

## **Preliminary Comments on the ISA from Dr. Matthew Campen**

The 2<sup>nd</sup> draft of chapter 4 represents a stronger, better organized chapter, and the authors were very responsive to the previous review. The addition of mode of action figures is helpful and justified, to better understand how the Agency is attempting to organize the disparate research information available into a pathophysiological mechanism.

### **Specific Charge Questions:**

*1. The dosimetry section (Section 4.2) expands on the description of the epithelial lining fluid in the tracheobronchial and alveolar regions. Further, the deficiencies and uncertainties associated with the lack of a validated NO<sub>2</sub> dosimetry model are more explicitly described. Please comment on the adequacy and clarity of these expanded discussions. To what extent does Section 4.2 address the reactive nature of NO<sub>2</sub> and its ability to pass beyond the epithelial lining fluid?*

The revised draft is much clearer and better organized in the dosimetry section. The chapter contains an appropriate level of detail. What questions remain – regarding the reactive intermediates – are largely unknown.

*2. Section 4.3 discusses mode of action for specific outcome groups and also includes new figures that describe what scientific information is available on the key events and endpoints that make up the pathophysiological changes that lead to particular health effects. What are the Panel's views on the effectiveness of the organization around the outcomes of interest? To what extent do the new figures facilitate integration with the health effects evidence in Chapters 5 and 6?*

The figures are really quite nice for this integration. Additional details of pathways would be unjustified based on the current literature. In Figure 4-3, “Vascular Activation” might be changed to Endothelial Inflammatory Activation”.

### **General comments:**

Figure 2-19 and 2-20 do not reproduce well in grayscale. Consider changing some lines to dashed.

Page 4-32, nitrite is dismissed as a potential mediator of NO<sub>2</sub> toxicity, with justification from several therapeutic in vivo studies. I would recommend detailing specific NO<sub>2</sub>/3 concentrations in serum and intracellularly, and noting the relative potential increase from inhaled NO<sub>2</sub>, based on reports.

Eicosanoids are formed after NO<sub>2</sub> – are these due to enzymatic processes only, or as a reaction between NO<sub>2</sub> and arachidonic acid?

In the section on ANS (4-32 to 4-34), respiratory rate changes are used as a surrogate for neural activation. While there is certainly logic to this conclusion, it would be justified to note that many of the exposure studies did not specifically include permutations with a pharmacological inhibitor of the ANS, such as propranolol or hexamethonium. Without these, the respiratory rate changes are not mechanistically linked to ANS modulation. On 4-34, lines 12-13, and appropriate statement is made

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regarding atropine and vagal tone. Something similar should be noted earlier in the section for sympathetic activity.

Page 4-35, last paragraph, notes activation of NFkB and later about IL-6 and IL-8, but does not specifically note that these cytokines are under NFkB transcriptional regulation, which would tie the concepts together a bit better.

Pages 4-37 and 4-38, the alterations of selenium in the diet may alter glutathione, but was that measured and might other proteins be impacted by selenium availability? Just noting the limitation of the study may be worthwhile.