.

1.5. Evaluation of Independent Effects of NO2. This section provides good documentation with ample references to key studies since the last review and before.

1-40. Indoor NO2. The influence of outdoor NO2 on indoor NO2 is not described in this short section. Neither is there any discussion of the health of effects of indoor NO2 affecting responses to outdoor NO2 exposure.

1-50. At-risk populations. Since there is a major concern about the interface of air pollution and obesity, diabetes and the metabolic syndrome, recent studies (or lack of studies) on NO2 exposure and these newly identified at-risk populations should be addressed.

1-47. Last paragraph does not give support to changing the causality level of the respiratory effects of long-term NO2 exposure.

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

Comments on the Executive Summary

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

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

Dr. Michael Jerrett

Overall I found the review to be of high quality, but I noted several important omissions and points that need further clarification, including:

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

2. There is a growing literature on metabolic effects of air pollution and several studies have found associations between NO2 and diabetes (Coogan et al., Chen et al. and others that I can identify later). There should be a separate section dealing with metabolic outcomes.

3. Given the high level of spatial variability in NOx, it seems that some priority should be given to studies that use within-city exposure estimates, rather than those using central site monitors, for the long-term studies. It was not always clear from reading Chapter 1 if the adequate weighting was being given when studies using central site vs. within city estimates of NO2 were being compared (e.g. ACS vs. Harvard Six Cities). There is likely to be a much higher level of measurement error when the central sites are being used for exposure assessment when compared to the within city studies. If these comparisons treated the exposure assessments equally and were used as a factor in determining causality, there should be a reweighing than de-emphasizes the studies using central monitors.

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

5. More emphasis should be given to understanding the micro-environment concentrations as was done in the HEI Health Effects of Traffic Report.

6. Inhalation differences based on physical activity need to be acknowledged as another source of exposure error, particularly for walkers and bikers who use busy roads. There is enough literature on this to indicate likely higher rates of inhalation that go beyond the simple level changes in difference micro-environments.

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

8. Stress as an effect modifier. There are several studies (e.g., Shankardass et al. 2009 PNAS) that suggest an important effect modification role of stress. This could be another avenue of susceptibility.

9. Occupational exposures. There is no mention of these exposures or their potential confounding effect as a co-exposure or as a modifier or as a basis for evidence of effects from NO2. This should be added to the ISA.

10. There is substantial evidence that NO2 exposures are not equally distributed among the population, but instead follow an inverse social gradient such that the socially disadvantaged groups face generally higher exposures. Since these groups are also potentially more susceptible, this has been referred to as double jeopardy. Some recognition of this literature and its potential for generating great health effects is needed (IOM 1999 and several others have made this point in general).

Dr. Tim Larson

Charge Questions 1 & 2

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

- The Executive Summary should be the first thing that people read. Instead we have a Preamble that is basically the same for all criteria pollutants and may be more information than what most people want who choose to read this document. I suggest making the Preamble a separate document that can be referenced by all subsequent documents (not just those for NOx).
- There is also a tendency to group separate topics into the same paragraph or even the same sentence
- When referring to concentrations, concepts such as 'average daily 1-hour maximum' will not be understood by a lot of readers whereas annual average makes general sense. May wish to consider another way to present this or simply refer to a percentage of the current standard.
- Reference to 'microscale' or 'neighborhood scale' is potentially confusing. Most readers won't know what these scales represent
- Need to better describe the relevance of indoor exposures and near road exposures to the health studies. A clear statement is needed on how this information was used to arrive at the key findings.
- The entire discussion on exposure measurement error could be shortened. Phrases like 'spatially resolved' are unclear. The whole issue of biased estimates versus more uncertain estimates needs clarification.
- At the beginning of the Health Effects section, the statement is made that relevant concentrations are no more than two orders of magnitude above peak ambient levels. These high levels refer to animal exposures, not relevant human studies cited in the document. This needs to be clarified.
- The discussion of biological mechanisms does not make it clear that the secondary oxidation products and subsequent reactions refer to respiratory effects. At the end of this paragraph there are a few sentences speculating on cardiovascular mechanisms. This appears to get equal time with the better understood respiratory mechanisms, although it is clear from the main document that the cardiovascular mechanisms are not well understood. There is also a final sentence in this section that states "it is not clear by what processes inhaled NO may induce health effects" although earlier in the summary it was concluded that there is no evidence for inhaled NO induced health effects.

- The paragraph at the top of page lxxv would be difficult to follow for the average informed reader
- The following paragraph on page lxxv implies that epi studies that adjust for confounding by other pollutants is the main reason for going from 'likely causal' to 'causal'. Although this is an important factor, it was not the only reason for this change. This is stated more clearly in the conclusions section.
- The last paragraph in the Summary cites a relevant distance of 500 meters from roadways, whereas earlier in the summary the relevant distance was 15 meters (presumably from the I-710 experiments in Southern California). This should be clarified.

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

- For the general public, the executive summary is much too long. I assume EPA will issue a summary that is no more than 2 or 3 pages max.
- For the general air pollution community, a shorter (~5-7 page) summary would still be useful perhaps organized around Table ES-1 with a brief rationale that focuses on what evidence was necessary to go from suggestive to causal (e.g. epi results robust to confounders, epi results consistent across cities and across different NO2 exposure metrics, human clinical results consistent with epi outcomes, and animal tox mechanisms consistent with both human clinical and epi metrics.). I would shorten Chapter 1 if possible, using the same subheadings.

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

It should be very useful during panel discussions of key studies. I will be interested in what others think.

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

Chapter 1 provides an excellent summary of the ISA. Section 1.5 should be kept here in its entirety.

2d. What are the Panel's thoughts on the rationale presented for forming causal determinations for NO2 exposure only and considering epidemiologic results for associations between NOX and health effects in causal determinations for NO2 (Sections 1.4.1 and 1.4.3)?

The statement that 'NO or NOx exposures are also found to be associated with health effects' implies that these studies have passed the same high bar as the NO2 evidence. Maybe this needs to be reworded. The rationale seems OK and the fact that it is part of a larger discussion of plausible biological mechanisms is informative.

2e Section 1.5 discusses available information that is not necessarily included in the health effect chapters on potential confounding by copollutants and other factors as well as the potential for NO2 to serve primarily as an indicator of traffic-related pollutants and traffic proximity. This discussion is in Chapter 1 because it integrates information across Chapters 2, 4, and 5. Please comment on the extent to which this discussion is informative in describing how the evidence of independent effects of NO2 is evaluated in this ISA. Does the discussion accurately reflect the available evidence? If this discussion is informative, what information could be added or removed to improve the discussion. Should the discussion remain in Chapter 1 or should it be moved to another part of the ISA?

I think this section is very informative and a more complete discussion of these issues than is currently in the Executive Summary. An important point to the informed reader who is not an epidemiologist is how confounding is assessed- not by the confidence intervals crossing zero, but rather the change in the magnitude of the main effect. When I was looking at the relevant co-pollutant discussion section in Chapter 4, my interpretation of Figures 4-10 and 4-11 would have been enhanced if part of this discussion were also repeated there. My simple reading of these two figures (and others like them in the chapter) is that most confidence intervals cross zero when the model is adjusted for co-pollutants and the magnitude of the effect moves toward the null but usually remains positive. In the absence of section 1.5, this might have led me to conclude that the effects are hopelessly confounded.
The point raised about the differences in near-road gradients in NO2 versus UFPs or BC is somewhat moot given that the epidemiological studies relied on monitors placed away from the road where these gradient differences are not pronounced. The panel studies with personal monitoring do not appear to support a copollutant confounding, an important point made here.

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

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

General Comments

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

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

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

P2-59, line 15 also might want to refer to models that include building wake effects such as OSPM or Austal2000

P2-61, line 31 See also Yuval et al Atm Env 79 261-270 2013 (non linear optimization model); Wilton et al STOTEN 408, 1120-1130, 2010 (hybrid dispersion, LUR model for NOx); Lindstrom et al (2013) Environmental and Ecological Statistics doi:10:1007/s10651-013-0261-4 (NOx spatio-temporal model with disperson-based covariate)

P4-188 Fig4-11 results shown for vonKlot et al for beta-agonist is not obviously consistent with those reported in the original paper

Dr. Jeremy Sarnat

Generally, I believe that the draft ISA presents a comprehensive collection of the science regarding NO2. The interpretation of this body of work is largely coherent and I support many of the recommended changes that may affect future policy decisions aimed at regulating this pollutant. My main comments on the draft ISA center primarily on the weight given to results from two-pollutant epidemiologic models (co-pollutant models) in decisions related to causal determination status. Although my comments may be broadly applicable to determination decisions across the ranges of exposures and effects, I believe the implications are most pronounced for the science and uncertainties related to short-term NO2 exposures and respiratory effects, which are the focus of my observations below.

The evidence from the 2008 NO2 ISA and findings published since, continue to implicate NO2 as a likely *independent* causal factor of acute adverse respiratory response. Despite this, I find the justification to change the status to 'causal' based largely on the use and application of epidemiologic results from co-pollutant models to be unjustified, with results that do not 'rule out…confounding, and other biases' as stipulated in the causal framework guidelines. Specifically, I don't believe the co-pollutant results presented in this draft ISA sufficiently preclude the possibility that either: a) NO2 is serving as a surrogate of traffic pollution mixtures or traffic components more causally associated with short-term respiratory response; or that b) NO2 may play some role in independently eliciting short-term respiratory response within a complex mixture, but that this effect is minor relative to the effect attributable to its other correlated co-pollutants.

Within the ISA, there also appears to be some ambivalence about this potential source of confounding as well. There the following statements, for example, in Section 1xxv of the Preamble: '*In the current ISA, the causal determination is strengthened from likely to be a causal relationship to causal relationship because the recent epidemiologic evidence reduces the previously identified uncertainty regarding confounding by other traffic-related pollutants.*' This assertion could be juxtaposed with the following (from Sec. 4-4): '*Although there was coherence of evidence across related outcomes and disciplines, a major uncertainty that remained regarding the respiratory effects of short-term ambient NO2 exposure was the high correlations of NO2 with other traffic-related pollutants and the potential for NO2 to serve primarily as an indicator for another or mixture of combustion-related pollutants.*' I found it interesting that sections of the draft IRP continue to note this fundamental question as an outstanding source of uncertainty as well. (Draft IRP, pages 4-13, 4-16).

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

a) *Confounding of NO2 by other 'criteria' pollutants*. The 2008 ISA results, as well as more recent findings, provide strong evidence that the NO2-related health risk estimates are unlikely confounded by other, ubiquitous urban air pollutants (e.g., O3, SO2, PM, CO). The population-based epidemiologic modeling examining short-term respiratory and, especially the mortality results are numerous and convincing. Despite this, very few co-pollutant analyses have examined confounding from other traffic-related pollutants, including VOCs, particulate organic, and

transition metal species. The results presented in Chapter 4 examining short-term NO2 exposures and corresponding changes in lung function, serve as an example. Of the 53 short-term NO2 and acute respiratory studies cited in Table 4-7, including numerous panel and small cohort designs with excellent exposure and health characterizations, only 9 studies (17%) specifically measured non-criteria pollutant components we typically associate with traffic emissions (i.e., UFP, BC/EC, BTEX, particulate organic species). Of these, only a couple included comprehensive chemical speciation of the exposure measurements. With the exception of a very small number of these findings (Delfino et al., 2008, for example), it was not clear whether NO2 was an independent driver of lung function response. While these outcomes deal with lung function exclusively, similar trends exist for other acute endpoints, including AHR and pulmonary inflammation. The relative dearth of NO2 and traffic related co-pollutant results is also noted in several sections of the ISA (Page 1-14, for example).

Finally, I feel the results from the few measurement studies including specific traffic trace components (Brook et al., 2007, for example), highlight the potential that strong collinearity exists between NO2 and other traffic species. Since, we hypothesize that these traffic species elicit respiratory responses (as well as responses in other organ systems) via similar biological pathways as NO2, this further raises the concern that they may serve as confounders.

b) *Model specification*. Specification for most of the co-pollutant models examining acute respiratory outcomes primarily focuses on the issue of confounding solely (i.e., what is the effect estimate of NO2, while controlling for another pollutant), rather than the potential for joint effects or effect modification. These latter scenarios appear to me to be equally plausible in characterizing NO2 short-term health respiratory effects, and that NO2 along with a complex suite of particles and gases, may elicit response via inflammation-mediated pathways. A key area of uncertainty is whether epidemiologic models more properly designed to assess the effects of pollutant **mixtures**, either in a more properly specified joint effects or effect modification setting, that may include interaction terms among the pollutants, are more efficient and provide better fits to the C-R relationship than model with two, independent pollutant terms. Currently, there are a very limited number of studies who have attempted to model NO2 a part of a mixture. In revisions to the final ISA draft, I would recommend a greater discussion of alternative approaches for characterizing NO2 within a mixture (i.e., Bayesian modeling as done with the mortality results or various factor analytical and source apportionment approaches). Of particular interest are the APHENA findings (Katsouyanni et al., 2003), where greater PM risks were observed in cities with high NO2 concentrations, and whether similar patterns exist for short-term NO2 and acute respiratory response.

A related source of uncertainty regarding specification of the co-pollutant models is the potential non-linearity of associations between NO2 and its co-pollutants. The use of linear expressions, within a co-pollutant setting, to control for confounding of non-linearly correlated co-pollutants could lead to imprecision and/or bias; an appearance of effects associated with NO2, where they do not exist. Modeling NO2 with higher order pollutant terms could be a more appropriate means of addressing confounding in these circumstances. NO2 formation and NOx chemistry differs between low and high O3 regimes (as noted on Page 2-7). It makes sense, therefore, that

epidemiologic models with both terms may also want to consider non-linear terms when formally assessing confounding.

c) *Limits of assessing confounding through co-pollutant models*. There is acknowledgement in various parts of the ISA that co-pollutant models may have limitations in assessing potential confounding (Page 4-2, for example), and there is some very limited discussion of unspecified or residual confounding. I believe this discussion deserves greater attention. What specifically are the implications for the observed epidemiologic results from improper modeling of confounding? Is bias likely to occur, or a lack of precision? Which pollutants may be more susceptible to potential bias and errors resulting from this modeling approach? A number of investigators have approached this from a biostatistical modeling framework (e.g., L. Sheppard and her group, for example) and could offer insight into framing this source of uncertainty. At the very least, greater attention to the shortcomings of co-pollutant models would enhance transparency.

Correlations between NO2 and other pollutants. There is a useful discussion about the potential for confounding from correlated co-pollutants in the NO2 exposure assessment sections of the ISA (Pages 2-69 through 2-83). Along with the epidemiologic results and the controlled exposures and toxicology, these exposure and measurement findings can inform the question of whether NO2 is a potential confounder or indicator of specific sources. Despite this, there is limited integration of these results as they relate to potential confounding, as addressed throughout Chapters 4 and 5.

Section 2.6.4.1 (Page 2-70) is vague about the role of averaging time on observed strengths of association between NO2 and its co-pollutants. The results generally describe correlations over 24h integrated periods, with some daily 1h max correlations as well. Are there any studies who have examined more temporally resolved associations? I suspect that we will see stronger correlations between NO2 and especially the traffic components. If acute health effects are also occurring on these scales, then these associations will be useful to study.

Dr. Richard Schlesinger

Comments on Chapter 3

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

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

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

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

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

6. p 3-17, lines 21-26. This is aimed at indicating why endogenous NO2 levels will not be affected by inhaled NO2. However, while endogenous NO2 may not be systemically distributed per the discussion, there could potentially be an increase in reaction products in the tissues due to changes in levels of endogenous NO2.

7. p 3-18, lines 16-25. This part of the paragraph should be in Section 3.2.3. On page 3-17, it is noted that NO2 reacts with some antioxidants resulting in production of nitrite, yet there is no indication of whether this would affect toxicity of inhaled NO2. However, on p 3-18, it seems to be inferred that there may be toxicity of nitrite from NO or NO2. In addition, the last sentences which indicate uncertainty about the relative contribution of endogenous NO2 with low level inhalation exposure seem to contradict the comment noted in # 5 above that endogenous oxidants will likely not affect toxicity of inhaled oxidants.

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

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

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

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

12. p 3-19, Endogenous NO2. The discussion seems to be about NO rather than NO2.

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

14. p 3-43, line 14. Is it correct to say that the NO2 exposure enhanced "..preexisting emphysema in animal models" or would it be better to say "preexisting emphysema-like conditions…."?

15. p 3-46, line 23-25. Here again it seems to contradict statements about the relative roles of endogenous and exogenous NO2.

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

17. p 3-57. Summary. The last sentence noted that inhaled NO2 may contribute to the endogenous body burden of NO2 species, yet in many places earlier it is stated or inferred that this does not occur. There needs to be some consistency about this issue.

Comments on Chapter 4

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

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

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

4. P. 4-183, line 22-25. There seems to be somewhat of a disconnect between this statement and prior statements in Section 4.2.9. For example, here it indicates that there are associations between NO2 and hospital admissions for all respiratory causes, but on page 4-181 line 13-14 it is noted that evidence suggests a causal relationship between NO2 and respiratory effects primarily evidenced only by asthma morbidity. Then, on page 4-185 lines 27-38, again the main evidence is noted as referring to asthma exacerbation. Thus, it is not clear whether causality is being proposed for just asthma or for all respiratory causes.

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

6. p. 4-242, line 10-13. The first paragraph on page 4-241 indicates that there was little evidence for CV effects based upon studies in the 2008 ISA. However, here it states that epi data continues to support an association between NO2 and CV effects. Continues from what?

7. p. 4-249, line 16-19. Here it is noted that inconsistencies across studies and limited evidence does not support effects observed in hospital admissions and CV mortality. However, on p. 4-247 line 30 it is noted that epi studies consistently demonstrate NO2 associated hospital visits for CV effects and mortality. The two statements seem contradictory.

8. p. 4-282 line 12-18. There seems to be a contradiction here. In the first sentence, it is noted that the NO2 mortality association is robust in copollutant models, but this is followed by the statement that it is hard to disentangle independent effects of NO2 from those of other measured or unmeasured pollutants, adding to uncertainty. So, what exactly is robust and what is not.