Disposition of Peer Review Comments for Toxicological Profile for Ethylene Oxide

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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ATSDR assembled two groups of peer reviewers to review the third pre-public draft of the Toxicological Profile for Ethylene Oxide. In this formal disposition of peer review comments, Group 1 includes three peer reviewers charged with reviewing the toxicological profile; Group 2 includes three additional peer reviewers charged with reviewing the toxicological profile and unpublished studies.

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Comments Provided by Group 1 Peer Reviewer #1

Annotated Comments on the Toxicological Profile

COMMENT 1: Regarding Figure 1-1, the Reviewer states "Figure 1-1 below does not seem to mention Mammary tumors in animals, as per IARC Mono 100-F 2012".

RESPONSE: *The list of cancer types in the 33–50 ppm section of Figure 1-1 was revised to include mammary gland and Harderian gland.*

COMMENT 2: Regarding the paragraph in Section 1.2 that reads "In laboratory animals exposed by inhalation, ethylene oxide was associated with a variety of cancer types (leukemia, mesotheliomas, lymphomas, tumors of lungs, brain, harderian gland, and female reproductive organs) (Lynch et al. 1984a, 1984b; NTP 1987; Snellings et al. 1984b). Forestomach cancer (at the application site was reported in rats administered ethylene oxide by gavage (Dunkelberg 1982)", the Reviewer states "Mice and breast cancer? See IARC Mono 100-F, 2012".

RESPONSE: *The discussion of cancer effects in Section 1.2 was revised to include the female mammary gland as a cancer type:*

> In laboratory animals exposed by inhalation, ethylene oxide was associated with a variety of cancer types (leukemia, mesotheliomas, lymphomas, tumors of lungs, brain, Harderian gland, and female mammary gland and reproductive organs) (Lynch et al. 1984a, 1984b; NTP 1987; Snellings et al. 1984b).

COMMENT 3: Regarding the paragraph in Section 1.2 that reads "The Department of Health and Human Services (HHS) has classified ethylene oxide as *known to be a human carcinogen* (NTP 2016). The EPA characterized ethylene oxide as "carcinogenic to humans" by the inhalation exposure route (EPA 2016a). The International Agency for Research on Cancer (IARC) has designated ethylene oxide as *carcinogenic to humans (Group 1)* (IARC 1987, 2012)", the Reviewer states "I suggest you mention here – or somewhere (see comment below as well) on the risk assessment and URE developed by EPA."

RESPONSE: *The EPA inhalation unit risk information is summarized in Section 2.19:*

> Based on results from the National Institute for Occupational Safety and Health (NIOSH) study (Steenland et al. 2003, 2004) that evaluated cancer risk in a cohort of workers exposed to ethylene oxide, EPA derived adult-based unit risk estimates of $2.6x10^{-3}$per µg/m^3 ($9.1x10^{-3}$ per ppb) for lymphoid cancer, $7.0x10^{-4}$ per µg/m^3 ($1.3x10^{-3}$ per ppb) for female breast cancer, and $3.0x10^{-3}$ per µg/m^3 ($5.5x10^{-3}$ per ppb) for both cancer types combined. Application of standard age-dependent adjustment factors yields a full lifetime total cancer unit risk estimate of $5.0x10^{-3}$ per µg/m^3 ($9.1x10^{-3}$ per ppb) (EPA 2016a).

The unit risk was added to Table 7-1.

COMMENT 4: Regarding the Cancer bullet in Section 2.1, the Reviewer states "Again, add breast cancer as per quote from IARC Mono 100-F, Treatment-related increases in lymphomas, Harderian gland, mammary gland carcinomas, and uterine adenocarcinomas were also seen in B6C3F1 mice (NTP, 1987; Picut et al., 2003)."

RESPONSE: *The list of cancer endpoints for animals exposed by inhalation was revised to include the mammary gland:*

> In laboratory animals exposed by inhalation, ethylene oxide was associated with a variety of cancer types (leukemia, mesotheliomas, lymphomas, tumors of lungs, Harderian gland, and female mammary gland and reproductive organs).

COMMENT 5: Regarding presentation of the forest plots (Figures 2-4 through 2-9), the Reviewer states "How about a table with exposure-response results rather than exposed vs non exposed. It is the exp-response data which drove the assessment the ETO is carcinogenic by IARC and NTP?" The Reviewer further states "You may want to take a look at Marsh et al 2019 meta analysis of lympho-hematopoetic and breast cancer for ETO studies."

RESPONSE: *Meaningful exposure-response data were not generally available for epidemiological studies examining the carcinogenicity of ethylene oxide. Therefore, it was determined that available data should be presented as exposed versus nonexposed. ATSDR considered the forest plots a more visual way to provide an overview of the risk ratios for the different tumor types.*

The meta-analysis of Marsh et al. (2019) was added to Section 2.19:

> Marsh et al. (2019) conducted a systematic literature review of occupational exposure to ethylene oxide and risk of lymphohematopoietic cancer and breast cancer and identified 13 studies that were included in a meta-analysis. Overall meta-relative risks (meta-RRs) were 1.48 (95% CI 1.07–2.05) for lymphohematopoietic cancer and 0.97 (95% CI 0.80–1.18) for breast cancer. For lymphohematopoietic cancer, the study authors reported meta-RRs of 1.46 (95% CI 0.85–2.50) among ethylene oxide production workers and 1.07 (95% CI 0.87–1.30) among ethylene oxide sterilization workers. Higher risks of lymphohematopoietic cancer were noted for earlier published studies, compared to more recent studies.

COMMENT 6: Regarding the statement in Section 2.19 that reads "EPA (2016a) characterized ethylene oxide as 'carcinogenic to humans' by the inhalation exposure route, based on the total weight of evidence including epidemiological evidence of lymphohematopoietic cancers and breast cancer in ethylene oxide-exposed workers, lymphohematopoietic cancers in rats and mice and mammary carcinomas in mice,…", the Reviewer states "Yup here you do have breast ca in animals".

RESPONSE: *No response is necessary.*

COMMENT 7: Regarding presentation of IARC's cancer characterization for ethylene oxide in Section 2.19, the Reviewer stated "Should you not also give the exposure response data for lymphoid cancer here?"

RESPONSE: *The referenced discussion of IARC's review of the breast cancer data was deleted from the last paragraph in Section 2.19.*

COMMENT 8: Regarding presentation of EPA cancer characterization for ethylene oxide in Section 2.19, the Reviewer stated "Why not present in this section or somewhere - the EPAs IRIS unit risk estimate and ATSDR's own risk assessment (CREG) based on EPAs work."

RESPONSE: *Statements regarding EPA's unit risk estimates were already presented in Section 2.19. The following text regarding EPA's cancer unit risk estimates was added:*

> Based on results from the National Institute for Occupational Safety and Health (NIOSH) study (Steenland et al. 2003, 2004) that evaluated cancer risk in a cohort of workers exposed to ethylene oxide, EPA derived adult-based unit risk estimates of $2.6x10^{-3}$per µg/m^3 ($9.1x10^{-3}$ per ppb) for lymphoid cancer, $7.0x10^{-4}$ per µg/m^3 ($1.3x10^{-3}$ per ppb) for female breast cancer, and $3.0x10^{-3}$ per µg/m^3 ($5.5x10^{-3}$ per ppb) for both cancer types combined.

COMMENT 9: Regarding information in Section 5.5.1 cited to Parod (2014), the Reviewer stated "More details needed here from Parod, not accessible as it is in a book,. What was the median or geo mean?. How many samples? How many below LOD? What was LOD?"

RESPONSE: *The following statement was added to the reported data from Parod (2014):*

> Limitations of the report include lack of information regarding the number of samples evaluated, limit of detection, number of samples below the limit of detection, and whether the mean value was geometric or arithmetic.

ATSDR Charge Questions and Peer Reviewer Comments

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 10: Partly. Need more reporting of exposure-response data, as they have been important in assessing causality and deriving UREs.

RESPONSE: *In Section 6.2 (Epidemiology and Human Dosimetry Studies), ATSDR has identified a data need for additional studies of occupational cohorts for whom reliable historical exposure levels can be determined. The following statement was added to Section 2.19 concerning the Steenland et al. (2003, 2004) study that was used to derive the unit risk estimates:*

> The authors found positive exposure responses for breast cancer mortality in females for log cumulative exposure and a 20-year lag. There was also a positive exposure response for lymphoid tumors (non-Hodgkin's lymphoma, myeloma, and lymphocytic leukemia) in both sexes for cumulative exposure.

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 11: Regarding cancer, animal and human data are largely concordant.

RESPONSE: *No response is necessary.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 12: Yes

RESPONSE: *No response is necessary.*

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 13: Yes, human data in low dose range are sparse.

RESPONSE: *No response is necessary.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 14: Agree, but this is outside my area of expertise

RESPONSE: *No response is necessary.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 15: Reviewer made no comment.

RESPONSE: *No response is necessary.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 16: Partly, although the Figures emphasizing exposed vs nonexposed results for cancer are not as informative as providing intormation of exposure-response data.

RESPONSE: *As noted in the Response to Comment 5, ATSDR presented the data as exposed versus nonexposed since meaningful exposure-response data were not generally available or consistent between studies.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 17: Yes

RESPONSE: *No response is necessary.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 18: Not sure, outside my area of expertise, but IARC has summarized these data recently so they should be easy to assemble.

RESPONSE: *ATSDR considers the presentation of animal studies to adequately portray available animal data.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 19: Not sure, outside my area of expertise, but IARC has summarized these data recently so they should be easy to assemble

RESPONSE: *ATSDR considers the presentation of animal studies to adequately portray available animal data.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 20: Not for human data, see my comment above

RESPONSE: *As noted in the Response to Comments 5 and 16, ATSDR notes that meaningful exposure-response data were not generally available for human studies.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 21: No

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 22: No

RESPONSE: *No response is necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 23: Not sure, essentially these ae coming only from animal studies, with which I am not familiar

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 24: Again these are restricted to animal data, not my area.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 25: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 26: In the summary of health effects regarding cancer at the beginning, the draft says "Some investigators suggested that exposure to ethylene oxide increased the risk of selected cancer types (e.g., lymphohematopoietic cancer, leukemia, breast cancer". Then this sections ends by noting that that IARC and NTP and EPA have determined that ETO is a definite human carcinogen. This seems a bit of a disconnect. Why not summarize the evidence that IARC and EPA thought was most compelling, which includes – for epidemiology – exposure-response data rather than exposed/non-exposed comparisons. Why not also cite the work done by EPA in the IRIS report regarding a URE? I note ATSDR did cite this in its report on Willowbrook/Sterigenics, where ATSDR Cancer Risk Evaluation Guide (CREG) is presented for ETO.

RESPONSE: *The statement "Some investigators suggested that exposure to ethylene oxide may increase the risk of selected cancer types (e.g., lymphohematopoietic cancer, leukemia, breast cancer)" was revised:*

> Results from some cohort studies suggest that exposure to ethylene oxide may increase the risk of selected cancer types (e.g., lymphohematopoietic cancer, leukemia, breast cancer).

The cancer bullet in Section 2.1 is a brief summary statement only. More detailed information regarding the carcinogenicity of ethylene oxide is presented in Section 2.19. The information regarding EPA's inhalation unit risk was revised to the following:

> EPA derived adult-based unit risk estimates of $2.6x10^{-3}$per µg/m^3 ($4.8x10^{-3}$ per ppb) for lymphoid cancer, $7.0x10^{-4}$ per µg/m^3 ($1.3x10^{-3}$ per ppb) for female breast cancer, and $3.0x10^{-3}$ per µg/m^3 ($5.5x10^{-3}$ per ppb) for both cancer types combined. Application of standard age-dependent adjustment factors yields a full lifetime total cancer unit risk estimate of $5.0x10^{-3}$ per µg/m^3 ($9.1x10^{-3}$ per ppb) (EPA 2016a). The commensurate lifetime chronic (lower bound) exposure level of $2x10^{-4}$ µg/m^3 ($1x10^{-4}$ ppb) corresponds to an increased cancer risk of 10^{-6} (1 in 1,000,000). The unit risk estimate was developed for environmental ethylene oxide exposures up to about 40 µg/m^3 (20 ppb) and is not applicable to higher exposure levels that may occur in occupational exposure scenarios. Maximum likelihood estimates of extra risk of lymphoid cancer and breast cancer (combined) for occupational exposure scenarios in the range of 0.1–1 ppm for an 8-hour TWA for 35 years range from 0.037 to 0.11 (upper bound estimates 0.081–0.22).

EPA's inhalation unit risk value for ethylene oxide was also added to Table 7-1.

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

The Peer Reviewer commented that Chapter 3 was "Outside my area of expertise" and did not provide response to the charge questions.

Chapter 4. Chemical and Physical Information

The Peer Reviewer commented that Chapter 4 was "Outside my area of expertise" and did not provide response to the charge questions.

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 27: Seems reasonably done, not my area

RESPONSE: *No response is necessary.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically

sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 28: Seems OK, not my area

RESPONSE: *No response is necessary.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 29: Seems OK, not my area

RESPONSE: *No response is necessary.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 30: The text does not include the most recent EPA data on ambient levels, These data are quite surprising, showing levels about 1/3 or 1/5 of the levels near the Sterigenics plant in Chicago. It would be good to note the implications of the recent EPA data, which are potentially quite serious regarding health risk to the general population, assuming EPA recent IRIS report conclusions and their URE estimate are correct.

RESPONSE: *Table 5-8 was updated with the most recent data (October 2018–September 2019).*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 31: See comment above

RESPONSE: *See the Response to Comment 30.*

Chapter 6. Adequacy of the Database

COMMENT 32: I would suggest that updating the NIOSH cohort would be quite useful

RESPONSE: *The following statement was added to the Epidemiology and Human Dosimetry Studies section of Section 6.2:*

The ethylene oxide NIOSH cohort should continue to be followed.

Chapter 7. Regulations and Guidelines

COMMENT 33: This seems fine but again, why not report the EPA's URE for cancer as well as current regulations

RESPONSE: *EPA's inhalation unit risk information has been added to Table 7-1.*

Comments provided by Group 1 Peer Reviewer #2

Annotated Comments on the Toxicological Profile

COMMENT 1: Regarding the statement in Section 1.1 "In water, ethylene oxide will either break down or be destroyed by bacteria within a few days", the Reviewer stated "By what process? Hydrolysis?"

RESPONSE: *The statement in Section 1.1 was revised:*

In water, ethylene oxide will either evaporate quickly, break down by hydrolysis, or be destroyed by bacteria.

COMMENT 2: Regarding the green highlighting of selected studies in LSE Table 2-1, the Reviewer stated "Not clear to me why this row is highlighted in green."

RESPONSE: *The green highlighting is intended to make it easy to locate studies in LSE tables that serve as a basis for MRL derivation. Footnotes identify the critical effect that serves as a basis for a given MRL.*

COMMENT 3: In Section 2.12, the Reviewer suggested that the word "postinstallation" should be hyphenated.

RESPONSE: *ATSDR convention is to not hyphenate such prefixes unless the unhyphenated word could have multiple interpretations.*

COMMENT 4: Regarding the beginning of Section 3.1 (TOXICOKINETICS), the Reviewer stated "It is important and useful to provide a brief summary paragraph before provided a bulleted list toxiokinetic issues."

RESPONSE: *It is consistent with ATSDR toxicological profile guidance to begin Section 3.1 with bulleted text to provide a summary statement regarding absorption, distribution, metabolism, and excretion. ATSDR will consider the Reviewer's suggestion in future updates of the toxicological profile guidance.*

COMMENT 5: Regarding the statement in Section 3.1 that reads "The toxicokinetics of exogenous ethylene oxide is typically measured using a radiolabeled isotope (typically ^{14}C-ethylene oxide)", the Reviewer suggested replacing the first iteration of "typically" with "commonly" to eliminate using "typically" twice in the same sentence.

RESPONSE: *The requested word change was made in Section 3.1:*

The toxicokinetics of exogenous ethylene oxide is commonly measured using a radiolabeled isotope (typically ^{14}C-ethylene oxide).

COMMENT 6: Regarding the statement in Section 3.1 that reads "Kirman and Hays (2017) designed a study to evaluate relative contributions of exogenous and endogenous sources of the ethylene oxide to the

production of the adduct hemoglobin N-(2-hydroxyethyl)-valine (HEV)", the Reviewer inserted "human-subject" between "designed a" and "study".

RESPONSE: *The statement in Section 3.1 was revised:*

Kirman and Hays (2017) designed a human study to evaluate relative contributions of exogenous and endogenous sources of the ethylene oxide to the production of the adduct hemoglobin N-(2-hydroxyethyl)-valine (HEV).

COMMENT 7: Regarding the Section 3.1.1 statement that reads "In a study of hospital workers exposed to ethylene oxide in the workplace air at concentrations ranging from 0.2 to 24.1 mg/m^3 (0.11–13.2 ppm), approximately 75% of the inhaled ethylene oxide was absorbed (Brugnone et al. 1985, 1986)", the Reviewer suggested adding "into the blood stream" to indicate where the inhaled ethylene oxide was absorbed.

RESPONSE: *The sentence in question in Section 3.1.1 was revised:*

In a study of hospital workers exposed to ethylene oxide in the workplace air at concentrations ranging from 0.2 to 24.1 mg/m^3 (0.11–13.2 ppm), approximately 75% of the inhaled ethylene oxide was absorbed into the bloodstream (Brugnone et al. 1985, 1986).

COMMENT 8: Regarding the Section 3.1.1 sentences that read "Blood:air coefficients were 2.5–3.3 measured from venous blood samples collected 4 hours after the workshift. Inhaled ethylene oxide was rapidly absorbed through the respiratory system of the rat (Filser and Bolt 1984; Koga et al. 1987; Matsuoka 1988; Nakashima et al. 1987; Tardif et al. 1987), mouse (Cumming et al. 1981; Ehrenberg et al. 1974; Tardif et al. 1987), and rabbit (Tardif et al. 1987)", the Reviewer stated "You are jumping from human studies to rat studies. I would be useful to have a transition sentence here to make that clear."

RESPONSE: *The statements in Section 3.1.1 were separated by creating a new paragraph for the animal data.*

COMMENT 9: Regarding the statement in Section 3.1.1 that reads "No studies were located regarding absorption of ethylene oxide after oral exposure", the Reviewer asked if this statement refers to both humans and animals.

RESPONSE: *The statement in Section 3.1.1 was revised to:*

No human or animal data were located regarding absorption of ethylene oxide after oral exposure.

COMMENT 10: Regarding the Section 3.1.2 heading "Distribution", the Reviewer suggested revising the heading to "Tissue Distribution following Abosorption".

RESPONSE: *No change was made. ATSDR will consider changing the title of this section of the profile in future updates of the toxicological profile guidance.*

COMMENT 11: Regarding the Section 3.1.2 statement that reads "Krishnan et al. (1992) demonstrated relatively similar ethylene oxide tissue distribution for male Fischer rats based on calculated *in vitro* tissue:air partition coefficients for fat (44.1), brain (48.3), lung (60.9), liver (61.6), blood (64.1), and testes (83)", the Reviewer suggested deleting the word "relatively".

RESPONSE: *The suggested deletion was not made. The spread of* in vitro *tissue:air partition coefficients necessitates the inclusion of the word "relatively."*

COMMENT 12: In the Section 3.1.3 sentence that reads "The other pathway involves glutathione conjugation to form mercapturic-acid and meththio metabolites, some of which are converted to thiodiacetic acid", the Reviewer suggested deleting the hyphen associated with the word "methio".

RESPONSE: *The suggested deletion was made.*

COMMENT 13: In the Section 3.1.3 sentence that reads **"**The order of areas under the curve (AUC) values was humans>rats>mice", the Reviewer suggested adding the word "values" to follow the term "(AUC)".

RESPONSE: *The sentence in Section 3.1.3 was revised:*

The order of area under the curve (AUC) values was humans>rats>mice.

COMMENT 14: Regarding the Section 3.1.4 sentence that reads "In the rat exposed to ^{14}C-ethylene oxide by inhalation, 59% of the ^{14}C-activity was recovered in the urine, 12% expired as $^{14}CO_2$, 4.5% in feces, and 1% expired unchanged (Tyler and McKelvey 1982)", the Reviewer suggested revision to "In a study of rats exposed to ^{14}C-ethylene oxide by inhalation, 59% of the ^{14}C-activity was recovered in the urine, 12% expired as $^{14}CO_2$, 4.5% in feces, and 1% expired unchanged (Tyler and McKelvey 1982)."

RESPONSE: *The referenced sentence in Section 3.1.4 was revised:*

In a study of rats exposed to ^{14}C-ethylene oxide by inhalation, 59% of the ^{14}C-activity was recovered in the urine, 12% expired as $^{14}CO_2$, 4.5% in feces, and 1% expired unchanged (Tyler and McKelvey 1982).

COMMENT 15: In the Section 3.1.4 sentence that reads "Ehrenberg et al. (1974) reported an approximate biological half-life of 9 minutes for absorbed ethylene oxide; approximately 78% of an administered dose of radioactivity from radiolabeled ethylene oxide was eliminated in the urine within 48 hours", the Reviewer stated "This 70% elimination time does not seem to be consistent with a 9-min biological half live".

RESPONSE: *The statement in Section 3.1.4 was revised:*

In a study of mice exposed to radiolabeled ethylene oxide for 60–75 minutes, an average of 78% of the absorbed radioactivity was eliminated in the urine within 48 hours (Ehrenberg et al. 1974).

COMMENT 16: In the Section 3.1.5 sentence that states **"**The epoxide hydrolase pathway is simulated as a capacity-limited process governed by a maximum (Vmax, mmol/hour/kg liver) and a Michaelis half-saturation constant (Km, mmol/L)", the Reviewer suggested that the term "epoxide hydrolase" should be hyphenated and that the word "rate" should be inserted following the word "maximum".

RESPONSE: *These requested changes were made in Section 3.1.5:*

The epoxide-hydrolase pathway is simulated as a capacity-limited process governed by a maximum rate (Vmax, mmol/hour/kg liver) and a Michaelis half-saturation constant (Km, mmol/L).

COMMENT 17: In the Section 3.1.5 portion describing the Fennel and Brown (2001) models, the Reviewer suggested that the sentence stating "Absorption from the respiratory tract is simulated as a flow-limited transfer from a gas exchange compartment of the lung to a metabolic lung compartment, governed by an uptake fraction, concentration difference across the lung compartments, alveolar ventilation rate, cardiac output, and the blood:air partition coefficient" should include addition of the word "a" between "as" and "flow-limited".

RESPONSE: *The requested addition of "a" was made:*

Absorption from the respiratory tract is simulated as a flow-limited transfer from a gas exchange compartment of the lung to a metabolic lung compartment, governed by an uptake fraction, concentration difference across the lung compartments, alveolar ventilation rate, cardiac output, and the blood:air partition coefficient.

COMMENT 18: In the Section 3.1.5 portion that reads **"**The epoxide hydrolase pathway is simulated as a capacity-limited process governed by a maximum (Vmax, mmol/hour) and a Michaelis half-saturation constant (Km, mmol/L)", the Reviewer suggested adding the word "rate" to follow the word "maximum".

RESPONSE: *The word "rate" was added as suggested:*

The epoxide hydrolase pathway is simulated as a capacity-limited process governed by a maximum rate (Vmax, mmol/hour) and a Michaelis half-saturation constant (Km, mmol/L).

COMMENT 19: Regarding Section 4.1, the Reviewer stated "This chapter should begin with a summary statement that identifies the goals and methods of the chapter".

RESPONSE: *The following statement was added to the beginning of Section 4.1:*

Ethylene oxide is both a manmade substance and a natural substance produced in the body via oxidation of absorbed or endogenously produced ethylene during normal oxidation processes.

COMMENT 20: Regarding Chapter 4, the Reviewer stated "This chapter should begin with a summary statement that identifies the goals and methods of the chapter".

RESPONSE: *ATSDR believes that most readers will anticipate the type of information that will be presented in Chapter 4 based on its title: Chemical and Physical Information. ATSDR also notes that none of the chapters in the toxicological profile begin with a statement that identifies the goals and methods of the chapter.*

COMMENT 21: Regarding the first bullet in Section 5.1, the Reviewer stated "No mention of any water ingestion or dermal contact pathways".

RESPONSE: *No information was located regarding ethylene oxide in drinking water sources. The following bullet was added to the Section 5.1 bullets discussing exposure pathways:*

Dermal contact during sterilization/fumigation practices

ATSDR Charge Questions and Reviewer Comments

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 22: I believe that the report is complete with regard to identifying all ethylene oxide health effects in humans and animals that have been reported in the literature.

RESPONSE: *No response is necessary.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 23: I believe the report is adequate in explaining the relevance to human health of any effects that have been only observed in animals

RESPONSE: *No response is necessary.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 24: I believe that all relevant exposure conditions are covered in Chapter 1.

RESPONSE: *No response is necessary.*

Minimal Risk Levels (MRLs)

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 25: There is no MRL derived for cancer in humans. Given that HHS has classified ethylene oxide as "known to be a human carcinogen", EPA has characterized it as "carcinogenic to humans" and IARC has designated it as "carcinogenic to humans" (Group 1) there should be an explanation for why there is no MRL for cancer as an endpoint.

RESPONSE: *As stated at the beginning of Appendix A, "An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered."*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose. Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 26: I believe the proposed MRL values have been adequately documented. I agree with uncertainty factor values

RESPONSE: *No response is necessary.*

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 27: No additional comments

RESPONSE: *No response is necessary.*

Chapter 2. Health Effects

COMMENT 28: As a reviewer, I need to preface my comments on this chapter by noting that my expertise is primarily in exposure science, toxicokinetics, and risk assessment and no so much in toxicology and epidemiology. Nevertheless, I did read through this chapter in detail and base my comments on my familiarity with toxicology and epidemiology as they relate to risk assessment.

RESPONSE: *No response is necessary.*

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 29: As far as I can determine the health effects summaries provided in this chapter reflect findings in the published literature. In addition, for the most part, the health effects conclusions provided in Chapter 2 adequately reflect the literature findings. I do question though why the extensive literature findings on observations of cancer in both humans and animals along with significant amounts of exposure data were not used to provide either a cancer dose-response assessment or cancer benchmark dose.

RESPONSE: *ATSDR does not perform risk assessment evaluations or assign classifications for cancer endpoints. ATSDR relies on available cancer assessments of EPA, the Department of Health and Human Services (via the National Toxicology Program [NTP]), and the International Agency for Research on Cancer (IARC) for cancer assessments and classifications.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 30: Based on my review, I believe adequately designed human studies were identified and summarized and characterized in terms of study limitations.

RESPONSE: *No response is necessary.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 31: Based on my review, I believe adequately designed animal studies were identified and summarized and characterized in terms of study power limitations.

RESPONSE: *No response is necessary.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 32: The animal species selected are appropriate

RESPONSE: *No response is necessary.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 33: No. As noted above I believe the authors missed an opportunity to provide a dose-response characterization for human cancer incidence based on the amount of data available for observations of cancer in both humans and animals

RESPONSE: *As noted in the Response to Comment 29, ATSDR does not perform risk assessment evaluations or assign classifications for cancer endpoints.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 34: I am not aware of any such studies

RESPONSE: *No response is necessary.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 35: I am not aware of any such studies

RESPONSE: *No response is necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 36: I believe all appropriate NOAELs and/or LOAELs were identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 37: I found the categorization of “less serious” and “serious” for the effects cited in the LSE tables effective, useful, and informative to the reader.

RESPONSE: *No response is necessary.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 38: I believe all possible mechanisms of action are covered.

RESPONSE: *No response is necessary.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 39: I believe the conclusions are generally appropriate, but I recommend that the report include a conclusion about the cancer dose-response.

RESPONSE: *ATSDR does not perform risk assessment evaluations or assign classifications for cancer endpoints. ATSDR relies on available cancer assessments of EPA, the Department of Health and Human Services (via the National Toxicology Program [NTP]), and the International Agency for Research on Cancer (IARC) for cancer assessments and classifications.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

Toxicokinetics

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 40: The discussion of absorption, distribution, metabolism and excretion is extensive and useful . It is very helpful for making clear the structure and application of the pharmacokinetic models. There is one issue that caught my attention—in section 3.2.4 the authors describe an approximate biological half-life of 9 minutes for absorbed ethylene oxide, but then say 78% of an administered dose of radioactivity from radiolabeled ethylene oxide was eliminated in the urine within 48 hours. These seem inconsistent, so an explanation is needed.

RESPONSE: *The statement regarding a biological half-life of 9 minutes for absorbed ethylene oxide was deleted. It appears that the study author was describing the half-life in blood, but it was not clearly stated.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 41: As far as I can determine, the authors have presented all relevant pharmacokinetic/ pharmacodynamic models and supporting data.

RESPONSE: *No response is necessary.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 42: In section 3.1.6, which summarizes animal-to-human extrapolations, there is limited but likely adequate discussion of differences in toxicokinetics. However, with regard to a summary discussion of the relevance of animal toxicokinetic information for humans there is only the rather vague statement—"Interspecies extrapolation should include identification of the proximate toxicant(s)." I am not sure what this statement means—so that should be explained. In addition, there is a need here for a. bit more discussion of when and how animal toxicokinetic information is relevant for humans. detoxification pathways

RESPONSE: *The statement in question in Section 3.1.6 was revised:*

> Interspecies extrapolation would need to account for species differences in metabolic pathways, species-specific contribution of exposure concentration, and the identity of the toxicant or toxicants (ethylene oxide itself and/or its metabolite[s]) responsible for a particular toxic effect.

Children and Other Populations that are Unusually Susceptible

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 43: I agree that there are very limited data on child-specific risk factors. I am not aware of any data that has been excluded from the report. The authors point out correctly that, even though the issue is not explicitly discussed in the current literature, children are likely to be more susceptible to ethylene oxide toxic effects because they have incomplete development of detoxification pathways needed to remove this substance from the body.

RESPONSE: *No response is necessary.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 44: Yes, there is discussion of whether any populations (other than children) are at higher risk of susceptibility. The authors report and document that, based on limited data, there is no indication of significant sex-related differences. The also observe and document with appropriate citations that Individuals with genetic deficiencies in activities of detoxification enzymes would likely be at increased risk of ethylene oxide toxicity/carcinogenicity.

RESPONSE: *No response is necessary.*

Biomarkers of Exposure and Effect

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 45: The authors have identified and documented with references the currently know biomarkers of exposure for ethylene oxide.

RESPONSE: *No response is necessary.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 46: The authors report that there is no information available on biomarkers of effect for ethylene oxide and I am not aware of any literature that could contradict this observation.

RESPONSE: *No response is necessary.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 47: The authors report that there is no information available on toxicologically-relevant interactions between ethylene oxide and other substances and I am not aware of any literature that could contradict this observation.

RESPONSE: *No response is necessary.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 48: This question is not relevant because there are no known interactions.

RESPONSE: *No response is necessary.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 49: I believe that the information provided in the chemical and physical properties tables is complete and correct. However, for me it is odd that this chapter consists only of tables, I believe it needs to begin with a summary statement that identifies the goals and methods of the chapter.

RESPONSE: *The following statement was added to Section 4.1:*

Ethylene oxide is both a manmade substance and a natural substance produced in the body via oxidation of absorbed or endogenously produced ethylene during normal oxidation processes.

The following statement was added to the beginning of Section 4.2:

Ethylene oxide is a colorless, flammable gas. It is highly soluble in water ($1x10^6$ mg/L at 20°C) and possesses a high vapor pressure ($1.095x10^3$ mm Hg at 20°C).

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 50: Yes information is provided on the physical form of the substance.

RESPONSE: *No response is necessary.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 51: Based on my experience with ethylene oxide, the information on production, import/export, use and disposal is complete.

RESPONSE: *No response is necessary.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 52: As far as I can determine the text provides sufficient and technically sound information on occurrence at NPL. However, I found that the tracking of transport could be expanded to better address multimedia transport. For the multimedia fate assessment the authors rely primarily on the 1983

Conway reference. I believe they should also consult the more recent fate assessments by Staples and Gulledge (2006) and the details of the ChemCAN model simulation, that is described in one of the report's cited references (WHO, 2003).
Staples, CA, Gulledge, W (2006) An environmental fate, exposure and risk assessment of ethylene oxide from diffuse emissions. Chemosphere, 65 (4): 691-698, https://doi.org/10.1016/j.chemosphere.2006.01.047.
WHO. 2003. Ethylene oxide. Concise International Chemical Assessment Document 54. Geneva, Switzerland: World Health Organization. CICADS 54.

RESPONSE: *The results of the Staples and Gulledge multi-media fate modeling exercise were summarized and Section 5.4.1 was revised to include the following statement:*

> Staples and Gulledge (2006) used a level III multi-media fate model to calculate ethylene oxide concentrations in air, water, soil, and sediment given an estimated annual emission rate using six different environmental scenarios meant to represent different climatic regions of the United States. Mass transport parameters such as the erosion and runoff mass transport velocities as well as rainfall rates and composition of the four main environmental compartments were varied in these six scenarios. The modeled output concentrations in air, water, and sediment were not highly sensitive to the changes in environmental parameters. However, modeled soil levels were shown to be fairly sensitive to changes in the input parameters, most likely the 10-fold changes in the soil erosion and runoff mass transport parameters. In all cases, hazard quotients calculated for target species were much lower than 1, suggesting low adverse risk to aquatic and terrestrial wildlife at the given emissions used in the model.

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 53: For the most part, the information provided on transport, partitioning, transformation, and degradation of the substance in all media is complete. However, the authors report in section 5.4.1 that no data on the accumulation and/or fate of ethylene oxide in the soil environment are available. However, estimates of fate factors are provided in the ChemCAN model used in (WHO, 2003) and the fate analysis of Staples and Gulledge (2006). These fate factors and their relationship to soil and sediment fate from these two references should be consulted and discussed.
Staples, CA, Gulledge, W (2006) An environmental fate, exposure and risk assessment of ethylene oxide from diffuse emissions. Chemosphere, 65 (4): 691-698, https://doi.org/10.1016/j.chemosphere.2006.01.047.
WHO. 2003. Ethylene oxide. Concise International Chemical Assessment Document 54. Geneva, Switzerland: World Health Organization. CICADS 54.

RESPONSE: *The summary of the pertinent output of Staples and Gulledge (2006) was captured in Section 5.4.1. As stated elsewhere in the document; ethylene oxide is unlikely to accumulate or be detected in soil barring an accidental release due to its tendency to volatilize and potential reactivity with mineral surfaces.*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 54: The Concise International Chemical Assessment Document 54 (WHO, 2003) discusses measurements of Ethylene Oxide in indoor environments and suggests that indoor exposures are an exposure pathway of note. This should be considered here. With regard to other issues, proper units are used for each medium, form of the substance is reported and there is adequate discussion of information quality.
WHO. 2003. Ethylene oxide. Concise International Chemical Assessment Document 54. Geneva, Switzerland: World Health Organization. CICADS 54.

RESPONSE: *Ethylene oxide levels in indoor air are discussed in Section 5.5.1. A sentence was added regarding the WHO (2003) document:*

> Ethylene oxide was detected in only 1 of 50 samples of indoor air in a study of residences by Health Canada; the concentration was determined to be 4 $\mu g/m^3$ (2.2 ppb) in the 24-hour sample (WHO 2003). It was also detected at levels of 5 $\mu g/m^3$ in 3 of 24 personal air samples collected from an occupant of each of the 50 residences.

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 55: The text does describe sources and pathways of exposure for the general and occupational populations as well as populations with high exposures. I agree with the selection of these populations but have a comment on exposure pathways for the general population and a comment on dermal exposure in occupational settings.. In a study of comparing cumulative multimedia exposures to chemical releases to air and surface water, Bennett et al (2002) report for Ethylene Oxide that the intake fraction for water releases is similar to the intake fraction for air releases. Although reported releases to water are much lower than releases to surface waters, it should be noted in the report that the efficiency of the population intake from water releases are comparable to those from air releases. In addition there is no discussion of dermal contact exposures in Section 5.1 even though dermal toxicity is discussed in Chapter 2 and later in section 5.7 of Chapter 5.
Bennett, DH, Margni, MD McKone, TE, Jolliet O. (2002) Intake fraction for multimedia pollutants: a tool for life cycle analysis and comparative risk assessment. Risk Analysis 22(5):903-916.

RESPONSE: *In Section 5.1, a bulleted exposure pathway was added:*

> Dermal contact during sterilization/fumigation practices

The following statement was added to Section 5.5.2:

> The intake fraction (*iF*) is the modeled portion of chemical mass releases into the environment that will be inhaled, ingested, or absorbed by the population. The water *iF* for ethylene oxide is $1.32x10^{-5}$ compared to the air intake fraction of $1.99x10^{-5}$, suggesting similar efficiency of population intake via water compared to air (Bennett et al. 2002).

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 56: I am not aware of any additional studies.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 57: I agree with the identified data needs. However, I believe it would be useful to suggest some priority among the data needs in terms of what new data could have the most impact on risk assessments.

RESPONSE: *The purpose of the data needs discussion in the toxicological profile is to identify data gaps. The data needs are not prioritized in this document.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 58: Yes the results are presented in a neutral, non-judgmental fashion .I did not detect any bias in the text or format of the presentation.

RESPONSE: *No response is necessary.*

Chapter 7. Regulations and Guidelines

COMMENT 59: I am not aware of any additional regulations or guidelines that should be included. I do not believe that any of the listed guidelines and regulations should be removed.

RESPONSE: *No response is necessary.*

Appendices

COMMENT 60: No comments

RESPONSE: *No response is necessary.*

Comments provided by Group 1 Peer Reviewer #3

Annotated Comments on the Toxicological Profile

COMMENT 1: Regarding the statement in Section 1.1 that reads "When ethylene oxide is produced or used, some of the gas is released to air and water. If it is released into the air, it will break down within a few days", the Reviewer states "The persistence of EtO in the air is subject to much uncertainty; "breaking down within a few days" is an inaccurate statement based on the best available knowledge. Note the statements in section 5.4.2 of this document;

Air. There is limited information on the fate of ethylene oxide in the atmosphere. EPA (1984b) reported that the most probable path of atmospheric degradation of ethylene oxide is oxidation via free-radical formation, and estimated its half-life in air at 25°C to range from 69 to 149 days, based on data (rate constants and the concentration of OH radicals) obtained by Fritz et al. (1982). Atmospheric half-lives based on reaction with hydroxyl radicals were also estimated as ranging from 38 to 382 days (WHO 2003).

Recommend that the opening statement reflect the current thinking that EtO is persistent in the atmosphere for a period of several months, but that more research is needed to better delineate the extent under controlled lab conditions as well as real-world atmospheric conditions."

RESPONSE: *The text in Section 1.1 was revised to indicate that it will breakdown in several months:* If it is released into the air, it will break down within several months.

COMMENT 2: Regarding Section 3.3.1 (Biomarkers of Exposure), the Reviewer states "This is not my area of expertise but what about HbEO? Recently there has been publicity about a Univ of IL – Chicago study looking at HbEO markers in the blood of residents living near two EtO sources – Medline and Vantage in Lake County IL. ATSDR and CDC staff have been involved. Is this too new to mention here and/or because it's unpublished? Dr. Susan Buchanan is the PEHSU contact."

RESPONSE: *The use of the ethylene oxide hemoglobin adduct hydroxylated N-terminal valine is discussed in Section 3.3.1. The data from the biomonitoring study of residents living near the Illinois sites have not been published and were not added to the profile.*

COMMENT 3: Regarding the statement in Section 5.1 that reads "There are no data to indicate that ethylene oxide is a common constituent of air or water sources of any type in any geographic location within the United States.", the Reviewer states "EPA has made six months of ambient EtO data available from 18 sites located in 9 states - https://www.epa.gov/hazardous-air-pollutants-ethylene-oxide/ethylene-oxide-data-summary-national-air-toxics-trends.
The current plan is to expand this network to 34 sites in 21 states + District of Columbia. Certainly the available data can be updated into Table 5-6.
It might be more accurate to say that "only a limited amount of data are available that show current levels of ethylene oxide in the air" and reference EPA's effort to make data available."

RESPONSE: *The text in Section 5.1 was revised to include a note that EPA is monitoring for ethylene oxide:*

> There is a limited amount of ethylene oxide air monitoring data in the United States; EPA has begun to measure ethylene oxide at the National Air Toxics Trends Stations and the Urban Air Toxics Monitoring Program networks.

The results from the EPA monitoring program was added to Section 5.5.1:

> EPA (2019a) reported ethylene oxide average concentrations from ambient air samples collected at 8 National Air Toxics Trends Stations (NATTS) and 10 Urban Air Toxics Monitoring Program (UATMP) sampling sites between October 2018 and September 2019. Results are summarized in Table 5-8. Measured average ethylene oxide concentrations ranged from 0.136 µg/m^3 (0.075 ppb) in Pinellas Park, Florida to 0.407 µg/m^3 (0.224 ppb) in Phoenix, Arizona.

COMMENT 4: Regarding the statement in Section 5.5 that reads "The samples were collected in areas with no known ethylene oxide sources and ranged from below the reporting level to 1.68 µg/m^3 (0.92 ppb).", the Reviewer states "Not true – the data from Colorado, Michigan, and presumably Illinois (Willowbrook?) are from networks located around EtO sources. Were the referenced data chosen from the upwind and/or most distant residential sites? This is not explained in Table 5-6. But clearly the referenced data are from the Terumo (CO) and Viant (MI) sterilizer air studies. I assume the IL data are from the Sterigenics network (May 2018 initial study?)."

RESPONSE: *Table 5-6 was revised to only include monitoring data collected from areas without ethylene oxide sources. Thus, the data from Michigan were deleted. Regarding the Colorado data, only data from background sites were used; this is noted in a footnote added to the table. The Illinois data are from areas without ethylene oxide sources.*

COMMENT 5: Regarding Table 5-6, the Reviewer states "You could add the data summary from the NATTS data set in AQS. James Durant has done a lot of analysis on the data for ATSDR. The AQS web page can serve as a citation if needed to allow access."

RESPONSE: *As noted in the Response to Comment 3, the data from the National Air Toxics Trends Stations were added to Section 5.5.1.*

COMMENT 6: Regarding the Colorado entry in Table 5-6, the Reviewer states "https://environmentalrecords.colorado.gov/HPRMWebDrawer/RecordView/1272508

It appears that only the data from "background" sites is being used. So your footnote C should indicate what subset of data you are using. A conc > 2 ug/m3 is noted in the post-control data set so be clear what data you are choosing to document in this table.

The same specificity should be applied to the MI and IL data sets; be clear about what data are being used in this table."

RESPONSE: *Footnote "c" in Table 5-6 was revised:*

> [c]Post-control samples only from background sites.

As noted in the Response to Comment 4, the Michigan data were deleted from Table 5-6. A footnote was not added to the Illinois data since these data are all background levels.

COMMENT 7: Regarding the citation of EPA 2019 in Section 5.5.1, the Reviewer states "I don't see this citation in the reference listing."

RESPONSE: *The citation was added to the reference list.*

COMMENT 8: Regarding the statement in Section 6.2 (Exposure Levels in Environmental Media) that reads "Ambient concentrations of ethylene oxide in high density urban and industrial areas that have potentially large sources of ethylene oxide would be helpful in determining the ambient concentrations of ethylene oxide so that exposure estimates can be made for the general population.", the Reviewer states "I don't disagree with the premise but I think it's equally (if not greater) importance to understand EtO in additional locations away from sources (especially rural/remote locations). Getting a sense if EtO is internationally transported would be important as well. If the preliminary NATTS EtO air data are representative of "true" background, many people will be getting their exposures in places where there are no known EtO sources."

RESPONSE: *Section 6.2 (Exposure Levels in Environmental Media) was revised to include a need for monitoring data in rural/remote areas:*

> Additionally, monitoring data from rural and/or remote locations would provide valuable information on background levels.

General Comments

Chapter 1. Relevance to Public Health

COMMENT 9: Page 1, Lines 14-16: The general statement about ethylene oxide (EtO) is incorrect; note your own language in section 5.4.2. This is an area of significant uncertainty but clearly "**breaking down within a few days**" is not consistent with current thinking. EPA/ORD researchers are beginning to study this issue although no updates will be available for some time.

RESPONSE: *As noted in the Response to Comment 1, The text in Section 1.1 was revised to indicate that it will break down in several months.*

COMMENT 10: Page 1, Line 18: I did not comment specifically in the document but I do wish to point out that the statement, **"You are not likely to be exposed to high levels of ethylene oxide in the general environment"** is simplistic. I understand that the statement refers to acute/short-term exposure but I'll point out that longer-term exposure at EtO concentrations measured around certain EtO emitting facilities (e.g., commercial sterilizers) might be considered high in the context of acceptable cancer risk. Based on the 2016 URE value for EtO arising from EPA's 2016 review in the IRIS program, exposure around these facilities (or even due to background EtO levels that have been detected so far) would exceed EPA's screening level of 1×10^{-4} cancer risk.

RESPONSE: *The text in Section 1.1 was revised to include living near a facility producing or using ethylene oxide may also be a source of high exposure:*

> Residents living near facilities producing or using ethylene oxide may also be exposed to higher than background levels via emissions or accidental releases.

Chapter 2. Health Effects

COMMENT 11: The Reviewer stated "Chapter 2 comments: None."

RESPONSE: *No response is necessary.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT 12: Page 88, section 3.3.1: Note my reference to the biomarker HbEO adducts study currently being conducted by the University of Illinois – Chicago. This is not mentioned in sections 3.3.1 – 3.3.2."

RESPONSE: *As noted in the Response to Comment 2, the results of the biomonitoring study of residents living near the Illinois sites have not been published and were not added to the profile.*

Chapter 4. Chemical and Physical Information

COMMENT 13: The Reviewer stated "Chapter 4 comments: None."

RESPONSE: *No response is necessary.*

Chapter 5. Potential for Human Exposure

COMMENT 14: Page 93, section 5.1, lines 17-20: Caution should be exercised concerning definitive statements about the fate of EtO in the atmosphere. Available references are very dated so there is no current research about key atmospheric reactions and their relationship to the persistence of EtO. These statements would best be qualified based on the state of the research although the reaction times may indeed be correct. There is an apparent disconnect with the information provided in section 5.4.2. although the duration of underlying reaction times is not the same as EtO's ultimate lifetime in the atmosphere. Nevertheless, the two sets of statememts may be confusing.

RESPONSE: *The text in Section 5.1 discussing environmental fate in air has been revised:*

> In the air, oxidation via free radical formation is the most probable degradation pathway; the estimated half-life of this reaction ranges from 2 to 5 months. The half-life estimates for other degradation pathways for atmospheric ethylene oxide vary widely.

COMMENT 15: Page 93, section 5.1, lines 33-34: Regarding "**No data available to determine the general population's exposure levels to ethylene oxide**", please note my comments about the availability of ambient EtO monitoring data from EPA's National Air Toxics Trends Stations (NATTS) network – see https://www.epa.gov/hazardous-air-pollutants-ethylene-oxide/ethylene-oxide-data-summary-national-air-toxics-trends. While these data only represent 6 months of data from a subset of the NATTS, their availability should be noted in the text. Also, the statement "**Environmental exposures may include ethylene oxide from car exhaust and tobacco smoke,** is likely based on dated research. Anything currently available is possibly the result of industry-financed, non-peer-reviewed

work, so caution is advised. These topical areas will be addressed later in 2020 by ongoing research being conducted by EPA's ORD and OTAQ offices.

RESPONSE: *The discussion of atmospheric levels in Section 5.1 has been revised to indicate that EPA has started to monitor levels:*

> There is a limited amount of ethylene oxide air monitoring data in the United States; EPA has begun to measure ethylene oxide at the National Air Toxics Trends Stations and the Urban Air Toxics Monitoring Program networks.

The statement that there are no data to determine general population exposure was also revised:

> There are limited data to evaluate general population's exposure levels to ethylene oxide.

In the absence of new information on exposure sources, the statement that environmental exposure may include ethylene oxide from car exhaust and tobacco smoke was not revised.

COMMENT 16: Page 103, section 5.4.2, lines 23-26: Note my previous comments on the dated nature of information on the atmospheric life of EtO. Statements in the Tox profile should at least be internally consistent and note the dated nature of the source material. Certainly the disconnect between the ranges noted in lines 23-26 and the statement in line 30 (..indicate an ethylene oxide half-life of about 1,400 years) should give the reader pause. This is a high priority research area for EPA as the current availability of ambient EtO data from the NATTS data do suggest enough persistence to support long-range transport.

RESPONSE: *ATSDR did not identify more recent information on the atmospheric life of ethylene oxide. The need for additional information on the environmental fate of ethylene oxide is discussed in the data needs section of the profile (Section 6.2).*

COMMENT 17: Page 106, section 5.5, lines 1-2 and Table 5-6: As noted in my specific comments in the draft, the statement "**The samples were collected in areas with no known ethylene oxide sources ……"** is inaccurate as a number of the cited studies were conducted around commercial sterilizers (CO, IL, MI). I believe the intent of referenced data is to demonstrate the EtO concentrations observed at either background sites, or those located upwind of the source(s) based on prevailing meteorology. If that is the case, the citations should explain that use of a subset of the available data. Table 5-6 could also be revised to include the NATTS data as previously mentioned.

RESPONSE: *Table 5-6 was revised; the Michigan data were deleted since the monitoring sites were near a point source. For the Colorado data, ATSDR only reported the values for the four background monitoring sites; this is noted in a footnote that was added to the table. The Illinois data are background levels.*

Chapter 6. Adequacy of the Database

COMMENT 18: Page 119, section 6.2, lines 22-25: Note my comments that an additional need is ambient EtO in rural areas as well as locations suitable for characterizing potential internation transport."

RESPONSE: *As noted in the Response to Comment 8, Section 6.2 (Exposure Levels in Environmental Media) was revised to include a need for monitoring data in rural/remote areas.*

Chapter 7. Regulations and Guidelines

COMMENT 19: Chapter 7 comments: None.

RESPONSE: *No response is necessary.*

Appendices

COMMENT 20: Appendices comments: None.

RESPONSE: *No response is necessary.*

Comments provided by Group 2 Peer Reviewer #1

Specific Comments on the Toxicological Profile

COMMENT 1: Regarding the statement in Section 1.2 that states "A systematic review of noncancer endpoints resulted in the following hazard identification conclusions", the Reviewer states "It is difficult to understand whether cancer endpoints were also included in the systematic review based on the description in Chapter 1."

RESPONSE: *Cancer endpoints were not included in the systematic review. As noted in Section 1.2, a systematic review was done for noncancer endpoints.*

COMMENT 2: The Reviewer states "Figure 1- 1 describes health effects find in animals. It is particularly challenging for the reader since all species are lumped together."

RESPONSE: *The intent of Figure 1-1 is to provide an overview of health effects and to identify the lowest exposure levels associated with these effects. A more detailed discussion of health effects, including information on animal species, is included in Chapter 2.*

COMMENT 3: Regarding the statement in the Neurological Effects portion of Section 1.2 that states "In studies using several animal species…", the Reviewer states "Which species?"

RESPONSE: *The statement in Section 1.2 was revised to include the animal species:*

> In studies using several animal species (monkeys, rats, mice, rabbits) at moderately high levels of ethylene oxide (200–375 ppm) for 6–7 months, hind leg paralysis and atrophy, abnormal knee and extensor reflexes, and diminished pain perception were reported (Hollingsworth et al. 1956).

COMMENT 4: Regarding the cancer portion of Section 1.2, the Reviewer states "While I understand the need for brevity and this chapter, I think the discussion of cancer effects is particularly abbreviated. Lines 4-6, it is particularly challenging to understand which investigators suggested that ethylene oxide increased the risk of which types of cancer."

RESPONSE: *A more detailed discussion of the results of the epidemiological studies evaluating carcinogenicity is outside the scope of Section 1.2. The intent is to let readers know that some studies have found associations between ethylene oxide exposure and cancer and to list the types of cancer found. A more detailed discussion of the epidemiological carcinogenicity data is presented in Section 2.19.*

COMMENT 5: Regarding Chapter 1, the Reviewer states "In this chapter there is no mention of genetic toxicity and mutations and heritable genetic effects."

RESPONSE: *ATSDR does not typically discuss genotoxicity in Chapter 1, a detailed discussion is included in Section 2.20.*

COMMENT 6: Regarding Chapter 2, the Reviewer states "While the instructions to reviewers indicate that ATSDR does not include detailed descriptions of every relevant study in this chapter, There are areas where inclusion of slightly more detail would help the reader. For example, page 20 lines 33-35 "Endocrine Endpoints: Adverse effects (gross and/or histopathologic changes) have been observed in rats and guinea pigs following acute- and/or chronic-duration exposure to ethylene oxide vapor." It would be helpful to identify which tissues were affected. On page 21, line 3 the text describes histopathological lesions observed among laboratory animals. it would be helpful to identify which species."

RESPONSE: *The text in Section 2.1 was revised to indicate that the lesions were observed in the adrenal gland:*

> **Endocrine Endpoints:** Adverse effects (gross and/or histopathologic changes in the adrenal gland) have been observed in rats and guinea pigs following acute- and/or chronic-duration exposure to ethylene oxide vapor.

COMMENT 7: Regarding Table 2-1, footnotes b and c, the Reviewer states "These kind of footnotes make it so hard on the reader to find the particlular study in a 10 page table. It would be helpful to note that these are the two studies highlighted in green."

RESPONSE: *A footnote was added that the green highlight is to indicate the principal studies for MRLs.*

COMMENT 8: Regarding the statement in Section 2.16 that reads "Increased incidence of resorptions, decreased numbers of pups per litter, and decreased numbers of fetuses born relative to numbers of implantation sites were reported in a study of rats intermittently exposed to ethylene oxide vapor at 150 ppm for 3 weeks premating and during gestation days 1–16 (NIOSH 1982)", the Reviewer states "Was exposure to the dams only, or to the dams and sires?"

RESPONSE: *The text was revised to clarify that female rats were exposed:*

> Increased incidence of resorptions, decreased numbers of pups per litter, and decreased numbers of fetuses born relative to numbers of implantation sites were reported in a study of female rats intermittently exposed to ethylene oxide vapor at 150 ppm for 3 weeks premating and during gestation days 1–16 (NIOSH 1982).

COMMENT 9: Regarding Table 2-3, the Reviewer states "This table is particularly hard to follow with the various cohorts and sub cohorts being reported all in one table."

RESPONSE: *The table was designed to provide some study design details for the occupational exposure studies discussed in Section 2.19. The initial study of a cohort and subsequent follow-up studies of the cohort or a subcohort were grouped together so that the reader was aware that they were the same populations rather than unique cohorts.*

COMMENT 10: Regarding the statements in the animal studies portion of Section 2.19 that read "Significantly increased incidence of spleen mononuclear cell leukemia was observed in female rats exposed to ethylene oxide vapor for up to 2 years at ≥33 ppm (Garman et al. 1985, 1986; Snellings et al. 1984b). When incidences among rats dying early or killed in moribund condition were included, there were significant mortality-adjusted trends for mononuclear cell leukemia in males and females. Male rats of the 100 ppm exposure group exhibited significant increases in peritoneal mesothelioma and subcutis

fibroma. A significant trend for primary brain neoplasms was observed in males and females.", the Reviewer states "why not include the tumor incidence for these studies."

RESPONSE: *The incidence data were added to the discussion of this study in Section 2.19:*

Significantly increased incidence of spleen mononuclear cell leukemia was observed in female rats exposed to ethylene oxide vapor for up to 2 years at 100 ppm (58% versus 8 or 11% controls; $p<0.01$) (Garman et al. 1985, 1986; Snellings et al. 1984b). Although the incidence was only statistically significantly elevated at 100 ppm, increased incidences were also observed at 10 ppm (11/54 versus 11/115 in controls) and 33 ppm (14/48). When incidences among rats dying early or killed in moribund condition were included, there were significant mortality-adjusted trends for mononuclear cell leukemia in males and females at ≥33 ppm (incidence data not reported). Male rats of the 100 ppm exposure group exhibited significant increases in peritoneal mesothelioma when rats that died or were killed in moribund condition were included (no incidence reported) and subcutis fibroma (36% versus 2 and 4% control; $0.01>p>0.001$). A significant trend for primary brain neoplasms was observed in males ($p<0.01$) and females ($p<0.05$).

COMMENT 11: The Reviewer noted a typo in a citation (Norman et all. 1995) in the last paragraph of Section 2.19.

RESPONSE: *The referenced discussion was deleted from the last paragraph in Section 2.19.*

COMMENT 12: Regarding a statement in Section 3.1 that reads "Kirman and Hays (2017) designed a study to evaluate relative contributions of exogenous and endogenous sources of the ethylene oxide to the production of the adduct hemoglobin N-(2-hydroxyethyl)-valine (HEV).", the Reviewer states ")." I believe the purpose of their study was to evaluate endogenous production of ethylene oxide, and to produce an endogenous equivalent value for exposure, to support risk assessment determinations and risk management decisions."

RESPONSE: *ATSDR agrees that as written, the text implies that Kirman and Hays (2017) designed the study for a purpose other than what the study authors explicitly say. The text in Section 3.1 has been modified:*

Kirman and Hays (2017) evaluated the relative contributions of exogenous and endogenous sources of the ethylene oxide to the production of the adduct hemoglobin, N-(2-hydroxyethyl)-valine (HEV).

COMMENT 13: Regarding a statement in Section 3.1 that reads "Fixed and random models predicted mean and standard deviation values of 20.5±14.0 pmol/g hemoglobin and 21.1 ± 14.6, respectively", the Reviewer states "This text is not clear."

RESPONSE: *The referenced text was revised:*

For smokers and occupationally exposed workers, mean HEV values ranged from 19.2 to 15,472 pmol/g hemoglobin. Fixed and random models predicted mean and standard deviation HEV values of 20.5±14.0 and 21.1±14.6 pmol/g hemoglobin, respectively.

COMMENT 14: Regarding a statement in Section 3.1 that reads "These results indicate that endogenously-produced ethylene oxide provides a substantial proportion of ethylene oxide in the body", the Reviewer states "This is not adequately explained. Do we mean for the general population?"

RESPONSE: *The investigators did not indicate whether this statement applied to workers, smokers, nonsmokers, or all populations.*

COMMENT 15: Regarding a summary of a study by Brown et al. (1996) in Section 3.1.2, the Reviewer states "This paragraph refers to Brown et al., 1996, which is the first of two papers by Brown et al. The discussion is missing the key study that followed on from this and generated the data for demonstrating the apparent saturation of metabolism in mouse, which is really a depletion of glutathione. Brown et al., 1998. Brown, C. D., Asgharian, B., Turner, M. J., & Fennell, T. R. (1998). Ethylene oxide dosimetry in the mouse. *Toxicology and Applied Pharmacology*, 148(2), 215–221. https://doi.org/10.1006/taap.1997.8349. A copy is provided."

RESPONSE: *The Brown et al. (1998) was added to Section 3.1.2:*

> Brown et al. (1998) reported a linear increase in ethylene oxide levels in blood with increasing exposure concentrations in mice exposed to 50–200 ppm ethylene oxide for 4 hours; steady state was achieved with the first 2 hours of exposure. At higher concentrations (300 and 400 ppm), blood ethylene oxide levels increased sublinearly and continued to increase during the 4-hour exposure. The sublinear increase in blood levels correlated with tissue glutathione depletion.

COMMENT 16: Regarding Section 3.1.5, the Reviewer states "The order of the presentation of the PBPK models starts with the most recent model, and works backwards, which is different from the order normally used."

RESPONSE: *ATSDR's profile guidance does not prescribe a set order to present the PBPK models.*

COMMENT 17: The Reviewer noted locations in the toxicological profile where the author name "Fennel" is misspelled (should be Fennell).

RESPONSE: *The misspelling of Fennell was corrected throughout the profile.*

COMMENT 18: Regarding the description of the PBPK models of Filser and Klein (2018a, 2018b) in Section 3.1.5, the Reviewer states "The description of the revisions to the model is not correct: Item (2) including suicide substrate (ethylene) inhibition of liver epoxide hydrolase. It should be inhibition of ethylene oxidation. CYP2E1-mediated metabolism of the suicide substrate ET"

RESPONSE: *The text in Section 3.1.2 (Filsner and Klein 2018a, 2018b Models of Human, Mouse, and Rat) was revised:*

> The major enhancements made to the Csanady et al. (2000) model were as follows: (1) including glutathione transferase activity to extra-hepatic tissues; (2) including suicide substrate (ethylene) inhibition of hepatic CYP2E1-mediated metabolism…

COMMENT 19: Regarding the citation Filser and Klein (2018a, 2018b) in Section 3.1.5, the Reviewer states "I think that this should be one citation. The 2018b is the supplementary data from 2018a."

RESPONSE: *Consistent with ATSDR guidance, the supplementary data have a separate citation from the main study.*

COMMENT 20: Regarding the citation of Thier et al. (1999) in Section 3.3.1 (and other loc, the Reviewer states "The text cites Thier et al., 1999 several times, discussing HEVal blood levels. This is an in vitro paper, measuring the metabolism of ethylene oxide by GST-T1. The Bono et al., 1999 paper does show a relationship between smoking status and HEVal. The Muller et al., 1998 paper shows no relationship between HEVAl and the hGSST1 or the hGSTM1 in smokers, only in nonsmokers. A missing paper indicates that HEVal in smokers is higher in GSTT1 null individuals. Fennell et al., 2000 Fennell, T. R., MacNeela, J. P., Morris, R. W., Watson, M., Thompson, C. L., & Bell, D. A. (2000). Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-null and M1-null genotypes. Cancer Epidemiol Biomarkers Prev, 9(7), 705–712." The Reviewer provided a copy of Fennell et al. (2000).

RESPONSE: *The incorrect Thier et al. (1999) study was listed in Chapter 8, the reference has been corrected:*

Thier R, Lewalter J, Kempkes M, et al. 1999. Haemoglobin adducts of acrylonitrile and ethylene oxide in acrylonitrile workers, dependent on polymorphisms of the glutathione transferases GSTT1 and GSTM1. Arch Toxicol 73:197-202.

The text in Section 3.3.1 was revised to indicate that GSTM1 status did not influence HOEtVal levels and to add the Fennell et al. (2000) paper:

In humans, HOEtVal blood levels are influenced by endogenous production of ethylene oxide, genetic status of the polymorphic glutathione transferases *hGSST1* (Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999, 2001; Yong et al. 2001), and smoking status (Bono et al. 1999; Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999). *hGSTM1* genotypes did not influence HOEtVal blood levels (Fennell et al. 2000; Müller et al. 1998; Their et al. 1999).

COMMENT 21: Regarding the statement in Section 3.3.1 that reads "Ethylene oxide can be measured in blood (Bailey et al. 1987; Brugnone et al. 1986; Farmer et al. 1986) and alveolar air (Brugnone et al. 1986).", the Reviewer states "Please check the accuracy of the descriptions and the associated citations. I don't have copies of these, but the abstracts suggest that Bailey et al. and Farmer et al. measured adducts in the blood and not ethylene oxide in blood. Brugnone measured both ethylene oxide in blood and in alveolar air."

RESPONSE: *Section 3.3.1 was revised to correct the error in the citation for ethylene oxide measurement in blood:*

Ethylene oxide can be measured in blood and alveolar air (Brugnone et al. 1986).

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

Toxicokinetics

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 22: See comments above about missing reference on Brown et al. 1998.

There is little discussion of the reactivity of ethylene oxide and its formation of DNA adducts. There are 18 instances of "DNA adducts" in the text. Most of them are in Tables or in the cited references. One is on page 98, lines 10-11. No discussion of how ethylene oxide may cause DNA adducts and indicate dose in tissues. Or how HEVal may indicate dose in blood.

RESPONSE: *As noted in the Response to Comment 15, the Brown et al. (1998) citation was added to Section 3.1.2.*

A discussion of DNA adduct formation was added to Section 2.20:

> In addition to these genotoxic effects, *in vitro* studies in mammal tissues, *in vivo* studies in rats and mice, and studies in humans have demonstrated the formation of DNA adducts. Ethylene oxide is an alkylating agent that forms adducts with DNA, ribonucleic acid (RNA), and proteins. The primary DNA adduct formed is N7-(2-hydroxyethyl)guanine (7-HEG). Other DNA adducts have been found in lesser amounts, these include N3-(2-hydroxyethyl)adenine and O^6-(2-hydroxyethyl)guanine (EPA 2016a; IARC 2012). 7-HEG has been detected in various tissues of rats exposed via inhalation to ethylene oxide for up to 4 weeks (Walker et al. 1990). Duration-related increases were observed in the brain, lung, spleen, kidney, leukocytes, liver, and testis.

The text in Section 3.3.1 has been revised to include a discussion of DNA adducts and to expand the discussion of hemoglobin adducts:

> Ethylene oxide is a direct acting alkylating agent that can form adducts with macromolecules such as DNA and hemoglobin. The primary DNA adduct formed is 7-HEG (EPA 2016a; IARC 2012). Studies in rats and mice have found concentration- and duration-related increases in 7-HEG levels following inhalation exposure (Rusyn et al. 2005; Walker et al. 1993). EPA (2016a) notes that DNA adducts are less reliable measures of exposure because they can be repaired or fixed as mutations. The ethylene oxide hemoglobin adduct, hydroxylated *N*-terminal valine (HOEtVal), has been widely used as a biomarker of occupational exposure to ethylene oxide (see Angerer et al. 1998; Boogaard 2002; Boogaard et al. 1999). Occupational exposure studies have found a correlation between ambient air levels of ethylene oxide and HOEtVal levels (Angerer et al. 1998; Boogaard 2002). Studies in rats and mice have reported increases in HOEtVal levels following a single inhalation exposure (Walker et al. 1992) or intraperitoneal injection (Tates et al. 1999; Walker et al. 1992) or repeated inhalation (Tates et al. 1999; Walker et al. 1992, 1993) or drinking water (Tates et al. 1999) exposures. A 4-week inhalation exposure to 3–33 ppm resulted in a linear increase in HOEtVal levels; at 100 ppm, the slope estimated from the 3–33 ppm exposure underpredicted the HOEtVal levels by 20 and 25% in rats and mice, respectively (Walker et al. 1992). In humans, HOEtVal blood levels are influenced by endogenous production of ethylene oxide, genetic status of the polymorphic glutathione transferases *hGSST1* (Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999, 2001; Yong et al. 2001), and smoking status (Bono et al. 1999; Fennell et al. 2000; Müller et al. 1998; Thier et al. 1999). *hGSTM1* genotypes did not influence HOEtVal blood levels (Fennell et al. 2000; Müller et al. 1998; Their et al. 1999).

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 23: There is a model developed by Hattis that exists, but I am not sure if it was published. See Pharmacokinetic/mechanism-based analysis of the carcinogenic risk of ethylene oxide, https://www.osti.gov/biblio/7067804

The phenomenon of wash in – wash out, which reduces the uptake of water soluble gases and volatile chemicals was not discussed. Mentioned in several of the PB-PK models – Fennell and Brown, 2000, and Filser and Klein 2018.

RESPONSE: *A literature search did not identify a published paper by Hattis regarding a pharmacokinetic/mechanism-based analysis of ethylene oxide carcinogenicity.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 24: The Reviewer provided no comment to this charge question.

RESPONSE: *No response is necessary.*

Children and Other Populations that are Unusually Susceptible:

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 25: While I am not aware of health and developmental effects, I think it is pertinent to identify areas where we can verify that exposure takes place in children.

Bono, R., Vincenti, M., Schilirò, T., Traversi, D., Pignata, C., Scursatone, E., … Gilli, G. (2005). Cotinine and N-(2-hydroxyethyl)valine as markers of passive exposure to tobacco smoke in children. *Journal of Exposure Analysis and Environmental Epidemiology*. https://doi.org/10.1038/sj.jea.7500344

Farmer, P. B., Cordero, R., & Autrup, H. (1996). Monitoring human exposure to 2-hydroxyethylating carcinogens. *Environmental Health Perspectives*, *104*(SUPPL. 3), 449–452. https://doi.org/10.2307/3432802

Tavares, R., Ramos, P., Palminha, J., Bispo, M. A., Paz, I., Bras, A., … Bailey, E. (1994). Transplacental exposure to genotoxins. Evaluation in haemoglobin of hydroxyethylvaline adduct levels in smoking and non-smoking mothers and their newborns. *Carcinogenesis*. https://doi.org/10.1093/carcin/15.6.1271

Von Stedingk, H., Vikström, A. C., Rydberg, P., Pedersen, M., Nielsen, J. K. S., Segerbäck, D., … Törnqvist, M. (2011). Analysis of hemoglobin adducts from acrylamide, glycidamide, and ethylene oxide in paired mother/cord blood samples from Denmark. *Chemical Research in Toxicology*, *24*(11), 1957–1965. https://doi.org/10.1021/tx200284u

RESPONSE: *A discussion of exposure of newborns and children was added to Section 5.6:*

> Exposure to ethylene oxide from environmental tobacco smoke and via maternal transfer has been demonstrated in several studies. In a study of 3–13-year-old children, the levels of HOEtVal (ethylene oxide hemoglobin adduct) was correlated to the number of cigarettes passively smoked by the children (Bono et al. 2005). The levels of HOEtVal were also correlated to urinary cotinine (a nicotine metabolite) levels. Similarly, studies of newborns of mothers have found correlations between HOEtVal levels in maternal blood and cord blood (Farmer et al. 1996; Von Stedingk et al. 2011) or newborn blood levels (Tavares et al. 1994). The cord blood levels were 5 times higher in

smokers than nonsmokers (Farmer et al. 1996) and newborn blood levels were about 3 times higher in smokers (Tavares et al. 1994).

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 26: The Reviewer provided no comment to this charge question.

RESPONSE: *No response is necessary.*

Biomarkers of Exposure and Effect:

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 27: Hydroxyethyl valine is a marker that is derived from ethylene oxide. HEMA, S-[2-hydroxyethyl]mercapturic acid, is a metabolite of ethylene oxide. However, it is not specific to ethylene oxide, and can be derived from other substances, such as by oxidative metabolism of acrylonitrile.

RESPONSE: *The text in Section 3.3.1 was revised to indicate that HEMA is not specific to ethylene oxide:*

However, HEMA is not specific to ethylene oxide; it is also a metabolite of acrylonitrile and vinyl chloride.

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 28: DNA adducts are indicated as a biomarker of effect in Section 3.3, page 98, lines 10-11. However, these aren't even mentioned in Section 3.3.2 Biomarkers of Effect. Other potential effects are mutations, and while not specific, may be important in understanding the progression from exposure to an adverse effect. At high exposure concentrations, ethylene oxide causes depletion of glutathione in mice, and while not specific for ethylene oxide, it may have consequences in non-linearity of the dose response, and it may contribute to species differences.

RESPONSE: *ATSDR disagrees that the DNA adducts are biomarkers of effect since an association between DNA adducts and carcinogenicity has not been established in humans. As noted in the Response to Comment 22, DNA adducts were added to the discussion of biomarkers of exposure. Mutations and depletion of glutathione were not considered biomarkers of effect since they are nonspecific.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 29: Chemicals that deplete glutathione may interfere with the metabolism of ethylene oxide and cause increased internal dose.

RESPONSE: *The following statement was added to Section 3.4:*

Although no specific data were identified, chemicals the deplete glutathione levels may interfere with the metabolism of ethylene oxide and could result in an increased internal dose and toxicity.

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 30: The Reviewer provided no comment to this charge question.

RESPONSE: *No response is necessary.*

Chapter 5. Potential for Human Exposure

COMMENT 31: The one aspect that appears to be missing is the potential to be exposed to ethylene oxide by metabolism of ethylene.

RESPONSE: *The endogenous production of ethylene oxide from the oxidation of ethylene is discussed in Section 3.1. Since this is not an environmental exposure, it was not added to Chapter 5.*

Chapter 6. Adequacy of the Database

COMMENT 32: A general comment is that some text from previous sections is repeated here. For example, the text on Page 127, line 26 and 27 are repeated from Page 98, lines 31-32. Please update after addressing comments in other sections.

RESPONSE: *The text in the Biomarkers of Exposure and Effect section of Section 6.2 has been revised so that it does not replicate the text in Section 3.3.1:*

Several biomarkers of exposure have been identified for ethylene oxide. These include the hemoglobin adduct HOEtVal, DNA adduct 7-HEG, and the urinary metabolite HEMA. The HOEtVal and 7-HEG are specific to ethylene oxide, whereas HEMA is a metabolite for several compounds. There are no known biomarkers of effect that are unique to ethylene oxide exposure. Additional data on the biomarkers of exposure, particularly HOEtVal, would be valuable for animal to human dose extrapolation.

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT 33: Appendix A. Page 172, line 14. Human Equivalent Concentration. The PBPK modeling approach was rejected due to a lack of experimental data regarding the proper dose metric (proximate toxicant) for ethylene oxide induced neurotoxicity. Presumably the default option would be to consider ethylene oxide itself as a toxicant. Was this considered and evaluated?

RESPONSE: *ATSDR considered using the PBPK model with the assumption that ethylene oxide was the causative agent. However, the Agency determined that this approach decreased the confidence in the MRL and thus, opted to not use the PBPK model.*

COMMENT 36: Page 172, lines 23-24. I don't understand why a blood/air partition coefficient was not used for the mouse. There is one reported by Fennell and Brown 2001. The Reviewer provided a Table in which a blood/air partition coefficient was reported to be 78 (Table 2 of Fennell and Brown 2001).

RESPONSE: *The mouse blood:gas coefficient reported in Fennell and Brown (2001) was added to the profile. The default value of 1 was still used since the animal coefficient exceeded the human coefficient.*

Comments provided by Group 2 Peer Reviewer #2

Annotated Comments on the Toxicological Profile

COMMENT 1: Regarding the statement in Section 1.1 that reads "Factory workers where ethylene oxide is produced or used to make other chemicals, and those working in sterilization facilities, may have contact with ethylene oxide", the Reviewer states "Residents near industrial facilities that make EO may also be at risk. Accidental releases may occur into air and water. Take the example of natural disasters in highly industrialized and populated areas (e.g., Houston and Hurricane Harvey)"

RESPONSE: *The text in Section 1.1 was revised to include residents living near point sources:*

Residents living near facilities producing or using ethylene oxide may also be exposed to higher than background levels via emissions or accidental releases.

COMMENT 2: Regarding the statement in Section 1.1 that reads "It is not known if food crops are a source of exposure to ethylene oxide for the general public", the Reviewer states "Add that EO may be a product of fumigation of crops, rather than just naturally occurring in crops? The latter is implied here."

RESPONSE: *The text in Section 1.1 was revised:*

The U.S. Environmental Protection Agency (EPA) has determined that there is reasonable certainty that dietary and drinking water risks from supported registered uses of ethylene oxide will not harm any population subgroup (EPA 2006).

COMMENT 3: Regarding the first sentence in Section 1.2, the Reviewer states "The first two sentences imply that cancer studies in animals are not important part of the overall database. Perhaps something along the lines of: 'Many animal studies have tested for both cancer and non-cancer effects of ethylene oxide and have been conducted in a variety of exposure durations: acute, sub-acute, sub-chronic and chronic 2-year studies. However, studies in humans primarily focused on carcinogenicity or carcinogenesis-related endpoints'?"

RESPONSE: *The beginning of Section 1.2 has been revised:*

Information on the toxicity and carcinogenicity of ethylene oxide comes from epidemiological studies and studies conducted in experimental animals. Most human studies evaluated only cancer endpoints; inhalation was the presumed exposure route. These studies evaluated the carcinogenicity of ethylene oxide in cohorts involved in ethylene oxide production and/or workers in areas where ethylene oxide was used as a sterilizer. Information on noncancer health effects primarily come from experimental animal studies.

COMMENT 4: Regarding the statement in Section 1.2 that reads "A total of 55 studies evaluated the toxicity of ethylene oxide in humans; most studies evaluated only cancer endpoints", the Reviewer states "The number of studies is a fluid value as studies get published all the time. Why mention the number of human studies but NOT mention the number of animal studies? If the point is that "the database is sufficiently robust and includes a large number of human and animal studies" than say so…"

RESPONSE: *The text in Section 1.2 has been revised and no longer includes the number of epidemiological studies (see the Response to Comment 3 for the revised text).*

COMMENT 5: Regarding the statement in Section 1.2 that reads "Nearly 90% of the animal studies employed the inhalation exposure route", the Reviewer states "And what about human studies? The arguments overall appear to be not presented in a balanced manner…"

RESPONSE: *The text was revised to indicate that inhalation was the likely exposure route:*

Most human studies evaluated only cancer endpoints; inhalation was the presumed exposure route.

COMMENT 6: Regarding the statement in Section 1.2 that reads "Results from selected inhalation studies of animals indicated species and sex differences in ethylene oxide toxicity", the Reviewer states "This implies there were more differences than similarities and thus it clouds the interpretation of the data. I disagree. There are more similarities and this sentence needs to be removed from the summary or re-phrased."

RESPONSE: *The referenced statement was removed from Section 1.2.*

COMMENT 7: Regarding the sentences in Section 1.2 that read "Limited information was available regarding ethylene oxide toxicity following dermal exposure. Ethylene oxide is a contact dermal and ocular irritant in humans and animals", the Reviewer states "These two sentences are contradictory. Fiurst you say that there are few studies and then you say it is a contact (which implies dermal as well) irritatnt…"

RESPONSE: *The referenced statement in Section 1.2 was revised:*

The limited information available regarding ethylene oxide toxicity following dermal exposure suggests that it is a contact dermal and ocular irritant in humans and animals.

COMMENT 8: Regarding the statement in Section 1.2 that reads "As illustrated in Figures 1-1 and 1-2, the most sensitive targets of ethylene oxide toxicity appear to be hematological, endocrine, and neurological endpoints", the Reviewer states "Non-cancer? Or cancer? effects". This comment refers to the terms "toxicity" and "endpoints".

RESPONSE: *The statement in Section 1.2 was revised to clarify that these are noncancer effects:*

As illustrated in Figures 1-1 and 1-2, the most sensitive noncancer targets of ethylene oxide toxicity appear to be hematological, endocrine, neurological, reproductive, and developmental endpoints; cancer effects also occur at lower exposure levels.

COMMENT 9: Regarding the statement in Section 1.2 that reads "A systematic review of noncancer endpoints…", the Reviewer states "This should be its own paragraph or section 'Non-cancer effects' as the short paragraphs that follow appear to be all non-cancer effects."

RESPONSE: *The suggested revision was made to Section 1.2; the results of the systematic review are discussed in its own paragraph.*

COMMENT 10: Regarding the summary of respiratory effects in Section 1.2 that reads "Bronchitis, pulmonary edema, and emphysema have been reported in workers after acute high-level exposure (Theiss 1963), but respiratory problems have not been reported to occur with relatively low-level chronic exposure (estimated long-term average of 5–10 ppm) (Joyner 1964). Adverse respiratory effects such as

labored breathing, nasal discharge, dyspnea, histopathologic pulmonary lesions, rhinitis, and/or pulmonary edema were observed in multiple animal species exposed to ethylene oxide vapor once or intermittently for up to 2 years (Hollingsworth et al. 1956; Jacobson et al. 1956; NTP 1987)", the Reviewer states "The sequence of information here is confusing. Usually, there are more studies in animals than in humans and the effects in animals are detailed first in a summary. That is followed by information on whether in humans some (or all, or none) of these have also been detected with a brief discussion of the doses at which effects were seen in animals vs humans. THIS COMMENT APPLIES TO ALL SUMMARIES BELOW". The Reviewer also states "State concentrations tested? If you state concentrations for humans…" in reference to the statement regarding exposure of multiple animal species to ethylene oxide vapor.

RESPONSE: *It is ATSDR's practice to discuss human data before animal data.*

COMMENT 11: Regarding the discussion in Section 1.2 of respiratory effects observed in humans, the Reviewer states "State concentrations tested? If you state concentrations for humans…"

RESPONSE: *The exposure level resulting in respiratory effects in animals was added to Section 1.2:*

> Adverse respiratory effects such as labored breathing, nasal discharge, dyspnea, histopathologic pulmonary lesions, rhinitis, and/or pulmonary edema were observed in multiple animal species exposed to 113–841 ppm ethylene oxide vapor once or intermittently for up to 2 years (Hollingsworth et al. 1956; Jacobson et al. 1956; NTP 1987).

COMMENT 12: Regarding the statement in Section 1.2 that reads "The Department of Health and Human Services (HHS) has classified ethylene oxide as *known to be a human carcinogen* (NTP 2016). The EPA characterized ethylene oxide as "carcinogenic to humans" by the inhalation exposure route (EPA 2016a). The International Agency for Research on Cancer (IARC) has designated ethylene oxide as *carcinogenic to humans (Group 1)* (IARC 1987, 2012)", the Reviewer states "I suggest adding information on what information was used by each one of these to derive the classification. For example, the NTPs RoC arrived at the classification based on "sufficient evidence of carcinogenicity from studies in humans, including epidemiological studies and studies on mechanisms of carcinogenesis". The IARC (volume 100F, 2012) was based on limited evidence in humans, sufficient evidence in experimental animals, and "compelling data in support of the genotoxic mechanism"… This is useful information to balance ATSDR's conclusions in two preceding paragraphs." The Reviewer also states "Volume 97 (2008) should also be cited" in reference to the IARC cancer designation.

RESPONSE: *The statement in Section 1.2 regarding the cancer classifications is intended to give a high level summary of cancer classifications. A more detailed discussion of HHS, EPA, and IARC cancer classifications is presented in Section 2.19. ATSDR also notes that it does not include the volume number in short citations within the text; this information is presented in the full citation in Chapter 8.*

COMMENT 13: Regarding the statement in Section 1.3 that reads "The inhalation database was considered adequate for derivation of provisional acute- and intermediate--duration inhalation MRLs for ethylene oxide", the Reviewer states "State here that animal data are meant here."

RESPONSE: *ATSDR considers all epidemiological and experimental animal data in evaluating whether the database is adequate for MRL derivation.*

COMMENT 14: Regarding the statement in Figure 1-3 that reads "Summary of Sensitive Targets of Ethylene Oxide – Inhalation", the Reviewer states "Non-cancer effects." This is a suggestion to state that the "targets" are noncancer targets.

RESPONSE: *The subtitle of this figure was revised to indicate that it is for noncancer endpoints:* Hematological, endocrine, and neurological endpoints are the most sensitive noncancer targets of ethylene oxide inhalation exposure.

COMMENT 15: Regarding the statement in Figure 1-3 that reads "Hematological, endocrine, and neurological endpoints are the most sensitive targets of ethylene oxide inhalation exposure.", the Reviewer states "Text above also includes 'developmental'"

RESPONSE: *As per ATSDR guidance, the three most sensitive targets are included in this subtitle. The lowest LOAEL for developmental is higher than the LOAELs for hematological, endocrine, and neurological effects.*

COMMENT 16: Regarding the LOAELs identified as bubbles in Figure 1-3, the Reviewer states "Why not include footnotes to the actual studies from which these LOAELs were extracted from? So that the reader doesn't have to go deep into the document to find out?"

RESPONSE: *Figure 1-3 is intended to illustrate how the lowest LOAELs for individual endpoints compare. It is not intended to be a full presentation of available data; these data are presented in Chapter 2.*

COMMENT 17: Regarding Figure 1-4 that presents information regarding sensitive targets of ethylene oxide by the oral exposure route, the Reviewer states "No information on this was included in the summary section 1.2… I suggest include very brief paragraphs, otherwise this is completely new information with no summary…". The Reviewer also states "Same as above – make it clear what studies these LOAELs are from."

RESPONSE: *Given the limited amount of data for oral exposure, ATSDR has deleted Figure 1-4 from the profile.*

COMMENT 18: Regarding the statement in Section 2.1 that reads "The health effects of ethylene oxide have been evaluated in a number of occupational cohorts and a variety of animal studies. As illustrated in Figure 2-1, the inhalation exposure route was employed in the majority of animal studies; inhalation was assumed to be the predominant exposure route in the occupational cohort studies. The most examined endpoints in animal studies were body weight (20% of the animal studies) and neurotoxicity (10% of the animal studies). Cancer was the most examined endpoint in epidemiological studies (32% of the human studies)", the Reviewer states "The purpose of this information is not clear. A summary has already been included in section 1.2 and here it appears to be an even shorter summary with the same issues as I have pointed out for Section 1.2". The Reviewer also states "I am highly dubious on the value of these %. They are uninterpretable by themselves and may confuse the reader."

RESPONSE: *Chapter 1 is an executive summary-type overview on the potential for human exposure and the health effects associated with exposure to ethylene oxide. Chapter 2 provides a more detailed discussion on health effects. The intent of Section 2.1 is to provide a more detailed overview of endpoints*

examined in available epidemiological and experimental studies. ATSDR acknowledges that there is an overlap in the information presented in Section 1.2 and 2.1; however, the two sections have different intended audiences and purposes.

Regarding the Reviewer's comment on the percentage of studies examining a particular endpoint, this information was deleted from the text:

> The most examined endpoints in animal studies were body weight and neurotoxicity. Cancer was the most examined endpoint in epidemiological studies.

COMMENT 19: Regarding the statement in Section 2.1 that reads "Animal studies suggest that relatively sensitive noncancer targets of ethylene oxide include hematological, endocrine, neurological, reproductive, and developmental endpoints", and the subsequent bullets describing the types of endpoints, the Reviewer states "But no human relevance is mentioned in these short summaries. Which implies human data are not available on many of these endpoints, which is not correct…"

RESPONSE: *Available human data are presented in these summary paragraphs; the neurological, reproductive, and cancer summaries present human data. ATSDR added a bullet for respiratory effects that also presents human and animal data. The text for the hematological, endocrine, neurological, and reproductive effects were revised to include the relevance of the endpoint to humans.*

- **Respiratory Endpoints:** Respiratory effects are a presumed health effect for humans based on a moderate level of evidence in workers and a high level of evidence in experimental animal studies. Compromised respiratory function has been reported in workers exposed to high levels of ethylene oxide. Inhalation studies in experimental animals have reported several respiratory effects including labored breathing, nasal discharge, pulmonary lesions, rhinitis, and pulmonary edema.

- **Hematological Endpoints:** Hematological effects are a suspected health effect for humans based on a moderate level of evidence in animal studies. Repeated exposure of experimental animals to ethylene oxide vapor has resulted in hematological effects such as decreases in hemoglobin, hematocrit, erythrocyte count, packed cell volume, and/or reticulocytes, and splenic extramedullary hematopoiesis.

- **Endocrine Endpoints:** Adrenal gland effects are a suspected health effect in humans based on a moderate level of evidence in animal studies. Adverse effects (gross and/or histopathologic changes in the adrenal gland) have been observed in rats and guinea pigs following acute- and/or chronic-duration exposure to ethylene oxide vapor.

- **Neurological Endpoints:** Neurological effects are a presumed health effect in humans based on a low level of evidence in occupational exposure studies and a high level of evidence in animal studies. Clinical signs of neurotoxicity (e.g., neuropathy, weakness in extremities, impaired hand-eye coordination, cognitive dysfunction, memory loss, headache, lethargy) were reported among workers exposed to ethylene oxide for various durations. Sural nerve biopsies revealed axonal degeneration and regeneration in two studies. Neurological effects such as ataxia, impaired sensory reflexes, hindlimb paralysis, and/or degenerative histopathologic lesions have been observed among laboratory animals exposed to ethylene oxide by inhalation.

- **Reproductive Endpoints:** Male reproductive effects are a presumed health effect in humans based on a moderate level of evidence in occupational exposure studies and a high level of evidence in animal studies. Limited human data suggest possible ethylene oxide-related effects

on sperm production. Animal studies provide convincing evidence of ethylene oxide-induced effects on the male reproductive system (e.g., decreases in male reproductive organ weights, germ cell survival, and sperm count; histopathologic lesions).

COMMENT 20: Regarding the identified serious LOAELs of 400 ppm for rhinitis and 600 ppm for lymphocyte necrosis in thymus presented in Figure key 15 of LSE Table 2-1, the Reviewer states "In my opinion this is 'less serious'"

RESPONSE: *The rhinitis observed in the NTP (1987) study (Figure key 15) was revised to be a less serious LOAEL. The lymphocyte necrosis in the thymus of mice exposed to 600 ppm was graded by NTP (1987) as 3.8 (on a scale of 0 to 4); given the severity of the lesion, ATSDR considered 600 ppm to be a serious LOAEL for immunological effects.*

COMMENT 21: Regarding the identified LOAELs of 100 and 200 ppm for renal tubular degeneration presented in Figure key 39 of LSE Table 2-1, the Reviewer states "In my opinion this is 'serious'"

RESPONSE: *The renal tubular degeneration observed at 100 ppm in male mice and 200 ppm in female mice in the NTP (1987) study (Figure key 39) was not considered a serious effect because the lesions were graded as minimal (0.7 in males and 1.0 in females, based on a scale of 0–4).*

COMMENT 22: Regarding the paragraphs in Section 2.2 that present information regarding death in experimental animals, the Reviewer suggests adding the statements "The following LC50 values were reported for studies in experimental animals by inhalation" to the beginning of the paragraph that describes death following inhalation exposure and "The following LD50 values were reported for studies in experimental animals by oral route of exposure" to the beginning of the paragraph that describes death following oral exposure. The Reviewer also states "Consider similar edits for all sub-chapters below to make it clear that each paragraph contains studies of a particular exposure route. This may not be self-obvious to a non-expert reader."

RESPONSE: *The referenced sentence states that the animals were exposed to ethylene oxide vapor; this implies that they were exposed via inhalation. In other sections of Chapter 2, the exposure route was reported, a statement was made that data were only available for the inhalation route, or that the animals were exposed to ethylene oxide vapors.*

COMMENT 23: Regarding the statement in Section 2.2 that reads "Dunkelberg (1982) reported decreased survival among 50 female rats gavaged at 30 mg/kg/day, 2 times/week, for up to 150 weeks", the Reviewer states "State % here".

RESPONSE: *The text in Section 2.2 was revised to indicate that a decrease in the length of survival was observed; the investigators did not report the average survival length:*

Dunkelberg (1982) reported decreased length of survival among 50 female rats gavaged at 30 mg/kg/day, 2 times/week, for up to 150 weeks.

COMMENT 24: Regarding the statement in Section 2.3 that reads "Hollingsworth et al. (1956) reported "markedly subnormal growth" among rats, mice, rabbits, guinea pigs, and/or monkeys intermittently

exposed to ethylene oxide vapor at 204–357 ppm for up to 226 days", the Reviewer states "State % here, if available".

RESPONSE: *Hollingsworth et al. (1956) did not report the magnitude of the growth reductions. The text was revised to indicate this data deficiency:*

> Hollingsworth et al. (1956) also reported "markedly subnormal growth" (magnitude not reported) among rats, mice, rabbits, guinea pigs, and/or monkeys intermittently exposed to ethylene oxide vapor at 357 ppm for up to 85 days.

COMMENT 25: Regarding the statement in Section 2.4 that reads "Inhalation exposure of workers to high concentrations of ethylene oxide for brief periods has resulted in bronchitis, pulmonary edema, and emphysema (Theiss 1963)", the Reviewer states "State the numbers here for exposure levels".

RESPONSE: *The Thiess (1963) paper did not report exposure levels.*

COMMENT 26: Regarding the statement in Section 2.7 that reads "Limited information is available regarding hematological effects in experimental animals following inhalation or oral exposure to ethylene oxide", the Reviewer states "I question the descriptor 'limited'. There are 4 studies that show these effects. This is no different from the evidence for musculoskeletal, for example…"

RESPONSE: *The text in Section 2.7 was revised:*

> Information is available regarding hematological effects in experimental animals following inhalation or oral exposure to ethylene oxide. No information was located for the dermal exposure route.

COMMENT 27: Regarding the statement in Section 2.10 that reads "Information regarding renal effects in humans after inhalation exposure to ethylene oxide is limited to a report by Joyner (1964) which indicates that there was no evidence of nephritis or other parenchymal disease among workers exposed to ethylene oxide at 5–10 ppm for a mean exposure time of 10.7 years", the Reviewer states "Please standardize the language describing Joyner 1964 study across all section. Here the duration is 10.7 year while in other sections it is listed as 10 years…"

RESPONSE: *The text in Section 2.9 was revised to indicate that the exposure duration was 10.7 years:*

> The results suggested that workers exposed to about 5–10 ppm for 10.7 years did not have major signs of hepatic toxicity such as jaundice or palpable liver.

COMMENT 28: Regarding the statement in Section 2.10 that reads "Limited information is available regarding renal effects in experimental animals following inhalation exposure to ethylene oxide", the Reviewer states "I question the descriptor 'limited'. There are several studies that show these effects. This is no different from the evidence for musculoskeletal, for example…"

RESPONSE: *The text in Section 2.10 was revised:*

> Information is available regarding renal effects in experimental animals following inhalation exposure to ethylene oxide.

COMMENT 29: Regarding the statement in Section 2.13 that reads "Limited information is available regarding endocrine effects in experimental animals following inhalation exposure to ethylene oxide", the

Reviewer states "I question the descriptor 'limited'. There are several studies that show these effects. This is no different from the evidence for musculoskeletal, for example…"

RESPONSE: *The text in Section 2.13 was revised:*

> Information is available regarding endocrine effects in experimental animals following inhalation exposure to ethylene oxide.

COMMENT 30: Regarding the statement in Section 2.16 that reads "Limited information is available regarding reproductive effects in experimental animals following inhalation exposure to ethylene oxide", the Reviewer states "I question the descriptor 'limited'. There are several studies that show these effects. This is no different from the evidence for musculoskeletal, for example…"

RESPONSE: *The text in Section 2.16 was revised:*

> Information is available regarding reproductive effects in experimental animals following inhalation exposure to ethylene oxide.

COMMENT 31: Following the first sentence in Section 2.17, the Reviewer suggests adding the statement "Information is available regarding developmental effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes." The Reviewer also suggests deleting the statement "No information was located regarding developmental effects in experimental animals following oral or dermal exposure to ethylene oxide." This statement is the final sentence in Section 2.17.

RESPONSE: *Section 2.17 was revised to add a statement before the discussion of the animal study results that the database consists of only inhalation studies and to delete the statement regarding the lack of oral and dermal studies at the end of the section:*

> Information is available regarding developmental effects in experimental animals following inhalation exposure to ethylene oxide. No information was located for oral or dermal exposure routes.

COMMENT 32: Regarding the statement in Section 2.19 that reads "Epidemiological data for ethylene oxide include limitations such as lack of personal estimations of ethylene oxide exposure levels, concomitant exposure to other toxic substances, small numbers of subjects, and/or small numbers of selected cancer deaths", the Reviewer states "Why not mention 'strengths' as well here if you mention the 'limitations'? This gives an impression of a weakness in the database, when in fact this is a very strong dataset that has led other agencies (EPA, NTP and IARC) to conclude that EO is a known human carcinogen…"

RESPONSE: *ATSDR did not conduct a weight-of-evidence evaluation of the carcinogenicity database, which would have included evaluating the relative strengths and weaknesses of individual studies. The statement at the beginning of Section 2.19 is a general statement regarding the limitations of the epidemiological studies.*

COMMENT 33: Regarding the statement in Section 2.19 that states "IARC and EPA have evaluated ethylene oxide for carcinogenicity (EPA 2016a; IARC 2008, 2012)", the Reviewer states "Why is evaluation by NTP/RoC not mentioned here?" The Reviewer further states "Include this 2018 publication by the EPA: Jinot et al Toxicol Mech Methods. 2018 Jun;28(5):386-396. doi:

10.1080/15376516.2017.1414343". The Reviewer also states "EO was also evaluated by IARC in 1976, 1985, 1987 and 1994)".

RESPONSE: *Unlike IARC and EPA, the NTP Report on Carcinogens does not identify which cancer site has the most convincing evidence; thus, it was not included in the referenced paragraph in Section 2.19. The NTP classification is mentioned in Section 1.2 and in the Carcinogenicity Assessment portion of Section 2.19. The Jinot et al. (2018) paper was reviewed by ATSDR, it was not included in the profile because it is a review article. Regarding the comment about citing older IARC evaluations, all of the IARC cancer evaluations for ethylene oxide are cited in other parts of Section 2.19. In the referenced statement, ATSDR is citing the two most recent evaluations that support the Agency's approach of focusing on the cancer discussion on lymphohematopoietic cancers and female breast cancer.*

COMMENT 34: Regarding the statement in Section 2.19 that reads "Both agencies concluded that the most convincing evidence for increased risk among workers exposed to ethylene oxide is for lymphohematopoietic cancers and female breast cancer", the Reviewer states "State that these sites were concordant with animal cancer studies. This strengthens the overall weight of evidence."

RESPONSE: *The intent of the referenced statement is to provide support for why ATSDR's review of the epidemiological data focused on lymphohematopoietic cancers, leukemia, myeloma, non-Hodgkin's lymphoma, lymphosarcoma/reticulosarcoma, and breast cancer. The animal data are discussed later in this section.*

COMMENT 35: Regarding the statement in the comment field of the Steenland et al. 2004 entry in Table 2-3 that reads "Positive trend for hematopoietic cancers in males with a 15-year lag time (driven by lymphoid tumors); positive trend for breast cancer using log of cumulative exposure and 20-year lag time", the Reviewer states "Not clear where this information is presented in Figures. Also, you need to acknowledge re-analysis of the Steenland 2004 data for quartiles of exposure that showed significant associations for highest exposed workers (Jinot et al 2018 – supplemental Tables and EPA 2016 IRIS)."

RESPONSE: *Figures 2-4 through 2-9 in Section 2.19 are forest plots of risk estimates (and confidence intervals) for several cancer types. The results of trend tests are presented in Table 2-2. Because ATSDR did not conduct a qualitative analysis of the epidemiological cancer data, it did not include EPA's re-analysis of the Steenland et al. (2003, 2004) data in the profile.*

COMMENT 36: Regarding the statement in the comment field of the Steenland et al. 2003 entry in Table 2-3 that reads "Significant trend (p=0.002) for increased breast cancer incidence with increasing cumulative ethylene oxide exposure using a 15-year lag period; breast cancer incidences evaluated through 1998; referent rates from non-exposed population in NCI's SEER Program", the Reviewer states "Not clear which figures include this information, if any…"

RESPONSE: *The results of trend tests are not included in the forest plots; this information is presented in Table 2-3.*

COMMENT 37: Regarding Figure 2-6 (Summary of Epidemiological Studies Evaluating Associations between Inhaled Ethylene Oxide and Breast Cancer), the Reviewer states "EPA re-analysis of the Steenland 2003 and 2004 and Mickoczy et al 2011 studies for quartiles of exposure showed significant

associations for highest exposed workers (Jinot et al 2018 – supplemental Tables and EPA 2016 IRIS). This needs to be acknowledged in text."

RESPONSE: *Because ATSDR did not conduct a qualitative analysis of the epidemiological cancer data, it did not include EPA's re-analysis of the Steenland et al. (2003, 2004) data in the profile.*

COMMENT 38: In the case reports portion of Section 2.19, the Reviewer states "Need to add Kardos et al 2003 Environ Mol Mutagen, 42, 59-60".

RESPONSE: *The Kardos et al. (2003) study is not a case report; it is a cross-sectional study evaluating cancer mortality among female hospital workers. Given the volume of literature evaluating the carcinogenicity of ethylene oxide, only select occupational studies were included in the profile. The Kardos et al. (2003) study was not added to the profile because it examined a small number of workers and did not include any adjustments for potential confounders.*

COMMENT 39: In the animal studies portion of Section 2.19, the Reviewer states "I find this section's presentation of the data confusing. Some paragraphs include incidence, others do not. Some endpoints are mentioned, others are not… It would be more logical to organize the data by the organ/cancer type and summarize all data from various studies. OR, if the summary is by study, all endpoints need to be mentioned. Cross-referencing the information below with Table 3.1 of IARC monograph volume 100F (pages 385-387) shows a number of inconsistencies that I pointed below. Knowing how much scrutiny goes into preparation and fact-checking of those tables by the working groups and IARC monographs staff, I tend to trust those numbers above anything else."

RESPONSE: *The discussion of the animal data in Section 2.19 was revised to include incidence data (when available) for all studies. See the Responses to Comments 40–46 for other revisions to this discussion.*

COMMENT 40: In the first paragraph of the animal studies portion of Section 2.19, the Reviewer notes that a p-value, stated as "$p<0.5$", should be "$p<0.05$".

RESPONSE: *The typographical error in Section 2.19 was corrected:*

Significantly increased incidence of brain gliomas (5/79 versus 0/76 controls; $p<0.05$)

COMMENT 44: For the Lynch et al. 1984a, 1984b statement in the first paragraph of the animal studies portion of Section 2.19, the Reviewer states "mononuclear cell leukemia were significant at 50 ppm exposure".

RESPONSE: *The text in Section 2.19 was revised to include mononuclear cell leukemia:*

An increase in the incidence of mononuclear cell leukemia was also observed at 50 ppm (38/79 versus 24/77 controls, $p<0.01$).

COMMENT 42: Regarding the statement in the first paragraph of the animal studies portion of Section 2.19 that reads "Significantly increased incidence of spleen mononuclear cell leukemia was observed in female rats exposed to ethylene oxide vapor for up to 2 years at ≥33 ppm (Garman et al. 1985, 1986; Snellings et al. 1984b)", the Reviewer states "Male rats in the same studies also had significant increased

trends for the same endpoint". The Reviewer also states "The lowest dose was 10 ppm in these studies and trend tests included 10 ppm. So why mention 33 ppm here?"

RESPONSE: *The increase in the incidence of mononuclear cell leukemia was significantly increased only in female rats at 100 ppm (this was corrected in the profile). The text in Section 2.19 was revised to indicate that nonsignificant increases were also observed in females at 10 and 33 ppm. The findings for the male rats are discussed in the text.*

> Although the incidence was only statistically significantly elevated at 100 ppm, increased incidences were also observed at 10 ppm (11/54 versus 11/115 in controls) and 33 ppm (14/48). When incidences among rats dying early or killed in moribund condition were included, there were significant mortality-adjusted trends for mononuclear cell leukemia in males and females at ≥33 ppm (incidence data not reported).

COMMENT43: Regarding the summary of the NTP 1987 study in the animal studies portion of Section 2.19, the Reviewer states "Trend test are not mentioned".

RESPONSE: *In the discussion of the NTP (1987) study in Section 2.19, ATSDR opted to only discuss the findings in which there were significant increases in the neoplasm incidence.*

COMMENT 44: Regarding the summary of Adkins et al. 1986 in the animal studies portion of Section 2.19, the Reviewer notes that the "lung tumors" were "adenomas".

RESPONSE: *The text in Section 2.19 was revised to indicate that the tumors observed in the Adkins et al. (1986) study were adenomas:*

> Significantly increased incidences of lung adenomas were reported among female A/J mice intermittently exposed to ethylene oxide vapor for 6 months at 70 or 200 ppm; lung adenoma incidences were 16/28 and 25/29, respectively, compared to 8/30 controls (Adkins et al. 1986). Incidences were statistically significantly increased in both ethylene oxide-exposed groups. In a replicate study that included controls and 200 ppm groups, incidences of lung adenomas in surviving mice were 9/29 (29%) and 12/28 (42%), respectively.

COMMENT 45: Regarding the summary of Dunkelberg 1982 in the animal studies portion of Section 2.19, the Reviewer states "A study of Dunkelberg 1981 that was subcutaneous injections is not mentioned. While it is not a relevant route of exposure for humans, it does show that EO is a point of contact carcinogen (sarcomas at the site of injection). This study may be at least acknowledged as not relevant."

RESPONSE: *Because the carcinogenicity of ethylene oxide has been evaluated following inhalation, oral, and dermal exposure, ATSDR opted to not include parenteral administration studies in the Section 2.19 discussion.*

COMMENT 46: Regarding the statement in the summary of Dunkelberg 1982 in the animal studies portion of Section 2.19 that reads "There was no indication of ethylene oxide exposure-related increased incidences of tumors at sites away from the point of administration", the Reviewer states "This statement is odd as no other paragraph states this. It is obvious that all positive findings are reported and none of the other sites are mentioned."

RESPONSE: *The referenced statement was deleted from Section 2.19.*

COMMENT 47: In the portion of Section 2.19 that reads "The HHS has classified ethylene oxide as *known to be a human carcinogen* (NTP 2016) based on sufficient evidence of carcinogenicity from studies in humans (increased risk of cancer in workers exposed to ethylene oxide during its synthesis, production, and use), and evidence for a common mechanism of carcinogenesis in humans and experimental animals (similar genetic damage in cells of animals and workers exposed to ethylene oxide)", the Reviewer states "A sub-heading is needed as this appears to fit into 'Animal studies' sub-section". The preceding statement reads 'In a lifetime skin painting study, application of a 10% solution of ethylene oxide to the backs of mice did not result in skin tumors (Van Duuren et al. 1965).'"

RESPONSE: *A subheading "Carcinogenicity Assessments" was added to Section 2.19.*

COMMENT 48: In the first paragraph of Section 2.20 that reads "Extensive reviews are available regarding the genotoxicity of ethylene oxide (see EPA 2016a; IARC 1994, 2008)", the Reviewer states "Add IARC 2012 reference here as mechanistic evidence of genotoxicity is also extensively reviewed there."

RESPONSE: *The IARC (2012) citation was added to the string reference:*

Extensive reviews are available regarding the genotoxicity of ethylene oxide (see EPA 2016a; IARC 1994, 2008, 2012).

COMMENT 49: Regarding the introductory bullets at the beginning of Section 3.1, the Reviewer states "This is a good summary, but an introductory sentence or two stating that it is a summary may be needed. Also, the summary may need to include information about species differences/similarities. Otherwise it is not clear if this is a summary of human, animal, or any mammalian organism TK…"

RESPONSE: *Consistent with ATSDR's toxicological profile guidance document, this section starts off with the summary bullets. ATSDR will consider the Reviewer's suggestion in future revisions of the profile guidance.*

COMMENT 50: Regarding the initial statement in Section 3.1.2 that reads "No studies were located regarding the distribution of ethylene oxide in human tissue", the Reviewer suggested that the word "tissue" be replaced by "body".

RESPONSE: *The text in Section 3.1.2 was revised:*

No studies were located regarding the distribution of ethylene oxide in humans.

COMMENT 51: In the summary of the Filser and Klein (2018a, 2018b) models of human, mouse, and rat (Section 3.1.5) that reads "The model also predicts that exposure concentrations exceeding 200 ppm in mice would result a supra-linear increase in blood ethylene oxide concentrations (i.e., an increase in blood concentration larger than the proportional increase in exposure concentration)", the Reviewer suggests that the word "super-linear" be replaced with "supra-linear".

RESPONSE: *The blood concentration-exposure level curve was described in Section 3.1.5 as super-linear:*

> The model also predicts that exposure concentrations exceeding 200 ppm in mice would result a super-linear increase in blood ethylene oxide concentrations (i.e., an increase in blood concentration larger than the proportional increase in exposure concentration).

COMMENT 52: In the portion of Section 3.2 that reads "However, because detoxification of ethylene oxide occurs via hydrolytic and glutathione-S-transferase pathways, very young children with incomplete development of these detoxification pathways may exhibit increased susceptibility to ethylene oxide toxicity", the Reviewer states "This statement needs a reference. Perhaps http://dmd.aspetjournals.org/content/46/8/1118.long or http://jpet.aspetjournals.org/content/300/2/361 or similar?"

RESPONSE: *The statement was revised to include the McCarver and Hines (2002) and Zhong et al. (2018) citations:*

> However, because detoxification of ethylene oxide occurs via hydrolytic and glutathione-S-transferase pathways, very young children with incomplete development of these detoxification pathways (McCarver and Hines 2002; Zhong et al. 2018) may exhibit increased susceptibility to ethylene oxide toxicity.

COMMENT 53: In the portion of Section 5.1 that reads "Soil organisms may also convert it to glycols", the Reviewer suggests that "organisms" be replaced with "microorganisms".

RESPONSE: *The suggested revision was made to Section 5.1:*

> Soil microorganisms may also convert it to glycols.

COMMENT 54: Regarding the units associated with ethylene oxide production in Section 5.2.1, the Reviewer states "You may wish to be consistent in the units and convert everything to pounds or tonnes (more relevant to the worldwide units of measure)".

RESPONSE: *Since most of the production data is reported in pounds, this unit was used throughout Section 5.2.1. For the data reported in kilotons or metric tons, the pound equivalents were added:*

> According to the American Chemistry Council (ACC) Economics and Statistics Department, in 2018, it was reported that there were 15 process plants in the United States that produced ethylene oxide with a total production volume of 2.92 million metric tons (6,400 million pounds) (ACC 2019). Total production capacity is about 3.5 million metric tons (7,700 million pounds) but is expected to increase by another 1.8 million metric tons (4,000 million pounds) by 2023 due to high market demand (ACC 2019).

COMMENT 55: Regarding units reported in Table 5-1, the Reviewer states "Convert to tonnes? Throughout…".

RESPONSE: *Since the TRI data are presented in pounds, this unit was used in Table 5-1.*

COMMENT 56: Regarding the statement in Section 5.2.2 that reads "Imports of ethylene oxide can vary by year", the Reviewer states "I assume this is "into the US"? Please state so."

RESPONSE: *The text in Section 5.2.2 was revised:*

Due to its high reactivity, most ethylene oxide that is produced is also used on site to create other products (ethylene oxide derivatives). There are little import or export volumes of ethylene oxide; however, there is substantial trade involving ethylene oxide derivatives such as monoethylene glycol (MEG) (ACC 2019). In 2018, approximately one-third of the MEG produced in the Unties States from ethylene oxide was exported to other nations. In 2018, the United states exported 0.831 million metric tons of ethylene oxide derivatives and also imported 1.2 million metric tons of ethylene oxide derivatives.

COMMENT 57: Regarding the statement in Section 5.2.2 that reads "Shell Petroleum exported 825,320 pounds of ethylene oxide in 2010; however, the other producers reported either zero export and import volumes or declared this as CBI (EPA 2014a, 2017a)", the Reviewer states "from the US?"

RESPONSE: *The text in Section 5.2.2 was revised to specify U.S. producers:*

Shell Petroleum exported 825,320 pounds of ethylene oxide in 2010; however, the other U.S. producers reported either zero export and import volumes or declared this as CBI (EPA 2014a, 2017a).

COMMENT 58: Regarding the statement in Section 5.2.3 that reads "Greater than 99% of ethylene oxide production involves its use as a chemical intermediate for the production of other chemicals", the Reviewers suggests replacing "various" with "other".

RESPONSE: *The suggested revision was made to Section 5.2.3:*

Greater than 99% of ethylene oxide production involves its use as a chemical intermediate for the production of other chemicals.

COMMENT 59: Regarding the statement in Section 5.2.3 that reads "Small quantities of ethylene oxide are also used in the production of ethanolamines, polyethylene and polyalkylene glycols, polyols, and glycol ethers, as well as for fumigant, disinfectant, and medical sterilant applications (ICIS 2013)", the Reviewer states "I would refrain from using 'small' descriptor here. Very large quantities of EO are produced overall, so 'small' refers to the overall EO production, but the amounts may be large nonetheless compared to other chemicals. So please avoid misleading the public by using 'small' that may be mi-interpreted as 'insignificant'"

RESPONSE: *The referenced statement in Section 5.2.3 was revised:*

The ACC reported the following consumption patterns of ethylene oxide in 2018: 34% to produce MEG; 28% in the production of ethoxylates; 16% to produce ethanolamines; 6% in the production of glycol ethers; 4% to produce polyether polyols; and 12% for other uses, which include medical sterilization and microbial reduction in spices (ACC 2019).

COMMENT 60: Regarding the statement in Section 5.2.3 that reads "Relatively small amounts of ethylene oxide are used as a fumigant, a sterilant for food (spices) and cosmetics, and in hospital sterilization of surgical equipment and plastic devices that cannot be sterilized by steam (EPA 2017b; Parod 2014; Ribeiro 1994; WHO 2003)", the Reviewer states "See above". This is in reference to the preceding comment.

RESPONSE: *The referenced statement in Section 5.2.3 was revised:*

Ethylene oxide is used as a fumigant, a sterilant for food (spices) and cosmetics, and in hospital sterilization of surgical equipment and plastic devices that cannot be sterilized by steam (EPA 2017b; Parod 2014; Ribeiro 1994; WHO 2003).

COMMENT 61: Regarding the statement in Section 5.2.3 that reads "According to the EPA (2008) RED, approximately 8.2 million pounds of ethylene oxide are used annually in the United States for commercial fumigation/sterilization", the Reviewer states "This is NOT a 'small' amount…".

RESPONSE: *As noted in the Response to Comment 59, the text was revised to delete the statement that small amounts are used as a fumigant.*

COMMENT 62: Regarding the statement in Section 5.2.4 that reads "Because ethylene oxide is listed as a hazardous substance, disposal of wastes containing this compound is controlled by a number of federal regulations", the Reviewer states "By who?". The Reviewer also states "List those regulations? Or refer the reader to Chapter 7".

RESPONSE: *Toxicological profiles do not typically include information on regulations pertaining to disposal of wastes. The profile focuses on regulations and guidelines that deal with protecting human health.*

COMMENT 63: Regarding the unit of measure (kkg) in the statement in Section 5.3.1 that reads "Other known sources of ethylene oxide air emissions include its production from combustion of hydrocarbon fuels and its release from commodity-fumigated materials, estimated to be about 10 million pounds (4,500 kkg)", the Reviewer states "Yet another unit of measure used…".

RESPONSE: *In the referenced sentence in Section 5.3.1, the kkg unit was deleted.*

Other known sources of ethylene oxide air emissions include its production from combustion of hydrocarbon fuels and its release from commodity-fumigated materials, estimated to be about 10 million pounds annually (EPA 1980), and losses during disinfection of hospital equipment (EPA 2017b).

COMMENT 64: Regarding decimals (fractions) for amount of ethylene oxide emitted in air in Table 5-3, the Reviewer states "Do you really need the decimals in this table?"

RESPONSE: *Table 5-3 was revised to eliminate the decimals for numbers >1.*

COMMENT 65: Regarding the statement in Section 6.1 that reads "Most of the information concerning health effects in humans is reported in occupational studies that are difficult to interpret because of limitations in study design (e.g., exposure levels and duration cannot be quantified and concurrent exposure to other toxic substances cannot be ruled out)", the Reviewer suggested adding the word "typical" in front of the word "limitations".

RESPONSE: *ATSDR disagrees with the Reviewer that "typical" should be added to the referenced sentence in Section 6.1. Some of these limitations are not typical of occupational exposure studies.*

COMMENT 66: Regarding the statement in the chronic-duration MRLs portion of Section 6.2 that reads "The inhalation database was not considered adequate for derivation of a chronic-duration inhalation MRL for ethylene oxide", the Reviewer states "I disagree with this statement. See comments on the MRL sections."

RESPONSE: *Although there were adequate data to derive a chronic-duration inhalation MRL, the candidate MRL value was higher than the intermediate-duration MRL and therefore, the database was considered inadequate; see Responses to Comments 72, 73, 81 and 82.*

COMMENT 67: Regarding the statement in the Children's Susceptibility portion of Section 6.2 that reads "However, very young children with incomplete development of ethylene oxide detoxification pathways might be at increased susceptibility to ethylene oxide exposure-related effects", the Reviewer suggests revising the statement to "However, very young children with incomplete development of detoxification pathways that are known to metabolize ethylene oxide might be at increased susceptibility to ethylene oxide exposure-related effects".

RESPONSE: *The suggested revision was made in Section 6.2:*

> However, very young children with incomplete development of detoxification pathways that are known to metabolize ethylene oxide might be at increased susceptibility to ethylene oxide exposure-related effects.

COMMENT 68: Regarding Table 7-1, the Reviewer suggested adding TCEQ derived Effects Screening Level values for ethylene oxide in 2003. The Reviewer included a URL (https://www.tceq.texas.gov/toxicology/esl/list_main.html/#esl_2)

RESPONSE: *Table 7-1 does not typically include state regulations and guideline values.*

COMMENT 69: Regarding the lines in Figure A-1 that represent the BMD and BMDL, the Reviewer noted that the BMD was indicated as a yellow line in the figure legend and the BMDL as a blue line in the figure legend. The Reviewer states "There are no yellow or blue lines in this chart. Please update the legend to make it more clear what vertical line is a BMD and what is BMDL".

RESPONSE: *Figure A-1 was revised so that the BMD and BMDL lines match the color in the legend.*

COMMENT 70: Regarding the modifying factor of 3 for lack of neurotoxicity data (including a functional observational battery of testing in experimental animals) used for the intermediate-duration inhalation MRL in Appendix A, the Reviewer states "I am not sure why include this. The NOEL is based on a functional test in absence of histological observations. As stated below, there is ample data on neurotoxicity of EO, albeit little dose-response data. I would not characterize this as a deficient database and because the study used had an actual NOEL, I would not include this additional factor of 3. Therefore **sub-chronic MRL should be 0.06 ppm.**"

RESPONSE: *The discussion of the modifying factor was expanded in the Appendix A MRL worksheets for intermediate-duration inhalation MRL:*

> The $NOAEL_{HEC}$ of 1.8 ppm was divided by a total uncertainty factor of 30 and a modifying factor of 3 for insufficient animal data assessing functional neurological endpoints. Case studies of workers

have reported a number of neurological effects such as cognitive dysfunction and memory loss, which have not been evaluated in animal studies. The epidemiological studies do not provide adequate exposure-response data. The modifying factor of 3 is used to address concern that these effects could occur at lower concentrations than those associated with gait alterations and decreased locomotor activity.

COMMENT 71: Regarding the statement in the Rationale for Not Deriving an MRL in the Appendix A worksheet for the chronic-duration inhalation MRL discussion that reads "In cynomolgus monkeys intermittently exposed to ethylene oxide vapor for 2 years, decreased sperm count (28% less than controls) and motility (32% less than controls) were noted at the lowest exposure level tested (50 ppm) (Lynch et al. 1984a). These changes were of relatively low magnitude and of questionable toxicological significance", the Reviewer states "This is a bold statement without justification. I would suggest you delete of rephrase this, or provide justification for calling 'toxicological significance' into question."

RESPONSE: *The statement regarding the toxicological significance of the alterations was deleted from the profile. A statement was added to note that it is not known if these alterations would result in alterations in reproductive function:*

It is not known whether these alterations would result in decreases in fertility as the study did not evaluate reproductive function.

COMMENT 72: For the hematological effects entry in Table A-15 (Lynch et al. 1984b), the Reviewer states "In my opinion, this study should be considered for dose-response calculations because of the consistency of the adverse effects on the spleen and hematopoiesis across studies of different durations."

RESPONSE: *The Lynch et al. (1984b) rat study was considered as the basis of a chronic-duration inhalation MRL. However, the potential chronic-duration inhalation MRL derived from this study was slightly higher than the provisional intermediate-duration inhalation MRL.*

COMMENT 73: Regarding the statement in the chronic-duration inhalation MRL discussion of Appendix A that reads "The LOAEL of 50 ppm for splenic extramedullary hematopoiesis was initially considered as a candidate point of departure", the Reviewer states "This target tissue appears to be a consistent target across studies. Effects on hematopoiesis have been observed in 10-/11-week studies and in a chronic study. I suggest use this POD is used for derivation of a chronic MRL (with total UF=300 to arrive at 0.034 ppm chronic MRL".

RESPONSE: *As noted in the Response to Comment 72, derivation of an MRL based on the hematological effects observed in the Lynch et al. (1984b) rat study resulted in an MRL that was higher than the provisional intermediate-duration MRL.*

COMMENT 74: Regarding the uncertainty factor of 3 for lack of neurotoxicity data (including a functional observational battery of testing in experimental animals) used for the chronic-duration inhalation MRL in Appendix A, the Reviewer states "I am not sure why this factor is applied as the adverse effect chosen has little to do with this limitation of the database. I would NOT include this additional factor."

RESPONSE: *A modifying factor of 3 was used to account for the lack of animal studies evaluating the potential neurotoxicity of ethylene oxide following chronic-duration exposure. The Appendix A chronic inhalation MRL worksheet was revised to expand the support for the modifying factor:*

The $BMCL_{10HEC}$ of 2.3 ppm was divided by an uncertainty factor of 30 and a modifying factor of 3 to account for lack of data regarding the neurological effects of chronic-duration inhalation exposure to ethylene oxide. Intermediate-duration studies in animals and case studies of workers have reported a number of neurological effects; however, the potential neurotoxicity of ethylene oxide following chronic-duration exposure has not been evaluated in animals.

COMMENT 75: Regarding the number "73" in the statement in C.2.2 that reads "In the second step in the literature screening process for the systematic review, a full text review of the 73 health effects studies identified in the update literature was performed", The Reviewer states "How did you get to 73 if the previous paragraph says that 64 studies were selected?"

RESPONSE: *Section C.2.2 was revised so that the number of studies meeting the health effects inclusion criteria matches the number of studies undergoing a full text review:*

Title and Abstract Screen. In the Title and Abstract Screen step, 4,680 records were reviewed; 57 studies were considered to meet the health effects inclusion criteria in Table C 1 and were moved to the next step in the process.

Full Text Screen. In the second step in the literature screening process for the systematic review, a full text review of 87 health effect documents (documents identified in the update literature search and documents cited in older versions of the profile) was performed. From those 87 documents, 126 studies were included in the qualitative review.

COMMENT 76: Regarding C.8 information on neurological function in the presentation of "Presumed", the Reviewer states "Add here the study of Tyler and McKelvey 1982".

RESPONSE: *ATSDR believes that the Reviewer is referring to the unpublished study by Snelling (Ethylene oxide ten-to eleven week vapor inhalation probe study on mice. Snellings, W.M., Project report 45-158) that is included in the unpublished report referenced to Tyler and McKelvey (1982). The Tyler and McKelvey (1982) report contains a number of studies sponsored by Union Carbide. The 10–11-week study in mice has been published and is referenced in the profile as Snellings et al. (1984a). Snellings et al. (1984a) is included in Section C.8. The paper that ATSDR references to Tyler and McKelvey (1982) (Dose dependent disposition of 14C labeled ethylene oxide in rats. Union Carbide Corp. Submitted to the U.S. Environmental Protection Agency under TSCA section 8D. OTS0206060. EPA Document No. 878212056, 251-271) is a toxicokinetic study, which is discussed in Sections 3.1.2 and 3.1.4.*

COMMENT 77: Regarding C.8 information on hematological effects in the presentation of "Suspected", the Reviewer states "Add here the study of Tyler and McKelvey 1982".

RESPONSE: *As noted in the Response to Comment 76, ATSDR believes that the Reviewer is referring to the data from the Snellings et al. (1984a) study, which is included in Section C.8.*

ATSDR Charge Questions and Responses and Reviewer Comments

Chapter 1. Relevance to Public Health

QUESTION: Do you agree with those effects known to occur in humans as reported in the text? If not, please explain why and provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

COMMENT 78: Yes. A number of suggestions were made to streampline and standardize the manner in which information is presented.

RESPONSE: *See responses to specific comments.*

QUESTION: Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

COMMENT 79: Most of the effects are relevant to humans. I suggested how the information may be presented to make those points stronger. See specific comments in the text.

RESPONSE: *See the Response to Comment 19; the text in Section 2.1 was revised to include information from the systematic review regarding the relevance of the effects to humans.*

QUESTION: Have exposure conditions been adequately described? If you disagree, please explain.

COMMENT 80: This section does not list exposure ranges/levels consistently. See specific comments in the text.

RESPONSE: *Exposure levels were added to Section 1.2, see Response to Comment 11.*

QUESTION: If no MRLs have been derived, do you agree that the data do not support such a derivation? Please explain.

COMMENT 81: Yes, I agree there aren't sufficient data for deriving oral MRLs. For chronic inhalation MRL, I disagree with UF selections in the intermediate inhalation MRL derivation, and the choice of a study for deriving a candidate chronic inhalation MRL, see responses to #5 below.

RESPONSE: *See the Response to Comment 82.*

QUESTION: If MRLs have been derived, do you agree with the proposed MRL values? Explain. If you disagree, please specify the MRL value that you would propose.

a) Do you agree/disagree with each component of the total uncertainty factor? Explain. If you disagree, please specify the uncertainty factor(s) that you propose.

COMMENT 82: I agree with how the studies and PODs were selected for the acute and intermediate inhalation MRLs. Great job on the detailed explanation and considerations of multiple candidate studies; however, I disagree with the extra factor of 3 applied for intermediate MRL and the choice of the candidate endpoint/study for derivation of chronic inhalation MRL.

Intermediate inhalation MRL:
For inhalation intermediate MRL, I disagree that additional factor of 3, on top of UF 30, is needed. See more detailed comments in the text itself. If that factor is removed, the intermediate inhalation MRL will be 0.06 ppm.
Chronic inhalation MRL:
If the intermediate inhalation MRL number is revised to 0.06 ppm, then the chronic inhalation MRL derivation should be revisited as well.
The agency included additional factor of 3 here similar to the process used to derive intermediate inhalation MRL. It is unclear why that was done as the endpoints used for deriving the chronic MRL are not the same as for the intermediate MRL. If the factor of 3 is removed, the candidate chronic inhalation MRL based on the changes in the adrenal gland would be 0.09 ppm. However, I believe that the adverse effects on the spleen (Lynch et al 1984b) should have been used as a basis for deriving the chronic MRL. With the adjusted LOEL of 10.4 ppm and uncertainty factors for extrapolation from animals to humans using dosimetric adjustment (3x), for human variability (10x) and for extrapolation from LOEL to NOEL (10x), the candidate chronic inhalation MRL would be 0.034 ppm. Because it would be lower than the intermediate MRL of 0.06 ppm, the chronic MRL of 0.034 ppm would stand.

RESPONSE: *As noted in the Response to Comment 70, the modifying factor of 3 was used to account for the insufficient assessment of functional neurological endpoints, particularly for tests evaluating neurological outcomes reported in workers (e.g., cognitive dysfunction and memory loss).*

The alterations in the adrenal gland and in the spleen were observed at the same LOAEL value (50 ppm). Benchmark dose modeling was attempted for the splenic extramedullary hematopoiesis, adrenal cortical hyperplasia, and adrenal cortical vacuolation; none of the models provided adequate fit for the splenic extramedullary hematopoiesis. Three PODs were considered for deriving the potential chronic inhalation MRL: $BMCL_{10}$ of 11.11 ppm for adrenal cortical vacuolation, $BMCL_{10}$ of 15.03 ppm for adrenal cortical hyperplasia, and LOAEL of 50 ppm for splenic extramedullary hematopoiesis. The $BMCL_{10}$ was selected as the POD over the LOAEL because the BMCL is based on dose-response information over a range of exposure levels compared to the LOAEL which is based on a single data point.

QUESTION: Please comment on any aspect of our MRL database assessment that you feel should be addressed.

COMMENT 83: Consistency of the observations that hematological effects (e.g., spleen) is a target tissue across study durations should be discussed. For example, in Chapter 6, it is stated that "Hematological effects. Ethylene oxide has been shown to affect the hematological system in animals exposed via inhalation. The effects on the hematological system appear to have been adequately addressed. Additional animal studies are not necessary at this time."

RESPONSE: *ATSDR agrees with the Reviewer that hematological alterations have been consistently observed and no additional studies are needed.*

Chapter 2. Health Effects

QUESTION: Do the health effect conclusions made in Chapter 2 adequately reflect the findings in the published literature? If not, please suggest appropriate changes.

COMMENT 84: See edits in the text of Chapter 2 for specific comments.

RESPONSE: *See responses to specific comments in the Annotated Comments on the Toxicological Profile section.*

QUESTION: Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? Were the major study limitations sufficiently described in the text without going into lengthy discussions? If study limitations were not adequately addressed, please suggest appropriate changes.

COMMENT 85: Yes, but the strengths may also need to be acknowledged, where appropriate.

RESPONSE: *See Response to Comment 32.*

QUESTION: Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

COMMENT 86: See edits in the text of Chapter 2 for specific comments.

RESPONSE: *See responses to specific comments in the Annotated Comments on the Toxicological Profile section.*

QUESTION: Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

COMMENT 87: Yes.

RESPONSE: *No response is necessary.*

QUESTION: Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

COMMENT 88: Yes, but for animal studies the trend tests have not been mentioned.

RESPONSE: *See Response to Comment 43.*

QUESTION: Are you aware of any studies that are not included in the profile that may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicate where in the text each study should be included.

COMMENT 89: See edits in the text of Chapter 2 for specific comments.

RESPONSE: *See responses to specific comments.*

QUESTION: Are you aware of any studies that are not included in the profile that may be relevant to deriving MRLs for any of the substance isomers? Please provide a copy if this is a new reference.

COMMENT 90: No

RESPONSE: *No response is necessary.*

QUESTION: Were all appropriate NOAELs and/or LOAELs identified for each study (both in the text and the Levels of Significant Exposure (LSE) tables and figures)? If not, did the text provide adequate justification for excluding NOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

COMMENT 91: Yes

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables? If not, please explain why and suggest appropriate changes.

COMMENT 92: Mostly yes, see specific comments in the text.

RESPONSE: *See responses to specific comments.*

QUESTION: Have all possible mechanisms of action been discussed within their relevant health effect section? If not, please explain. If citing a new reference, please provide a copy and indicate where (in the text) it should be included.

COMMENT 93: Genotoxicity really is the primary one to mention. This was done well.

RESPONSE: *No response is necessary.*

QUESTION: Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

COMMENT 94: Yes. Albeit I did not find ATSDRD making conclusions regarding cancer hazard.

RESPONSE: *ATSDR does not categorize the carcinogenic potential of a chemical in toxicological profiles; rather, it discusses the available data and cites carcinogenicity assessments conducted by HHS (NTP Report on Carcinogens), EPA, and IARC.*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

Toxicokinetics

QUESTION: Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

COMMENT 95: Yes, a very comprehensive section.

RESPONSE: *No response is necessary.*

QUESTION: Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

COMMENT 96: Yes, a very comprehensive section.

RESPONSE: *No response is necessary.*

QUESTION: Is there adequate discussion of the differences in toxicokinetics between humans and animals? Is there adequate discussion of the relevance of animal toxicokinetic information for humans?

COMMENT 97: Yes, this section overall is excellent.

RESPONSE: *No response is necessary.*

Children and Other Populations that are Unusually Susceptible:

QUESTION: Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be? Please provide any relevant references.

COMMENT 98: The text is comprehensive. I suggest adding a reference or two to explain developmental maturation of GST pathways (see text).

RESPONSE: *See Responses to Comment 52.*

QUESTION: Is there a discussion of populations at higher risk of susceptibility? Do you agree with the choice of populations? Please explain and provide any additional relevant references.

COMMENT 99: There are no other data of relevance to EO that I am aware of and the discussion is good as is.

RESPONSE: *No response is necessary.*

Biomarkers of Exposure and Effect:

QUESTION: Are the biomarkers of exposure specific for the substance? Please explain.

COMMENT 100: The biomarkers of exposure section is written well and addresses this point.

RESPONSE: *No response is necessary.*

QUESTION: Are the biomarkers of effect specific for the substance? Please explain.

COMMENT 101: No data are available

RESPONSE: *No response is necessary.*

Interactions with Other Chemicals

QUESTION: Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? Please explain and provide any additional references.

COMMENT 102: No data are available

RESPONSE: *No response is necessary.*

QUESTION: If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? Please explain and provide any additional references.

COMMENT 103: No data are available

RESPONSE: *No response is necessary.*

Chapter 4. Chemical and Physical Information

QUESTION: Are any of the values or information provided in the chemical and physical properties tables wrong or missing? Please explain and provide any additional references.

COMMENT 104: No, this is good as is. Most people probably wouldn't go to the ATSDR profile for this information, but to the EPA dashboard or some other database.

RESPONSE: *No response is necessary.*

QUESTION: Is information provided on the various forms of the substance? Please explain.

COMMENT 105: Not applicable.

RESPONSE: *No response is necessary.*

Chapter 5. Potential for Human Exposure

QUESTION: Is the information on production, import/export, use, and disposal of the substance complete? Please explain and provide any additional relevant references.

COMMENT 106: Yes, this section is very comprehensive. No further information. Some minor edits suggested in text.

RESPONSE: *See Responses to Comments 54 and 55.*

QUESTION: Has the text appropriately traced the substance from its point of release to the environment until it reaches the receptor population? Does the text provide sufficient and technically sound information regarding the extent of occurrence at NPL sites? Do you know of other relevant information? Please provide references for added information.

COMMENT 107: Yes, this section is very comprehensive. No further information. Some minor edits suggested in text.

RESPONSE: *See Responses to Comments 63 and 64.*

QUESTION: Does the text cover pertinent information relative to transport, partitioning, transformation, and degradation of the substance in all media? Do you know of other relevant information? Please provide references for added information.

COMMENT 108: Yes, this section is very comprehensive. No further information. Some minor edits suggested in text.

RESPONSE: *ATSDR notes that in the annotated comments on the toxicological profile, the Reviewer did not provide comments on Section 5.4 (Environmental Fate).*

QUESTION: Does the text provide information on levels monitored or estimated in the environment, including background levels? Are proper units used for each medium? Does the information include the form of the substance measured? Is there an adequate discussion of the quality of the information? Do you know of other relevant information? Please provide references for added information.

COMMENT 109: Yes, this section is very comprehensive. No further information. Some minor edits suggested in text.

RESPONSE: *ATSDR notes that in the annotated comments on the toxicological profile, the Reviewer did not provide comments on Section 5.5 (Levels in the Environment).*

QUESTION: Does the text describe sources and pathways of exposure for the general population and occupations involved in the handling of the substance, as well as populations with potentially high

exposures? Do you agree with the selection of these populations? If not, why? Which additional populations should be included in this section?

COMMENT 110: Yes, this section is very comprehensive. No further information. Some minor edits suggested in text.

RESPONSE: *ATSDR notes that in the annotated comments on the toxicological profile, the Reviewer did not provide comments on Section 5.5 (Levels in the Environment).*

Chapter 6. Adequacy of the Database

QUESTION: Do you know of other studies that may fill a data gap? Please provide any relevant references.

COMMENT 111: No, the data are comprehensive.

RESPONSE: *No response is necessary.*

QUESTION: Do you agree with the identified data needs? Please explain.

COMMENT 112: Yes. Well described

RESPONSE: *No response is necessary.*

QUESTION: Are the data needs presented in a neutral, non-judgmental fashion? Please note any bias in the text.

COMMENT 113: Yes, the writeup is good.

RESPONSE: *No response is necessary.*

Chapter 7. Regulations and Guidelines

QUESTION: Are you aware of any additional regulations or guidelines that should be included? Please provide citations.

COMMENT 114: TCEQ has inhalation values: listed on the most recent table that can be downloaded from https://www.tceq.texas.gov/toxicology/esl/list_main.html/#esl_2

RESPONSE: *ATSDR does not typically include state regulations and guidelines in Table 7-1.*

QUESTION: Are there any that should be removed? Please explain.

COMMENT 115: No.

RESPONSE: *No response is necessary.*

Appendices

QUESTION: Please provide any comments on the content, presentation, etc. of the included appendices.

COMMENT 116: Specific edits are included in appendices.

RESPONSE: *See Responses to Comments 69–77.*

Comments provided by Group 2 Peer Reviewer #3

Annotated Comments on the Toxicological Profile

COMMENT 1: Regarding the Figure 1-1 entry for depressed maternal body weight at the 100-150 ppm listed as acute inhalation, the Reviewer states "Depressed maternal body weight is listed here as Acute 100-150ppm. This effect is not supported elsewhere in the document at that concentration of length of exposure. In Table 2-1 pg14 (Saillenfait 1996 ref#9) this endpoint is listed as 1,200 ppm LOAEL and 800 ppm NOAEL. Is there another study showing a LOAEL of 100-150ppm for acute exposures?"

RESPONSE: *Depressed maternal weight was deleted from Figure 1-1.*

COMMENT 2: Regarding the statement in Section 1.2 (heading of Developmental Effects) that reads "the neonates were smaller in both length and weight and had reduced ossification of the skull and sternebrae (Snellings et al. 1982a)", the Reviewer states "The only significant exposure-relate change reported by Snellings 1982a is fetus weight. Crown-to-rump length was unaffected at any dose of EO (1982a, Table 2), and although ossification endpoints may be numerically elevated compared to 0ppm controls, no statistically significant differences were detected (1982a, Table 3)."

RESPONSE: *The text was revised to expand the dose range and include additional citations:*

> However, embryo and fetal toxicity were reported in the offspring of rats exposed to 100–150 ppm during gestation; the neonates were smaller in both length and weight and had reduced ossification of the skull and sternebrae (Neeper-Bradley and Kubena 1993; NIOSH 1982; Snellings et al. 1982a).

COMMENT 3: Regarding the Figure 1-3 entry for body weight changes with an identified LOAEL of 100 ppm under chronic inhalation exposure, the Reviewer states "LOAEL for chronic body weight changes should be 50ppm to agree with Table 2-1 pg 22 for ref#46 Lynch etal 1984b".

RESPONSE: *The Lynch et al. (1984a, 1984b) study found a decrease in body weight at 100 ppm; the error in Table 2-1 was corrected.*

COMMENT 4: Regarding the statement in Section 2.2 that reads "Mice and dogs were more sensitive than rats; reported 4-hour LC_{50} values were 835 and 960 ppm, respectively (Jacobson et al. 1956)", the Reviewer states "However rats are consistently more sensitive than mice with intermediate and chronic exposures. In (Hollingsworth 1956 key#35 only deaths at 204ppm for 6 weeks are rats, and similarly only rats experience mortality with 100ppm after 104 weeks."

RESPONSE: *The text in Section 2.2 was revised to indicate that this statement is referring to acute lethality:*

> Mice and dogs were more sensitive to acute lethality than rats; reported 4-hour LC_{50} values were 835 and 960 ppm, respectively (Jacobson et al. 1956).

COMMENT 5: Regarding the statement in Section 2.3 that reads " Hollingsworth et al. (1956) reported "markedly subnormal growth" among rats, mice, rabbits, guinea pigs, and/or monkeys intermittently exposed to ethylene oxide vapor at 204–357 ppm for up to 226 days", the Reviewer states "The intermediate LOAEL for body weight should be included in here: 113ppm for male rats (Hollingsworth 1956 ref key#34). It is correctly indicated in Figure 1-3 and Table 2-2."

RESPONSE: *The text in Section 2.3 was revised to include the LOAELs for the decreases in final body weight:*

> Decreases in final body weights (10–20%) were observed in rats and guinea pigs exposed to 113–204 ppm ethylene oxide for up to 226 days (Hollingsworth et al. 1956).

COMMENT 6: Regarding the statement in Section 2.3 that reads "In 2-year rat studies that employed repeated exposure to ethylene oxide vapor, approximately 13% depressed body weight gain was reported at an exposure level of 100 ppm (Garman et al. 1986; Lynch et al. 1984a, 1984b)", the Reviewer states "In Lynch 1984b (Fig1, mean BW) and in Table 2-1 (Pg22, fey#46, weight gain), the LOAEL is indicated at 50 ppm with 16% decrease. In Table 2-1 and Table 2-2 (pg30) this change is described as Less Serious. The text here might be changed to agree with the Tables. Garman et al., 1986 reports a LOAEL of 100ppm and NOAEL of 33 ppm."

RESPONSE: *As noted in the Response to Comment 3, the LOAEL reported in Table 2-1 for body weight effects observed in the Lynch et al. (1984a, 1984b) study was changed to 100 ppm. The text in Section 2.3 was revised to expand the magnitude of body weight effects observed in the chronic rat studies:*

> In 2-year rat studies that employed repeated exposure to ethylene oxide vapor, 12–18% depressed body weight gain was reported at an exposure level of 100 ppm (Garman et al. 1986; Lynch et al. 1984a, 1984b).

COMMENT 7: Regarding the statement in Section 2.4 that reads "Inhalation of ethylene oxide is irritating to mucous membranes including those associated with the respiratory system. Inhalation exposure of workers to high concentrations of ethylene oxide for brief periods has resulted in bronchitis, pulmonary edema, and emphysema (Thiess 1963)", the Reviewer states "Are data available for the exposure concentrations? Frequency of exposure?"

RESPONSE: *The Thiess (1963) study did not report exposure levels or the frequency of exposure.*

COMMENT 8: Regarding the statement in Section 2.4 that reads "Acute bronchopneumonia, chronic pneumonia, pulmonary edema, and suppurative rhinitis were observed in rats exposed at 50 ppm for 104 weeks (Lynch et al. 1984a, 1984b)", the Reviewer states "Are these the same rats that were used for the LOAEL for endocrine, body weight, and hematological effects for chronic exposures (Figure 1-3, page 7, and Table 2-1, key#46)? Were 100ppm groups also infected and treated? Did the study authors comment on how extrapulmonary responses to ethylene oxide might have been altered due to the infections?"

RESPONSE: *All groups of rats were treated with antibiotics. The investigators noted that the respiratory infection and treatment did not 'compromise the major findings of the rat study."*

COMMENT 9: Regarding the discussion of animal data in Section 2.4, the Reviewer states "However pulmonary effects are seen at 113ppm in rats. This information is lost amongst the several strains mentioned in lines 1-2 above."

RESPONSE: *The discussion of pulmonary effects following acute and intermediate-duration exposure has been revised:*

> Adverse respiratory effects (e.g., dyspnea, pulmonary edema, pulmonary hemorrhage and congestion, "severe lung injury") were reported for experimental animals (rats, mice, and/or guinea pigs) exposed to 357–841 ppm ethylene oxide vapor for acute durations (Hollingsworth et al. 1956; NTP 1987). Rhinitis was also observed in mice exposed to 400 ppm ethylene oxide for up to 2 weeks (NTP 1987). Intermediate-duration studies reported labored breathing and nasal discharge in rats exposed to 406 ppm for 6 weeks (Jacobsen et al. 1956), an increase in relative lung weight in rats and guinea pigs exposed to 113–204 ppm for up to 226 days (Hollingsworth et al. 1956), rhinitis in mice exposed to 200 ppm for up to 14 weeks (NTP 1987), and pulmonary congestion, and alveolar collapse in dogs exposed to 292 ppm for 6 weeks (Jacobsen et al. 1956).

COMMENT 10: Regarding the summary of respiratory effects from the NTP (1987) study in Section 2.4 (Respiratory), the Reviewer states "Should rhinitis effects at 200ppm (LOAEL) be included for completeness (NTP 1987)?"

RESPONSE: *As noted in the Response to Comment 9, the text in Section 2.4 was revised and includes the rhinitis observed in mice exposed to 200 ppm.*

COMMENT 11: Regarding the summary of animal data in Section 2.7, the Reviewer states "Normochromic anemia in dogs at 102 ppm @ 6 months (Jacobson 1956;key#41) is indicated in Figure 1-3 and Table 2-2 Intermediate exposure. It should be included here for completeness."

RESPONSE: *The results of the Jacobsen et al. (1956) study were added to Section 2.7:*

> Hematological alterations indicative of normochromatic anemia were also observed in dogs exposed to 102 ppm for 6 months (Jacobson et al. 1956).

COMMENT 12: Regarding the statement in Section 2.8 that reads "Histopathologic indicators of muscular atrophy were reported for experimental animals (rats, rabbits, monkey, dogs) repeatedly exposed to ethylene oxide vapor for 6 weeks to 226 days at exposure levels in the range of 204–357 ppm (Hollingsworth et al. 1956; Jacobson et al. 1956)", the Reviewer states "A minor issue with comparative anatomy terms: muscular effects in Table 2-1 refer to hindlegs or hindlimbs. Monkeys have two arms and two legs and should be referred to separately from term 'hindlimbs' used for rodents and dogs in the 'Effects' column in Table 2-1 (Pg 20 key#35; Pg 19 key#33; Page 16 key#20)."

RESPONSE: *ATSDR disagrees with the Reviewer; the term* hindlimbs *is used for nonhuman primates. This is likely because most primates exhibit quadrupedal walking and running, which contrasts to humans who are bipedal walkers.*

COMMENT 13: Regarding the statement in Section 2.8 that reads "There was no indication of musculoskeletal effects in mice repeatedly exposed to ethylene oxide vapor for up to 14 weeks at 250 ppm (Snellings et al. 1984a)", the Reviewer states "It may be worth mentioning that both histological and functional effects were assessed. This is also another endpoint where mice are less sensitive than rat."

RESPONSE: *The functional effects are discussed in Section 2.15 (neurological); the following statement was added to Section 2.8:*

See Section 2.15 for a discussion of neuromuscular alterations.

COMMENT 14: Regarding the statement in Section 2.10 that reads "At 200 ppm, 8/10 females exhibited renal tubular degeneration", the Reviewer states "This is already stated above in line 14. Did the authors mean to state that 0/10 females had tubular necrosis at 100 and 200ppm?"

RESPONSE: *The referenced sentence was deleted from Section 2.10.*

COMMENT 15: Regarding the statement in Section 2.10 that reads "Lethal exposure levels (400 and 600 ppm) resulted in increased incidences of necrosis in both sexes", the Reviewer states "… and occurred within 2-4 weeks."

RESPONSE: *The text in Section 2.10 was revised to include the exposure duration:*

Lethal exposure levels (400 and 600 ppm) resulted in increased incidences of necrosis in both sexes exposed for up to 2 weeks.

COMMENT 16: In the first paragraph of Section 2.14, the Reviewer suggests that "Atmospheric" be replaced with "Workplace".

RESPONSE: *The suggested revision was made in Section 2.14:*

Workplace concentrations were generally <0.05 ppm (the detection limit of the analytical method) with occasional peaks of 8 ppm during the 4 years that the air was monitored.

COMMENT 17: Regarding the statement in Section 2.16 that states "Intermittent inhalation exposure of monkeys to ethylene oxide vapor at 50 ppm (the lowest exposure level tested) for 24 months resulted in decreases in sperm count (28% less than controls) and motility (32% less than controls); however, reproductive function was not tested (Lynch et al. 1984a)", the Reviewer states "Descriptions of these effects are absent from Table 2-1 (key#44) and Fig 2-2. Were effects on sperm not deemed significant?"

RESPONSE: *The reproductive effects (decreased sperm count and motility) from the Lynch et al. (1984a) study were added to the LSE table (Table 2-1; Figure key #44).*

COMMENT 18: Regarding the statement in Section 2.20 that states "*In vivo* studies using experimental test species and available human data are summarized in Tables 2-4 and 2-5, respectively", the Reviewer states "Switch the order : human data is in Table 2-4 and animal data is Table 2-5".

RESPONSE: *The sentence calling out Tables 2-4 and 2-5 was corrected:*

Studies evaluating the genotoxicity of ethylene oxide in humans and *in vivo* studies using experimental test species are summarized in Tables 2-4 and 2-5, respectively.

COMMENT 19: Regarding the statement in Section 5.5 that reads "The samples were collected in areas with no known ethylene oxide sources" in reference to data in Table 5-6, the Reviewer states "This

Michigan data in this table is from MDEQ monitor near a known source (ViantMedical in Grand Rapids, MI)".

RESPONSE: *The Michigan data were deleted from Table 5-6.*

COMMENT 20: Regarding the Table 5-6 entry for Michigan, the Reviewer states "Oleguer et al., 2020 (IntJEnvResPubHealth 17,42) reports ambient level of 1.83 ug/m3 near this facility in Grand Rapids, MI."

RESPONSE: *Table 5-6 presents data from samples collected in areas with no known ethylene oxide source. As noted in the Response to Comment 19, the Michigan data were deleted from the table.*

COMMENT 21: Regarding the statements in Section 5.5.1 that read "A statistical breakdown of the calculated concentrations of ethylene oxide in ambient outdoor air is provided in Table 5-7. The mean calculated national concentration of ethylene oxide in ambient air was 2.92×10^{-4} µg/m^3 (1.61×10^{-4} ppb) and the maximum level measured was 0.144 ug/m^3 (0.079 ppb).", the Reviewer states "Newer data released from EPA for October 1, 2018 – March 31, 2019 report national average of 0.297 ug/m3and peak of 0.397 (Phoenix, AZ). https://www.epa.gov/hazardous-air-pollutants-ethylene-oxide/ethylene-oxide-data-summary-national-air-toxics-trends"

RESPONSE: *Information from NATTS monitoring was added to the text in Section 5.5.1:*

EPA (2019a) reported ethylene oxide average concentrations from ambient air samples collected at 8 National Air Toxics Trends Stations (NATTS) and 10 Urban Air Toxics Monitoring Program (UATMP) sampling sites between October 2018 and September 2019. Results are summarized in Table 5-8. Measured average ethylene oxide concentrations ranged from 0.136 µg/m^3 (0.075 ppb) in Pinellas Park, Florida to 0.407 µg/m^3 (0.224 ppb) in Phoenix, Arizona.

Additional Comments

The Reviewer provided critique and comments on each chapter of the toxicological profile in a summary report. Numerous comments were similar to specific comments provided in annotated pages addressed in the section of annotated comments on the toxicological profile above. However, there were some differences in these additional comments, as well as some comments that were not presented in the annotated pages. Therefore, these additional comments are presented below verbatim from the summary report of the Reviewer.

Chapter 1. Relevance to Public Health

COMMENT 22: Noncancer endpoints known to occur in humans are appropriately enumerated and described in Chapter 1 (respiratory, neurological, reproductive, and developmental). Likewise the adverse effects reported in experimental animals but that have less evidence in humans are also appropriately identified and justified (hematological and endocrine). The hazard categories for each health effect from ethylene oxide exposure are either 'presumed' or 'suspected' (p2). Derivation of these categories are well-detailed in Appendix C (Section C8), and would edify the reader if reference to this appendix were made here in Chapter 1.

RESPONSE: *A call out to Appendix C was added to the discussion of the systematic review in Section 1.2:*

> A systematic review of noncancer endpoints (see Appendix C for details) resulted in the following hazard identification conclusions.

COMMENT 23: On Page 8 Figure 1-1 (Health Effects Found in Animals Following Inhalation Exposure to Ethylene Oxide), "depressed maternal body weight", is listed as the first effect for the Acute dose range of 100-150ppm. This effect is not supported elsewhere in the document at that concentration and length of exposure. In Table 2-1 pg14 (Saillenfait 1996 ref#9) this endpoint is listed as 1,200 ppm LOAEL and 800 ppm NOAEL. Is there another study showing a LOAEL of 100-150ppm acute exposure?

RESPONSE: *As noted in the Response to Comment 1, maternal body weight effects were deleted from Figure 1-1.*

COMMENT 24: In summarizing the Developmental Effects on page 5, the neonatal outcomes attributed to Snellings et al. 1982a for smaller length and reduced ossification of the skull and sternebrae are not consistent with the original report. The only significant exposure-relate change reported by Snellings 1982a is fetus weight. Crown-to-rump length was unaffected at any dose of EO (Snellings 1982a, Table 2), and although ossification endpoints may be numerically elevated compared to 0ppm controls, no statistically significant differences were detected (Snellings 1982a, Table 3).

RESPONSE: *As noted in the Response to Comment 2, **t**he text in Section 1.2 was revised to include additional citations for the developmental effects.*

COMMENT 25: In the Summary of Sensitive Targets figure (Fig 1-3), body weight changes with chronic exposures should be changed from a LOAEL of 100 ppm to 50 ppm. The correct LOAEL of 50ppm is listed in Table 2-1 pg 22 for ref#46 Lynch et.al. 1984b. Indeed, all chronic LOAELs listed in Figure 1-3 are from the same report by Lynch 1984b.

RESPONSE: *As noted in the Response to Comment 3, the correct LOAEL for the body weight effects in the Lynch et al. (1984a, 1984b) study is 100 ppm; the error in Table 2-1 was corrected.*

COMMENT 26: Other minor revisions or comments are made directly in the text of the document but are not necessary to enumerate here.

RESPONSE: *The referenced "minor revisions or comments" provided on annotated pages of the toxicological profile are addressed in the section titled* **Annotated Comments on the Toxicological Profile** *above.*

MRL Derivations

COMMENT 27: The proposed acute MRL of 0.4ppm is derived from a robust study that describes fetal body weight changes in F344 rats (Snellings et al., 1982a), and strong supporting studies in SD rats (Neeper-Bradley and Kubena, 1993). Data from Snellings, which reported a NOAEL of 33ppm and LOAEL of 100ppm was appropriately used to derive a benchmark change of 5% from control of

45.12ppm for 6h exposure (later adjusted to 11.28ppm). Comparative modeling as presented in Table A-3 shows the strong rational for selection of this point of departure. Derivations using data from Neeper-Bradley and Kubena result in a higher BMCL of 87.08ppm and are a result of the dose range used in the study.

Two modifications for uncertainty are justified: extrapolation dosimetry from animals to human (3) and for human variability (10) provide a conservative adjustment to the benchmark response of 30-fold. While development effects (especially teratological) are serious health effects that could warrant a further uncertainty factor of 3, the NOAEL and LOAEL for fetal body weight effects used in these analysis provide a sufficient basis for an acute MRL.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is needed.*

COMMENT 28: The proposed intermediate MRL of 0.02 was derived from neurotoxicity outcomes in mice with NOAEL of 10 and LOAEL of 50 (Snellings et al., 1984a). These results are the lowest dose effects seen among respiratory, hematological, renal and other neurological outcomes that reported LOAELs of 100-500ppm. The Snelling study assessed a wide dose range (0, 50, 100, 250 a) and multiple neurologic, biochemical, and histological endpoints, but was limited by low animal numbers (n=5). The key data is observational (subjective) of hunched posture during gait and of reduced motor function, but the demarcation of responses between 10 and 50ppm was definitive (Table A-8). These data appear to be extracted from the larger original source report by Tyler and McKelvey (1982) where multiple neurologic endpoints were collected as part of broad observational screening (Irwin Screen), and with only two subjective measures having significant, exposure-related changes. Tyler and McKelvey suggest the dose-dependent responses of these endpoints be interpreted cautiously.

An alternative study that might be considered is Snellings 1982b that describes reproductive effects associated with 19-week exposures with a NOAEL of 33 and LOAEL of 100ppm. Depending on the uncertainty factors applied, the analysis might produce the same MRL as the acute value that was derived from the same NOAEL and LOAEL values but from different endpoints (Snellings 1982a). Both male and female animals were exposed prior to conception and may be viewed as a confounding the interpretation.

For the neurological data, adjustments to the 10ppm are appropriately applied for Human Equivalence (1), extrapolation from animals to humans (3), and for human variability (10). The need for an additional factor of 3 for 'lack of neurotoxicity data" needs more explanation/justification as it is not clear at the bottom of page 22 Appendix A. The NAOEL/LOAEL from this study is already marginal (and may be too low?) so application of another UF favors an even lower concentration.

RESPONSE: *In the discussion portion of the Snellings et al. (1984a) paper, the investigators made the following comment regarding the neurological effects "Although a dose-response was apparent, with the small sample size it was difficult to establish what level could be regarded as a threshold." ATSDR agrees that the small number of animals tested (n=5/sex) decreases the statistical power of the study and notes that statistically significant differences were observed for hunched posture during gait and reduced locomotor activity between the 50 ppm group and the controls. If more animals were tested, the decreased locomotor activity observed in the 40% of the males exposed to 10 ppm may have been statistically different from the response in controls (0%). Given the high response rates (≥80% at the LOAEL) and other studies reporting gait alterations (Kaido et al. 1993; Matsuoka et al. 1990; Ohnihsi et al. 1986), ATSDR considered the neurological effects to be related to ethylene oxide exposure.*

The reproductive effects reported in the Snellings et al. (1982b) study was not considered for MRL derivation because the LOAEL of 100 ppm is higher than the LOAEL for neurological effects (50 ppm) identified in the Snellings et al. (1984a) study.

In the Appendix A MRL worksheet for the intermediate-duration inhalation MRL, ATSDR has expanded the rationale for the modifying factor:

> The $NOAEL_{HEC}$ of 1.8 ppm was divided by a total uncertainty factor of 30 and a modifying factor of 3 for insufficient animal data assessing functional neurological endpoints. Case studies of workers have reported a number of neurological effects such as cognitive dysfunction and memory loss, which have not been evaluated in animal studies. The epidemiological studies do not provide adequate exposure-response data. The modifying factor of 3 is used to address concern that these effects could occur at lower concentrations than those associated with gait alterations and decreased locomotor activity.

COMMENT 29: A chronic MRL of 0.03 ppm was calculated from endocrine responses in F344 rats exposed for 2 years to 0, 50 or 100ppm (Lynch et al., 1984b). Some (most?) rats in this study contracted *Mycoplasma pulmonis* as early as 8 months and despite colony-wide treatment with tetracycline, ethylene exposures were suspended during month 16 for 2 weeks because of the infection. The study authors do not indicate the numbers or groups affected. Although multiple dose-dependent effects are described, pulmonary infections and resultant inflammation could compromise uptake and dosimetry of ethylene oxide to affect systemic sites such as adrenal gland, spleen and muscle that are indicated as in Table A-15.

Two other chronic studies of F344 rats by Garman et al., 1986 and Snellings et al., 1984b reported body weight changes at 100ppm with NOAEL at 33 ppm. The only other potential study for MRL analysis finds no effects in mice exposed up to 100 ppm (NTP 1987). As such the Lynch et al., 1984b study is the best available dataset of hematological and endocrine outcomes for calculating the chronic MRL.

Because of the limitations in the available chronic studies and because the derived MRL of 0.03ppm is close to the intermediate MRL, it is appropriate that no chronic MRL is derived for ethylene oxide. Furthermore, as stated in Appendix A, the ATSDR considers the intermediate-duration MRL to be protective for chronic exposures.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is needed.*

Chapter 2. Health Effects

COMMENT 30: Ethylene oxide elicits a constellation of adverse responses in a number of biological systems. Descriptions of the respiratory, neurological, reproductive, developmental, hematological and endocrine responses from exposure was for the most part accurate and representative of the literature. No obvious omissions of critical studies were apparent. Identification of exposure concentrations, NOAELs and LOAELs and study limitations were consistently reported with some exceptions as noted below. Additional comments and revisions have been made directly in the document but are minor and not included here.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is needed.*

COMMENT 31: Some clarifications/corrections are required for the discussion of body weight effects after intermediate and chronic exposures on page 37, first paragraph. As written, the intermediate LOAEL is suggested to be 204 ppm. The correct intermediate LOAEL for body weight is 113ppm for male rats (Hollingsworth 1956 ref key#34). This LOAEL for intermediate exposure is correctly indicated in Figure 1-3 and Table 2-2. Later in the paragraph it is suggested that the chronic LOAEL is 100ppm, when the correct level is 50 ppm. In Lynch 1984b (Fig1, mean BW) and in Table 2-1 (Pg22, weight gain), effects are indicated at 50 ppm with 16% decrease (i.e., LOAEL is 50 ppm, not 100ppm as suggested here). In Table 2-1(ref key#46) and Table 2-2 (pg30) this change is described as Less Serious. The text here might be changed to agree with the Tables.

RESPONSE: *As noted in the Responses to Comments 5 and 6, the text in Section 2.3 has been revised and includes the LOAEL of 113 ppm identified in rats (Hollingsworth et al. 1956). The LOAEL for body weight effects in the Lynch et al. (1984b) study is 100 ppm; this was corrected in Table 2-1.*

COMMENT 32: In the discussion of respiratory effects on page 38, airway inflammatory responses with 50ppm chronic exposure in rats are explained as secondary to bacterial infections in all study groups (Lynch 1984a, 1984b). While that consideration for airway responses is appropriate, the extrapulmonary responses in these same rats are also apparently used as the LOAEL for body weight, endocrine, and hematological effects for chronic exposure (Figure 1-3, page 7, and Table 2-1, key#46). Did the study authors comment on how extrapulmonary responses to ethylene oxide might have been altered due to the infections? Should these systemic LOAEL effects be viewed with caution?

RESPONSE: *As noted in the Response to Comment 8, the investigators noted that the respiratory infection and treatment did not "compromise the major findings of the rat study."*

COMMENT 33: In the discussion of hematological effects on page 38, intermediate effects should be included for normochromic anemia in dogs at 102 ppm @ 6 months (Jacobson 1956;key#41) is indicated in Figure 1-3 and Table 2-2 Intermediate exposure.

RESPONSE: *As noted in the Response to Comment 11, **t**he results of the Jacobsen et al. (1956) study were added to Section 2.7.*

COMMENT 34: A minor issue with comparative anatomy terms: muscular effects in Table 2-1 refer to hindlegs or hindlimbs. Monkeys have two arms and two legs and should be referred to separately from terms 'hind----' used for rodents and dogs in the 'Effects' column in Table 2-1 (Pg 20 key#35; Pg 19 key#33; Page 16 key#20).

RESPONSE: *As noted in Response to Comment 12, ATSDR disagrees with the Reviewer; the term hindlimbs is used for nonhuman primates.*

COMMENT 35: In the summary of reproductive effects (bottom of pg 46, top of pg 47), effects of decreases sperm count and motility with 50 ppm in monkeys are mentioned, but these effects are not included in TABLE 2-1 (key#44) or Fig 2-2. These effects would represent a lower LOAEL than the current 100ppm for reproductive effects. Were the effects on sperm from this particular study not deemed significant or were there weaknesses in the study?

RESPONSE: *The reproductive effects reported in the Lynch et al. (1984a) study (decreased sperm count and motility) were added to the LSE table (Table 2-1) and figure (Figure 2-2).*

Chapter 3. Toxicokinetics, Susceptible Populations, Biomarkers, Chemical Interactions

COMMENT 36: Discussions of ethylene oxide absorption, distribution, metabolism, biomarkers, and excretion are informative and provided in the sufficient detail.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is needed.*

COMMENT 37: Although there are interesting discussions of the differences in toxicokinetics across animal species and between humans and animals, there is no translation of these differences to observed health outcomes with regard to relevant clearance mechanisms (epoxide hydrolase vs. glutathione conjugation). A number of rat/mouse health effects differences in dose responses (with rat more susceptible than mice) are presented in Chapter 2 that may be explained by species-specific clearance mechanisms. Likewise, differences in clearance mechanisms are not incorporated in discussion of the tumor-dose relationship and translation for rodents to humans.

RESPONSE: *The available data suggest some species differences in toxicokinetic properties (disposition and metabolism); however, data are not available to assess how these differences could lead to toxicity differences. In Section 6.2 (Comparative Toxicokinetics), ATSDR notes that the proximal toxicant(s) responsible for ethylene oxide-induced noncancer effects needs to be identified in order to apply PBPK models that could account for species differences. ATSDR did not conduct a weight-of-evidence analysis of the carcinogenicity data or evaluate possible species differences for carcinogenicity. In Section 2.19, there is a statement that HHS (NTP 2016) notes that there is evidence for a common mechanism of carcinogenesis in humans and experimental animals.*

Chapter 4. Chemical and Physical Information

COMMENT 38: All tabular data appears to be accurate and complete.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is necessary*

Chapter 5. Potential for Human Exposure

COMMENT 39: There are more recent data for airborne concentrations than reported in Section 5.5, Tables 5-6 and 5-7 (pp107-108). A recent report from Oleguer et al., 2020 (Int J Env Res Pub Health 17,42) reports ambient level of 1.83 ug/m3 that is greater than the value for this Michigan site in Table 5-6 (0.018 ug/m3, if this is indeed the same monitoring site). Secondly, a summary of ambient data collected from the National Air Toxics Trends Stations and the Urban Air Toxics Monitoring Program networks from October 1, 2018 – March 31, 2019 report national average of 0.297 ug/m3and peak of 0.397 (Phoenix, AZ), which is greater than the values reported in Table 5-7.

https://www.epa.gov/hazardous-air-pollutants-ethylene-oxide/ethylene-oxide-data-summary-national-air-toxics-trends.

RESPONSE: *Table 5-6 was revised and the Michigan data were deleted because the monitoring data were from areas near a point source. As noted in the Response to Comment 21, data from the National Air Toxics Trends Stations and the Urban Air Toxics Monitoring Program networks were added to Section 5.5.1. The Olaguer et al. (2020) data were not added to Table 5-6 because the table includes background ambient air levels and Olaguer et al. (2020) measured ethylene oxide levels near a point source.*

Chapter 6. Adequacy of the Database

COMMENT 40: Overall the data needs were presented in a comprehensive and non-bias fashion.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is necessary.*

COMMENT 41: Human data needs of more robust study design in occupational cohorts, dermal sensitization, and extending animal findings to human studies for hematological, endocrine, neurotoxicity, and reproductive toxicology would strengthen the framework from which to derive more accurate MRLs.

RESPONSE: *ATSDR agrees with the Reviewer. The need for additional studies evaluating hematological, endocrine, neurological, and reproductive endpoints and epidemiological studies is discussed in Section 6.2.*

COMMENT 42: Relevant animal data from which to derive chronic inhalation MRL and all oral MRLs are appropriately identified as a needs. The chronic respiratory effects from Lynch et al., 1984 were confounded by respiratory infections and treatments throughout the 2 year study. LOAEL effects would likely have occurred at 50ppm for adverse chronic respiratory responses, and potential subchronic effects might be more robust than the subjective neurologic screening data used to derive the intermediate MRL. This is a critical knowledge gap and this section appropriately calls for further studies that assess in more detail respiratory and neurological effects.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is necessary.*

Chapter 7. Regulations and Guidelines

COMMENT 43: In addition to NIOSH, the American Conference of Government and Industrial Hygienist calculate a time-weighted average for occupational exposure. Current threshold limit value is 1 ppm.

RESPONSE: *ATSDR does not include the ACGIH threshold limit values if there are federal government occupational limits. Since NIOSH and OSHA have permissible exposure limits and recommended exposure limits for ethylene oxide, the ACGIH values were not added to Table 7-1.*

Appendices

COMMENT 44: Critical materials in the appendices were very helpful to supplement the narratives in Chapters 1 & 2; no suggestions for their improvement.

RESPONSE: *ATSDR thanks the Reviewer for the comment. No response is necessary.*

Peer Reviewer Comments on Unpublished Studies

General Comment

PEER REVIEWER #1 COMMENT: I am neither a veterinarian, a pathologist nor a statistician, so some of the questions posed about the individual studies are not within my domain of expertise.

Comments on Specific Studies and Responses to ATSDR Charge Questions

Celanese Chem Co 1972

PEER REVIEWER #3 COMMENT: This study compared responses of primary skin irritancy in albino rabbits to 18 chemically related alkyl epoxides that included ethylene oxide, formaldehyde, acetic acid, vinyl acetate and acrylates, among others. Test articles (0.5 ml wet or 0.5 g dry) were applied to intact and abraded skin on 6 animals for 4 hours and erythema and edema responses were graded 4, 24 and 72h after application.

Dermal exposure to ethylene oxide resulted in a Primary Irritation Score of 7.8 (maximal score = 8.0). Erythema and edema responses were maximal by 4h (score 7.2-7.5) in both intact and abraded tissue and characterized by subdermal hemorrhage. Injury persisted through 72h by 24h induced chemical burns that were considered irreversible.

Responses across the materials tested ranged from 2.2 to 8.0. There was no discussion of the results by the study authors.

Conclusions: These results provide clear evidence for dermal toxicity of undiluted ethylene oxide to produce acute chemical burns and tissue injury to intact skin. Skin sensitization and dermal uptake with systemic effects were not assessed in this study. The study was strengthened by the comparative nature of the study that assessed responses to multiple related chemicals.

ATSDR CHARGE QUESTION: Did the study use an adequate number of animals and practice good animal care?

PEER REVIEWER #1 COMMENT: 6 rabbits were used per chemical.

PEER REVIWER #2 COMMENT: The study included 6 animals per test substance. This appears to be a sufficient number as the results are highly consistent among animals. There is no guidance on the number of animals in test guideline OECD 404 which was adopted well after this study was done. There is little information in this study on animal care but the procedure detailed is largely consistent with current OECD 404 guideline.

ATSDR CHARGE QUESTION: Did the study account for competing causes of death?

PEER REVIEWER #1 COMMENT: No animals died on study.

PEER REVIWER #2 COMMENT: There were no deaths in this study.

ATSDR CHARGE QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

PEER REVIEWER #1 COMMENT: Only one dose group was reported, with 0.5 mL applied. It was not specified how exactly this was conducted.

PEER REVIWER #2 COMMENT: Only one dose was tested, again, consistent with OECD 404 guideline. This is adequate for the purpose of this study.

ATSDR CHARGE QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

PEER REVIEWER #1 COMMENT: No

PEER REVIWER #2 COMMENT: Not applicable.

ATSDR CHARGE QUESTION: Do you agree with the conclusions of the author? If not, please explain.

PEER REVIEWER #1 COMMENT: Yes. The authors concluded ethylene oxide was corrosive, and caused erythema and edema.

PEER REVIWER #2 COMMENT: Yes, Ethylene Oxide was classified as "corrosive" (see Tables 1 and VI).

Neeper-Bradley and Kubena 1993

PEER REVIEWER #3 COMMENT: This report describes developmental outcomes in CD rats exposed to 0, 50, 125, and 225 ppm ethylene oxide on gestational days (GD) 6 through 15 and sacrificed on GD 21. Maternal body weight and food intake were measured throughout the study and maternal and fetal tissues were evaluated post-necropsy.

Results: Maternal Toxicity: Compared to 0 ppm controls, rats exposed to 250ppm experienced decreased food consumption, body weight, and body weight gain at multiple times throughout the study and at necropsy (final BW 354+/-16.9 vs 380.17 +/-26.5 grams). Decreased body weight was also reported for rats exposed to 125 ppm (364.25 +/-31 grams). Reductions in food consumption in the mid-dose group were not deemed to be exposure related due to the lack of dose-response relationship. Relative, but not absolute, liver weights were increased with 125 and 225 ppm exposures. As such, with effects at 125ppm the authors conclude the NOAEL of 50 ppm for maternal toxicity.

However a caveat for this interpretation is the inclusion of an apparent outlier in the 125ppm group that is categorized as pregnant (P) but whose body and uterus weights suggests otherwise. In Table 2, Page 103 of 312 of Neeper-Bradley, Animal 8279 (top row) has a body weight (259g) and uterus weight (2.449g) that is more consistent with Non-pregnant (NP) in this exposure group. Although a mathematical outlier, these data were included in the Pregnant (P) group for statistical analysis, thus potentially skewing the difference in body and relative liver weights from the control group (0 ppm).

Fetal (Developmental) Toxicity: Compared to 0 ppm controls, fetal body weights were significantly reduced with 50ppm (3.67% decrease), 125ppm (5.23%) and 225ppm (10.02%). Twelve different forms of skeletal variations (ossification deficits) were identified with 225ppm while 3 variations were detected with 125ppm. A constellation of other developmental defects – ecchymosis, under-ossified metcarpals and phalanges and bilobed cervical centra were inconsistent across doses, including 50ppm, but authors concluded these were unrelated to treatment because they lacked a dose-dependent relationship. The authors interpret these findings as a NOAEL of less than 50ppm (i.e. LOAEL of 50 ppm) for developmental toxicity. However they also suggest that the depressed body weight is minor and has little or no biological consequence because it is not associated with skeletal endpoints.

Conclusions:

- Both of the lowest NOAELs from this study (maternal and fetal body weight) are Less Serious and have limitations that would preclude them from being used as a point of departure or to be the sole driver to derive MRLs. These data are however sufficient to be supportive of MRLs that are derived from other studies.
- Additional review of the maternal dataset of the original report (Table 2, Page 103 of 312) is warranted in order to confirm that the appropriate animals (pregnant/non-pregnant) are incorporated in the analysis to validate the NOAEL of 50ppm that the authors propose (detailed above).

Evidence of developmental toxicity at 50ppm is weak/inconclusive, and the data as a whole supports a NOAEL of 50ppm, LOAEL of 125ppm.

ATSDR CHARGE QUESTION: Did the study use an adequate number of animals and practice good animal care?

PEER REVIEWER #1 COMMENT: 25 timed mated pregnant rats were used per group, with exposure concentrations of 0, 50, 125 and 225 ppm ethylene oxide, 6 hours per day, 5 days per week. It appears that an adequate number of dams were used, and that appropriate animal care was used.

PEER REVIWER #2 COMMENT: The study included 25 female rats per dose group. This appears to be a sufficient number as the outcome and design are consistent with current test guideline OECD 414 which calls for “20 female animals with implantation sites at necropsy. Three concentrations, at least, should be used.” In the study, the number of pregnant females in each group was 20 or more and 3 dose groups plus a control group were investigated. This is a GLP study and all appropriate animal care and husbandry details are provided.

ATSDR CHARGE QUESTION: Did the study account for competing causes of death?

PEER REVIEWER #1 COMMENT: No females that died on study.

PEER REVIWER #2 COMMENT: There were no deaths in this study.

ATSDR CHARGE QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

PEER REVIEWER #1 COMMENT: Yes – they included a control and four treatment groups at 0, 50, 125, and 250 ppm.

PEER REVIWER #2 COMMENT: There were no deaths in this study.

ATSDR CHARGE QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

PEER REVIEWER #1 COMMENT: No

PEER REVIWER #2 COMMENT: Not applicable.

ATSDR CHARGE QUESTION: Do you agree with the conclusions of the author? If not, please explain.

PEER REVIEWER #1 COMMENT: Yes

PEER REVIWER #2 COMMENT: Yes, NOEL for developmental toxicity was concluded to be "les sthan 50 ppm".

Tyler and McKelvey 1982

PEER REVIEWER #1 COMMENT: The Reviewer stated "The pdf file labeled Tyler and McKelvey 1982 contained a substantial number of study reports from Union Carbide. I have provided comments or a notation of no comment for each one." The Reviewer identified the individual Union Carbide studies as:

- Ethylene oxide ten-to eleven week vapor inhalation probe study on mice. Snellings, W.M., Project report 45-158
- Ethylene oxide 7 –to eight week vapor inhalation probe study on rats and mice. Snellings, W.M., Project report 45-139
- Ethylene oxide dominant lethal mutagenicity inhalation test in rats. Snellings, W.M., Listed as a draft report, and only a summary was provided.
- Ethylene Oxide Metabolism Report, Intramural Report 40-18
- Metabolism Study on Ethylene Oxide in Conjunction with Dominant Lethal Test. Project Report 41-122
- A Closed, Recirculating, Inhalation Metabolism System for Radiotracer Studies. McKelvey and Tyler, Intramural Report 45-189
- Dose Dependent Disposition of ^{14}C Labeled Ethylene Oxide in Rats, Tyler and McKelvey, Intramural Report 45-190
- LONG-TERM ETHYLENE OXIDE INHALATION STUDY ON RATS: Report on Cytocenetic Studies of Bone Marrow Cells 12-month Sacrifice Interval Project Report 43-58
- ENVIRONMENTAL FATE AND EFFECTS OF ETHYLENE OXIDE. A manuscript submitted for publication
- "Environmental Impact Product Analysis. Acute Toxicity Testing". Progress Report. January 25. 1974. File No. 19133. Research and Development Department. Union Carbide Corporation
- "Environmental Impact Analysis, Product … Biodegradability Testing". Progress Report, August 12. 1974, File No. 19751, Research and Development Department, Union Carbide Corporation

ATSDR RESPONSE: *The citation of Tyler and McKelvey (1982) is for the study identified by the Reviewer as* "Dose Dependent Disposition of ^{14}C Labeled Ethylene Oxide in Rats, Tyler and McKelvey, Intramural Report 45-190".

PEER REVIEWER #1 COMMENT: Regarding the Tyler and McKelvey (1999) study, the Reviewer commented "This describes the use of a closed recirculating system to conduct exposure of 4 male rats to 10, 100, and 1000 ppm ^{14}C ethylene oxide."

PEER REVIEWER #3 COMMENT: This report describes toxicological outcomes in B63CF1 mice exposed to 0, 10, 50, 100 or 250ppm for 10 (male) and 11 (female) weeks. Body weight, food consumption, and neurobehavioral (Irwin Screen) endpoints were collected at interim times of the study, while additional clinical and pathological data were collected 18-24h after the last exposure.

Results: Irwin Screen Observations (Appendix, pp49-54): Abnormal outcomes for locomotor activity, righting reflex, and pinch reflex were documented after 5 weeks of exposure to 250ppm (only 0 and 250ppm groups were assessed at this timepoint). All groups were included in assessments conducted after 7 and 10 weeks of exposure. All animals in the 250ppm group (n=5) showed the same neurobehavioral deficits as observed at these later timepoints, while responses varied among other ethylene oxide groups over time. By the last week of exposure, reduced locomotor activity (males) and abnormal gait (females) were significantly reduced in 50, 100, and 250ppm groups with the control group having no responders (0/5).
LOAEL of 50 ppm and NOAEL = 10ppm

Low animal numbers per group (n=5) required responses in at least 4 animals to reach statistical significance. With some endpoints at some intermediate times, 1 or 2 control animals were documented as effected so even 5/5 responders in ethylene oxide-exposed groups were not significantly different from controls. However at necropsy when the control group was observed to have no adverse effects (0/5), a dose-dependent effect was evident. Furthermore the authors caution that interpretation is also problematic because assessing locomotor activity and gait is subjective.

Clinical: Body weight, urinalysis, and hematological endpoints were mildly affected in the 250ppm group.

Necropsy/Histopathology: Absolute and relative spleen weights were decreased after exposure to 100 (female) and 250 ppm (male and female). Hemoglobin-associated endpoints were also reduced in the 250ppm group. The authors deemed other organ weight changes (liver, brain, testes) with 250ppm as not biologically important. No remarkable histopathological findings were noted.

Conclusions: Neurological effects are clearly evident from these studies and are even suggestive of dose- and time-dependence. However, the current dataset has limitations of animal numbers and statistical power, the subjective nature of determining locomotor activity and gait analysis (two effects reported below 250ppm), and the lack of corresponding histopathological findings. While a number of Irwin Screening endpoints were negative, the two subjective endpoints were consistently affected and showed statistical significance for 50 ppm and above by the last measurement prior to necropsy.

ATSDR CHARGE QUESTION: Did the study use an adequate number of animals and practice good animal care?

PEER REVIEWER #2 COMMENT: The study included 10 animals per sex and dose group for each of the major sample collections at the end of the study. This appears to be consistent with the subchronic inhalation toxicity guidelines (OECD 413); however, duration of the studies was inconsistent – less than 10 weeks for males (first and last weeks of exposures were partial weeks) and less than 11 weeks for females which is inconsistent with recommebded 90-day exposure. This is a well-documented study which was consistent with GLP practice of documentation.

ATSDR CHARGE QUESTION: Did the study account for competing causes of death?

PEER REVIEWER #2 COMMENT: Some deaths occurred during the study but those were attributed to non-exposure related causes.

ATSDR CHARGE QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

PEER REVIEWER #2 COMMENT: Four doses plus controls was tested, exceeding the requirements of OECD 413 guidelines. This is adequate for the purpose of this study.

ATSDR CHARGE QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

PEER REVIEWER #2 COMMENT: Not applicable.

ATSDR CHARGE QUESTION: Do you agree with the conclusions of the author? If not, please explain.

PEER REVIEWER #2 COMMENT: The study did not derive a NOEL, only commented on the potential "biological" relevance of the results. The arguments for the lack of "biological relevance" are questionable for some of the effects. This reviewer believes the reduction in spleen weights and erythropoiesis effects in the hematology data are concordant and indicative of an adverse effect. Locomotor activity data are also pointing to a dose-related adverse effect. Other findings are more difficult to interpret.

Union Carbide Corp 1983:

PEER REVIEWER #1 COMMENT: The pdf file labeled Union Carbide Corp 1983 contained a single study report from Union Carbide. This report had been included in Tyler and McKelvey 1982. LONG-TERMETHYLENE OXIDE INHALATION STUDY ON RATS: Report on Cytogenetic Studies of Bone Marrow Cells 12-Month Sacrifice Interval Project Report 43-58. No further comment on this report

The Reviewer provided the following comment on this report "This is a limited report, and indicates that: 'A detailed description of the dosing methods. sample characterization, results and conclusions of the chronic inhalation study will be included in the final report of the two-year study.'"

PEER REVIEWER #3 COMMENT: This report describes genetic toxicity in bone marrow-derived cells from F344 rats exposed to 100ppm ethylene oxide for 1 year. Triethylenemelamine was used as a positive control for data of chromatid and chromosomal gaps/breaks, 6 categories of chromosomal aberrations and abnormal nuclear pathologies.

Results: Rates of chromosomal aberrations with ethylene oxide exposure were 4% (males) and 6.7% (females) and were not statistically different from air controls (3.1 and 3.2% respectively). By comparison, rats treated with triethylenemelamine had rates of 62.6 and 53.5%.

Conclusions:

- The authors express concern that background rates of aberrations for 0ppm (air) exposed rats is greater than historical data generated from other research labs. Factors of advanced age and isolation techniques are suggested as possible contributions to increases above 0.15-0.44% levels seen in control animals from other groups. However others had reported background values of 1 to 5%.

With up to 11 animals per group the statistical power of this study should be sufficient to accept the results despite the non-parametric nature of frequency data. Slides were adequately blinded and double coded to limit bias in counting procedures. Counting more cells (current cells/rat =50) would not likely improve the variability in the groups. Limitations of lack of dose response in both the ethylene oxide and positive control is minor. These data are acceptable and suggest that 100ppm ethylene oxide does not induce chromosomal aberration in bone marrow cells. This finding is consistent with Lynch et al 1984b which reports no neoplasia in bone marrow (myeloid leukemia) in F344 rats exposed to 50 or 100ppm for 2 years.

ATSDR CHARGE QUESTION: Did the study use an adequate number of animals and practice good animal care?

PEER REVIEWER #2 COMMENT: The study included 10-11 animals per sex. This appears to be a sufficient number as the current OECD test guideline 475 requires at least 5 animals per sex/dose. There is little information in this study on animal care and it is not clear whether the animals were treated with a metaphase-arresting agent before sacrifice. No details on husbandry or treatment have been provided in this report, albeit this was part of a 2-year study that was reported elsewhere and may have included necessary details. On its own merits, this report is not well documented.

ATSDR CHARGE QUESTION: Did the study account for competing causes of death?

PEER REVIEWER #2 COMMENT: No information was provided in this report on animal deaths.

ATSDR CHARGE QUESTION: Did the study include a sufficient number of dose groups, and sufficient magnitude of dose levels?

PEER REVIEWER #2 COMMENT: Only one dose was tested, 100 ppm.

ATSDR CHARGE QUESTION: If you think the study was not adequately designed or reported, does that negate the utility of the study? Please explain.

PEER REVIEWER #2 COMMENT: While the study was not adequately reported, the data are clear and useful

ATSDR CHARGE QUESTION: Do you agree with the conclusions of the author? If not, please explain.

PEER REVIEWER #2 COMMENT: There was clear response to positive control. EO treatment resulted in non-significant elevations in chromosomal aberrations in either males or females.