

# EtO Charge Question Responses

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# Charge Question 1: Lagged Exposure

- Draft report description of choices and implementation in modeling is clear
- Scientific/biological support is ultimately paramount and critical to statistical treatment
  - CDC 9-11 Working Group guidelines serves as a good model to define systematically define process of assessing latency. Not suggesting disease-specific discussion is relevant to this review.
- IRIS report should include in the appendix, sensitivity analysis for select candidate models and competitors
  - EPA should aim to define qualitatively how it will factor results of the sensitivity analysis in their assessment of model stability
- We recommend that draft report include a more complete description of the NIOSH cohort, including
  - The report (or an appendix) should provide description of the historical profile of exposures among cases and non-cases

# Charge Question 2a: Considerations for model selection for BC incidence

- The work is scientifically appropriate; the description can be improved with better organization and clarity of language
- The choice of a linear spline for estimating both the unit and excess risks is appropriate given the NIOSH data
  - Confirming the reasonableness of exposure predictions will help support this
- We request more exposure and background descriptive information that characterizes the cohort
  - Lack of description of the exposure in the cohort, including basic understanding of the duration, magnitude, and timing of exposure to individuals is one of our biggest concerns. More careful evaluation of Hornung et al by the Committee has alleviated some of the concerns, including those mentioned by the public
- The amount of model searching done is of concern but it can be reduced by reporting the target parameter of interest (e.g. unit risks) from multiple models as sensitivity analyses; we request unit risk estimates from all models be reported
- The IRR results reported by Mikoczy et al 2011 could be used to support the selected model; also it would be helpful to use these results in unit risk sensitivity analyses

## Charge Question 2b: Reasonable models for unit risks for BC incidence

- Even given the following suggestions and recommendations, the overall the approach was scientifically appropriate
- There should be more clarity in the text about the choices considered and their rationale
- Discarding a model because the estimate is “too steep” needs scientific justification
  - The use of the term “unstable” in this context must be clearly defined or omitted
- Use of AIC for selecting models is acceptable within some constraints; it is not a preferred way to characterize model fit
  - More details will be included in the final report

# Charge Question 2c: Knot identification approach

- This is scientifically appropriate and transparently described

## Charge Question 3a: Linear regression of categorical results - lymphoid cancer

- We suggest EPA revise the text, including more clearly motivating the methods that were used
- The linear regression of categorical estimates should not be selected unless the individual exposure model results are biologically implausible.
  - We recommend EPA give multiple estimates of the unit risk in sensitivity analyses and update their justification of one model over another
- If linear regression of categorical results is to be used we recommend the category medians rather than the means be used as they better represent the exposure in each category.

## Charge Question 3b: Model select for env & occ risk ests - lymphoid cancer

- The spline allows different functions of risk for low and high exposure, relies more on local rather than global behavior of the data, and allows a single model to be used for both the unit risk and excess risk estimates
  - We suggest EPA consider use one model for both environmental and occupational exposures
  - Using different models is acceptable with revised justification
- Report results of all models and on the sensitivity of the results, for all models.

## Charge Question 3c: Lifetable calcs for incidence - lymphoid cancer

- These are explained transparently and are scientifically appropriate
- We suggest EPA put the extra lifetime risk in terms of the number of lymphoid cancers are due to the exposure to EtO in the cohort

# Charge Question 4: Qualitative discussions of uncertainty

- We recommend that EPA expand the quantitative assessment of uncertainty to improve the qualitative discussion by reporting and addressing a broader array of sensitivity analyses that report the target parameter of interest (i.e. unit risks, excess risks). Details can be in an appendix. Consider:
  - The range of reasonable models
  - Various lags
  - Increased number of bins in the linear regression of categorical results
- We encourage EPA to expand the qualitative summary through discussion of
  - Exposure estimation and exposure characteristics of the NIOSH cohort
  - Dose metric
  - Biologic plausibility
- Discussions of estimates based on external standards (e.g. SIR, SMR) should be downweighted (e.g., moved to an appendix with minor comments in the main document) due to the importance of healthy worker effect in this population

# Charge Question 5a

- Section 3.33 and Appendix C of the USEPA draft document presents an accurate, objective and transparent summary of the results of research studies published up to July, 2013 on ethylene oxide (EtO) genotoxicity. The Scientific Advisory Board agrees that the weight of the scientific evidence from epidemiological studies, laboratory animal studies and in vitro studies supports the general conclusion that the carcinogenicity of EtO in laboratory animals and humans is mediated through a mutagenic mode of action (MOA). Indeed, the genotoxicity database has established that EtO it is a direct-acting agent as evidenced by the formation of DNA adducts and highly reproducible, positive effects in a variety of in vitro and in vivo mutation and clastogenesis assays.

# Charge Question 5a

- However, several areas of the draft report require revision to provide a more detailed interpretation of findings within the context of recent advances in our understanding of the biology of cancer as well as enhanced clarity of presentation. Specific recommendations and suggestions for revision include:

# Charge Question 5a

## Recommendations

- Table 3.6 should be revised to specify the sites involved and the relative weights assigned to individual studies. In addition, a new table should be added to show the dose-response relationships for DNA adduct and in vivo genotoxic effects in humans and comparative model systems.
- The rationale for decisions made regarding model selection for calculations of unit risk should be presented in this section, and elsewhere, within the context of MOA considerations and initial key biological events involved in mutagenesis and carcinogenesis. The evidence for mutagenic MOA can be used to explain the behavior of the data in low dose regions and the subsequent extrapolation for risk assessment.

# Charge Question 5a

- While the description of the database was found to be adequate, the synthesis of information to support a mutagenic MOA should be presented in a more systematic and complete manner. Section 3.4 should be reorganized around a broader evidence base for a mutagenic MOA in order to establish a framework for defining mutagenic MOA. Key elements of this framework, as informed by a recent review by Eastmond (2012) should include:
  - - Characterization of Molecular Alterations – does the chemical interact with protein and/or DNA, undergo redox cycles, modulates cell cycle/rates of cell replication, apoptosis, signaling pathways, and the doses required to induce these changes. In the case of EtO, the primary effect appears to be direct interaction with DNA to produce a variety of adducts; other effects occur including protein adducts and some evidence of oxidative stress;
    - Does the chemical induce mutagenic or clastogenic effects – which biological systems and what doses are required for adduct formation. In the case of EtO, it has been shown that genotoxic effects occur at doses below those required to induce cytotoxicity or tumorigenesis. While this information would be helpful to clarify whether specific DNA adducts are associated with genotoxic effects, the absence of these data does not negate a mutagenic MOA for EtO.

# Charge Question 5a

- In the absence of further mechanistic information, evidence for DNA interactions coupled to consistency in the occurrence of mutagenic/clastogenic effects provides a sound basis for applying a mutagenic MOA to risk assessment. Additional data that may be informative in revising the draft to support a mutagenic MOA includes:
  - Genotoxic Effects in Cancer Target Organs – these effects can include DNA adducts (weight increased if they are known to be promutagenic DNA adducts), mutational and clastogenic effects in the target organ. In the case of EtO there is evidence for mutational effects in breast tissue from mouse cancer bioassays (altered mutational spectra of tumor tissue – e.g., Houle et al. 2006), as well as altered mutational spectra in lung and other target tissue tumors (Hong et al. 2007). The fact that EtO-induced mutational spectra changes occur in tumor suppressor genes and oncogenes provides additional weight for a mutagenic MOA. Regarding lymphoid tumors, there is evidence from several studies for genotoxic effects of EtO in bone marrow and PBL. On a more general basis, if target organ data did not exist, consideration should be given to toxicokinetic or physico-chemical factors that prevent access to the cancer target organ.

# Charge Question 5a

- Non-linearities – Are there non-linearities that would suggest that the mutagenic MOA does not continue to be operative at low or high dose levels? In the case of EtO, the DNA adduct dose response extends to very low doses, well below the cancer effect level (Marsden et al., 2009).
- Temporal Relationships – do DNA adducts and genotoxic effects precede the carcinogenic effect. In the case of EtO, as cited in the draft document, short-term and subchronic studies find evidence of genotoxic effects.
- Alternative Mechanisms – Are there other effects that might account for the oncogenic effects, at what doses do they occur, and how robust are these findings. In the case of EtO, cytotoxicity, oxidative stress, alterations of signaling pathways may occur, but evidence is lacking that these effects would become a primary effect at low doses.
- Summarization of the cancer MOA – via description of key events and whether they are consistent with a mutagenic MOA; for EtO these key events would appear to be: a) DNA adduct formation; b) mutation/clastogenesis; c) clonal expansion of altered cells; d) tumor formation. The statement of key events on Page 3-42 does a reasonable job of summarizing what is believed to occur with EtO but as I state above could be better supported.

# Charge Question 5a

- In light of the above discussion, the organization of the text should also be revised to discuss what is known about differences in mutagenic and carcinogenic pathways for EtO at different tumor sites, as well as the degree to which biochemical differences at the cellular or tissue level differentially impact MOA. Furthermore, references made in page 3-29 to the levels of different adducts were presented without making a clear and necessary distinction between the putative or assigned biological impact for N-7 versus O-7 DNA adducts.

# Charge Question 5a

- Suggestions
- A discussion of the relative contributions of indirect mechanisms to EtO carcinogenesis and their influence on dose-response relationships would help address issues raised regarding the features of dose-response relationships in animal and human studies.
- Inclusion of additional experimental details about the separation of endogenous from exogenous adducts as reported by Marsden et al., 2009 would help provide biological perspective for issues related to risk assessment considerations, especially within the context of linearity versus non-linearity of dose-response relationships.

# Charge Question 5a

- The genotoxicity section would be improved by consideration of the role that differences in DNA repair capacity between different target cells in different tissues plays in relative vulnerability to mutagenesis. For example, genes known to regulate vulnerability of breast cancer in women such as BRAC1 and BRAC2 and ---- are known to regulate DNA repair pathways in breast tissue (REF). This line of thinking can help to inform the biological bases to better understand the shape of the dose response in the low dose region of the NIOSH dataset.

# Charge Question 5b

- In general the agency was highly responsive to the 2007 SAB report
  - Addressed all major points in the SAB review
  - When divergent views were expressed in the SAB review, addressed both viewpoints
- The responses were transparent and for the most part accurate and objective (exceptions are noted)
- The agency accepted most of the recommendations and suggestions and made the appropriate changes. Changes are brief discussed in the Appendix with pointers to where in the body of the report they were made.
  - Our report can list changes and improvements

# Charge Question 5b

- The agency attempted to respond to a recommendation to obtain individual data from the NIOSH study and use those data to derive the unit risk, but was unsuccessful in implementing this recommendation
  - The problems with implementation are clearly explained
  - Panel’s opinions on the course of action taken are covered in responses to CQ 1-4.
- The agency did not accept and implement some recommendations/suggestions
  - Reasons for not accepting these is explained in the Appendix

# Charge Question 5b

- Did not include non-linear approach to developing a unit risk
  - This panel does not insist that a non-linear approach be included in the assessment, but a discussion of the issue needs to be much more balanced and objective.
- Did not make use of the UCC data
  - The panel suggests that the agency discuss the extent to which the UCC study and others (e.g., the Swedish study) corroborate the NIOSH study.
- Used a two-part spline model was used to fit the data
  - This panel accepts the two-part spline as a reasonable fit to the data as given (see CQ 2-3)
- Did not provide summary of key points at the beginning of Chapter 3 or move Appendix A contents to body of the report
  - Agree with not moving contents of Appendix A to the body, but summary of key findings at the beginning of Chapters 3 and 4 would improve the document

# Charge Question 5b

- CQ5b asks that particular attention be paid to responses to:
  - Comments on endogenous EtO (H-4)
    - Response points to added text on this issue
    - Panel agrees with the changes
  - Comments on nonlinear approach (H13-17)
    - Panel finds that existing data do not clearly indicate whether the low dose response to EtO is either linear or non-linear.
    - Panel accepts the agency approach that a linear extrapolation is used in the absence of compelling data to support a nonlinear extrapolation
    - Narrative on this issue needs to be more balanced and objective
  - Comments on cancer hazard identification
    - Agency notes majority of 2007 SAB concurred with classification of EtO as carcinogenic in humans and has strengthened arguments to support this classification in the current draft
    - Panel agrees with the changes

# Charge Question 5b

- Other suggestions on Appendix H
  - Rather than include extended quote from Steenland, the agency should use his input to synthesize and present their own response to the question.
- Note: Responses to public comments at the end of Appendix H are addressed in response to CQ7.

## CHARGE QUESTION 6

1. The logic and progression of the literature review are clearly supported
2. EPA should consider separating their interpretation of their findings from those of the studies' authors
3. EPA should consider additional expanded review of recent studies, including summary reviews, with specific focus on issues related to mode of action
4. EPA should emphasize the importance of internal comparisons in occupational studies.
5. Discussion of Kiran case-control study of lymphomas is thorough

6. Conclusions that the Kiran paper overall supports IRIS report is reasonable
7. Conclusion that small numbers of participants in the Morgan and Ambroise papers preclude more detailed analysis, but warrant inclusion in the review is reasonable
8. Summary of the Valdez study discussion is thorough . Perhaps relevant sections could be incorporated earlier in the main body of the report (for instance detailed discussions in J2 and J3)
9. Mikoczy study, with detailed exposure data at low doses with documented substantial effects on breast cancer, has stronger implications than suggested in the current EPA document

10. Strong breast cancer findings at low dose exposures in the Swedish study add greatly to the overall findings. The decision to base the final assessment on NIOSH rather than the Swedish study is warranted in part because of the more extensive analysis of data with confounders in the NIOSH study

## 11. Specific suggestions for expanded inclusion of the Swedish study cancer results for breast in the EPA document

- a) Discussion of Mikoczy study should be moved to a more central position in the document.
- b) EPA assessment should consider using the word “strong” for evidence of carcinogenicity
- c) EPA should consider the exposure assessment as high quality in light of the results of the exposure matrix for the early period of the study being validated by hemoglobin adduct levels (Hagmar 1991)
- d) The dose response analysis results for breast cancer indirectly adjust for lifestyle and other factors related to breast cancer

- e) EPA might consider a quantitative risk assessment based on the breast cancer data in the study
- f) Alternately, EPA should consider applying NIOSH estimates to Swedish study to assess the consistency of findings with
  - i. Low dose exposure.
  - ii. Attenuation of risk with higher exposures
  - iii. The observation of increased breast cancer risk with 16 more years of follow-up (latency)

# Ethylene Oxide Review: Charge Question #7

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# Charge Question 7:

- EPA solicited public comments on a July 2013 public comment draft of the IRIS carcinogenicity assessment of EtO and has revised the assessment to respond to the scientific issues raised in the comments. A summary of the major public comments and EPA's responses are provided in Appendix L.

# Charge Question 7 (cont'd):

- Has EPA adequately addressed the scientific issues raised in the public comments? For example, please comment on EPA's explanations for (i) its use of the lymphoid cancer grouping and (ii) combining unit risk estimates derived separately for the independent cancer types of lymphoid cancer and breast cancer to develop a total cancer unit risk estimate.

- Appendix L begins with a brief and clear summary of the comments received .
- 16 comments were received; because of repetition, some comments are grouped together, making 14 be the total number of unique comments to which the EPA responded.
- Several of the comments cite specific studies or make reference to the 2011 NRC report or the rules and regulations under which the EPA operates.

# Major Topics of Comments:

- Adherence to 2011 NRC report guidelines and previous SAB recommendations
- Evidence for breast cancer too weak
- EtO is a weak mutagen
- Mutagenic MoA not supported; consider other MoAs (oxidative stress, cell proliferation)
- Failure to incorporate UCC data
- Use of summary rather than individual data
- Models used by EPA over-predict cancer deaths in NIOSH study
- Reexamine risk determination given background and endogenous levels of EtO

- EPA has been very responsive to comments.
- Detailed references to discussion of issues raised in either main text or Appendices.
- Responses are thorough and clear.
- In some cases, additional analyses have been conducted or additional models used.

***Comment #3:* Lymphohematopoietic and lymphoid cancers should not be grouped because they are derived from different cells of origin.**

- Document clearly states the rationale for grouping these together and notes that the 2007 SAB panel agreed with the logic of that grouping for comparison purposes.

***Comment #12: Combining breast cancer and lymphoid cancer unit risk estimates is not justified, and EPA did not discuss competing risks, different background populations, incidence vs. mortality, and the use of different exposure-response models.***

- Breast cancer and lymphoid cancers were first modeled separately and then combined.
- Rationale for combining these unit risk estimates is explained in detail in the document.
- Standard practice in IRIS assessments is to estimate total cancer risk and not just the risk from individual cancer types; this practice is consistent with EPA guidelines and NRC recommendations.
- The subject of competing and background risks is also discussed in detail in the document.
- The distinction between cancer incidence and cancer status is also discussed.

# **Concluding evaluation of EPA response to public comments:**

- The responses provided by the EPA are focused, generally complete, and delivered in good faith.
- EPA response to Comment #1 is clear but could be even stronger. There are several places in the main document where the weight-of-evidence approach is discussed and justified.

## Evaluation of EPA Responses to Public Comments from 2007 (Appendix H):

- Some of the comments raised in 2007 were addressed by changes made in the revised assessment and its appendices.
- Several other comments were redundant with public comments made on the revised assessment.
- In summary, the previous EPA responses in Appendix H as well as the changes that were instituted in the revised assessment adequately and appropriately respond to the public comments from 2007.

# Additional Considerations

- Commend Agency for being highly responsive and transparent to 2007 Review
- Employ grey box approach as NRC to highlight main points
- Include a description of the Swenberg review article
- Give further consideration to the Swedish study (incl. Hagmar 1991)
  - Br CA uses latency
- Comment on shape of dose-response in light of the MoA

# Additional Considerations

- Point to other papers that show D-R flattening after initial steep D-R
- With further consideration of Swedish study all Hill Criteria are met (section 3.5)
- Use of internal rate ratios is appropriate
  - Cohort characteristics differ from general public
  - Health worker effect
- Summarize data using median rather than mean as a measure of central tendency

# Additional Considerations

- Avoid use of the term “Similar” when describing relationships between data
- Provide tables of descriptive data
- Suggest further exploration of the sensitivity of the findings to the lag parameter in the section on uncertainties
- Section D – “lag exposure data erases potential for health worker effect”
- CDC 9-11 paper – suggest EPA include the criteria for selection of lags

# Additional Considerations

- Tables 4-11 & 4-13 Use scientific notation to enhance clarity
- Suggest exploration of more bins in the categorical modeling of the NIOSH data
- Suggest EPA explore other possible dose metrics beyond cumulative dose (ppm-yrs)