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WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

December 21, 2007

EPA-SAB-08-004

Honorable Stephen L. Johnson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Subject: Review of Office of Research and Development (ORD) draft assessment entitled, "Evaluation of the Carcinogenicity of Ethylene Oxide".

Dear Administrator Johnson:

In response to a request from EPA's Office of Research and Development (ORD), the Science Advisory Board (SAB) convened an expert panel to conduct a peer review of EPA's draft assessment entitled, "Evaluation of the Carcinogenicity of Ethylene Oxide". EPA last published an assessment of the potential carcinogenicity of Ethylene Oxide (EtO) in 1985. The current assessment evaluates the more recent database on the carcinogenicity of EtO and focuses on lifetime cancer risk from inhalation exposure.

The SAB was asked to comment on three issues, including carcinogenic hazard, derivation of a cancer unit risk value for inhalation exposure to EtO, and uncertainties associated with the carcinogenicity assessment. The report contains a number of recommendations that are aimed at making the assessment more transparent and improve the scientific bases for the conclusions presented. Appendices authored by two panel members are also included to provide further discussion of the issues where the Panel had divergent opinions, e.g., low dose extrapolation and the healthy worker survivor effect. The Panel's key recommendations are highlighted below.

A majority of the Panel agreed with the conclusion in the draft document that the available evidence supports a descriptor of "Carcinogenic to Humans" although some Panel members concluded that the descriptor "Likely to be Carcinogenic to Humans" was more appropriate. There was consensus that the epidemiological data regarding ethylene oxide carcinogenicity were not in and of themselves sufficient to provide convincing evidence of a causal association between human exposure and cancer. Differing views as to the appropriate carcinogenicity descriptor for ethylene oxide were based on differences of opinion as to whether criteria necessary for designation as "Carcinogenic to Humans" in the absence of conclusive evidence from epidemiologic studies were met. The majority of Panel members thought that the combined weight of the epidemiological,

experimental animal, and mutagenicity evidence was sufficient to conclude that EtO is carcinogenic to humans.

The Panel concluded that the assessment would be improved by: 1) a better introduction to the hazard characterization section, including a brief description of the information that will be presented; 2) a clear articulation of the criteria by which epidemiologic studies were judged as to strengths and weaknesses; 3) addition of a more inclusive summary figure and/or table at the beginning of section 3.0; and 4) inclusion and expansion of material now provided in Appendix A of the draft assessment to within the main body of that assessment.

The Panel concurred that the NIOSH cohort is the best single epidemiological data set with which to study the relationship of cancer mortality to the full range of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly consider all of the epidemiological data in developing its final Assessment.

The Panel identified several important shortcomings in the linear regression modeling approach used to establish the point of departure for low dose extrapolation of cancer risk due to EtO. The Panel was unanimous in its recommendation that the EPA develop its risk models based on direct analysis of the individual exposure and cancer outcome data for the NIOSH cohort rather than the approach based on grouped data that is presently used.

The Panel was divided on whether low dose extrapolation of risk to environmental EtO exposure levels should be linear (following Cancer Guideline defaults for carcinogenic agents operating via a mutagenic MOA) or whether plausible biological mechanisms argued for a non-linear form for the low dose response relationship. With appropriate discussion of the statistical and biological uncertainties, several Panel members strongly advocated that both linear and nonlinear calculations be considered in the final EtO Risk Assessment.

The Draft Assessment characterizes the magnitude of the risk associated with EtO as “weak”. This finding is well substantiated by the epidemiologic evidence where a relatively small number of excess cancers are found above background even among highly exposed individuals. However, the magnitude of risk suggested by the unit risk estimate is somewhat at odds with this concept. In our report, we provide specific recommendations for addressing this apparent inconsistency.

In accordance with EPA guidance, the draft assessment applied an Age Dependent Adjustment Factor (ADAF) to adjust the unit risk for early life exposure. While the Panel felt that the application of a default value by the Agency was appropriate, the description in the Draft Assessment was not adequate, particularly for those not familiar with the EPA Guidance.

The Panel appreciates both the public health and economic significance of EPA’s EtO risk assessment. A more thorough discussion of the Panel’s recommendations is included in the body of the report. Some of the Panel’s recommendations, such as the reanalysis of the NIOSH cohort using data from individuals, will require significant effort.

The Panel encourages the Agency to devote sufficient resources to make implementation of these recommendations possible. We look forward to receiving the Agency's response and appreciate the opportunity to provide EPA with advice on this important subject.

Sincerely,

/Signed/

Dr. Stephen Roberts, Chair
SAB Ethylene Oxide Review Panel

/Signed/

Dr. Granger Morgan, Chair
EPA Science Advisory Board

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Ethylene Oxide Review Panel*

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EXECUTIVE SUMMARY

EPA's Office of Research and Development (ORD) requested that the Science Advisory Board (SAB) review its draft assessment entitled, "Evaluation of the Carcinogenicity of Ethylene Oxide." EPA last published a health assessment of the potential carcinogenicity of Ethylene Oxide (EtO) in 1985 (U.S. EPA, 1985). EPA'sORD completed a review of the more recent database on the carcinogenicity of EtO, pertinent data from the 1985 assessment, and several reviews and assessments issued by other organizations. The Agency's Draft Assessment focuses on lifetime cancer risk from inhalation exposure. The EtO Review Panel of the EPA Science Advisory Board met in January 2007 to deliberate on charge questions raised by ORD. These questions focused on three issues, including carcinogenic hazard, derivation of a cancer unit risk value for inhalation exposure to EtO, and uncertainties associated with the analysis.

This Executive Summary highlights the outcome of the Panel's deliberations. It includes the context for the charge questions and issues raised for consideration by EPA, and the conclusions reached by the SAB Review Panel. While the Agency requested that the Panel respond to three separate multi-part charge questions, the Panel has presented their response to the third charge question in the context of each of the other two charge questions. Therefore, this report is structured so that the comments concerning Uncertainty (Issue 3) are integrated in the responses to the Carcinogenic Hazard (Issue 1) and Risk Estimation (Issue 2) sections.

Issue 1: Carcinogenic Hazard (Section 3 and Appendix A of the EPA Draft Assessment)

1. Do the available data and discussion in the draft document support the hazard conclusion that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's 2005 Guidelines for Carcinogen Risk Assessment? In your response, please include consideration of the following:

1. a. EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but less than completely conclusive. Does the draft document provide sufficient description of the studies, balanced treatment of positive and negative results, and a rigorous and transparent analysis of the data used to assess the carcinogenic hazard of ethylene oxide (EtO) to humans? Please comment on the EPA's characterization of the body of epidemiological data reviewed. Considerations include: a) the consistency of the findings, including the significance of differences in results using different exposure metrics, b) the utility of the internal (based on exposure category) versus external (e.g., SMR and SIR) comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the epidemiological evidence.

A majority of the Panel agreed with the conclusion in the draft document that the available evidence supports a descriptor of "Carcinogenic to Humans" although some Panel members concluded that the descriptor "Likely to be Carcinogenic to Humans" was more appropriate. There was consensus that the epidemiological data regarding ethylene oxide carcinogenicity were not in and of themselves sufficient to provide convincing evidence of a causal association between human exposure and cancer. Differing views as to the appropriate descriptor for ethylene oxide were based on differences of opinion as to whether criteria necessary for designation as "Carcinogenic to Humans" in the absence of conclusive evidence from epidemiologic studies were met. The majority of Panel members thought that the combined

weight of the epidemiological, experimental animal, and mutagenicity evidence was sufficient to conclude that EtO is carcinogenic to humans.

The Panel concluded that the assessment would be improved by: 1) a better introduction to the hazard characterization section, including a brief description of the information that will be presented; 2) a clear articulation of the criteria by which epidemiologic studies were judged as to strengths and weaknesses; 3) addition of a more inclusive summary figure and/or table at the beginning of section 3.0; and 4) inclusion of material now provided in Appendix A of the draft assessment to within the main body of that assessment.

The Panel agreed with the EPA in their reliance on "internal" estimates of cancer rates rather than "external" comparisons (SMR, SIR) due to well recognized limitations to the latter method of analysis.

The Draft Assessment characterizes the magnitude of the unit risk estimate associated with EtO as "weak". This finding is substantiated by the epidemiologic evidence where a relatively small number of excess cancers are found above background even among highly exposed individuals. However, the magnitude of risk suggested by the unit risk estimate is somewhat at odds with this concept. Subsequent recommendations in our report try to address this apparent inconsistency.

1.b. Are there additional key published studies or publicly available scientific reports that are missing from the draft document and that might be useful for the discussion of the carcinogenic hazard of EtO?

The Panel agreed that the discussion of endogenous metabolic production of ethylene oxide and the formation of background adducts should be expanded.

The Panel believed that the description of studies of DNA adduct formation resulting from EtO exposure appears incomplete and superficial. This discussion should be expanded – both in terms of the number of studies cited and the depth of the discussion.

Since ethylene is metabolized to EtO, some members recommended the inclusion of the ethylene body of literature for consideration. Most members were hesitant about adding them to the document, but if added, they cautioned that a discussion of the caveats associated with their interpretation relative to ethylene oxide should be included.

1.c. Do the available data and discussion in the draft document support the mode of action conclusions?

The Panel agreed with the Draft Assessment conclusion of a mutagenic mode of action. However, an expanded discussion of the formation of DNA adducts and mutagenicity is warranted.

1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced and sound description that synthesizes the human, laboratory animal, and supporting (e.g., in vitro) evidence for human carcinogenic hazard?

While some members of the Panel found the hazard characterization section of the Draft Assessment to be satisfactory, a majority expressed concerns that this section did not achieve the necessary level of rigor and balance. An issue in this characterization, particularly in the face of epidemiological data that are not strongly conclusive, is whether the presumed precursor events leading to cancer in animals, such as mutations and/or chromosomal aberrations, are observed in humans. This issue needs to be addressed in greater detail.

Issue 2: Risk Estimation (Section 4 and Appendices C and D of the EPA Draft Assessment)

2. Do the available data and discussion in the draft document support the approaches taken by EPA in its derivation of cancer risk estimates for EtO? In your response, please include consideration of the following:

2.a. EPA concluded that the epidemiological evidence alone was strong but less than completely conclusive (although EPA characterized the total evidence - from human, laboratory animal, and in vitro studies - as supporting a conclusion that EtO as "carcinogenic to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003, 2004) data set, the most appropriate for estimating the magnitude of the carcinogenic risk to humans from environmental EtO exposures? Are the scientific justifications for using this data set transparently described? Is the basis for selecting the Steenland et al. data over other available data (e.g., the Union Carbide data) for quantifying risk adequately described?

The Panel concurred that the NIOSH cohort is the best single epidemiological data set with which to study the relationship of cancer mortality to the full range of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly consider all of the epidemiological data in developing its final Assessment. In particular, the Panel encourages the EPA to explore uses for the Greenberg et al. (1990) data including leukemia and pancreatic cancer mortality and EtO exposures for 2174 Union Carbide workers from its two Kanawha Valley, West Virginia facilities. (Also described in Teta et al. 1993; Teta et al., 1999).

The Panel encouraged the EPA to investigate potential instability that may result from interaction between the chosen time metric for the dose response model and the treatment of time in the estimated exposure (i.e., log cumulative exposure with 15 year lag) that is the independent variable in that dose-response model.

2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of a linear regression model fit to Steenland et al.'s categorical results for all lymphohematopoietic cancer in males in only the lower exposure groups scientifically and statistically appropriate for estimating potential human risk at the lower end of the observable range? Is the use of the grouping of all lymphohematopoietic cancer for the purpose of estimating risk appropriate? Are there other appropriate analytical approaches that should be considered for estimating potential risk in the lower end of the observable range? Is EPA's choice of a preferred model adequately supported and justified? In particular, has EPA adequately explained its reasons for not using a quadratic model approach such as that of Kirman et al. (2004) based? What recommendations would you make regarding low-dose extrapolation below the observed range?

The Panel identified several important shortcomings in the linear regression modeling approach used to establish the point of departure for low dose extrapolation of cancer risk due to EtO. The Panel was unanimous in its recommendation that the EPA develop its risk models based on direct analysis of the individual exposure and cancer outcome data for the NIOSH cohort rather than the approach based on published grouped data that is presently used. The suggested analysis will require EPA to acquire or otherwise access individual data and develop appropriate methods of analysis. The panel recommends that the Agency allocate the appropriate resources to conduct this analysis.

The Panel was divided on whether low dose extrapolation of risk due to environmental EtO exposure levels should be linear (following Cancer Guideline defaults for carcinogenic agents operating via a mutagenic MOA) or whether plausible biological mechanisms argued for a non-linear form for the low dose response relationship. With appropriate discussion of the statistical and biological uncertainties, several Panel members strongly advocated that both linear and nonlinear calculations be considered in the final EtO Risk Assessment.

In conjunction with its recommendation to use the individual NIOSH cohort data to model the relationship of cancer risk to exposures in the occupational range, the Panel recommended that the Agency explore the use of the full NIOSH data set to estimate the cancer slope coefficients that will in turn be used to extrapolate risk below the established point of departure. The use of different data to estimate different dose response curves should be avoided unless there is both strong biologic and statistical justification for doing so. The Panel believed this justification was not made in the Agency's draft assessment.

Although the analysis based on total lymphohematopoietic (LH) cancers might have value as part of a complete risk assessment, the rationale for this aggregate grouping needs to be better justified. The Panel recommends that data be analyzed by subtype of LH cancers (e.g. lymphoid, myeloid) and strong consideration be given to these more biologically justified groupings as primary disease endpoints.

The Panel was divided in its views concerning the appropriateness of estimating the population unit risk for LH cancer based only on the NIOSH data for males. Several Panel members pointed out that a standard approach in cancer epidemiology and risk analysis begins by conducting separate dose-response analyses on males and females and combining the data only if the results are similar. Conducting separate analyses for males and females is also the standard practice when analyzing data from animal carcinogenicity bioassays. A second approach to dealing with the possibility of gender differences in response is to include gender as a fixed effect in the statistical modeling of the data and determine whether gender or its interaction with other predictors (e.g., gender x exposure) are significant explanatory variables. If so, the combined model with the estimated gender effects could be used directly or separate, gender-specific dose-response analysis would be performed. If not, the gender effects could be dropped and the model re-estimated for the combined male and female data. In addition, the Agency should test whether the male/female differences are mitigated by use of alternate disease endpoints discussed in the previous paragraph.

2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk estimate, in accordance with EPA's Supplemental Guidance (U.S. 2005b), appropriate and transparently described?

In accordance with EPA guidance, the Draft Assessment applied an Age Dependent Adjustment Factor (ADAF) to adjust the unit risk for early life exposure. While the majority of the Panel felt that the application of a default value by the Agency was appropriate due to lack of data, the description in the Draft Assessment was not adequate, particularly for those not familiar with the EPA's Supplemental Guidance.

2.d. Is the use of different models for estimation of potential carcinogenic risk to humans from the higher exposure levels more typical of occupational exposures (versus the lower exposure levels typical of environmental exposures) appropriate and transparently described in Section 4.5?

While the method was transparently described, most of the Panel did not agree with the estimation based on two different models for two different parts of the dose response curve (see response to 2b). The use of different data to estimate different dose response models curves should be avoided unless there is both strong biological and statistical justification for doing so. The Panel believed this justification was not made in the Agency's draft report.

2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data appropriate and transparently described? Is the use of "ppm equivalence" adequate for interspecies scaling of EtO exposures from the rodent data to humans?

The ppm equivalence method is a reasonable approach for interspecies scaling of EtO exposures from rodent data to humans. If the use of animal data becomes more important (i.e., the principal basis for the ethylene oxide unit risk value), more sophisticated approaches such as PBPK modeling should be considered.

INTRODUCTION

This report was prepared by the Science Advisory Board (SAB) Ethylene Oxide (EtO) Review Panel (the "Panel") in response to a request by EPA's Office of Research and Development (ORD) to review their draft Evaluation of the Carcinogenicity of Ethylene Oxide. According to the document, EPA last published an assessment of the potential carcinogenicity of EtO in 1985. The current assessment reviews the more recent database on the carcinogenicity of EtO.

EtO is a gas at room temperature. It is manufactured from ethylene and used primarily as a chemical intermediate in the manufacture of ethylene glycol. It is also used as a sterilizing agent for medical equipment and as a fumigating agent for spices. The largest sources of human exposure are in occupations involving contact with the gas in plants (facilities) and in hospitals that sterilize medical equipment. EtO can also be inhaled by residents living near production or sterilizing/fumigating facilities. The Draft Assessment describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on human epidemiological data.

ORD identified 3 issues where they were seeking the SAB's advice and recommendations. These included the proposed carcinogenic hazard, risk calculations and uncertainty. The SAB EtO Review Panel was asked to comment on the scientific soundness of this risk assessment.

The Panel deliberated on the charge questions during their January 18-19, 2007 face-to-face meeting and during a conference call on May 29, 2007. The responses that follow represent the views of the Panel. In all cases, there was agreement by a majority of the Panel members as to a particular recommendation. In some cases, there were some Panel members that had a differing point of view. These instances have been noted throughout the report and are described in more detail in appendices authored by two panel members to provide further discussion of the issues where the Panel had divergent opinions, e.g., low dose extrapolation and the healthy worker survivor effect (Appendix A- Discussion of the Resurgent Controversy over Thresholds for Genetically Acting Agents and Appendix B- Illustration of a Simple Approach for Approximately Assessing the Effect of Measurement/Estimation Uncertainties for Individual Worker Exposures on Estimates of Dose Response Slopes and Appendix C- Framework Analysis of Genotoxicity and Risk Assessment).

The specific charge questions to the Panel are as follows:

Charge Questions

The memo requesting this review along with the complete charge to the Panel can be found in its entirety in Attachment 1. Below is an abbreviated version of the charge questions.

Issue 1: Carcinogenic Hazard (Section 3 and Appendix A of the EPA Draft Assessment)

1. Do the available data and discussion in the draft document support the hazard conclusion that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*? In your response, please include consideration of the following:

1.a. EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but less than completely conclusive. Does the draft document provide sufficient description of the studies, balanced treatment of positive and negative results, and a rigorous and transparent analysis of the data used to assess the carcinogenic hazard of ethylene oxide (EtO) to humans? Please comment on the EPA's characterization of the body of epidemiological data reviewed. Considerations include: a) the consistency of the findings, including the significance of differences in results using different exposure metrics, b) the utility of the internal (based on exposure category) versus external (e.g., SMR and SIR) comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the epidemiological evidence.

1.b. Are there additional key published studies or publicly available scientific reports that are missing from the draft document and that might be useful for the discussion of the carcinogenic hazard of EtO?

1.c. Do the available data and discussion in the draft document support the mode of action conclusions?

1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced and sound description that synthesizes the human, laboratory animal, and supporting (e.g., *in vitro*) evidence for human carcinogenic hazard?

Issue 2: Risk Estimation (Section 4 and Appendices C and D of the EPA Draft Assessment)

2. Do the available data and discussion in the draft document support the approaches taken by EPA in its derivation of cancer risk estimates for EtO? In your response, please include consideration of the following:

2.a. EPA concluded that the epidemiological evidence alone was strong but less than completely conclusive (although EPA characterized the total evidence - from human, laboratory animal, and *in vitro* studies - as supporting a conclusion that EtO as "carcinogenic to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003, 2004) data set**,** the most appropriate for estimating the magnitude of the carcinogenic risk to humans from environmental EtO exposures? Are the scientific justifications for using this data set transparently described? Is the basis for selecting the Steenland et al. data over other available data (e.g., the Union Carbide data) for quantifying risk adequately described?

2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of a linear regression model fit to Steenland et al.'s categorical results for all lymphohematopoietic cancer in males in only the lower exposure groups scientifically and statistically appropriate for estimating potential human risk at the lower end of the observable range? Is the use of the grouping of all lymphohematopoietic cancer for the purpose of estimating risk appropriate? Are there other appropriate analytical approaches that should be considered for estimating potential risk in the lower end of the observable range? Is EPA's choice of a preferred model adequately supported and justified? In particular, has EPA adequately explained its reasons for not using a quadratic model approach such as that of Kirman et al. (2004) based? What recommendations would you make regarding low-dose extrapolation below the observed range?

2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk estimate, in accordance with EPA's Supplemental Guidance (U.S. 2005b), appropriate and transparently described?

2.d Is the use of different models for estimation of potential carcinogenic risk to humans from the higher exposure levels more typical of occupational exposures (versus the lower exposure levels typical of environmental exposures) appropriate and transparently described in Section 4.5?

2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data appropriate and transparently described? Is the use of "ppm equivalence" adequate for interspecies scaling of EtO exposures from the rodent data to humans?

Issue 3: Uncertainty (Sections 3 and 4 of the EPA Draft Assessment)

1. EPA's *Risk Characterization Handbook* requires that assessments address in a transparent manner a number of important factors. Please comment on how well this assessment clearly describes, characterizes and communicates the following:
a. The assessment approach employed;
b. The use of assumptions and their impact on the assessment;
c. The use of extrapolations and their impact on the assessment;
d. Plausible alternatives and the choices made among those alternatives;
e. The impact of one choice versus another on the assessment;
f. Significant data gaps and their implications for the assessment;
g. The scientific conclusions identified separately from default assumptions and policy calls;
h. The major risk conclusions and the assessor's confidence and uncertainties in them, and;
i. The relative strength of each risk assessment component and its impact on the overall assessment.

RESPONSES TO THE CHARGE QUESTIONS

Specific responses to each of the charge questions are presented below. The Panel has responded to Charge Questions 1 and 2 and has tried to incorporate their comments regarding Charge Question 3 within those responses. A separate response for Charge Question 3 was not deemed necessary since issues of uncertainty were addressed in the responses to charge questions 1 and 2.

Charge Question 1- Hazard Descriptor

The Agency's assessment concludes that in accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EtO was characterized as carcinogenic to humans based on the total weight of evidence. This evidence, as assessed by EPA, included: a) strong, though less than completely conclusive, evidence of carcinogenicity from human studies; b) sufficient evidence of carcinogenicity in laboratory animals; c) EtO is a direct-acting alkylating agent with clear evidence of mutagenicity/genotoxicity, and there is sufficient evidence that DNA adduct formation and the resulting mutagenic/genotoxic effects are key events in the mode of action of EtO carcinogenicity; d) evidence of chromosome damage in humans exposed to EtO, supporting the inference that the same mode of action for EtO carcinogenicity is operative in humans.

1. Do the available data and discussion in the draft document support the hazard conclusion that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's 2005 Guidelines for Carcinogen Risk Assessment? In your response, please include consideration of the following:

1.a. Qualitative Characterization of Epidemiology Data

EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but less than completely conclusive. Does the draft document provide sufficient description of the studies and transparent analysis of the data used to assess the carcinogenic hazard of EtO to humans? Please comment on the EPA's characterization of the body of epidemiological data reviewed. Considerations include:

a) the consistency of the findings, including the significance of differences in results using different exposure metrics, b) the utility of the internal (based on exposure category) versus external (e.g., SMR and SIR) comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the epidemiological evidence.

A majority of the Panel agreed with the conclusion in the draft document that the available evidence supports a descriptor of "Carcinogenic to Humans" but some of Panel members concluded that the descriptor "Likely to be Carcinogenic to Humans" was more appropriate. The consensus of the Panel was that the epidemiological data regarding ethylene oxide carcinogenicity did not provide convincing evidence of a causal association between human exposure and cancer. The differing views as to the appropriate descriptor for ethylene

oxide were based on whether all of the requirements for designation as "Carcinogenic to Humans" in the absence of convincing epidemiological evidence were met. Panel members favoring a descriptor of "Carcinogenic to Humans" found the epidemiological evidence for an association between ethylene oxide exposure and cancer to be adequate, albeit not strong enough to assert causality. Other Panel members found the epidemiological evidence to be weak, lacking consistency across multiple studies, and they concluded that the data were currently insufficient to conclude that key precursor events were observed in humans.

The Panel believes that the document would be improved by a better introduction to the hazard characterization section, including a brief description of the information that will be presented. EPA has provided a comprehensive review (when the Draft Assessment as a whole is considered) of the existing epidemiologic evidence relevant to ethylene oxide and a fair, transparent, and critical assessment of this evidence for purposes of classifying EtO as a human carcinogen. Presentation of the epidemiologic evidence would be strengthened by including a summary figure and/or table at the beginning of section 3.0. In particular, the authors should include the material now provided in Appendix A of the Draft Assessment to within the main body of that Assessment. These tables should also provide clearer information on the observed endpoints, in particular any information regarding cancer type within the broad category of lymphohematopoietic cancers.

Based on this review, the Agency's assessment that the evidence is "strong but less than completely conclusive" is supported although a characterization of the epidemiologic evidence as "strong" is questionable. This ambiguity and the "less than completely conclusive" assessment is appropriate given the uncertainties and inconsistencies in the occupational epidemiology as is accurately summarized on page 11 of the Draft Assessment "3.1.1 Conclusions Regarding the Evidence of Cancer in Humans." EPA has both appropriately applied the Hill criteria (Bradford-Hill, 1965) to assess causality and correctly interpreted their application to the existing data. EPA's determination of EtO as a human carcinogen is robust in that this conclusion is sustained by the largest and highest quality study (i.e., the NIOSH study) under a variety of approaches to exposure classification. EPA appropriately identifies Steenland et al. as the critical study for establishing human carcinogenicity. We agree with EPA in their reliance on "internal" estimates of cancer rates rather than "external" comparisons (SMR, SIR) due to well recognized limitations to the latter method of analysis. The Draft Assessment characterizes the magnitude of the risk associated with EtO as "weak". This finding is well substantiated by the epidemiologic evidence where a relatively small number of excess cancers are found above background even among highly exposed individuals. A more comprehensive discussion with additional perspective can be provided by comparing EtO's unit risk to other similar carcinogens such as benzene, 1,3-butadiene, and/or formaldehyde.

The EPA's reliance on the NIOSH studies in providing a robust basis for assessment is well justified based on the sample size and available quantitative exposure data. In this study, the strongest exposure response associations were found with log cumulative exposure rather than average or peak exposure. Such a basis for exposure classification is well supported for a chronic effect such as cancer. The Draft Assessment describes both the internal and external cancer rates reported within the literature. This is appropriate both for providing an accurate summary and for addressing the different dimensions of EPA's evaluation, i.e. strength of evidence and unit risk estimate. There was a strong sense among the Panel that the EPA's risk

characterization could be improved by additional analyses of the raw NIOSH data, taking into account the individual exposure data in the dose-response model

1.b. Relevant Additional Key Studies

Are there additional key published studies or publicly available scientific reports that are missing from the draft document and that might be useful of the discussion of the carcinogenic hazard of EtO?

Although the Draft Assessment generally provides a clear and concise summary of the literature regarding EtO, the Panel identified two areas that deserve a more expansive treatment. First, endogenous production of EtO results in some measure of background DNA adducts and this issue should be addressed more fully in the document. The presentation of data from a single reference (Bolt, 1996) giving background levels of 7-HEG in unexposed humans suggests that (i) these values are the most reliable and (ii) the potential impact of spontaneous hydroxyethylation of DNA by endogenously formed EtO has little to no importance in the estimation of human cancer risk for this chemical. However, it has been known for nearly 20 years that endogenous formation of ethylene and conversion to EtO leads to 2-hydroxyethylation of DNA yielding background levels of 7-HEG in unexposed humans and rodents (Föst et al., 1989; Walker et al., 1992b, 2000; Cushnir et al., 1993; Farmer et al., 1993; van Delft et al., 1993, 1994; Leutbecher, 1995; Bolt et al., 1997; Wu et al., 1999; Zhao et al., 1999). Table V in Walker et al. (2000) lists a series of studies of background levels of these adducts in differing tissues of unexposed humans (see references therein), showing that lower spontaneous levels of 7-HEG have been typically found using more sensitive detection methods than those used in reports cited in Bolt's commentary (1996) (see references therein). In another commentary/review, Farmer and Shuker (1999) suggest that in order to estimate the increase in cancer risk attributable to a given external exposure, it is clearly important to establish and consider background levels of corresponding DNA damage so that the scale of the incremental increase can be calculated. It is mainly for this reason that more sensitive and specific analytical methods have been developed for the measurement of background and EtO treatment-induced levels of 7-HEG than for any single other DNA adduct (supporting references available). Because the levels of background 7-HEG are fairly substantial, and there are no chemical differences in DNA damage by endogenous versus exogenous EtO, the Draft Assessment requires a section considering the potential impact of endogenous versus exogenous EtO exposure that carefully lays out (i) why the current evidence of background levels of 2-hydroxyethylation of DNA does not constitute a threshold and (ii) whether the magnitude and variability in endogenous EtO-induced damage may overwhelm any contribution from exogenous EtO exposure (other than some acute high-dose exposure).

Second, a more comprehensive discussion of the production of DNA adducts by EtO exposure would be appropriate. For the last paragraph of section 3.3.1 (page 21), a report by Dan Segerbäck (1990) showed that treatment of calf thymus DNA with ^{14}C-labelled EtO resulted in the formation of N7-HEG, N3-HEA, and O^6-HEG at a ratio of 200:8.8:1. The Draft Assessment suggested that this ratio of DNA adducts was found in a study of EtO-exposed rats by Zhao et al. (1997); however, N7-HEG was the only product of EtO-induced hydroxyethylation measured in this study. Instead, Walker et al. (1992b) found that the ratio of the steady-state concentrations of 7-HEG, 3-HEA, and O^6-HEG was 300:1.2:1 following repeated exposures of rats to EtO,

indicating that 3-HEA and O^6-HEG do not accumulate in vivo to the levels predicted by the in vitro ratios of these adducts and 7-HEG. The same misquoting of Zhao et al., (1997) about the ratio of these three DNA adducts is present beginning on the last line of page 21 of the Draft Assessment.

Finally, some Panel members supported the inclusion of the cancer bioassay results for ethylene exposure and believed they were relevant and should be discussed in the Draft Assessment. However, others on the Panel were less enthusiastic about this addition and felt that, were the ethylene results to be included, a careful discussion of the caveats to their interpretation relative to EtO carcinogenicity would be essential. The rationale for including the bioassay results for inhalation exposure of F344 rats to ethylene (Hamm et al., 1984) is as follows. There were no treatment- or dose-dependent increases in the induction of neoplasms following 2 years of exposure to 0, 300, 1000, or 3000 ppm ethylene, suggesting that the levels of in vivo formation of EtO during exogenous exposures to ethylene were insufficient to have carcinogenic effects. In vivo metabolism of ethylene at high exogenous exposures (>1000 ppm) is saturated and EtO is formed at the highest rate possible in the rat, with ethylene concentrations higher than 1000 ppm corresponding to exogenous exposure to approximately 6 ppm EtO based upon N7-(2-hydroxyethyl)valine values and a two-compartment model (Bolt and Filser, 1987; Czanády et al., 2000; Walker et al., 2000). Measurements of N7-(2-hydroxyethyl)guanine (7-HEG) adduct levels in rats exposed to ethylene or directly to EtO indicate that 3000 ppm ethylene exposures yield equivalent EtO levels of 6.4 to 9.5 ppm in various tissues except for liver (Walker et al., 2000). The resulting reactions with nucleic acids and proteins following in vitro or in vivo exposures to EtO are purely chemical in nature. In terms of potential differences in the nature and/or the degree of DNA damage produced by hydroxyethylcarbonium ions resulting from (i) in vivo conversion of endogenously formed ethylene to EtO, (ii) in vivo formation of EtO following exogenous exposure to ethylene, and (iii) exogenous exposures to EtO – there is no biological, chemical, or theoretical basis for believing that hydroxyethylation arising from these three different sources is different and imposes more or less genetic risk. Furthermore, EtO arising from metabolism of endogeous/exogenous ethylene or from exogenous EtO exposures is rapidly and evenly distributed to all tissues (except for testis) in vivo (Wu et al., 1999; Walker et al., 2000). Thus, under standard cancer bioassay conditions using 63 to 80 rats per group, the ethylene equivalent of approximately 6 ppm EtO appears to be below the limit of detection for a tumor response over the spontaneous background in the F344 rat.

For the first paragraph of section 3.3.2.1, increased frequencies of *Hprt* gene mutations were also observed in lymphocytes of rats at concentrations of EtO used in cancer studies with this species (Tates et al., 1999; van Sittert et al., 2000; Walker et al., 2000). Likewise, for the sentence beginning on page 24, line 27, the underlined changes are suggested: "Increases in the frequency of gene mutations in the lung, in T-lymphocytes, in bone marrow, and/or in the testes have been observed in transgenic mice and in rats exposed to EtO by inhalation.....…" For the remainder of this section, it should be noted that *Hprt* refers to the rodent gene while *HPRT* is reserved for the human counterpart in discussing data about this reporter gene.

Relevant references which were not included in the draft on Evaluation of the Carcinogenicity of EtO:

1	Albertini, R.A., and Sweeney, L.M. (2007) Propylene oxide: genotoxicity profile of a rodent nasal carcinogen. Crit Rev Toxicol. 37(6):489-520.
2	Applegren, L.E., Eneroth, G., Grant, C., Lanström, L.E., and Tenghagen, K. (1978) Testing of ethylene oxide for mutagenicity using the micronucleus test in mice and rats. Act Pharmacol. Toxicol. 43: 69-71.
3	Bastlová, T., Andersson, B., Lambert, B., and Kolman, A. (1993) Molecular analysis of ethylene oxide-induced mutations at the HPRT locus in human diploid fibroblasts. Mutat. Res. 287: 283-292.
4	Bolt, H.M. and Filser, J.G. (1987) Kinetics and disposition in toxicology. Example: carcinogenic risk estimate for ethylene. *Arch. Toxicol.* 60: 73-76.
5	Conan, R.A., Waggy, G.T., Spiegel, M.H., and Berglund, R.L. (1979) Study of the mutagenic action of ethylene oxide, ethylene glycol, and 2-chloroethanol residues in plastic material sterilized by ethylene oxide. Ann. Falsif. Expert. Chim. 72: 141-151.
6	Eisenbrand, G., Muller, N., Denkel, E., and Sterzel, W. (1986) DNA adducts and DNA damage by antineoplastic and carcinogenic N-nitrosocompounds. J. Cancer Res. Clin. Oncol. 112: 196-204.
7	Farmer, P.B., Bailey, E., Naylor, S., Anderson, D., Brooks, A., Cushnir, J., Lamb, J.H., Sepai, O., and Tang, Y.-S. (1993) Identification of endogenous electrophiles by means of mass spectrometric determination of protein and DNA adducts. Environ. Health Perspect. 99: 19-34.
8	Farmer, P.B., and Shuker, D.E.G. (1999) What is the significance of increases in background levels of carcinogen-derived protein and DNA adducts? Some considerations for incremental risk assessment. Mutat. Res. 424: 275-286.
9	Farooqi, Z., Tornqvist, M., Ehrenberg, L., and Natarajan, A.T. (1993) Genotoxic effects of ethylene oxide and propylene oxide in mouse bone marrow cells. Mutat. Res. 299: 223-228.
10	Fomenko, V.N., Strekalova, E. Ye (1973) The mutagenic effect of some industrial toxins as a function of concentration and exposure time. Toksikol Nov Prom Khim Veshchestv 13: 51-57.
11	Golberg, L. (1986) Hazard Assessment of Ethylene Oxide. CRC Press, Boca Raton, FL, pp 3-7.
12	Hamm, T.E. Jr., Guest, D., and Dent, J.G. (1984) Chronic toxicity and oncogenicity bioassay of inhaled ethylene in Fischer-344 rats. *Fund. Appl. Toxicol.* 4: 473-478.
13	Hurst, D.T. (1980) An Introduction to the Chemistry and Biochemistry of Pyrimidines, Purines, and pteridines. Wiley, New York, pp 5-8.

14	Jenssen D., and Ramel, C. (1980) The micronucleus test is part of a short-term mutagenicity test program for the prediction of carcinogenicity evaluated by 143 agents test. Mutat. Res. 75: 191-202.
15	Kelsey KT, Wiencke JK, Eisen EA, Lynch DW, Lewis TR, Little JB. 1988. Persistently elevated sister chromatid exchanges in ethylene oxide-exposed primates: the role of a subpopulation of high frequency cells. Cancer Res. 48(17):5045-50.
16	Kligerman, A.D., Erexson, G.L., Phelps, M.E., and Wilmer, J.L. (1983) Sister-chromatid exchange induction in peripheral blood lymphocytes of rats exposed to ethylene oxide by inhalation. Mutat. Res. 120:37-44.
17	Lambert, B., Andersson, B., Bastlova, T., Hou, S.-M., Hellgren, D., and Kolman, A. (1994) Mutations induced in the hypoxanthine phosphoribosyl transferase gene by three urban air pollutants: acetaldehyde, benzo[a]pyrene diolepoxide, and ethylene oxide. Environ. Health Perspect 102 (Suppl. 4): 135-138.
18	LaMontagne AD, Kelsey KT. 1998. OSHA's renewed mandate for regulatory flexibility review: in support of the 1984 ethylene oxide standard. Am J Ind Med. 34(2):95-104.
19	LaMontagne AD, Kelsey KT. (1998) Ethylene Oxide. In Rom, W.N. (ed.): Occupational and Environmental Medicine, 3rd ed. Philadelphia: Lippincott/Raven Press.
20	Lin, J.-S., Chuang, K.-T., Huang, M.-S., and Wei, K.-M., (2007) Emission of ethylene oxide during frying of foods in soybean oil. Food and Chemical Toxicology 45: 568–574.
21	Lorenti Garcia, C. Darroudi, F., Tates, A.D., and Natarajan, A.T. (2001) Induction and persistence of micronuclei, sister-chromatid exchanges and chromosomal aberrations in splenocytes and bone-marrow cells of rats exposed to ethylene oxide. Mutat. Res. 492: 59-67.
22	Marsden DA, Jones DJ , Lamb JH, Tompkins EM, Farmer PB, Brown K (2007) Determination of endogenous and exogenously derived N7-(2-hydroxyethyl)guanine adducts in ethylene oxide-treated rats. Chem Res Toxicol 20:290-299.
23	Mayer J, Warburton D, Jeffrey AM, Pero R, Walles S, Andrews L, Toor M, Latriano L, Wazneh L, Tang D, et al. 1991. Biologic markers in ethylene oxide-exposed workers and controls. Mutat Res. 248(1):163-76.
24	Ong, T., Bi H.K., Xing, S., Stewart, J., and Moorman, W. (1993) Induction of sister chromatid exchange in spleen and bone marrow cells of rats exposed by inhalation to different dose rates of ethylene oxide. Environ. Mol. Mutgen. 22: 147-151.
25	Ribeiro, L.R., Tabello-Gay, M.N., Salvadori, D.M., Pereira, C.A., and Becak, W. (1987) Cytogenetic effects of inhaled ethylene oxide in somatic and germ cells of mice. Arch. Toxicol. 59: 332-335.
26	Rusyn, I., Asakura, S., Li, Y., Kosyk, O., Koc, H., Nakamura, J., Upton, P.B., and Swenberg, J.A. (2005) Effects of ethylene oxide and ethylene inhalation on DNA adducts, apurinic/apyrimidinic sites and expression of base excision DNA repair gene in rat brain, spleen, and liver. DNA Repair (Amst.) 4: 1099-1110.

27	Segerbäck, D. (1990) Reaction products in hemoglobin and DNA after in vitro treatment with ethylene oxided and *N*-(2-hydroxyethyl)-*N*-nitrosourea. *Carcinogenesis* 11:307-312.
28	Schulte PA, Walker JT, Boeniger M, Tsuchiya Y, and Halperin WE. (1995) Molecular, cytogenetic, and hematologic effects of the ethylene oxide on female hospital workers. J. Occup. Environ. Med 37:313-320.
29	Shore RE, Gardner MJ, Pannett B. 1993. Ethylene oxide: an assessment of the epidemiological evidence on carcinogenicity. Br J Ind Med. 50(11):971-97.
30	Streklova, E. Ye, Chirkova, E.M., and Golubovich, E. (1975) Mutagenic action of ethylene oxide on sex and somatic cells in male white rats. Toksikol Nov Prom Khim Veshchestv 14: 11-16.
31	Tates, D., van Dam, F. J., Natarajan, A. T., van Teylingen C. M.M., de Zwart, F. A., Zwinderman, A. H., van Sittert, N. J., Nilsen, A., Nilsen, O. G., Zahlsen, K., Magnusson, A.-L., and Tornqvist, M. (1999) Measurement of *HPRT* mutations in splenic lymphocytes and haemoglobin adducts in erythrocytes of Lewis rats exposed to ethylene oxide. Mutation Research 431: 397–415
32	Van Delft J.H.M., van Winden M.J.M., van den Ende, A.M.C., and Baan R.A. (1993) Determining N7-alkylguanine adducts by immunochemical methods and HPLC with electrochemical detection: application in animal studies and in monitoring human exposure to alklylating agents. Environ. Health Perspect. 99: 25-32.
33	Van Sittert, N.J., Boogaard, P.J., Natarajan, A.T., Tates A.D., Ehrenberg, L.G., and Tornqvist, M.A. (2007) Formation of DNA adducts and induction of mutagenic effects in rats following 4 weeks inhalation exposure to ethylene oxide as a basis for cancer risk assessment. Mutation Research 447: 27–48.
34	Walker, V.E., Wu, K.-Y., Upton, P.B., Ranasinghe, A., Scheller, N., Cho, M.-H., Vergnes, J.S., Skopek, T.R., and Swenberg, J.A. (2000) Biomarkers of exposure and effect as indicators of potential carcinogenic risk arising from *in vivo* metabolism of ethylene to ethylene oxide. *Carcinogenesis*, 21: 1661-1669.

1.c. Mode of Action

Do the available data and discussion in the draft document support the mode of action conclusions?

The Panel agrees with the conclusion in the draft assessment that the available data strongly support the action of EtO as a genotoxic agent producing DNA adducts as well as cytogenetic and small-scale mutagenic effects. However, a more careful discussion of the sequence of events that are presumed to lead to EtO-induced mutagenesis is warranted. In the Draft Assessment, the description of the events leading to gene mutations and chromosome damage presume that 7-HEG and *N*-alkylated bases are indirectly responsible, or primarily responsible, for genetic changes. The section on the mode of action does not consider any other possibilities to explain the genotoxicity of EtO, which include (but are not limited to) the

potential consequences of (i) formation of minor promutagenic adducts, (ii) hydroxyethylation of the DNA backbone, and (iii) the formation of secondary reactive species including reactive oxygen species. The sentence beginning on line 4 of page 22 states that "HEG adducts result in various types of cytogenetic damage, including gene mutations, which have been observed in mice and rats". However, there is currently limited evidence to directly support this statement.

As discussed in a recent review by Albertini and Sweeney (2006), N7-alkylguanine adducts formed from small epoxides such as EtO and propylene oxide do not cause distortion of the double helix and do not interfere with hydrogen bonding; rather, they are hypothesized to result in mutation via loss of N7-alkylguanine via depurination or the action of DNA glycosylases, leaving an apurinic site in the DNA. The action of apurinic endonuclease indeed creates a DNA single-strand break which, if unresolved, can lead to DNA double-strand breaks. Furthermore, depurination of N7-alkylguanine can result in preferential insertion of an adenine (according to the A-rule) or another base leading to mispairing/mutations. Based upon the initial mutational spectra data for EtO in mice (Walker and Skopek, 1993), it was hypothesized that formation of apurinic (AP) sites might be involved in the mutagenesis of EtO. In order for these mutagenic events to occur at a rate sufficient to result in an EtO-induced changes in mutational spectra (including increases in double-strand breaks and changes in mutant fractions for point mutations), then accumulation of AP sites arising from high levels of 7-HEG would be expected to occur over time. A study was recently completed to test the hypothesis that EtO exposure results in the accumulation of AP sites and induces changes in the expression of genes for base excision DNA repair, predisposing to point mutations and chromosomal aberrations in F344 rats exposed by inhalation for 4 weeks to 0 or 100 ppm EtO, or 0 to 3000 ppm ethylene, (Rusyn et al., 2005). The resulting data demonstrated that DNA damage induced by exposure to EtO is repaired without accumulation of AP sites, and that the mechanisms proposed above play a minor role in the mutagenicity of EtO. The same conclusions would apply to the accumulation of 3-HEA formed in minor amounts in EtO-exposed rats (Walker et al., 1992b), and the induction of strand breaks or point mutations at A:T base pairs. Rusyn et al. (2005) have suggested that the mutagenic effects of EtO were likely to be the result of minor promutagenic adducts, such as O^6-HEG, N1-HEAdenine, or possibly ring-opened 7-HEG.

Drs. Lars Ehrenberg and Timothy Fennell have independently proposed that EtO may induce strand breaks and chromosomal alterations via 2-hydroxyethylation of the DNA backbone. 2-Hydroxyethylation of phosphate groups introduces extreme instability into the sugar-phosphate backbone because the resulting phosphotriester breaks down through a dioxaphospholane ring intermediate (Eisenbrand et al., 1986). This alternative mechanism for EtO-induced strand breaks and chromosomal damage is not mentioned in the Draft Assessment.

In summary, the overall genetic toxicology data strongly support the consistent action of EtO as a relatively weak mutagen and clastogen, but the underlying mechanisms for its mode of action as a genotoxin are not known with a high degree of certainty. The paucity of knowledge about the fundamental ways in which EtO acts to induce large- and small-scale mutations is not reflected in the mode-of-action section; rather this section is presented as if there is a good basic understanding (which does not currently exist).

1.d. Hazard Characterization

Does the hazard characterization discussion for EtO provide a scientifically-balanced and sound description that synthesizes the human, laboratory animal, and supporting (e.g., *in vitro*) evidence for human carcinogenic hazard?

While some members of the Panel found the hazard characterization section of the Draft Assessment to be satisfactory, a majority expressed concerns that this section did not achieve the necessary level of rigor and balance. As discussed above, a majority of Panel members agreed with the overall characterization of EtO as a human carcinogen. However, a critical issue in this characterization, in particular in the face of epidemiological data that are not strongly conclusive, is whether the precursor events leading to cancer in animals are observed in humans at the levels to which they are exposed to EtO.

The mode of action for EtO carcinogenicity involves the key events of DNA alkylation and the induction of point mutations and/or chromosomal changes. Evidence for genotoxicity of EtO in humans is largely based on cytogenetic analyses. The frequency of cells with chromosomal aberrations and micronuclei in peripheral blood cells are two of the most accepted cytogenetic biomarkers used in human population studies because they were the first indicators of effect shown to be early predictors of cancer risk. However, the micronucleus data in EtO-exposed humans are weak, with very small increases reported, and the abundant data on chromosomal aberrations in EtO-exposed people have not demonstrated, with confidence, the occurrence of stable chromosome changes leading to mutations. As indicated at the bottom of page 20 of the Draft Assessment, chromosome painting/FISH are needed to detect and quantify stable chromosomal aberrations, which would provide more conclusive evidence for classifying EtO as a human carcinogen. A problem in the hazard characterization in the Draft Assessment is the lack of an adequate review of the cytogenetic data for EtO in exposed rodents and head-to-head comparisons to corresponding data in humans. The sections concerning sister chromatid exchanges (SCEs) (3.3.2.2) and chromosomal aberrations (3.3.2.3) in the Supporting Evidence present only data from human studies and overlook contradictory or equivocal findings from studies of EtO-exposed rodents. Furthermore, there is no discussion of findings related to micronuclei in humans or rodents in the Supporting Evidence section. In brief, several studies have shown that repeated exposures of rats to high concentrations of EtO induces dose-related increases in SCEs (Kligerman et al., 1982; Ong et al., 1993; van Sittert et al., 2000; Lorenti Garcia et al., 2001). Treatment of rats and mice with high acute doses of EtO by i.p./i.v. injection or oral dosing (i.e., routes of exposure not relevant to humans) also caused increases in the frequencies of micronuclei or chromosomal aberrations (Strekalova et al., 1971; Applegren et al., 1978; Conan et al., 1979; Jensen and Ramel, 1980; Farooqui et al., 1993). In contrast, following inhalation exposures (i.e., a route of exposure relevant to humans), no increases in the frequencies of micronuclei or chromosomal aberrations were found in peripheral blood/splenic lymphocytes from rats exposed at concentrations of 50 to 450 ppm EtO for 1 or 3 days (Kligerman et al., 1982) or 50 to 200 ppm EtO for 4 weeks (5 days/week, 6 h/day) (van Sittert et al., 2000; Lorenti Garcia et al., 2001). Furthermore, two studies showed that 4 weeks of exposure of rats to 200 ppm EtO failed to cause an increase in translocations (van Sittert et al., 2000; Lorenti Garcia et al., 2001) (e.g., the % translocation in controls and 200 ppm rats were 0.1% and 0.09%, respectively, in the latter study). In the study by van Sittert et al. (2000), the authors concluded that "The absence of effects on reciprocal translocations (assessed by FISH)

demonstrates that 4 weeks of inhalation exposure to EO at high levels does not produce genetically transmissible chromosome aberrations in the rat." A single study reported that repeated exposures of mice at 200 to 600 ppm EtO for two weeks induced chromosomal aberrations in bone marrow cells (Ribeiro et al., 1987), but no studies have been performed to assess whether this chemical causes transmissible chromosome aberrations in somatic cells in this species.

In contrast to lack of data supporting induction of chromosome aberrations and reciprocal translocations at EtO concentrations used in rodent carcinogenicity studies of this chemical, there are unequivocal data from three research groups (cited reports by Les Recio, Ad Tates, and Vernon Walker) showing that EtO causes dose-related increases in point mutations in multiple tissues of mice and rats exposed by inhalation to 50, 100, or 200 ppm EtO, or concentrations used in the cancer bioassays of EtO, as well as in oncogenes and tumor suppressor genes of various EtO-induced cancers in the mouse (Houle et al., 2006; Hong et al., 2007). In these rodent studies using the *Hprt* and/or *lacI* reporter genes, EtO was consistently a weak point mutagen. However, as noted in the Draft Assessment, studies of the induction of *Hprt* mutations in EtO-exposed humans have been inconclusive.

Thus, studies of both humans and rodents exposed to EtO have yielded evidence consistent with the genotoxic mode of action of EtO, but different types of genetic alterations are demonstrated in the two species.

Charge Question 2- Dose-Response Analysis

The Agency's assessment describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on the human data. An EC_{01} of 44 $\mu g/m^3$ (0.024 ppm) was calculated using a life-table analysis and linear modeling of the categorical Cox regression analysis results for excess lymphohematopoietic cancer mortality in males reported in a high-quality occupational epidemiologic study (Steenland et al., 2004). Linear low-dose extrapolation from the LEC_{01} yielded a lifetime extra cancer mortality unit risk estimate of 5.0×10^{-4} per $\mu g/m^3$ (0.92 per ppm) of continuous EtO exposure. According to EPA's assessment,applying the same linear regression coefficient and life-table analysis to background male lymphohematopoietic cancer *incidence* rates yielded an EC_{01} of 24 $\mu g/m^3$ (0.013 ppm) and a preferred lifetime extra cancer unit risk estimate of 9.0×10^{-4} per $\mu g/m^3$ (1.6 per ppm). The preferred estimate was greater than the estimate of 5.0×10^{-4} per $\mu g/m^3$ (0.91 per ppm; EC_{01} = 44 $\mu g/m^3$) calculated, using the same approach, from the results of a breast cancer incidence study of the same worker cohort (Steenland et al., 2003), and was recommended as the potency estimate for Agency use.

According to the Agency's assessment, the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity. The Draft Assessment then concludes that, in the absence of chemical-specific data on early-life susceptibility, an increased early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens,* hereinafter referred to as "EPA's Supplemental Guidance" (U.S. EPA, 2005b). Applying the ADAFs to the unit risk estimate of 9.0×10^{-4} per $\mu g/m^3$ yields

an adjusted full lifetime unit risk estimate of 1.5×10^{-3} per $\mu g/m^3$, and the commensurate lifetime chronic exposure level of EtO corresponding to an increased cancer risk of 10^{-6} is $0.0007 \mu g/m^3$. [Note that for less-than-lifetime exposure scenarios (or for exposures that vary with age), the unadjusted (adult-based) potency estimate of 9.0×10^{-4} per $\mu g/m^3$ should be used, in conjunction with the ADAFs as appropriate, in accordance with EPA's Supplemental Guidance.]

In the Agency's assessment, unit risk estimates were also derived from the three chronic rodent bioassays for EtO reported in the literature. These estimates, ranging from 2.2×10^{-5} per $\mu g/m^3$ to 4.6×10^{-5} per $\mu g/m^3$, are about an order of magnitude lower than the estimates based on human data [unadjusted for early-life susceptibility]. The Agency takes the position that human data, if adequate data are available, provide a more appropriate basis than rodent data for estimating population risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from rodents to humans are avoided. Although there is a fairly sizable difference between the rodent- and human-based estimates, the assessment infers that the similarity between the unit risk estimates based on the male lymphohematopoietic cancer and the female breast cancer results increases confidence in the use of the unit risk estimate based on the male lymphohematopoietic cancer results. According to the Agency assessment, the unit risk estimates were developed for environmental exposure levels and are not necessarily applicable to higher-level occupational exposures, that appear to be subject to a different exposure-response relationship. However, occupational exposure levels are of concern to EPA when EtO is used as a pesticide (e.g., fumigant for spices). Therefore, it is appropriate that EPA presents unit risk estimates for occupational exposure scenarios.

2. Do the available data and discussion in the draft document support the approaches taken by EPA in it derivation of cancer risk estimates for EtO? In your response, please include consideration of the following:

2.a. Selection of Epidemiology Studies

EPA concluded that the epidemiological evidence alone was strong but less than completely conclusive (although EPA characterized the total evidence - from human, laboratory animal, and in vitro studies - as supporting a conclusion that EtO as "carcinogenic to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003, 2004) data set, the most appropriate for estimating the magnitude of the carcinogenic risk to humans from environmental EtO exposures? Are the scientific justifications for using this data set transparently described? Is the basis for selecting the Steenland et al. data over other available data (e.g., the Union Carbide data) for quantifying risk adequately described?

The Panel agreed that the epidemiological evidence is less than completely conclusive. The data are somewhat consistent in showing a weak to moderate excess carcinogenic response. It is not unusual for epidemiological evidence to be strong but in and of itself not provide conclusive evidence of causation. It is appropriate in light of conclusive evidence in animals to use sound human epidemiological studies to determine the dose response even though in and of themselves these studies may not provide conclusive evidence of carcinogenicity.

The Panel agreed that the NIOSH retrospective cohort study with observations on in excess of 18,000 workers from 13 sterilizing facilities is the best single source of data for determining the dose-response relationship for evaluating the risk of low level EtO exposure in human populations (Steenland et al, 2004).

As a single source, the epidemiological data for the NIOSH cohort has the following distinct advantages:

1) A large (18,000+) sample of workers with long periods of exposure to EtO;

2) A roughly 55%/45% female to male gender ratio, similar to the general population;

3) Multiple distinct facilities with worker exposure estimates. (Facility intra-class correlation is never considered in any of the models applied to the NIOSH data);

4) Limited coincidental exposure in the occupational cohort to other compounds, e.g. ethylene dichloride, that might confound the interpretation of the relationship of EtO exposure to cancer outcomes.

5) Careful mortality follow-up using multiple sources; and finally

6) Continuity of the investigators who have been building and analyzing the data set.

A primary disadvantage of the NIOSH data , common to all retrospective epidemiological data, is the need to apply a model to estimate the profile (time, intensity) of individuals' exposure to EtO (Hornung, et al., 1994). The model of EtO exposure over time is needed to support the use of different exposure metrics, e.g. cumulative (time integrated), peak, duration. Random errors in estimating exposures are certainly present and systematic bias resulting from errors in model inputs or model mispsecification are certainly also present to some extent. Ideally, estimation biases are small relative to the variance of the predictions and the assigned exposure profiles result in acceptable classifications of individual exposure levels (see below). Given the importance the estimated exposures to the use of these data in ultimately modeling the dose-response relationship, the Panel noted several important features of the NIOSH exposure estimation model and the exposure predictions for individual NIOSH cohort members.

The worker exposure observations used to fit the model were not a random sample (effect unknown) of workers or work environments but were designed to represent the typical range of exposure conditions that occur in the contemporary work place. A total of 2350 full-shift charcoal tube measurements were collected from workers in twelve plants. By design, the observations were distributed across workers involved in eight exposure activity types (e.g. sterilizer area, production, maintenance) and five product types (e.g. spore strips, plastics, etc.). In addition to these two main effects, the multivariate regression model for predicting exposures for the NIOSH cohort workers includes additional covariates for age of product, year (of exposure), and size and ventilation characteristics of the work area. A random sub-sample of observations of the worker measurements was set aside as a cross-validation sample for purposes of evaluating the predictive potential of the fitted model. The final model produced an R^2 to the

cross-validation exposure measurements (cross validation sample) of 0.85. There was consensus among the Panel that the exposure model development for the NIOSH data was conducted in a rigorous fashion and it would be difficult to improve on the exposure estimates generated by the NIOSH exposure measurement study (Griefe et al., 1988, Steenland et al., 1987).

In its discussion of the predicted exposure measures in NIOSH cohort data, the Panel focused in some detail on the role of chronological time in the prediction of annual exposures for the individual cohort members. From Table VI of Hornung at al. (1994), the year in which the exposure occurred is highly predictive of the exposure concentration. Quoting from the paper, "We had hoped that some combination of engineering controls would eliminate the need for including calendar year in the model, ... However, no combination of variables could be found to allow removal of calendar year from the model. We attributed this finding to calendar year acting as a surrogate for improvement in work practices due to increased awareness of the potential health effects of ETO". The effect of chronological time is highly significant and quadratic---0.446 x (year-82) and 0.062 x $(\text{year-82})^2$. The model-based assignment of exposures to individuals in the NIOSH cohort truncates this highly significant time effect on exposure (quadratic) at 1978. That is, all exposures for work years prior to 1978 receive the same contribution to modeled exposure as in 1978—the year at which the trend in exposures is at a maximum for the quadratic time effect. The reason for assigning all pre-1978 exposures to the 1978 level is that prior to that time data on general work place exposures was scarce (7 activity x product combination mean exposures based on 23 individual samples collected in 1976-1978).

Regarding the handling of time in modeling annual exposures for individual NIOSH cohort members, the Panel's concern is less on the modeling decision but rather on how this estimation decision may interact with subsequent dose response model fitting in which the exposure metric is itself a function of time. For example, the 1978 truncation point is very close to the analysis censoring exposure point of t-15, or t-20 for the Steenland et al. (2004) dose response models that use time lagged exposure as the independent variable. Does introducing the lag into the cumulative exposure measurement alter the quality of the effective exposure model since the parameters for non-time factors (e.g. activity type, product type, engineering controls, size of workplace) have been estimated in the presence of a strong and dominant quadratic time trend? The Panel encourages the EPA to investigate potential instability that may result from interaction between the chosen time metric for the dose response model and the treatment of time in the estimated exposure (e.g. log cumulative, exposure with 15 year lag) that is the independent variable in that dose-response model.

While the advantages of the Steenland data set are described, the Draft Assessment contains no list of the criteria that were utilized to select studies for inclusion in the risk assessment process. For example, a description of what constituted adequate sample size, exposure assessment, minimum length of employment, length of follow-up, lag time for selected outcomes, etc., would be helpful. It is certainly appropriate to critique all available datasets and provide justification for excluding those who did not meet these criteria. While a review of most studies conducted between 1985 (when the last EtO assessment was conducted) and 2004 was included, it was not always clear why some studies were not considered in the process. For example, Steenland's dataset was deemed most appropriate because of the larger sample size (n=18,254), gender diversity (45% male, 55% female), lack of potentially confounding co-

exposures, and more developed measures of individual worker exposures. There were disadvantages, e.g., lack of information on age of the cohort members. The authors state that earlier exposures decreased markedly by the mid-1980's. Did the risk of cancer decrease in later time intervals, i.e., what is the risk for workers initiating employment in the 1980's when levels are lower over a similar time period (20 years)? Also, age at exposure, an increasingly recognized critical factor in environmental/occupational exposures and adverse health effects, could not be evaluated (e.g., younger workers with similar cumulative exposures may be at different risk than older workers).

Other cohorts consisted of smaller sample sizes, less precise measures of individual worker's exposure levels, and concurrent chemical exposures. Some of these studies were justifiably omitted from the risk assessment because of sufficient weaknesses in design and/or analysis. However, it seems it would be of value to examine some of these studies to determine the potential for interaction between EtO and other common workplace exposures.

To summarize, the Panel concurred that the NIOSH cohort is the best single epidemiological data set with which to study the relationship of cancer mortality to the full range of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly consider all of the epidemiological data in developing its Draft Assessment. In particular, the Panel encourages the EPA to explore uses for the Greenberg et al. (1990) data including leukemia and pancreatic cancer mortality and EtO exposures for 2174 Union Carbide workers from its two Kanawha Valley, West Virginia facilities. (Also described in Teta et al. 1993; Teta et al., 1999).

The Panel did not believe that it was necessary to use only one study to arrive at a single potency estimate or to limit the assessment to a single modeling approach. Panel members emphasized that the EPA's own cancer risk assessment guidelines support the consideration of the full range of available data as well as alternatives to the default exposure models. Quoting from the EPA's Guidelines for Cancer Risk Assessment,

> Section 1.3, p. 1-8, "[*T]hese cancer guidelines view a critical analysis of all of the available information that is relevant to assessing the carcinogenic risk as the starting point from which a default option may be invoked if needed to address uncertainty or the absence of critical information*".

2.b. Methods of Analysis

Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of a linear regression model fit to Steenland et al.'s categorical results for all lymphohematopoietic cancer in males in only the lower exposure groups scientifically and statistically appropriate for estimating potential human risk at the lower end of the observable range? Is the use of the grouping of all lymphohematopoietic cancer for the purpose of estimating risk appropriate? Are there other appropriate analytical approaches that should be considered for estimating potential risk in the lower end of the observable range? Is EPA's choice of a preferred model adequately supported and justified? In particular, has EPA adequately explained its reasons for not using a quadratic model approach such as that of Kirman et al. (2004) based? What

recommendations would you make regarding low-dose extrapolation below the observed range?

The Panel's discussion of this multi-part question was extensive. To simplify the presentation here, the written discussion is divided into seven segments: 1) linearity vs. nonlinearity in dose response modeling and extrapolation; 2) the linear regression methodology of grouped risk ratios employed in the EPA Draft Assessment; 3) exclusion of high exposure data points; 4) grouping lymphohematopoietic cancers in analysis; 5) using only male data; 6) justification of approach and alternatives; and 7) statistical and computational issues.

1. Linearity vs. non-linearity of low dose extrapolation

The Panel was "philosophically" and scientifically divided on the whether low dose extrapolation of risk to environmental EtO exposure levels should be linear (following Cancer Guideline defaults for mutagenic MOA) or whether plausible biological mechanisms argued for a non-linear form for the low dose response relationship. Some Panel members thought that the data on ethylene oxide imply a non-linear response despite a mutagenic mode of action. They encouraged the exploration of the use of non-linear models for low-dose extrapolation, such as the quadratic and linear quadratic. Others thought that non-linear extrapolation was inappropriate given the mutagenic mode of action. After considerable debate, the Panel was unable to arrive at consensus. Therefore the two distinct lines of argument are presented below.

The Linear Extrapolation Argument: In general a linear no-threshold interpolation to zero for ethylene oxide external exposure is consistent with available information about the mutagenic mode of action in this case. General arguments are that DNA repair and other genomic defense processes (e.g. apoptosis) are not likely to be perfect in the sense of repairing all incremental damage before the next cycle of DNA copying which would otherwise lead to miscoding errors or more extensive chromosome level changes including breakage, recombination, and nondisjunction events. Genomic defense processes have costs to the cell, and some are also known to create their own baseline damage, so that it is highly likely that the extent of the effectiveness of such processes has been subject to an evolutionary optimization that falls short of perfection. Thus, even with background and endogenous exposures there should be some expectation of ongoing equilibrium damage on a cellular stochastic basis. Such equilibrium damage is likely to contribute to the appreciable "background" of cancers of all types that humans suffer. A detailed review of the argument for "low dose linearity" in cancer risk assessment involving a mutagenic mode of action is given by Hattis (2007, Appendix A).

The Non-linear Low Dose Response Model Argument: Linear extrapolation of risk below the chosen point of departure (POD) to a zero baseline is a conservative assumption, given EtO's reactivity (which will diminish the amount reaching the nucleus), mutagenic mode of action, and that it is generated endogenously. Some repair seems likely and some threshold probably exists. Thus, the human risk estimates at the lower end of the observable range are likely to be exaggerated under a linear extrapolation. Furthermore, a linear model through zero (linear model per se at low doses is acceptable) assumes that other effects of EtO on the development of cancer are insignificant. This seems unlikely given the reactive nature of this compound and thus its ability to affect signaling pathways that may positively and negatively influence the development of cancer. Measuring such effects is problematic, but they must exist

and impact the incidence of cancer. Linear regression is for "extra" risk; but this still seems problematic given the endogenous level of EtO and base levels of damage and repair. In other words, is it justified to assume linear above baseline levels? At low doses, a reactive compound like EtO will react with cellular constituents before it ever gets to DNA. Linear defaults are not supported when a framework analysis is done of genotoxicity and this is even more strongly so for clastogenic agents, which are quadratic in dose response (Preston, 1999). Swenberg (2007, Appendix C) provides a framework analysis of Genotoxicity and Risk Assessment in support of an argument for a nonlinear low dose response mechanism for EtO.

At the conclusion of its discussion, the Panel was not in agreement on the linearity vs. non-linearity of the cancer response to EtO exposure levels in: 1) the occupational exposure data used to estimate the point of departure for the low dose extrapolation; and 2) in the form of the model used to extrapolate cancer risk below the POD to a zero or baseline exposure level. With appropriate discussion of the statistical and biological uncertainties, several Panel members advocated the consideration of both linear and nonlinear functional forms in the final EtO Risk Assessment. These Panel members pointed out that such an approach was consistent with the latest guidance in the EPA Guidelines for Cancer Risk Assessment. Quoting Section 1.3 p. 1-9,

> *"Significant risk management decisions will often benefit from a more comprehensive assessment, including alternative risk models having significant biological support."*

2. Linear regression model for categorical data

The Panel identified several important shortcomings in the linear regression modeling approach used to establish the point of departure for low dose extrapolation of cancer risk due to EtO. Based on its review of the methods and results presented at the January 17,18, 2007 meeting, the Panel was unanimous in its recommendation that the EPA develop its risk models based on direct analysis of the individual exposure and cancer outcome data for the NIOSH cohort. The Panel understands that these data are available to EPA analysts upon request to the CDC/NIOSH. The Panel recognizes the burden that a reanalysis of the individual data places on the EPA ORD staff but given the important implications of the risk assessment, this burden is well justified to achieve the best scientific and statistical treatment of all the available epidemiological data.

The following paragraphs present the statistical basis for the Panel's assessment of the linear regression model approach and the use of categorized exposure and outcome data.

The approach described in the Draft Assessment uses a model based on categories defined by cumulative exposure ranges for male subjects in the NIOSH cohort. Steenland et al. identified several models that provide a significant ($p<0.05$) fit to the exposure data; however, the EPA has elected to use model-based relative rate parameter estimates for categories of 15 year lagged, cumulative exposure. In Steenland, et al. (2004) this model was not one that provided a significant fit to the NIOSH data ($p=0.15$ for the likelihood ratio test of $\underline{\beta} = \{\beta_1, \beta_2, \beta_3, \beta_4\} = 0$). The use of the weighted least squares regression fit of a linear regression line through the three data points defined by the estimated rate ratios and mean cumulative exposures for the first three exposure categories of the Steenland, et al. 15 year lag,

cumulative exposure category model is not a robust application of this technique. The Panel identified four weaknesses in the approach.

a) Model-based dependent variable: The dependent variables are model-based estimates of rate ratios for exposure categories. The rate ratio values used in the weighted least squares regression are derived from a cumulative exposure model (15 year lag) in which the estimated regression parameters in the proportional hazards regression model are not significantly different from 0 at α=0.05 (p=0.15). In Steenland et al. (2004), the only individually based (proportional hazards) model that fits the data for males in the NIOSH cohort is a model for log of individual exposure through t-15 years.

b) Grouped data regression: The weighted least squares fit applies estimates of variance for the individual rate ratios under that assumption that these inverse weighting corrections correctly adjust for heteroscedasticity of residuals in the underlying regression model. Historically, models for grouped proportions applied adjustments of this type but it is by no means a preferred technique when the underlying individual data are available. The "ecological regression" model per Rothman (1998, Second edition) is subject to bias due to within group heterogeneity of predictors and unmeasured confounders. The heterogeneity in the grouped model involves the range of exposures within the collapsed categories. The unmeasured confounders include variables (other than gender) that affect the potency of exposure or may have produced gross misclassification based on the original exposure model estimation for the individual (Hornung, et al., 1994).

c) The model fitting does not conform exactly to the Rothman (1986) procedure:

The 1998 (Second edition) of Rothman (Rothman and Greenland, 1998) describes the technique for estimating this risk from grouped data in Chapter 23. In that updated version of the original monograph the model that is fitted is:

$$Expected(Rate \mid Exposure) = \hat{B}_0 + \hat{B}_1 \cdot Mean(Exposure)$$

The objective is to estimate the rate ratio (for exposure 0=no, 1=yes, or equivalently for a one unit increase in the exposure metric). That estimator is then:

$$rr = 1 + \hat{B}_1 / \hat{B}_0$$

The model estimated by the EPA method is:

$$Expected(rr \mid Exposure) = \hat{B}_1^* \cdot Mean(Exposure)$$

In the former, the variance in the estimation of the rate ratio is a function of the variance of the estimated slope and the variance in the estimated baseline hazard, represented by the estimated intercept. This variance is present in the estimation of the baseline hazard in the Steenland, et al. (2004) estimation of the rate ratios but is not present in the EPA adaptation to the linear rate ratio model. The EPA approach permits no intercept (>0) for the background exposure or any

allowance for an effect of true non-zero exposures in the internal control group (exposures less than 15 years).

In general, the use of categorical exposure ranges is not the optimal strategy for using epidemiologic data. When continuous data are categorized and then used in dose response modeling, it amounts to starting with a full range of exposures, collapsing that range into somewhat arbitrary boundaries, and then deriving a continuous dose response model for an even larger range of exposures.

Categorizing continuous variables results in a host of issues:

- Assumption that the risk within the category boundaries is constant
- It is not known whether a given categorization is representative of the data since there are many ways of categorizing.
- Loss of power and precision by spending degrees of freedom on each category
- Misclassification at category boundaries (this can be minimized by choosing cutpoints where relatively few observations are present)
- Categorizations can be manipulated to show the desired results

The Panel acknowledged that techniques such as the linear regression method described by Rothman (1998) or Poisson regression may be the most appropriate techniques when only grouped or categorized data are available for estimating the dose/response model. However, the original NIOSH cohort data are available at the individual level and this permits the use of models such as the Cox regression models employed by Steenland et al. (2004) that utilize the full information in the individual observations. If categories of exposure (as opposed to individual exposure estimates) must be used, the crude rates should be computed for a large number of equally spaced exposure ranges and the Rothman and Greenland (1998) model fitted to these multiple points.

3. Exclusion of high exposure groups

In conjunction with its recommendation to use the individual NIOSH cohort data to model the relationship of cancer risk to exposures in the occupational range (see 2.B.1 above), the Panel recommends that the EPA analysts explore the use of the full NIOSH data set to estimate the cancer slope coefficients that will in turn be used to extrapolate risk below the established point of departure. The use of different data to estimate different dose response curves should be avoided unless there is both strong biologic and statistical justification for doing so. The Panel believed this justification was not made in the Agency's draft report.

In the Draft Assessment, EPA analysts have faithfully adhered to the paradigm of cancer dose response analysis usually used for animal data in analyzing the human epidemiological data for this case. This is a useful step toward harmonizing the treatment of animal and human-derived information in carcinogenic risk estimation. However, while achieving operational consistency, the Agency's current analysis does not yet take into account some important differences between animal and human carcinogenic dose response data. These differences need to be factored in for designing a modern set of analytical procedures for human data to achieve more comparable types of risk inferences and a better analysis of uncertainties.

The most important differences in human vs. animal data that may require differences in analytical approach are:

- Animal exposures are the result of intentional and consistent administration of the test material at specific target levels, often reinforced with frequent empirical measurements of differences between target exposures and actual delivered doses. Human exposures, by contrast, are unintended, often variable over time, and at best estimated from occasional measurements of exposures of the subjects themselves or subjects considered to have similar exposures. Uncertainty in exposures is thus nearly always much greater for human than for animal data. Such uncertainties in human data lead to distortions in both central estimates and uncertainties in potency estimates that require at least discussion and preferably adjustment of ordinary dose response model fitting and "slope factor" estimation procedures. Procedures for adjusting traditional regression analyses for such effects are relatively well known in biostatistics under the general heading of "errors in variables' models,[*] but have rarely been applied to occupational cancer data in part because, unlike this case, exemplary quantitative analyses of likely errors in exposure estimates have not often been available. There are some examples of the use of errors in variables models in epidemiological studies of other effects (Stayner et al. 2003; Richardson et al. 2004; Brown et al. 2004; Choi, 2000; Carrothers and Evans, 2000; Kulathinal et al. 2002; Siebert et al. 2001). The current case, where very extensive efforts have been devoted to development of exposure estimates and quantitative errors in exposure estimates (Hornung et al. 1994), represents an invaluable opportunity to analyze and offset the distortion in dose response estimates from this type of problem. The analysis presented in Appendix C illustrates a relatively simple analytical approach to gauging the extent of the modification in the low-dose cancer risk for male lymphoid cancers that could be indicated for this case.

- The subjects of human epidemiological studies may be subject to a variety of selection biases, including the "healthy worker" effect, and the "healthy worker survivor" effect. The former complicates comparisons with general population mortality data, but the internal comparisons used for analyses of the Steenland et al. (2004) avoid these. The "healthy worker survivor" effect (HWSE) is a known phenomenon that produces established distortions in relationships between measured risks and measures of cumulative exposure, as shorter term workers suffer greater mortality than workers who work at exposure-producing jobs for longer periods of time (Steenland et al., 1996; Kolstad and Olsen, 1999; Garshick et al. 2004; Siebert et al. 2001; Steenland and Stayner 1991). Some Panel members believed that the HWSE may be applicable for workers exposed to Ethylene Oxide. Interestingly, in the light of the gender differences in the current analysis, in at least one study the healthy worker effect was found to be greater for women workers than for men (Lea et al. 1999). Adjustments for this effect are at the cutting edge of current practice for the treatment of human epidemiological data, but they are vital for achieving the best possible analysis of those data. The authors of some of the leading studies documenting the healthy worker survivor effect include authors involved in the Steenland et al. (2004) ethylene oxide mortality study (e.g., Steenland et al., 1996; Stayner et al. 2003). It might be useful for EPA to consult with Steenland and coworkers to judge whether analytical adjustments for this effect are possible in this case. Even if the data will not support the more complex analyses [and analyses of this sort are notoriously complex (Robins, 1986; Arrighi and

[*] Ordinary regression models minimize the sum of the squares of the distance of the points to the regression line only in the "y" dimension (representing the dependent variable), "errors in variables" models minimize the weighted sum of squares distance as measured in terms of the uncertainties in both the dependent and independent variables.

Hertz-Picciotto, 1996; Hertz-Picciotto, personal communication) EPA could provide at least some discussion of how large the distortions might be by citing previous cases such as the cancer risks from diesel particles (Garshick et al. 2004) and the approach that California risk assessors (and possibly others) have taken to risk analysis where the healthy worker survivor effect is even more prominent than it may be in this case. (For diesel particulates, the relative risk vs. cumulative dose curve even had a negative, rather than a positive slope.)

Another source of the dose estimation problem that is more distinctive for this case is the presence of a background of ethylene oxide exposure from endogenous generation. Conceivably this could be substantial enough to limit the potency of ethylene oxide that would be compatible with observations of "background" rates of lymphoid cancers in people without occupational exposures. Should the EPA analysts accommodate this possibility by adding estimates of exposure from this source (perhaps including variability and uncertainty) to the estimates of occupational exposures for all the groups in the Steenland et al. (2004) study? If so, how should such estimates be derived? In preliminary work, Hattis (see Appendix B) attempted to estimate this effect using the model parameters and data from Csanady et al. (2000). Generally, the exploratory work by Hattis (Appendix B) finds the effect to be small enough (about 1.8 ppb occupational equivalent ethylene oxide exposure, amounting to about 26 occupational equivalent ppm-days of cumulative exposure over a 60 year period) as not to be likely to appreciably distort the EPA analysis.

4. Cancer groupings

Although the analysis based on total LH cancers might have value as part of a complete risk assessment, the rationale for this aggregate grouping needs to be better justified. Certainly, a rational grouping of cancer types with a similar pathophysiology can lead to improved power to detect significant effects of EtO exposure. By the same turn, grouping cancers that affect a single organ system (e.g. blood) but with very different cancer etiology could produce a spurious and therefore misleading result. The Panel therefore recommends that data be analyzed by subtype of LH cancers with biological rationale for any groupings that are formed.

Lymphohematopoietic (LH) cancers are diverse diseases with diverse, and often multiple, etiologies, including exposure to ionizing radiation, viruses, and chemical carcinogens, and genetic predisposition. The Draft Assessment argues that (a) all LH cancers are a larger category, (b) there may be misclassification between LH categories, and (c) it could be a relevant category because of the existence of multipotent stem cells. However, a larger category that is made up of heterogeneous diagnoses is not desirable, one could aggregate bladder and kidney cancers because they were both urinary system cancers, but this would ignore their different etiologies. Even more marked differences exist between LH categories, and even between leukemia cell types. The misclassification of disease mentioned by the Draft Assessment (unspecified leukemias) would result in a slight loss of precision but not necessarily a bias. Also, misclassification between lymphoid, myeloid and other cancers is minimal. In addition, this issue exists within any organ system, e.g. there is some minimal misclassification between kidney and bladder cancers but it is not a good reason to aggregate the two cancers. While multipotent stem cells exist, research by Greaves (2004) suggests that the initiating event for many haematopoietic cancers does not occur in the multipotent stem cell. In addition, these cells

are few in number and are likely to be well-protected because of their critical function. Steenland et al's category of lymphoid tumors is more consistent with modern lymphohaematopoietic classification techniques (WHO 2001) and should be used as the preferred disease outcome.

5. Males Only

The Panel diverged in its views concerning the appropriateness of estimating the population unit risk based only on the NIOSH data for males.

Several Panel members pointed out that a standard approach in cancer epidemiology and risk analysis begins by conducting separate dose-response analyses on males and females and combining the data only if the results are similar. This approach assumes the possibility of known and unknown gender specific differences in the cancer etiology. Indeed, rates for Non-Hodgkin's Lymphoma, Multiple Myeloma, and Leukemias are 25 to 60% higher in men than in women (NCI, 2007). From a risk assessment perspective, it is protective against a gap in our biological knowledge of an underlying mode of action that is truly gender dependent. By the same turn, it is not a statistically conservative approach if in fact no gender difference exists. In the case of no gender effect, a sex-specific analysis will have reduced power to detect any common effect for women and men.

A second approach to dealing with the possibility of gender differences in response is to include gender as a fixed effect in the statistical modeling of the data and determine whether gender or its interaction with other predictors (e.g. gender x exposure) are significant explanatory variables. If so, the combined model with the estimated gender effects could be used directly or separate, gender-specific dose-response analysis would be performed. If not, the gender effects could be dropped and the model re-estimated for the combined male and female data.

Members of the Panel who argued for the second approach were concerned that there appears to be no genetic or other physiological basis for the observed differences in the trends observed for males and females (see overall SMRs). Women comprise 55% of the NIOSH cohort and in general have lower average levels of cumulative exposures to EtO than the males in the original cohort. From Steenland, et al. (2004), Table 4 the SMRs and observed deaths for males and females in 5 categories of lagged (10 year) cumulative exposure are:

Table 1: NIOSH Cohort. SMRs and observed lymphohematopoietic cancer deaths.
Source: Steenland et al. (2004)

Lagged (10) Cum Exp ppm-days	Male (n=7645)	Female (n=9885)
0 (prior to t-10)	1.15 (7)	0.31 (2)
1-1199	0.63 (5)	1.04 (13)
1200-3679	0.87 (5)	1.38 (10)
3680-13499	1.10 (7)	1.06 (9)
13500	1.46 (13)*	0.46 (3)

* 8 of 13 are NHL

Cox proportional hazards models with a random set of m=100 controls matched by race, birth year and gender found no significant effect of the cumulative exposure level on the all haematopoeitic cancer hazard for a combined analysis (p=0.20) or separately for males (p=0.12) or females (p=0.34). Steenland et al. (2004) refitted the models to indicator variables representing four quartiles of categorical exposure and again found no significant relationship between the cumulative exposure category and the lymphohematopoietic cancer hazard. Introducing time lags of 10, 15 and 20 years in cumulative exposure, Steenland et al. (2004) re-estimated the proportional hazards models using both actual estimated exposure values and the natural log transformation. For males only, the best fitting model (log cumulative exposure, 15 year lag) achieved a marginal significance level (p=0.02). In the broad class of models considered for females, the significance of the modeled relationship between lymphohematopoietic cancer hazard and the various exposure metrics did not approach significance under a nominal α =0.05 level.

Steenland, et al (2004) defend their model exploration steps against criticism of possible "data dredging" (and thus consideration of multiple testing criteria) by pointing out that prior work has shown latency (a lag in exposure measures) to be important in studies of cancer for occupational exposures and that empirically the log of cumulative exposure has provided a better fit to these types of time integrated data. To summarize, Steenland et al's work hints at a relationship between EtO exposure and all haematopoeitic cancer hazards, but only for men and not for women. In a statistical sense, this evidence for an exposure relationship in males is at best marginally significant and its estimated strength is influenced by the chosen exposure metric—the best metric being the log of estimated cumulative exposure (lagged to t-15 years). Similar sensitivity to the transformation of the exposure metric is seen in the Cox regression results relating breast cancer hazard to EtO exposure.

Given these results, the EPA should carefully consider the scientific justification for a "men only" model for its assessment of the risk of lymphohematopoietic cancer hazards. There should be a strong scientific argument for excluding the female data. Presently, the draft document identifies no basis for excluding the female data. In the data set, women on average have lower average levels of estimated exposure to EtO (possibly more relevant to the exposures of interest in the risk assessment). By the same turn, the results of the extensive modeling effort suggest that the significance of the model fit is influenced by the chosen exposure metric and the best fitting models are nonlinear with respect to exposure. Panel members are not challenging the statistical analyses presented in the Steenland et al (2004) paper. However, the Panel encourages the EPA to narrow its modeling efforts to functional forms, exposure metrics, and disease outcomes that make sense from a biological and risk assessment perspective. The process of model building should also include a challenge to any model (e.g. log cumulative exposure, 15 year lag) which yields results that differ substantially from a second model that only changes the scale of the exposure metric (e.g. cumulative exposure, 15 year lag).

6. Preferred model justified, alternative analytical approaches.

As discussed in 2.b.1-2.b.4 above, the Panel recommended exploration of use of a number of models, including non-linear models, to fit data within the observation range and calculate a point of departure (POD). Preference for biologically-based models was indicated.

7. Statistical issues

Pages 29-49 of the draft Evaluation outline the EPA's proposed approach to estimation of the Inhalation Unit Risk for EtO. In addition to the general issues of estimation and model-based extrapolation described above, there are a number of statistical assumptions and methods used in this approach that deserve mention.

Conditional on the cancer slope factor results from the weighted least squares regression analysis, the life table (BEIR IV) approach to the determination of the $LEC_{.01}$ is programmed correctly.

The life table methodology that is the basis for the BEIR IV algorithm is designed to estimate excess mortality and is not readily adapted to modeling excess risk for events (incidence) that do not censor observation on the individual in population under study. The methodology for substituting the mortality slope to an excess risk computation for HL cancer incidence requires the assumption of a proportional rate of incidence/mortality across the cancer types that are included in the grouped analysis. This is generally not a viable assumption. The Panel therefore discourages the use of the BEIR IV algorithm for extrapolation of the cancer mortality algorithm to estimation of excess cancer incidence.

Several Panel members commented on the use of the upper confidence limit for the estimated slope coefficient as the basis for estimating an $LEC_{.01}$. The Panel encourages the EPA to present unit risk estimates based on the range of $EC_{.01}$ values corresponding to the lower 95% confidence limit, the point estimate, and the upper 95% confidence limit for the estimated cancer slope coefficients from the final dose-response models.

2.c. Age-dependent Adjustment

Is the incorporation of age-dependent adjustment factors (ADAF) in the lifetime cancer unit risk estimate, in accordance with EPA's Supplemental Guidance (U.S. 2005b), appropriate and transparently described?

The Panel felt that the application of ADAF by the Agency was appropriate, but that the description in the Draft Assessment was not adequate, particularly for those not familiar with the EPA's Supplemental Guidance. A clear description of the ADAFs is important for the Draft Assessment. For example, on page 57 line 15 the lifetime unit risk calculation with ADAFs is presented. Unless the reader knows the binnings and associated uncertainty factors, this would not be understandable to the average reader. There was also discussion of the type of information that would be needed to address the issue of potential increased sensitivity of children.

While the Panel recognized the role of the childhood exposure uncertainty factors in the broader risk assessment process, it did not simply accept the defaults without first attempting to establish the biological arguments for their application in the case of EtO. The Draft Assessment states that because of the immaturity of detoxifying enzymes, a child's susceptibility may be

increased. This should, however, be extended to include the same comment about DNA repair enzymes being immature, and the presence of more DNA synthesis due to growth, and thus a further increased risk if exposure occurs during development.

In general, the Panel concluded that the lack of data examining the impact of prenatal and childhood exposure to ethylene oxide is a major limitation in the assessment of cancer risk (Kim et al., 2006; Olshan et al., 2000; Anderson et al., 2000). Given this uncertainty, the application of ADAFs in estimating cancer risks due to childhood exposure is warranted.
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2.d. Models For Occupational And Environmental Exposures

Is the use of different models for estimation of potential carcinogenic risk to humans from the higher exposure levels more typical of occupational exposures (versus the lower exposure levels typical of environmental exposures) appropriate and transparently described in Section 4.5?

The use of different data to estimate different dose response curves should be avoided unless there is both strong biologic and statistical justification for doing so. The Panel believed this justification was not made in the Agency's draft report.

The Panel believes that fitting separate models for occupational and environmental exposure levels, as proposed by EPA, requires some mechanistic justification. An alternative is to provide fits based on two known distortions from the expected linear shape of dose response relationships: the errors-in-variables issue as analyzed in Appendix C, and the healthy worker survivor effect discussed elsewhere in this report. Such analyses are not simple, but they do provide mechanistically justifiable ways to analyze the data without either arbitrarily dividing the data in to different regions of exposure or adopting a supralinear dose response form that does not have an obvious basis in plausible biological mechanisms."

2.e. Extrapolation from animal studies

Are the methodologies used to estimate the carcinogenic risk based on rodent data appropriate and transparently described? Is the use of "ppm equivalence" adequate for interspecies scaling of EtO exposures from the rodent data to humans?

The ppm equivalence method is a reasonable method for interspecies scaling of EtO exposures from rodent data to humans. If the use of animal data becomes more important (i.e., the principal basis for the ethylene oxide unit risk value), more sophisticated approaches such as PBPK modeling should be considered. The PBPK models that Filser's group (cite) and Fennell and Brown (cite) have developed are appropriate. One Panel member conducted a PBPK model some time ago and found that it gave very similar results to the ppm equivalence approach, although this should be revisited [in the event that animal data assume a greater role in the ethylene oxide unit risk].

All of the animal cancer data need to be presented as survival adjusted data.

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http://seer.cancer.gov/faststats/sites.php?stat=Incidence&site=Myeloma&x=18&y=14
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Appendix A

Discussion of the Resurgent Controversy over Thresholds for Genetically Acting Agents
Dale Hattis, Ph.D.

The roots of the historical controversy can be traced to a basic difference between different sets of disciplines in mental models of biological systems, and the ways that chemicals and other perturbing influences can cause effects. The disciplines of physiology, traditional toxicology and pharmacology tend to foster a view of biological systems as complex interacting webs of processes. These systems are seen as exquisitely designed so that perturbation of any one parameter automatically gives rise to countervailing adaptations that, if the perturbation is not too large, will keep the systems functioning within normal limits without serious or long lasting harm. This mental model leads directly to a general expectation that there should be thresholds in dose response; for any toxicant that acts by overwhelming some set of homeostatic processes there should be a dose below which the system can handle the perturbation without a meaningful adverse effect.

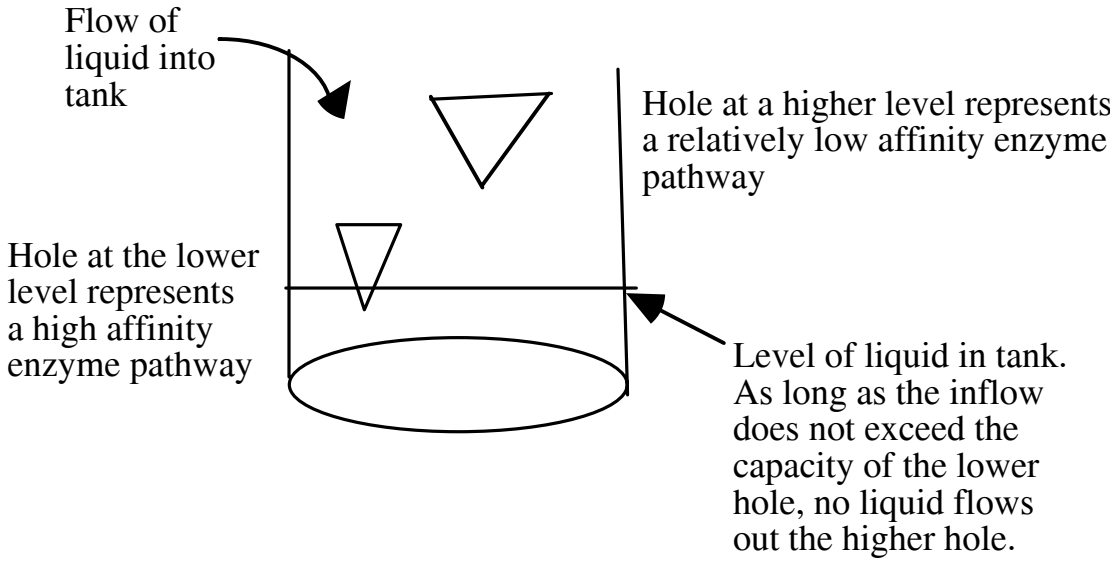
A different vision of some fundamental life processes arose from the ex-physicists who created the discipline of molecular biology in the decades after the end of World War II (e.g., Stent, 1963). This is the notion that there is a basic fragility in some functions that are central to life. When both somatic and germ cells divide, an enormous amount of information must be faithfully copied and distributed between the progeny cells. Mistakes can occur in this copying, and a change at even a single place in the DNA can give rise to important adverse (or, very rarely, beneficial) effects if by chance the mistake happens in just the wrong place in the DNA of the wrong cell. This leads to an intuition that even a single molecule of a DNA reactive chemical has a small but finite chance of doing lasting damage if it happens to react with the wrong place on DNA and if the DNA lesion is not repaired by the next time the DNA is copied.

In the 1970s and early 1980s it was recognized that basic bimolecular reaction kinetics require a fundamental linearity between the concentration of DNA reactants and relevant sites on DNA. However it was also recognized that there were many opportunities for at least high-dose nonlinearities both before and after DNA reaction in the sequence of events from intake of a DNA reactive agent (or a metabolic precursor) into the body to the ultimate manifestation of tumors (Hattis 1990).

In the 1970s some looked to pharmacokinetics as a potential source of threshold dose response relationships that might intervene between toxicant intake and the delivery of DNA reactive molecules to the nucleus of relevant cells. Figure 1 is an illustration similar to one that was published in Science (attributed to researchers at Dow Chemical) that attempted to make this pharmacokinetic-based threshold idea plausible. In the diagram, liquid (representing a continuous dosage of a toxicant) flows into a tank with two triangular holes. The level of liquid rises in the tank until some begins to flow out of the lower of the two holes (representing a high-affinity metabolic pathway producing a "safe" metabolite). A further rise occurs until the amount of liquid flowing out of the tank equals the amount flowing in. If the inflow is small enough that it can be completely balanced by flow out of the lower hole, then the liquid will not

Figure 1

Argument for the Plausibility that Thresholds Might Arise From the Competition Between Metabolic Pathways Producing Safe and Dangerous (DNA Reactive) Metabolites



rise to the level of the higher hole (representing the lower affinity enzyme producing the dangerous metabolite). Thus the analogy predicts a threshold of inflow into the tank, below which all of the metabolism is via the "safe" high affinity pathway.

Unfortunately, this representation of the competition between higher and lower affinity metabolic pathways is not compatible with conventional Michaelis-Menten enzyme kinetics (Hattis, 1990; Slikker et al. 2004). Using the basic Michaelis/Menten equation, the rate of the activating reaction (producing the dangerous metabolite, D) is:

$$\frac{dD}{dt} = \frac{V_{max}[C]}{K_m + [C]}$$

where [C] is the concentration of substrate (the form of the toxicant that is absorbed from the environment), Vmax is the maximum rate of the reaction that produces the dangerous metabolite, and Km (the Michaelis constant) is the substrate concentration at which the reaction proceeds at half of its maximum velocity (Vmax). Similarly the rate of the competitive detoxifying reaction (producing the safe metabolite, S) is:

$$\frac{dS}{dt} = \frac{V_{max}'[C]}{K_m' + [C]}$$

The [C]'s in the denominator of both equations can be neglected at low doses when they become small relative to the K_m's. At low doses we can therefore find the ratio of the substrate [C] that goes by the dangerous and safe metabolic pathways by simply dividing the two equations:

$$\frac{\text{rate of D production}}{\text{rate of S production}} = \frac{V_{max}[C]/K_m}{V_{max}'[C]/K_m'}$$

and because the numerator [C]'s now cancel, it can be seen that we are left with a ratio of four constants. This means that below the dose region where there is appreciable saturation of the enzymes producing either the safe or the dangerous metabolite, the fraction of the substrate taken by each pathway approaches a constant, independent of dose. There are no dose rate effects in this low dose region, there can be no thresholds, and indeed the system must operate linearly at the limit of low dosage, albeit with a different distribution of metabolism between "safe" and "dangerous" pathways than would be observed at higher doses. At the limit of high dose, the ratio of production of the dangerous to the safe metabolites is governed only by ratio of the two Vmax values; whereas at lower doses the Km's become progressively more involved. If the

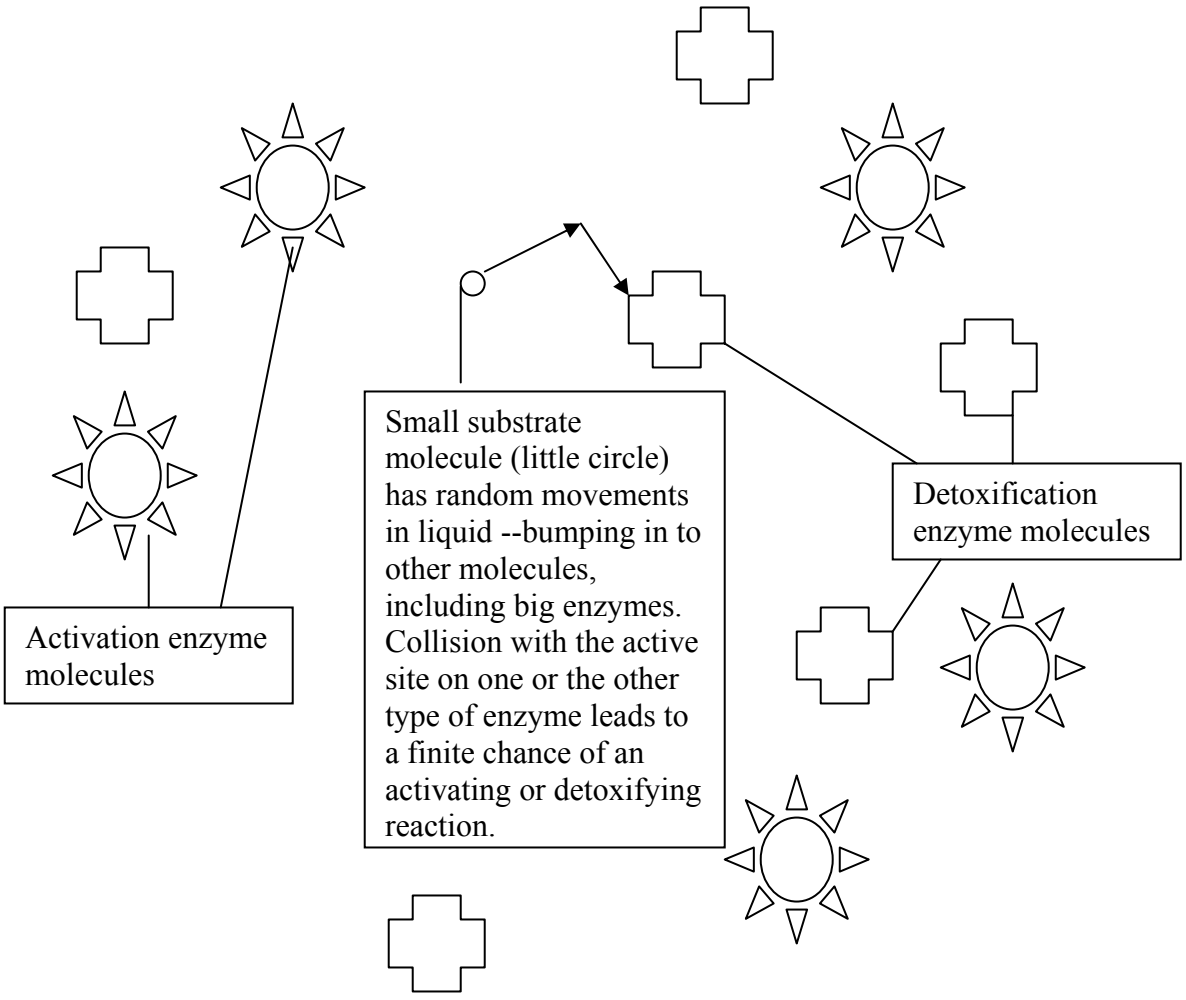
higher affinity (lower Km) pathway produces the dangerous metabolite, then the fraction of material metabolized by the dangerous pathway will be greater than at the highest saturating doses, resulting in a convex-upward dose response relationship for DNA damage (e.g. the pattern seen for vinyl chloride). On the other hand, if the safe pathway has the lower Km then the portion of the chemical processed by the safe pathway will be greater at lower doses than is seen at higher doses. In the abstract of a paper (Gehring et al 1978) describing a process model for carcinogenesis from electrophilic agents, Perry Gehring acknowledges that there should be an expectation for some "albeit negligible" carcinogenic risk from genetically acting chemicals at low doses.

It is well to emphasize that the basic Michaelis-Menten equation applied above is not simply an empirical formula. It is well grounded in fundamental mechanistic considerations of receptor association and dissociation kinetics with reasonably wide applicability (Hoel, 1985), The maximal velocity, Vmax, arises because there are a limited number of enzyme molecules available to catalyze the reaction, and each enzyme molecule is necessarily constrained to operate at a finite rate in converting its substrate into its product. The fact that the reaction proceeds linearly at low doses (with a rate constant of Vmax/Km) arises from the fact that the reaction is limited by the rate of diffusion of the substrate molecules into the active site of the enzyme—a rate that must be linear with substrate concentration at the limit of low doses. In the light of this Figure 2 offers a more accurate molecular-scale vision of the competition between enzyme-mediated activating and detoxifying processes. Each small substrate molecule has a "random walk" through a cellular compartment as it rebounds from collisions with other molecules. At the limit of low dosage, when there are few or no other similar substrate molecules around, the substrate molecule must have a finite chance of encountering the active site of each type of enzyme (or, similarly, a transport molecule taking it to a different compartment). Therefore each type of enzyme or macromolecular transporter must have finite opportunity to process the substrate molecule at the limit of low dosage.

The basic Michaelis-Menten equation form applies with equal force to active transport processes (in which specialized molecules utilize energy to pump specific molecules or ions into our out of cells), and to DNA repair processes. Thus the fundamental expectation for low dose linearity applies similarly to these other components of the causal chain between external exposure and the generation of somatic mutations that are components of carcinogenesis. At the limit of low dose the Michaelis Menten enzyme/transport reaction rates are limited by the rate of diffusion of substrate molecules into the active sites of the enzymes/transport molecules; and those diffusion processes, given a specific temperature, are linear functions of substrate concentrations.

Figure 2

A Molecular Vision of the Low-Dose Competition for Substrate between Activating and Detoxifying Enzyme Molecules



With this as background, we can now examine the bases for some more recent claims that thresholds should be expected at low doses for genetically acting agents. A convenient starting point for this examination is a special issue of Mutation Research published in 2000 by participants at a conference on threshold mechanisms of carcinogenesis sponsored by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Without going through the threshold claims from each of the papers in this collection individually (Kirshch-Volders et al. 2000; Schulte-Hermann et al. 2000; Muller and Kasper 2000; Moustacchi 2000; Parry et al. 2000; Swenberg et al. 2000; Lowell 2000; Madle et al. 2000; Henderson et al. 2000; Crebelli 2000; Kirkland and Muller 2000; Speit et al. 2000; Parry 2000), three main types of arguments stand out:

- Multiple targets. Some specific modes of genotoxicity are reported to depend on multiple interactions between chemicals and target macromolecules (rather than the single-DNA-reactant-molecule DNA adduct formation mechanism discussed above). If the number of target interactions required to produce an effect is large, the resulting low dose dose-response relationship can be expected to be highly upward-turning, and well approximated by a threshold.

- Multiple barriers. A molecule of a chemically reactive agent must pass multiple transport, potential detoxification, alternative targets for reaction other than DNA, and DNA repair hurdles in order to cause a permanent change in DNA sequence or chromosomal damage. The multiplicity of these hurdles makes it unlikely that any single molecule could cause an actual mutation along the pathway to carcinogenesis. If these multiple barriers do not produce an "absolute threshold" they can at least be expected to lead to a "pragmatic" or "practical" threshold below which exposure is of no real biological consequence.

- Inducible detoxification, apoptosis, and/or DNA repair processes. One result of exposure to a toxicant may be the induction of the levels of a variety of cellular and genomic defense processes. If this induction is effective enough, and occurs at low enough doses, it is possible that the prevention "good" that results from avoidance or repair of mutagenic damage from background processes may even be great enough to exceed the direct mutagenic harm done by the toxicant itself. This gives rise to "hormetic" dose response relationships in which the net mutagenesis and carcinogenesis is even reduced by some range of exposures to the toxicant compared to background (zero dose) levels.

Modes of Genetic Action Requiring Multiple Interactions with Macromolecular Target Molecules or Structures

The first paper in the Mutation Research special issue (Kirsch-Volders et al. 2000) gives a reasonable theoretical mathematical account of the dependence of the shape of the dose response curve on the number of macromolecular targets that must be "hit" in order to produce an effect. Essentially, if a single hit on DNA, an alpha or beta tubulin structure, or topoisomerase is sufficient to cause an effect (assuming imperfect repair) then the fundamental math calls for a single hit Poisson process:

$$\text{Probability of Effect/Target} = 1 - e^{-m} \qquad (1)$$

where m is the average number of "hits" per target. In cases where the number of hits per target needed to cause an effect is larger than 1 (e.g. according to the authors, where the target is the spindle drawing chromosomes to different progeny cells during mitosis, or the nuclear membrane, by a mechanism that is not detailed in the paper), then the appropriate Poisson term for n hits required per target is substituted:

$$\text{Probability of Effect/Target} = 1 - e^{-m}\frac{m^n}{n!} \qquad (2)$$

(where the notation n!, translated to English as "n factorial" means n X n-1 X …. All the way down to 1.)

The larger the n, the more steeply upward-turning the resulting curve will be—increasingly resembling, but not the same as a curve with a true threshold of zero probability of effect for a finite dose.

Later on, Kirsch-Volders et al (2000). add:

"To be able to clearly assess a threshold, the spontaneous frequency of the analysed endpoint should be very low, ideally equal to zero; indeed a too high spontaneous background will lead to additive effects and a difficult estimation of small increases at low dose level."

This comment undermines considerably the generality of the earlier application of multi-hit analysis to putative multi-target mutation/chromosomal damage mechanisms at very low doses. Essentially it says that in order for the multi-hit formulas to apply at the limit of low dosage, the genetically active agent must cause genetic changes by a mechanism that is somehow distinct from all the processes that cause the appreciable background of genetic changes from all other endogenous and exogenous agents, as well as the imperfections in functioning of the apparatus of polymerases, spindle proteins etc. that maintain, copy and transmit the genetic material.

The essential low-dose linearity of agents that act in concert with background processes was discussed in some of the foundational papers that derived the methods for inferring low dose cancer risks in the 1970s (e.g. Crump et al. 1976). This general expectation can be illustrated with a simple example of a two-stage mutation process in which there is a background of 1 arbitrary unit and an expectation for 1 additional induced unit per 1 mg/kg continuous dose of a mutagenic agent (Table 1, adapted from Hattis and Smith, 1986). It can be seen that at high doses, the dose response relationship between excess tumors over background vs dose of the inducing agent appears almost perfectly proportional to $(\text{dose})^2$. This is because at high doses, far above the background rate of the tumors, the agent predominantly acts by causing both mutations in the two-step process. As the dose is reduced to regions where it causes mutations that are a small fraction of background rates, the induced mutations predominantly cooperate with mutations that result from the background processes—leading to an increment in tumors over background that approaches linearity with dose of the added inducing mutagen.

Table 1

Effect of Background Mutation Rates on the Carcinogenesis Dose-Response Curve at Low Doses, Assuming a Hypothetical Two-Stage Carcinogenic Process

Dose	Rate of 1st Transition (1 extra unit per unit of dose)	Rate of 2nd Transition (1 extra unit per unit of dose)	Relative No. of Tumors (background = 1) (product of two previous columns)	Induced Excess Over Background
1000	1001	1001	1,002,001	1,002,000
100	101	101	10,201	10,200
10	11	11	121	120
1	2	2	4	3
.1	1.1	1.1	1.21	0.21
0.01	1.01	1.01	1.0201	0.0201
0.001	1.001	1.001	1.002001	0.002001

Source: Adapted from Hattis and Smith, 1986.

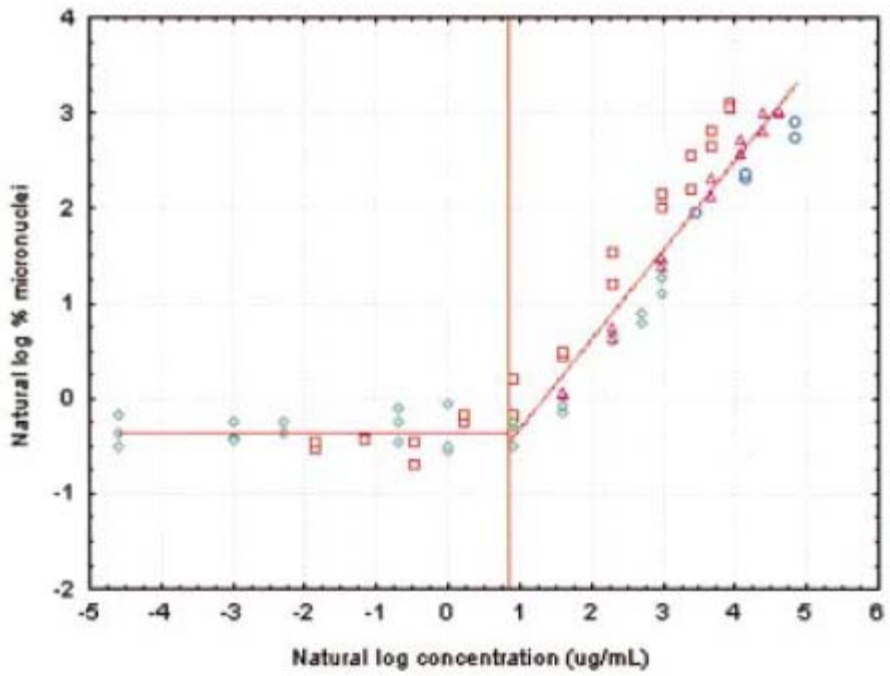
One example of a process that may involve multiple targets is the action of spindle poisons such as vinblastine and colcemid (Parry et al. 2000). Older observations by Elhajouji et al (1995, 1997) are often cited as evidence of thresholds for agents that inhibit spindle function. In vivo data are available in a recent report by Choudury et al. (2004). However even in this case, it is worth compiling data on background rates and mechanisms of spindle malfunction to assess the extent of potentially interacting background processes.

A more questionable example of a postulated threshold process that seems sometimes to be attributed to the multiple target type of theory is the inhibition of topoisomerase* (Lynch et al. 2003; Bolt and Degen 2004). From the available description of the mode of action of topoisomerases (see footnote) it is not completely clear that multiple targets are involved in the action of topoisomerase inhibitors to enhance single- and double-strand breaks at individual locations on DNA. A topoisomerase enzyme molecule apparently acts by itself in stabilizing a double strand break at a specific place in DNA. An inhibited enzyme molecule gives rise to a delay in religation with no mention of a need for cooperativity either between inhibitor molecules or between inhibited enzyme molecules. Nevertheless Lynch et al., after citing Kirsch-Volders et al (2000) and other papers in the same special issue of Mutation Research proceed to assume that because the interaction of inhibitor is not directly with DNA, a threshold theory for the topoisomerase inhibitor mode of action is biologically justified. They then go on to offer as experimental evidence, a particular kind of plot of dose response results for in vitro induction of micronuclei by three different topoisomerase inhibitors (Figures 3-5, but not Figure 6). In the first three of these plots the log of the % micronuclei is plotted vs the log of the concentration of the topoisomerase inhibitor in the culture

* In the last two decades an important role has become apparent for topoisomerase II in normal DNA replication. This enzyme gets its name from its function to change the topology of DNA during replication, transcription, and repair. It binds covalently to DNA in such a way as to produce a temporary double-strand break, allowing another DNA strand to pass through it. After this, normally the breaks in the two strands are rejoined (religated). However some compounds (DNA topoisomerase inhibitors) can stabilize the usually transient state of the complex with the double strand break unrepaired, and inhibit religation. This leads to chromosome breakage and rejoining events that in rare cases splice together inappropriate portions of different genes leading to uncontrolled cellular growth promotion signals.
Just such gene fusions are responsible for a key step in the development of several types of Acute Lymphocytic and Acute Myeloid Leukemia in children (Lightfoot and Roman 2004). By examining blood spots made at birth in children who later developed leukemia, it has been found that these events often happen before during fetal life; to be followed by one or more subsequent steps in leukemia development after birth (Gale et al. 1997).
Epidemiological studies indicate that cases of adult leukemias occur at increased frequency after chemotherapy with topoisomerase II inhibitors for other cancers (Greaves, 1999; Le Deley et al. 2007) compared to patients treated with other types of chemotherapy. Topoisomerases inhibitors are also used deliberately in the control of HIV, but activity of this type has also been reported from a metabolite of the headache remedy acetaminophen (Bender et al. 2004) and in a wide variety of foods and herbal medicines (Lightfoot and Roman, 2004). So far, there is limited evidence that maternal dietary consumption of specific DNA topoisomerase II inhibitors increases the risk of gene fusion related leukemias (Spector et al. 2005; Alexander et al. 2001; Ross 1998).

Figure 3

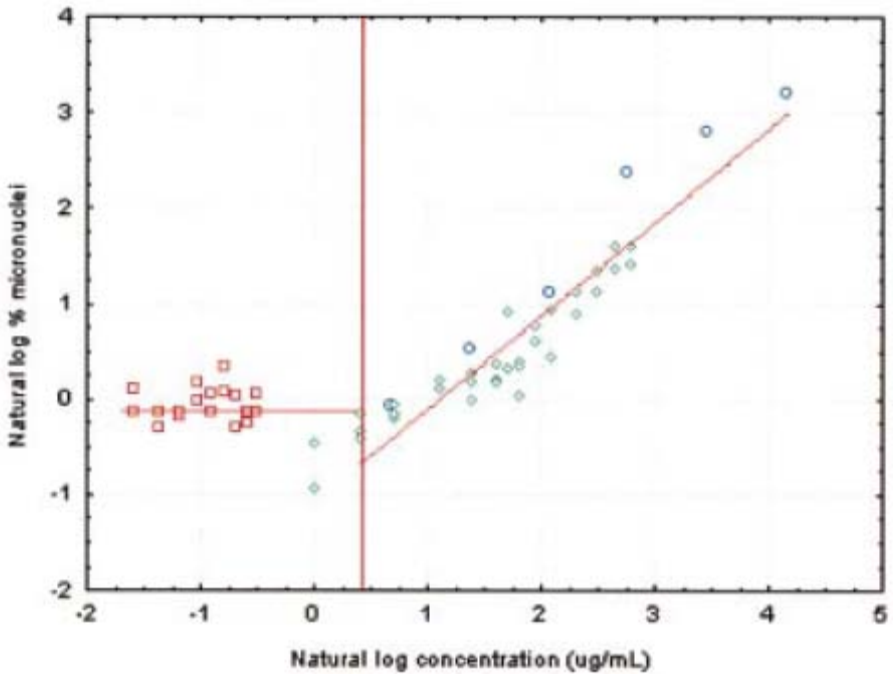
Log-Log Plot From Lynch et al. (2003) Offered in Support of a Threshold in the Dose Response Relationship for Induction of Micronuclei in L5178Y Mouse Lymphoma Cells by the Topoisomerase Inhibitor Etoposide*



* Source: Lynch et al. 2003. The different colored points represent different assays. The caption to this graph is "Broken stick model. The breakpoint was identified at ln(conc.) = -6.049 or 0.00236 µg/ml on the original concentration scale with ln(%MN) = -.364 fitted before the breakpoint and ln(%MN) = -5.234 + .94 X ln(conc) fitted afterwords. The goodness of fit (R^2) was 93.4%."

Figure 4

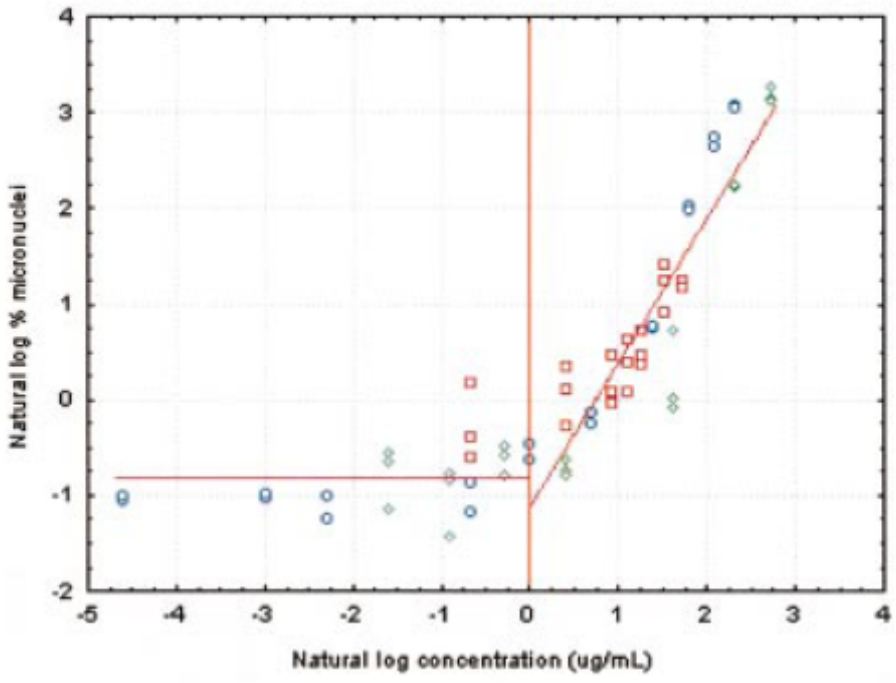
Log-Log Plot From Lynch et al. (2003) Offered in Support of a Threshold in the Dose Response Relationship for Induction of Micronuclei in L5178Y Mouse Lymphoma Cells by the Topoisomerase Inhibitor Doxorubicin*



* Source: Lynch et al. 2003. The different colored points represent different assays. The caption to this graph is "Broken stick model. The breakpoint was identified at ln(conc.) = -6.495 or 0.00151 µg/ml on the original concentration scale with ln(%MN) = -0.135 before the breakpoint and ln(%MN) = -5.674 +.979 X ln(conc) fitted afterwards. The goodness of fit (R^2) was 89.2%."

Figure 5

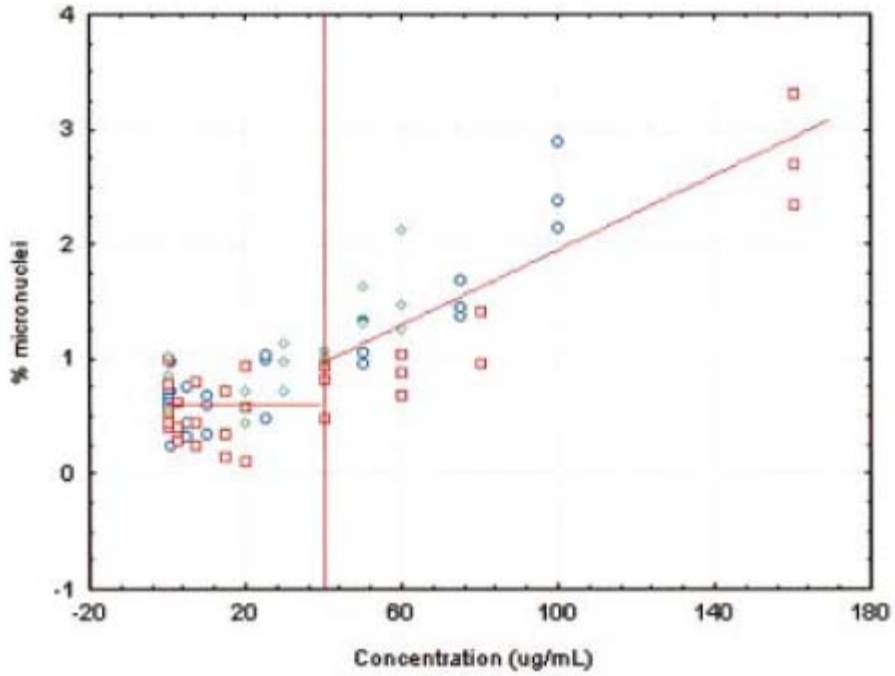
Log-Log Plot From Lynch et al. (2003) Offered in Support of a Threshold in the Dose Response Relationship for Induction of Micronuclei in L5178Y Mouse Lymphoma Cells by the Topoisomerase Inhibitor Genestein*



* Source: Lynch et al. 2003. The different colored points represent different assays. The caption to this graph is "Broken stick model. The breakpoint was identified at ln(conc.) = 0 or 1 µg/ml on the original concentration scale with ln(%MN) = -0.817 before the breakpoint and ln(%MN) = -1.117 + 1.508 X ln(conc) fitted afterwards. The goodness of fit (R^2) was 88%."

Figure 6

Linear %MN vs Linear(concentration) Plot From Lynch et al. (2003) Offered in Support of a Threshold in the Dose Response Relationship for Induction of Micronuclei in L5178Y Mouse Lymphoma Cells by the Topoisomerase Inhibitor Ciprofloxacin*



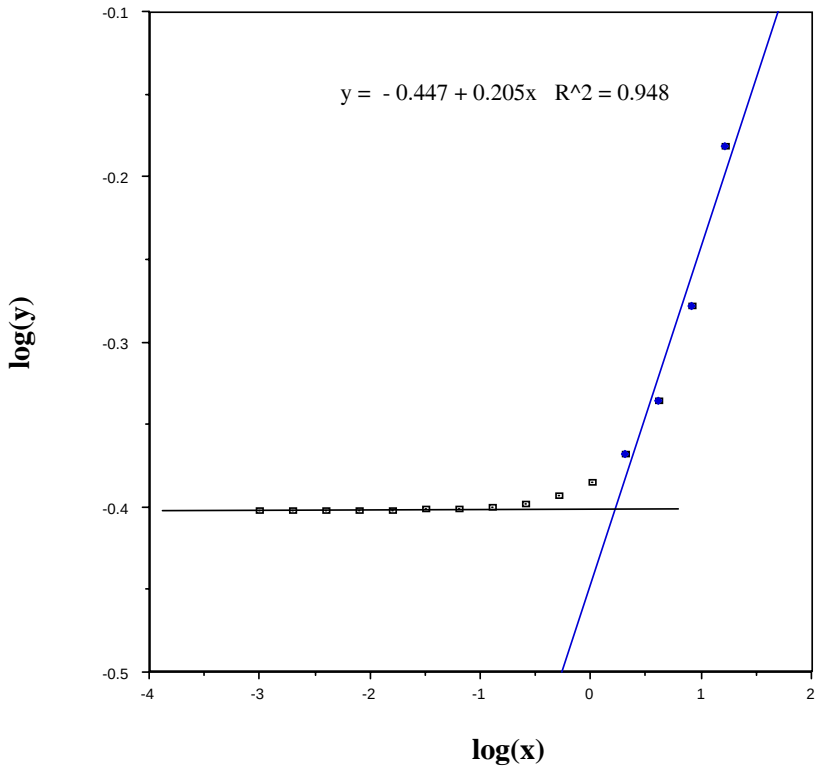
* Source: Lynch et al. 2003. The different colored points represent different assays. The caption to this graph is "Broken stick model. The breakpoint was identified at conc. =40 µg/ml with % micronuclei = 0.636 fitted before the breakpoint and % MN = 0.392 + 0.016 X conc. Fitted after the breakpoint. The goodness of fit (R^2) was 77.1%."

The log log plots of Figures 3-5 are an example of how disciplinary perspectives can shape the presentation and interpretation of evidence. From a toxicological perspective, there are two realms of concern – the realm of homeostasis and the realm in which homeostasis has broken down. Then using a logarithmic plot for the x axis seems appropriate since the logarithm of concentration makes available a great space on the graph for the very low doses that would be relevant if homeostatic effects were dominant. However the data in this low putative homeostatic realm are almost always completely uninformative. From a molecular biological perspective as well as an experimental biological perspective, the putative homeostatic region is one for which the data are consistent with a linear extrapolation to zero or to a fixed background as can be seen quite clearly in Figure 6 (despite the misleading line and commentary).

Whether or not thresholds are assumed to exist, log log plots such as 3-5 can serve to illustrate the state of the experimental data in that region. However, in general, because a log log plot expands (potentially indefinitely) the curve near zero, even a linear no-threshold function with a background level unaffected by the toxicant will exhibit a "broken-stick" appearance (Figure 7). And whenever there is lack of precision in measurements (giving rise to an implicit background and obscuring the fine curvature barely discernable in Figure 6), the cosmetic effect that gives the appearance of two lines will be enhanced. For that reason log log plots have no force as evidence for the existence of thresholds. Thus, contrary to the suggestions by Wadell (2006; 2003) for tumor data more generally, anyone starting from a molecular or experimental perspective is likely to regard such plots as distracting from a natural linear extrapolation. [See also the published critique of Haseman (2003).] In our view such plots may be used (if presented carefully) to characterize data availability in a low dose region; but any presentation as evidence for threshold behavior is fundamentally misleading.

Figure 7

Plot of Log(y) vs Log(x) for the Simple Linear Relationship, y = .392 + .016x With Regression Line Fit to Last 4 Points



Arguments for Absolute Thresholds or "Practical Thresholds" from the Presence of Multiple Transport, Detoxification, and Repair Processes

The "multiple barriers" argument appears to be mostly a rhetorical device since its mathematical implications reinforce linearity. By reciting the series of opportunities that a molecule of a DNA-reactive agent has to go astray rather than react with DNA and then have the adduct cause a mutation in a gene that matters in a cell that matters for carcinogenesis, a speaker can make it appear very unlikely that such a chain of events could occur (Parry et al. 2000). And indeed, from the standpoint of a single molecule, the probability is necessarily very small. However each physical barrier has some probability of being surmounted by each molecule; each alternate reaction opportunity or detoxification enzyme will divert a finite fraction of the molecules, each DNA repair process will repair the molecules/adducts at a finite rate and therefore a finite fraction of the DNA lesions will persist to the time of the next passage of the polymerase enzyme responsible for copying the DNA. Similarly not all cells with damaged DNA will be removed by apoptosis, and cell cycle checkpoint functions will not perfectly prevent the copying of damaged DNA. If there is a finite rate of DNA lesion generation and a finite rate of DNA repair or removal by apoptosis, then there must be a finite rate of mutation that, at the limit of low dosage where saturation effects are negligible, must be a linear function of the number of DNA reactive molecules (or their precursors) that enter the system. Moreover once an initial tumor cell is generated there must be a finite probability that it will escape repression by its normal neighbors through gap junction communication and by other immune-based defense processes. The presumption of some of the discussion in the Mutation Research special issue (Herman et al. 2000) seems to be that at low doses some or many of these processes can be assumed to be perfect; but this is just not possible. The dose response relationship for the combined process is a simple multiplicative combination of the component processes. If all of these are linear at low doses, then the combined dose response relationship must also be linear at low doses.

A final refuge of this set of arguments is to distinguish between an "absolute" threshold (a true zero response at a finite dose rate)[*] and a "pragmatic" or "practical" threshold. Lowell (2000) argues:

"A 'pragmatic' threshold can be considered as a concentration below which any effect is considered biologically unimportant (Figure 2)[**] (Lutz, 1998). This term is used in a somewhat similar way to how ECETOC defines a biological threshold except that it implies that there may be effects occurring because of treatment or exposure but these are considered below what might be considered biologically important. An example might be increases that did not exceed the range of responses seen in negative control material in a well-conducted series of experiments. Such a threshold may be defined, in part, with the help of statistical tests. The distinction

[*] Lowell (2000) quotes a somewhat different definition of "absolute" threshold attributed to ECETOC: "…a concentration below which a cell would not 'notice' the presence of the chemical. In other words, the chemical is present but does not interact with the cellular target." The precise identification of such a threshold, if it exists is difficult.

[**] The figure referred to is not reproduced here. It shows an upward turning dose response curve beginning at the origin (zero response at zero dose) but a region of response shaded and labeled as "Biologically unimportant". The point where the continuously increasing dose response curve emerges from this "unimportant" shaded region is labeled as the "Practical Threshold".

between the various classifications of thresholds can initiate a philosophical discussion but is not relevant to regulatory risk assessment."

It appears, therefore, that this line of argument reverts to treating an effect that may be present but which cannot be clearly demonstrated as above "the range of responses seen in negative control material" (with some undefined sample size and sensitivity/detection noise) as if it weren't there. It seems to us that risk assessment methods have been created precisely to make fair assessments of the likely magnitude of effects that cannot be directly measured but are still potentially substantial enough that decision-makers and the public may reasonably care about reducing them. Or, put another way, "practical" for a biologist to detect, may not mean the same as of "practical" concern because someone might get hurt. Our view is that terms such as "practical threshold" are inherently evasive and do a disservice to transparent public analysis and discussion of likely underlying realities.

Arguments for Thresholds or Hormetic Dose Response Relationships from Possible Inducible Detoxification or DNA Repair Processes

In contrast to some of the arguments reviewed above, this category of mechanisms does have some potential to produce changes to the low dose linear expectation under some circumstances. Up to this point we have discussed the several processes producing high dose nonlinearities in dose response relationships as if their levels were static—fixed at some baseline level of activity/efficiency in promoting or reducing damage to DNA or subsequent steps in the carcinogenic process. In fact, however, it is not unlikely that the levels of the enzymes that mediate these processes are themselves regulated by feedback mechanisms that respond to influences from the external and internal environment, as do many other components of biological systems (Schulkin, 2003). It is certainly possible, in theory, that under some circumstances the induction of detoxifying or DNA repair enzymes (e.g. from radiation--Schmerold and Wiestler 1986; Chan et al. 1992) could have the side-effect of preventing or repairing enough "background" damage as to outweigh the primary damage done by the inducing toxicant over some range of dosage. Whether such possible offsetting effects could extend all the way down to the limit of low dosage depends on the fundamental dose response relationships underlying the induction process(es) and the levels and types of "background" damage of the that are available to be prevented.

Specifying the requirements for this helps illuminate the special nature of the conditions that would be needed to produce a net biological benefit from a particular type of exposure to a genetically active agent:

- "Background damage" (e.g. from the DNA damaging free radicals produced as a byproduct of metabolism, other endogenous DNA reactive agents such as ethylene oxide and possibly formaldehyde, and other exogenous DNA reactive agents) must occur at sufficient rates that offsetting prevention benefits could occur,

- The usual "baseline" state of expression of the detoxification, DNA repair, apoptosis, or cell cycle check point mediators needs to be sub-optimal. Normally, one would expect that if it were net beneficial to have higher standing levels of a particular enzyme, then that would have been of selective advantage during evolution. Consequently people's normal constitutive should have

been adjusted to at least approximate optimality by natural selection. However, the types and levels of present-day exposures to mutagenic agents could conceivably be different enough from those present during the recent evolution of modern humans that prevailing constitutive levels of defensive enzymes are not perfectly tuned to current exposures. (For example cancer rates in wealthier industrialized countries tend to be very substantially higher than in poorer, less-developed countries. (Harris et al.1985)) In evaluating such possibilities, it is important to bear in mind that both detoxification enzymes and DNA repair enzymes can have adverse biological side-effects themselves. For example the same P450 "detoxification" enzyme that is induced by ethyl alcohol (Daiker et al. 2000; Feierman et al. 2003; Sato 1993) also is involved in the transformation of vinyl chloride to the activated form that reacts with DNA to induce the characteristic liver cancers produced by that compound. Epidemiologic data now exist indicating that high consumers of alcohol are much more susceptible to the carcinogenic effects of vinyl chloride (Mastrangelo et. al 2004). P450s also affect estrogen metabolism including some to genetically active agents. DNA excision repair enzymes, which repair DNA by cutting out small sections of DNA that has been damaged, also do damage themselves by making cuts at some finite rate in sections of DNA that do not contain pre-existing damage (Branum et al. 2001). Thus it is likely to be beneficial, biologically, to induce these enzymes above their baseline levels only when there is sufficient damage in a particular cell that the biological "costs" of the excision repair enzyme itself are outweighed by the need to repair an unusual amount of damage by a relatively rare exposure episode.

- The dose-response relationship(s) for induction of the detoxification and/or repair enzymes has to be steep enough, and the induction long lasting enough, that the prevention benefits are sufficient to offset the primary damage done by the inducing agent.

Appendix B

Illustration of a Simple Approach for Approximately Assessing the Effect of Measurement/Estimation Uncertainties for Individual Worker Exposures on Estimates of Dose Response Slopes*

Dale Hattis, Ph.D.

There are several steps in this analysis:

A. Use the cross-validation results for the Hornung et al. (1994) exposure estimation model to derive a preliminary quantitative estimate of the minimal likely measurement/estimation errors in the exposure levels used for calculating cumulative exposure for individual workers.

B. Derive an analytical expression for the observed distribution of individual male worker exposures in the reference group (that is, the non-lymphopoietic cancer cases) in the Steenland et al. (2004) study.

C. Remove the (assumed lognormal) variance attributable to random measurement/estimation errors from the lognormal variance of the observed worker exposures to derive an estimate of the lognormal variance of the real worker exposures.

D. Derive adjusted estimates of the likely real mean cumulative exposures of workers in each of the four categorical dose groups.

E. Redo the regression analyses of relative risk vs cumulative dose using the adjusted estimates of mean cumulative dose in each exposure group; assess the effects on the results of assuming larger estimates of measurement error than those directly derived from the Hornung et al. (1994) cross-validation analysis.

- **Implications of the Cross-Validation Results for the Hornung et al. (1994) Exposure Estimation Model**

Hornung et al. (1994), as part of the exemplary exposure assessment effort that led to the Steenland et al. (2004) study, gathered a total of 251 annual arithmetic means of measured ethylene oxide exposure levels in specific sets of job titles at 18 sterilization facilities based on 2700 individual full-shift charcoal tube samples. Before developing their exposure model, the data from 6 randomly selected plants (including 46 annual arithmetic means based on 350 individual charcoal tube samples) were set aside for later "validation" analysis of model performance. The model predicted individual annual exposures based on job/exposure category, product type, age of product, calendar year, rear exhaust, aeration procedure, and sterilizer volume. Table 1 summarizes the performance of the eventual exposure model—juxtaposing these 46 values with the eventual model predictions.

* An updated version of this case study will appear in a white paper on uncertainty in cancer risk assessment that is in process under separate EPA funding.

Table 1

Hornung et al. (1994) Comparison of Model-Predicted Annual Average 8-Hour Average Time-Weighted Average Exposure Levels with 46 Observations Not Included in the Data Used to Develop the Model

	Measured Level (ppm)	Model 2 "Prediction" (ppm)	Measured/ Model ratio
Gmean	2.22	1.5	1.48
Geom. Std. Dev.	3.8	5.09	.
Arith mean	4.62	3.5	1.32
Standard dev	5.76	3.79	.
Range	0.1-32.0	0.05-15.7	.

Bias = average of all 46 d_i's where d_i = prediction – measurement = 1.13 ppm

Precision = standard deviation of all d_i = 3.66 ppm

Overall the precision of the model predictions was superior (less) than the predictions of 11 different industrial hygienists with access to the same information, considered individually or collectively.

For purposes of this analysis it is desirable to separate the contribution of the indicated "bias" (apparent systematic underprediction of exposures relative to the measured exposures) to the random error represented by the "precision" measure of deviation. The minimum estimate of the contribution of the "bias" to the variance (square of the standard deviation) represented by the "precision" observation is

Prediction variance = $\text{Precision}^2 - \text{Bias}^2 = 13.4 - 1.3 = 12.1\ \text{ppm}^2$

In terms of an arithmetic standard deviation, the adjusted "precision" estimate is therefore $(12.1)^{0.5} = 3.48$ ppm. Dividing by the arithmetic mean of 4.62 ppm this yields a precision coefficient of variation of about 0.75.

It is clear from the relatively large ratio of the arithmetic standard deviations to the means of both the measured and model predicted values that the distributions will be better described by lognormal than arithmetic distributional statistics. (This is nearly always the case for exposure distributions.) Fortunately one can use a standard formula (Aitchison and Brown, 1957) to convert the "precision" coefficient of variation to an estimate of the geometric standard deviation (the antilog of the standard deviation of the log-transformed exposure values). Where CV is the coefficient of variation, an estimate of the geometric standard deviation is:

$$\text{Geometric Standard Deviation} = e^{\{\ln[CV^2+1]\}^{0.5}}$$

Applying this formula to the precision CV derived above yields a geometric standard deviation of 1.95 (about 2)—meaning that the random error in the exposure estimates is such that about 2/3 of the estimated values are expected to be within 2-fold of the actual values; and 95% of the estimates are expected to be within 4-fold of the true exposures.

In fact, however, there is reason to expect that the actual uncertainty may be larger than this. All of the exposure measurements used to derive and test the model were from the mid 1970s and later, whereas some of the exposures that were estimated appear to go back to the first regular use of ethylene oxide for sterilization in 1938 (Stayner et al. 1973). In the use of their exposure model, Hornung et al. assumed that exposures prior to 1978 were equal to the values that would apply to 1978. This creates some additional uncertainty in the exposure analysis that is not captured in the analysis of precision of exposure estimates from the 1970s and 1980s. Therefore the analysis below will include a hypothetical case assuming a much larger random error (3 times the estimated variance, corresponding to a geometric standard deviation of about 3.2) than that directly derived from the estimated imprecision in 1970s-1980s exposures.

- **Estimating the Cumulative Exposure Distribution for the Reference Group (Non-Lymphoid Cancer Cases)**

The main inputs for estimating the 15-year lagged exposure distribution for the reference group were the boundary lines for the exposure categories and the estimated numbers of workers in each category of accumulated exposure (Table 2). An initial probability plot of based only on the estimated numbers of workers with finite exposures in the different groups (Figure 1) led to the conclusion that the data (represented by the points) are reasonably described by a lognormal distribution (the fitted line) with a geometric mean of about $10^{0.336}$ = 2320 ppm-days and a geometric standard deviation of about $10^{1.22}$ = 16.6.

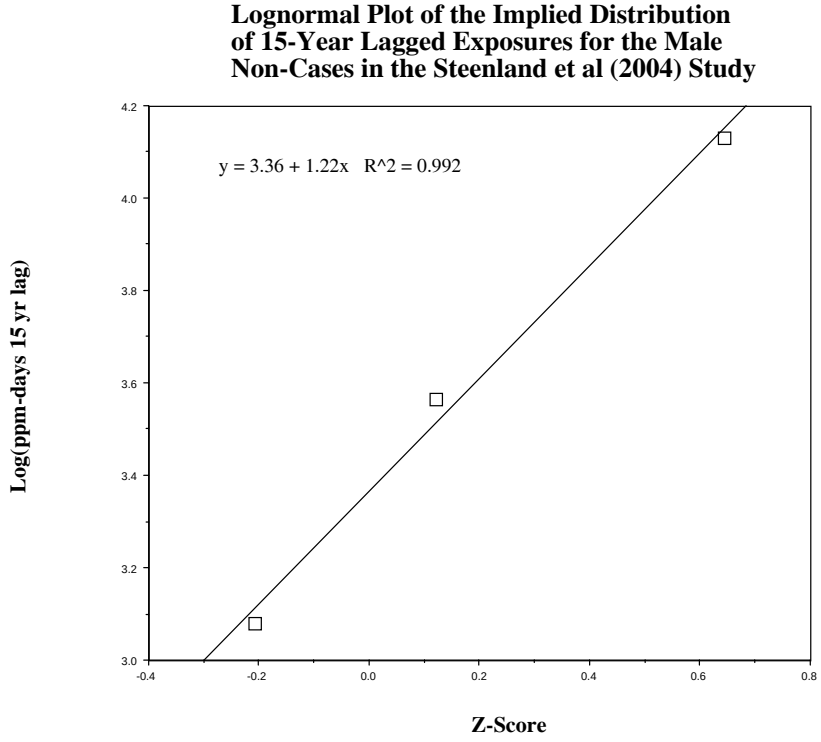
Table 2

Inputs for the Estimation of the Distribution of Exposures in Referent Workers (Non-Lymphohematopoietic Cancer Cases)

)sure Group	median exp	mean exp	RR
	(ppm-days)	(ppm-days)	
gged out)	0	0	1
)0 ppm-days	360	442	1.23
)-3679	2093	2191	2.52
)-13499	6230	7105	3.13
500	43212	60269	3.42

Figure 1

Initial Lognormal Probability Plot of the Exposure Distribution for Referent Workers Based on the Estimated Numbers within Each Group



A more extensive optimization analysis using the additional information on the within-group means and medians for the exposure groups from Table 2 led to an adjustment of the basic parameters for the lognormal distribution (geometric mean = 2910 ppm and geometric standard deviation = 9.86) and a conclusion that the exposure estimates were likely to be truncated at about 337,500 ppm-days (corresponding to 45 years of 250 day/year 8 hr/day occupational exposure at an average of 30 ppm, which would otherwise correspond to about the 98th percentile of a full lognormal distribution). This truncation point was chosen to avoid having key model parameters such as the mean of the highest exposure group being importantly dependent on cumulative exposures that are not likely to be present in the actual data. The resulting fit to the referent within-group mean and median cumulative exposure information is shown in Table 3.

Table 3

Fit of the Derived Referent Exposure Distribution to the Within-Group Mean and Median Exposure Data

	Observations		Lognormal Model "Predictions"[a]		Ratio: Model "Predictions"/Observations	
Exposure Group	median exp	mean exp	median exp	mean exp	median exp	mean exp
(ppm-days)	(ppm-days)	(ppm-days)	(ppm-days)	(ppm-days)	(ppm-days)	(ppm-days)
0 (lagged out)	0	0				
)0	360	442	342	426	0.949	0.964
)-3679	2093	2191	2121	2226	1.013	1.016
)-13499	6230	7105	6805	7376	1.092	1.038
500 [a]	43212	60269	36277[a]	62371[a]	0.840[a]	1.035[a]

[a]With truncation of the ≥13500 category at 337,500 ppm-days.

Overall, although the fit to the truncated lognormal distribution is not as close as might be hoped (particularly for the within-group median for the largest exposure group, the predictions for the key within-group means are not unacceptable, with no "prediction" departing from the reported observed mean by more than 4%.

- **Remove the (Assumed Lognormal) Variance Attributable to Random Measurement/Estimation Errors from the Observed Lognormal Variance to Estimate the Lognormal Variance of the Real Worker Exposures**

In the special case where both the distributions of the true worker exposures and the distribution of measurement/estimation errors are lognormal, then the lognormal variance of the observed distribution is just the sum of the real exposure variance and the error variance. This simplifying assumption allows us to estimate the lognormal variance of the real underlying worker exposures as:

Real lognormal variance [the square of the real log(geometric standard deviation)] = Observed lognormal variance – lognormal variance from measurement/estimation error
$= [\log(9.86)]^2 - [\log(1.95)]^2 = 0.903$

This lognormal variance implies a geometric standard deviation of about 8.92—slightly reduced from the geometric standard deviation of the observations of 9.86. Similarly, if we choose to assume that the measurement/estimation variance is as much as three times that derived from the Hornung et al. validation comparison the reduced estimate of the real variability in exposures corresponds to a geometric standard deviation of about 7.18.

Similar variance subtraction techniques have been used previously to quantify likely underlying real variability in a wide variety of parameters including survival and fecundity rates in ecological analyses and coal miner breathing rates (Akcakaya 2002; Hattis and Silver 1994).

For the more general case where the distributional forms of the real variation and measurement variation one needs "errors in variables" regression models (Stayner et al. 2003; Richardson et al. 2004; Brown et al. 2004; Choi, 2000; Carrothers and Evans, 2000; Kulathinal et al. 2002; Siebert et al. 2001 or more complex "deconvolution" procedures that are not yet in widespread use.

- **Derivation of Adjusted Estimates of the Real Distribution of Cumulative Exposures in Each of the Four Categorical Dose Groups**

Using the adjusted estimates of the geometric standard deviation and the same geometric mean and upper percentile truncation point derived earlier, it is straightforward to calculate a large number of percentiles of the indicated lognormal distributions and the mean values for exposures in each of the worker groups. The results in Table 4 are derived from dividing the original and error-variance corrected lognormal distributions into 3700 equal parts corresponding to the number of referent workers at a 100:1 ratio to cases (the exact number is not critical).

Table 4
Changes in Estimates of Mean Cumulative Exposures in Previously Defined Groups Using the Simple "Regression to the Mean" Effect

Exposure group (ppm-days for original observations)	Original Observations	Lognormal Fit to Original Observations	Error = GSD 1.95	Error = GSD 3.2
<1200	442	426	457	533
1200-3679	2191	2226	2247	2296
3680-13499	7105	7376	7060	6433
≥13500-337500	60269	62371	53629	38942

It can be seen in Table 4 that the narrowing of the distributions by subtraction of estimation error causes a reversion toward the mid point of the exposure distribution. The estimates of the mean cumulative exposures for the lower two groups are raised; and the estimated means for the higher two groups are lowered, with the greatest effects seen on the group with the largest exposures.

In addition to the regression to the mean effect shown in Table 4, there is an additional effect that results from what is classically known as "classification error". The error in estimating individual workers' exposures causes some estimated exposures to be 'scrambled"—that is, misclassified from their real exposure ranges to adjacent ranges. To model this, ten replicate Monte Carlo simulations were done of 3700 trials each for both assumed error level. On each trial, a random draw was made from the estimated underlying lognormal distribution of real exposures (after subtracting the variance attributed to measurement error) and then a random perturbation was added back corresponding to the lognormal distribution of measurement errors. The final two columns of Table 5 show the effects of this on the true mean exposures within each group for workers classified into exposure groups using their "observed"/estimated exposure levels. The calculation also took into account the censoring of both the "real" and estimated exposures at 337,500 ppm-days. Comparing Table 5 with Table 4, it can be seen that that undoing the effects of this scrambling misclassification leads to a much larger set of changes in

the estimated real exposures within each of the groups of workers classified by observed exposures.

Table 5
Changes in Estimates of Mean Cumulative Exposures (ppm-days) in Previously Defined Groups after Including the Scrambling of Individual Exposures among Exposure Groups

Exposure group (ppm-days for original observations)	Original Observations	Lognormal Fit to Original Observations	Error = GSD 1.95 After Scramble	Error = GSD 3.2 After Scramble
<1200	442	426	593	1264
1200-3679	2191	2226	2822	3836
3680-13499	7105	7376	8416	8415
≥13500-337500	60269	62371	51440	29290

These shifts have predictable effects on the estimates of the linear cancer potencies (Tables 6-7), using the same estimation methods (based on the same spreadsheet formulas) as were used by the EPA analysts. In the case of the 3 point calculation (Table 6), the overall effects are modest—slope factor estimates even decrease somewhat in the calculations with the scramble effect because of the increased estimated real mean exposure for workers with in the observed 3,680—13,499 ppm-day group. The full implications of the different amounts of estimation error are more apparent for the full analysis of all four points (Table 7). It can be seen that in this case the effect of the GSD 1.95 estimate of measurement error is to increase the slope factor by about 25%; whereas the effect of the larger GSD 3.2 assumption for estimation error is to slightly more than double the estimate of low dose risk. In the latter case, the ratio of the low-dose risks projected using the 3- vs the 4-point analysis is reduced to about 3-fold, compared with about 7.5-fold using the group means of the original observations. Thus, errors-in-variables distortion of the high dose point in particular seems to be a reasonable candidate explanation for some, but not all, of the convex nonlinearity seen in the data.

Table 6

Changes in Estimates of the Linear Slope Coefficient, ED10, and LED 10 for Dose Response Analyses Based on the Lowest 3 Exposure Groups

Risk Parmeter	Analysis With Group Means of the Original Observations	Analysis With Group Means of the Lognormal Fit to Original Observations	Analysis With Revised Means After Subtracting GSD 1.95 Estimation Error Without "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 3.2 Estimation Error Without "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 1.95 Estimation Error With "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 3.2 Estimation Error With "Scramble" Effect
est b	3.47E-04	3.34E-04	3.49E-04	3.80E-04	2.92E-04	2.75E-04
SE b	2.51E-04	2.43E-04	2.52E-04	2.71E-04	2.09E-04	1.93E-04
ucl b	7.60E-04	7.33E-04	7.62E-04	8.25E-04	6.36E-04	5.92E-04

Table 7

Changes in Estimates of the Linear Slope Coefficient, ED10, and LED 10 for Dose Response Analyses Based on All 4 Exposure Groups

Risk Parmeter	Analysis With Group Means of the Original Observations	Analysis With Group Means of the Lognormal Fit to Original Observations	Analysis With Revised Means After Subtracting GSD 1.95 Estimation Error Without "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 3.2 Estimation Error Without "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 1.95 Estimation Error With "Scramble" Effect	Analysis With Revised Means After Subtracting GSD 3.2 Estimation Error With "Scramble" Effect
est b	4.54E-05	4.38E-05	5.77E-05	7.31E-05	5.52E-05	1.04E-04
SE b	3.28E-05	3.17E-05	4.06E-05	5.03E-05	3.81E-05	6.42E-05
ucl b	9.94E-05	9.60E-05	1.25E-04	1.56E-04	1.18E-04	2.10E-04

Appendix C

Framework Analysis of Genotoxicity and Risk Assessment
James Swenberg, PhD

Slide 1

Framework Analysis of Genotoxicity and Risk Assessment

James Swenberg
University of North Carolina

Slide 2

2005 EPA Guidelines for Carcinogen Risk Assessment

- *Linear extrapolation* should be used when there are Mode Of Action data to indicate that the dose-response curve is expected to have a linear component below the POD.
- Agents that are DNA-reactive and have direct mutagenic activity

Slide 3

MOA Key Events

Genotoxic

↓

DNA Adducts

↓

Mutations in surrogate genes

↓

Mutations in cancer genes

↓

Cancer

Slide 4

Genotoxicity

- A chemical is defined as genotoxic if the weight of evidence is positive in a battery of genetic toxicology assays.
- This is not a quantitative data set.
- It represents Hazard Identification, not Risk Assessment.

Slide 5

MOA Key Events

Genotoxic

↓

DNA Adducts

↓

Mutations in surrogate genes

↓

Mutations in cancer genes

↓

Cancer

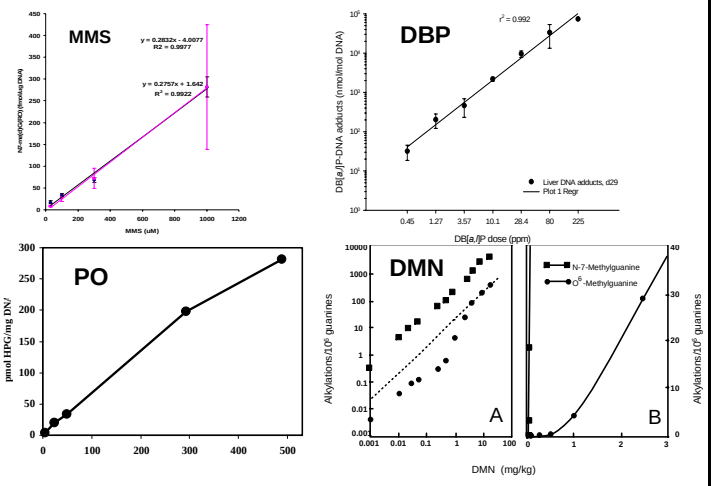
Slide 6

Molecular Dosimetry of DNA Adducts

- DNA adducts are expected to be linear at low doses.
- An exception to this is when identical adducts are formed endogenously.
- Many forms of endogenous DNA adducts have been identified and measured. These include direct oxidative adducts, exocyclic adducts, AP sites and deamination products.

Slide 7

Linear DNA Adducts at Low Doses



Slide 8

MOA Key Events

Genotoxic

↓

DNA Adducts

↓

Mutations in surrogate genes

↓

Mutations in cancer genes

↓

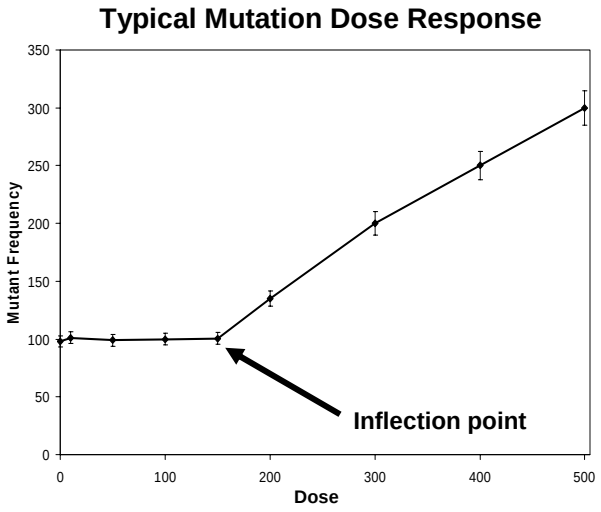
Cancer

Slide 9

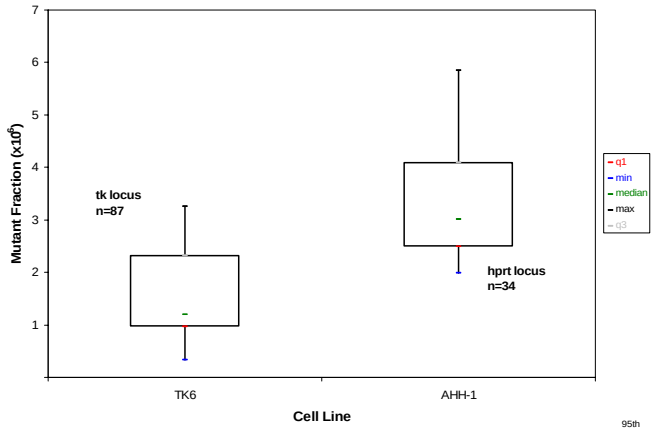
Mutations Do Not Go Through Zero

- In contrast to most DNA adducts, mutations do not go through zero.
- Rather, they reach a spontaneous level that reflects the summation of endogenous DNA damage and repair that occurs in cells.
- The inflection point for a dose response curve where the number of mutations increases above the spontaneous level represents the point at which the exogenous DNA damage starts driving the biology of mutations.

Slide 10



Slide 11



Penman and Crespi, *Environ Mol Mut* 10:35-60, 1987

Slide 12

SOURCES OF SPONTANEOUS MUTATIONS

Endogenous Sources | **Exogenous Sources**

Depurination | **Polymerase Errors** | **Alkylation & ROS** | **Environment Chemicals** | **Life Style** | **Radiation**

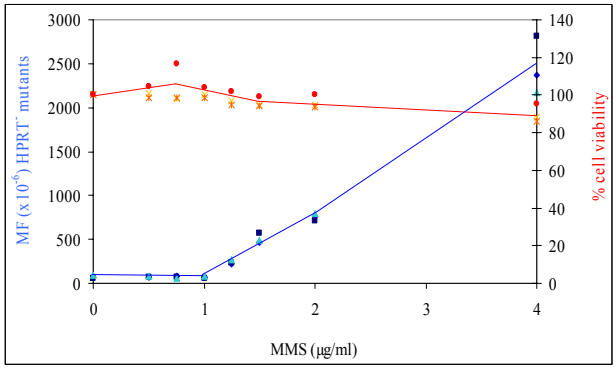
DNA DAMAGE

DNA REPAIR

MUTATIONS

Slide 13

HPRT Mutation in AHH-1 cells with MMS

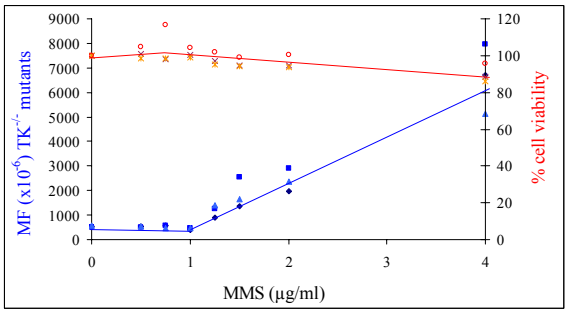


•Doses above **4µg/ml** MMS appear to be **cytotoxic**

•A possible threshold dose at **1µg/ml** MMS at the **HPRT** locus exists, with doses above 1µg/ml inducing significantly more mutants

Slide 14

MMS-induced TK $^{-/-}$ Mutants



•The NOEL for mutation at the TK locus is located at 1µg/ml MMS - same as seen with HPRT locus.

Slide 15

MNU and HPRT Mutation in AHH-1 Cells

•**Linear non-threshold** (LNT) dose response.

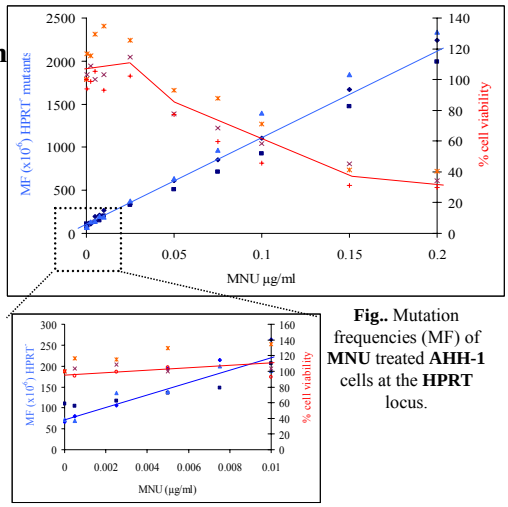
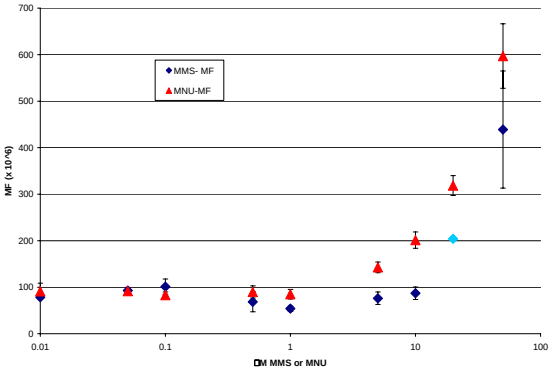


Fig.. Mutation frequencies (MF) of **MNU** treated **AHH-1** cells at the **HPRT** locus.

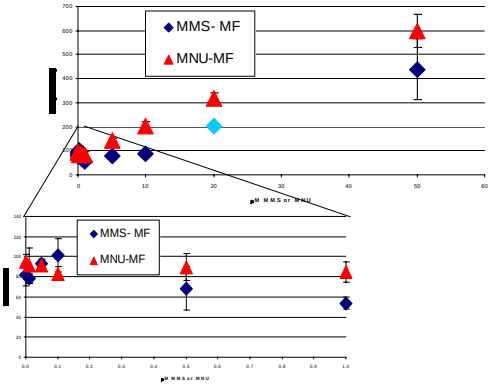
Slide 16

Mutant Frequency (TK$^-$) in Mouse Lymphoma Cells Treated with Equimolar MMS or MNU: Log-Linear Scale

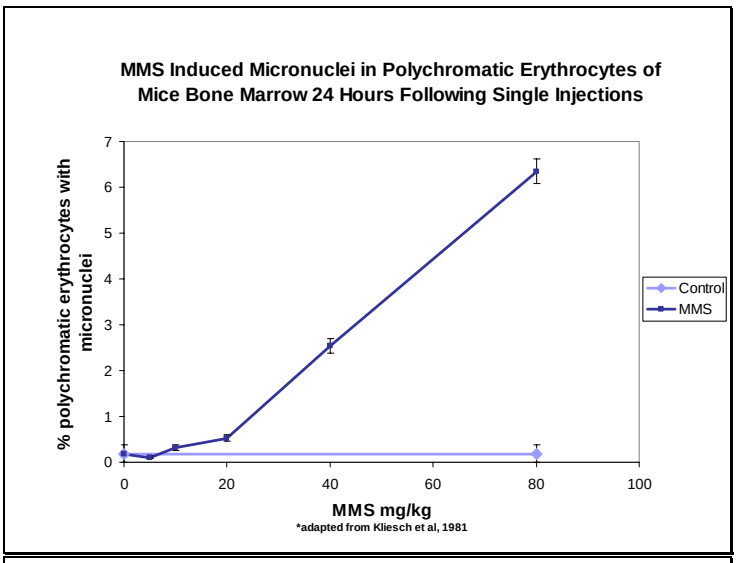


Slide 17

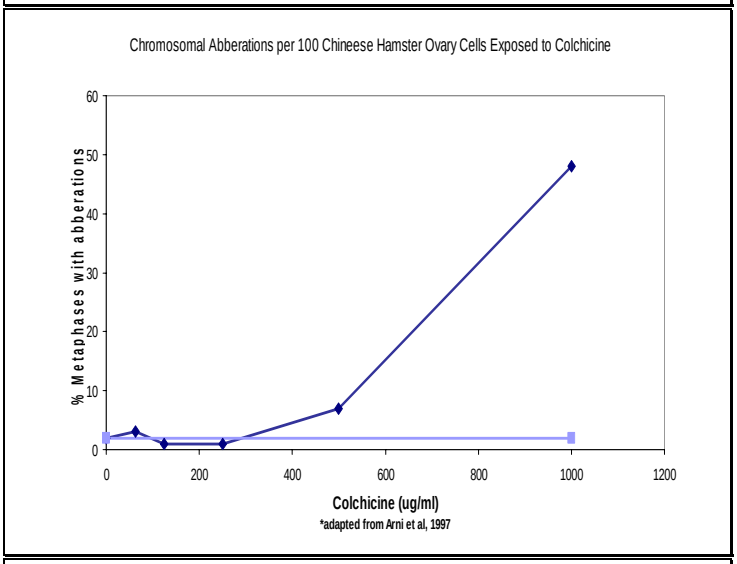
1) Mutant Frequency (TK$^-$) in Mouse Lymphoma Cells Treated with Equimolar MMS or MNU: Linear Scale



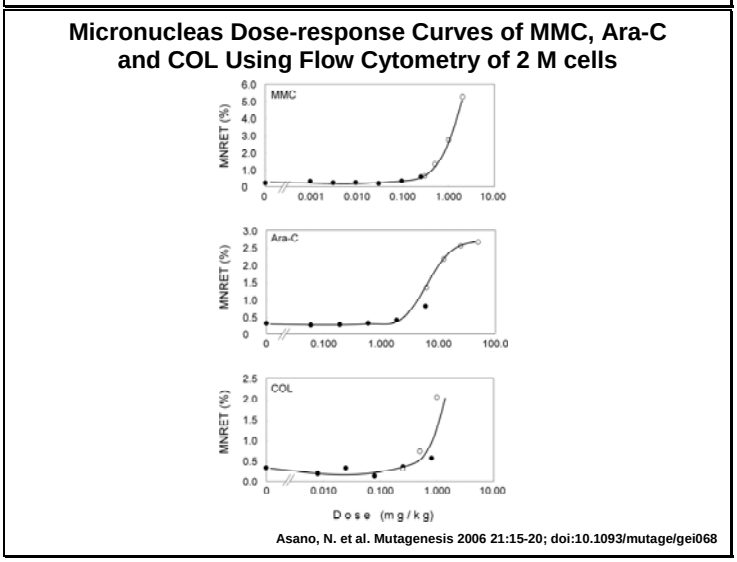
Slide 18



Slide 19



Slide 20



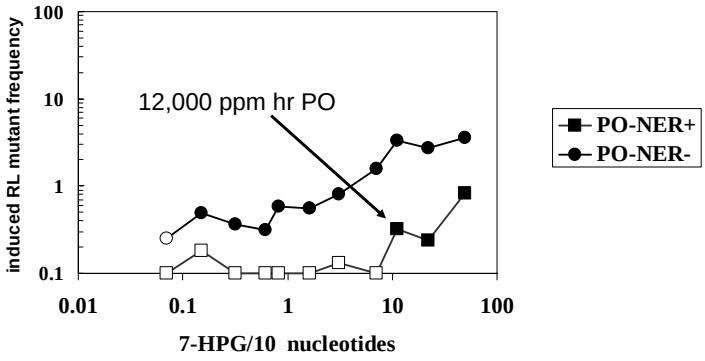
Slide 21

DNA Repair Can Modulate Where the Inflection Point Occurs

- If DNA repair is impaired or absent, the inflection point for mutations occurs at lower doses.
- This results from increased numbers of DNA adducts relative to a cell of individuals with normal DNA repair.

Slide 22

Exposure-response for Mutagenesis in Drosophila Exposed By Inhalation To Propylene Oxide



6 Nivard et al, Mut. Res. **529**: 95-107, 2003.

Slide 23

Ethylene Oxide

- Genotoxic in many systems including DNA adducts and *in vitro* and *in vivo* mutations.
- Known animal carcinogen.
- IARC Category 1 human carcinogen based on limited epidemiology data and human genetic toxicolgy.
- Formed endogenously in humans and animals from metabolism of ethylene.
- HEG is present in all human and animal cells.

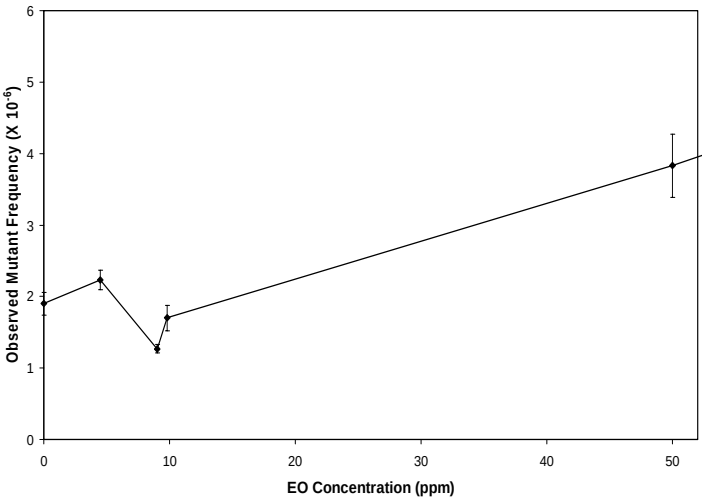
Slide 24

Observed N7-HEG (pmol/umol Guanine) following low-level EO exposure for 4 weeks

Tissue	ppm EO	Observed N7-HEG	
Spleen		Rats	Mice
	0	0.2	0.2
	3	2.5	0.5
	10	4.0	1.4
	33	8.8	5.6

Slide 25

***In vivo hprt* Mutations in Mice Exposed to Ethylene/Ethylene Oxide**



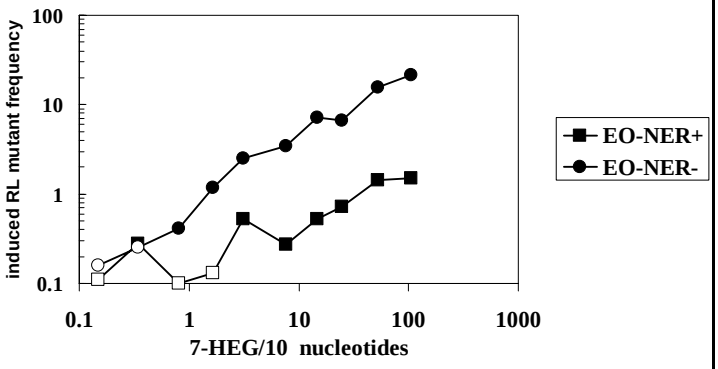
Slide 26

EO *hprt* Mutations in Mice

Ethylene Concentration (ppm)	EO Concentration (or Estimated EO Concentration) (ppm)	Observed Mutant Frequency (x 10^{-6}) Mean ± S.E. (n)
0.0	0.0	1.90 ± 0.16 (12)
40	(4.5 ± 2.0)	2.23 ± 0.14 (7)
1000	(9.0 ± 1.9)	1.27 ± 0.06 (7)
3000	(9.8 ± 3.0)	1.70 ± 0.18 (6)
	50	3.83 ± 0.44 (7)
	100	6.84 ± 0.86 (8)
	200	14.13 ± 1.13(9)

Slide 27

EO Drosophila Mutations



Slide 28

MOA Key Events

Genotoxic

DNA Adducts

Mutations in surrogate genes

Mutations in cancer genes

Cancer

Slide 29

Gaps in Knowledge

- Most mutation assays are done at high doses to establish that a compound is or is not genotoxic.
- There is a real need to generate dose response data at low exposures to establish NOAELs for mutation in CA, MN and surrogate genes such as *hprt.*
- These data will further establish the inflection points where the background number of mutations become increased.

Slide 30

Conclusions

- As our knowledge of carcinogenesis has expanded, concepts of "*one molecule → cancer* " have little to no scientific support.
- Mutations in genes controlling cell proliferation and cell death appear to play major roles in the induction of cancer.
- While these genes are difficult to monitor in noncancer tissues, surrogate mutations can be used to examine dose response in cells, animals and humans.

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Conclusions (cont.)

- Such mutations do not have linear relationships with exposure. Rather, they reach a spontaneous incidence that is driven by endogenous biological processes.
- The inflection point for mutagenesis represents a much more strongly supported Point of Departure for setting acceptable exposures.
- This could be accomplished by using a Margin of Exposure approach to protect susceptible individuals.

Appendix D [Additional References]

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ATTACHMENT 1 MEMO AND CHARGE QUESTIONS

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
National Center for Environmental Assessment
Washington, DC 20460

October 27, 2006

NCEA Washington Office (8623D)

MEMORANDUM

SUBJECT: Request for SAB review of the Draft Ethylene Oxide (EtO) Carcinogenicity Assessment

FROM: David A. Bussard, Director *David A Bussard*
National Center for Environmental Assessment-Washington (8623D)
Office of Research and Development

TO: Sue Shallal, Ph.D.
Designated Federal Officer
EPA Science Advisory Board Staff Office (1400F)

This is to request a review by the Science Advisory Board of the draft document entitled "Evaluation of the Carcinogenicity of Ethylene Oxide". This document is an assessment of the carcinogenicity of ethylene oxide (EtO). The assessment was prepared by the National Center for Environmental Assessment (NCEA), which is the health risk assessment program in the Office of Research and Development. The document has been made available for public comment on the Agency's NCEA web site at the following URL: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157664. The assessment broadly supports activities authorized in the 1990 Clean Air Act and is of particular interest to EPA's Office of Air and Radiation. However, the assessment should also be applicable to the needs of all program Offices and Regions in evaluating the carcinogenicity of EtO.

EPA last published an assessment of the potential carcinogenicity of EtO in 1985. The current assessment reviews the more recent database on the carcinogenicity of EtO. The scientific literature search for this assessment is generally current through June 2004, although a few later publications are included. This assessment focuses on lifetime cancer risk from inhalation exposure.

EtO is a gas at room temperature. It is manufactured from ethylene and used primarily as a chemical intermediate in the manufacture of ethylene glycol. It is also used as a sterilizing agent for medical equipment and as a fumigating agent for spices. The largest sources of human

exposure are in occupations involving contact with the gas in plants (facilities) and in hospitals that sterilize medical equipment. EtO can also be inhaled by residents living near production or sterilizing/fumigating facilities. This document describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on human epidemiological data.

Attached is a draft of a charge to the Science Advisory Board that identifies the questions and issues we want the Science Advisory Board to address in reviewing the document.

CHARGE QUESTIONS FOR EPA'S SCIENCE ADVISORY BOARD (SAB) REVIEW OF THE ETHYLENE OXIDE (EtO) CARCINOGENICITY ASSESSMENT

EPA's Office of Research and Development (ORD) has requested that the Science Advisory Board (SAB) review its document entitled "Evaluation of the Carcinogenicity of Ethylene Oxide". This document is EPA's draft of the evaluation of the carcinogenicity of ethylene oxide (EtO). The assessment was prepared by the National Center for Environmental Assessment which is the health risk assessment program in the Office of Research and Development. The assessment broadly supports activities authorized in the 1990 Clean Air Act and is of particular interest to EPA's Office of Air and Radiation. However, this review also should be applicable to the needs of all program Offices and Regions in evaluating the carcinogenicity of EtO.

EPA last published a health assessment of the potential carcinogenicity of EtO in 1985 (U.S. EPA, 1985). The current assessment reviews the more recent database on the carcinogenicity of EtO. The scientific literature search for this assessment is generally current through June 2004, although a few later publications are included. This assessment focuses on lifetime cancer risk from inhalation exposure.

EtO is a gas at room temperature. It is manufactured from ethylene and used primarily as a chemical intermediate in the manufacture of ethylene glycol. It is also used as a sterilizing agent for medical equipment and as a fumigating agent for spices. The largest sources of human exposure are in occupations involving contact with the gas in plants (facilities) and in hospitals that sterilize medical equipment. EtO can also be inhaled by residents living near production or sterilizing/fumigating facilities.

The DNA-damaging properties of EtO have been studied since the 1940s. EtO is known to be mutagenic in a large number of living organisms, ranging from bacteriophage to mammals, and it also induces chromosome damage. It is carcinogenic in mice and rats, inducing tumors of the lymphohematopoietic system, brain, lung, connective tissue, uterus, and mammary gland. In humans employed in EtO-manufacturing facilities and in sterilizing facilities, the greatest evidence of a cancer risk from exposure is for cancer of the lymphohematopoietic system. Increases in the risk of lymphohematopoietic cancer have been seen in several studies, manifested as an increase either in leukemia and/or in cancer of the lymphoid tissue. In one large epidemiologic study of sterilizer workers that had a well-defined exposure assessment for individuals, positive exposure-response trends for lymphohematopoietic cancer mortality in males and for breast cancer mortality in females were reported (Steenland et al., 2004). The positive exposure-response trend for female breast cancer was confirmed in an incidence study based on the same worker cohort (Steenland et al., 2003).

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EtO was characterized as carcinogenic to humans based on the total weight of evidence.

This evidence, as assessed by EPA, included:

a) strong, though less than completely conclusive, evidence of carcinogenicity from human studies

b) sufficient evidence of carcinogenicity in laboratory animals
c) EtO is a direct-acting alkylating agent with clear evidence of mutagenicity/genotoxicity, and there is sufficient evidence that DNA adduct formation and the resulting mutagenic/genotoxic effects are key events in the mode of action of EtO carcinogenicity
d) evidence of chromosome damage in humans exposed to EtO, supporting the inference that the same mode of action for EtO carcinogenicity is operative in humans

This document describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on the human data. An EC_{01} of 44 $\mu g/m^3$ (0.024 ppm) was calculated using a life-table analysis and linear modeling of the categorical Cox regression analysis results for excess lymphohematopoietic cancer mortality in males reported in a high-quality occupational epidemiologic study (Steenland et al., 2004). Linear low-dose extrapolation from the LEC_{01} yielded a lifetime extra cancer mortality unit risk estimate of 5.0×10^{-4} per $\mu g/m^3$ (0.92 per ppm) of continuous EtO exposure. Applying the same linear regression coefficient and life-table analysis to background male lymphohematopoietic cancer *incidence* rates yielded an EC_{01} of 24 $\mu g/m^3$ (0.013 ppm) and a preferred lifetime extra cancer unit risk estimate of 9.0×10^{-4} per $\mu g/m^3$ (1.6 per ppm). The preferred estimate is greater than the estimate of 5.0×10^{-4} per $\mu g/m^3$ (0.91 per ppm; $EC_{01} = 44$ $\mu g/m^3$) calculated, using the same approach, from the results of a breast cancer incidence study of the same worker cohort (Steenland et al., 2003), and is recommended as the potency estimate for Agency use.

Because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and in the absence of chemical-specific data on early-life susceptibility, this assessment finds that increased early-life susceptibility should be assumed and the age-dependent adjustment factors (ADAFs) should be applied, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to Carcinogens,* hereinafter referred to as "EPA's Supplemental Guidance" (U.S. EPA, 2005b). Applying the ADAFs to the unit risk estimate of 9.0×10^{-4} per $\mu g/m^3$ yields an adjusted full lifetime unit risk estimate of 1.5×10^{-3} per $\mu g/m^3$, and the commensurate lifetime chronic exposure level of EtO corresponding to an increased cancer risk of 10^{-6} is $0.0007 \mu g/m^3$. [Note that for less-than-lifetime exposure scenarios (or for exposures that vary with age), the unadjusted (adult-based) potency estimate of 9.0×10^{-4} per $\mu g/m^3$ should be used, in conjunction with the ADAFs as appropriate, in accordance with EPA's Supplemental Guidance.]

Unit risk estimates were also derived from the three chronic rodent bioassays for EtO reported in the literature. These estimates, ranging from 2.2×10^{-5} per $\mu g/m^3$ to 4.6×10^{-5} per $\mu g/m^3$, are about an order of magnitude lower than the estimates based on human data [unadjusted for early-life susceptibility]. The Agency takes the position that human data, if adequate data are available, provide a more appropriate basis than rodent data for estimating population risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from rodents to humans are avoided. Although there is a fairly sizable difference between the rodent- and human-based estimates, the assessment infers that the similarity between the unit risk estimates based on the male lymphohematopoietic cancer and the female breast cancer results increases confidence in the use of the unit risk estimate based on the male lymphohematopoietic cancer results.

The unit risk estimates were developed for environmental exposure levels and are not necessarily applicable to higher-level occupational exposures, which appear to be subject to a different exposure-response relationship. However, occupational exposure levels are of concern to EPA when EtO is used as a pesticide (e.g., fumigant for spices). Therefore, this document also presents extra risk estimates for cancer for a number of occupational exposure scenarios.

The SAB Ethylene Oxide Review Panel is being asked to comment on the scientific soundness of this carcinogenicity assessment. The specific charge questions to the Panel are as follows:

Issue 1: Carcinogenic Hazard (Section 3 and Appendix A of the Draft)

1. Do the available data and discussion in the draft document support the hazard conclusion that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*? In your response, please include consideration of the following:

1.a EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but less than completely conclusive. Does the draft document provide sufficient description of the studies, balanced treatment of positive and negative results, and a rigorous and transparent analysis of the data used to assess the carcinogenic hazard of ethylene oxide (EtO) to humans? Please comment on the EPA's characterization of the body of epidemiological data reviewed. Considerations include: a) the consistency of the findings, including the significance of differences in results using different exposure metrics, b) the utility of the internal (based on exposure category) versus external (e.g., SMR and SIR) comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the epidemiological evidence.

1.b. Are there additional key published studies or publicly available scientific reports that are missing from the draft document and that might be useful for the discussion of the carcinogenic hazard of EtO?

1.c. Do the available data and discussion in the draft document support the mode of action conclusions?

1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced and sound description that synthesizes the human, laboratory animal, and supporting (e.g., *in vitro*) evidence for human carcinogenic hazard?

Issue 2: Risk Estimation (Section 4 and Appendices C and D)

2. Do the available data and discussion in the draft document support the approaches taken by EPA in its derivation of cancer risk estimates for EtO? In your response, please include consideration of the following:

2.a. EPA concluded that the epidemiological evidence alone was strong but less than completely conclusive (although EPA characterized the total evidence - from human, laboratory animal, and

in vitro studies - as supporting a conclusion that EtO as "carcinogenic to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003, 2004) data set**,** the most appropriate for estimating the magnitude of the carcinogenic risk to humans from environmental EtO exposures? Are the scientific justifications for using this data set transparently described? Is the basis for selecting the Steenland et al. data over other available data (e.g., the Union Carbide data) for quantifying risk adequately described?

2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of a linear regression model fit to Steenland et al.'s categorical results for all lymphohematopoietic cancer in males in only the lower exposure groups scientifically and statistically appropriate for estimating potential human risk at the lower end of the observable range? Is the use of the grouping of all lymphohematopoietic cancer for the purpose of estimating risk appropriate? Are there other appropriate analytical approaches that should be considered for estimating potential risk in the lower end of the observable range? Is EPA's choice of a preferred model adequately supported and justified? In particular, has EPA adequately explained its reasons for not using a quadratic model approach such as that of Kirman et al. (2004) based? What recommendations would you make regarding low-dose extrapolation below the observed range?

2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk estimate, in accordance with EPA's Supplemental Guidance (U.S. 2005b), appropriate and transparently described?

2.d Is the use of different models for estimation of potential carcinogenic risk to humans from the higher exposure levels more typical of occupational exposures (versus the lower exposure levels typical of environmental exposures) appropriate and transparently described in Section 4.5?

2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data appropriate and transparently described? Is the use of "ppm equivalence" adequate for interspecies scaling of EtO exposures from the rodent data to humans?

Issue 3: Uncertainty (Sections 3 and 4)

1. EPA's *Risk Characterization Handbook* requires that assessments address in a transparent manner a number of important factors. Please comment on how well this assessment clearly describes, characterizes and communicates the following:

a. The assessment approach employed;
b. The use of assumptions and their impact on the assessment;
c. The use of extrapolations and their impact on the assessment;
d. Plausible alternatives and the choices made among those alternatives;
e. The impact of one choice versus another on the assessment;
f. Significant data gaps and their implications for the assessment;
g. The scientific conclusions identified separately from default assumptions and policy calls;
h. The major risk conclusions and the assessor's confidence and uncertainties in them, and;
i. The relative strength of each risk assessment component and its impact on the overall assessment.