

SAB 5/15/07 Draft

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR  
SCIENCE ADVISORY BOARD

May XX, 2007

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:

[TO BE DEVELOPED]

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Science Advisory Board  
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's Office of Research and Development (ORD) has now 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. This 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 cancer unit risk for inhalation exposure to EtO and associated uncertainty.

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

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1 oxide carcinogenicity were not in and of themselves sufficient to prove a causal  
2 association between human exposure and cancer. Differing views as to the appropriate  
3 descriptor for ethylene oxide were based on differences of opinion as to whether criteria  
4 necessary for designation as “Carcinogenic to Humans” in the absence of a causal  
5 association in humans were met. The majority of Panel members thought that the  
6 combined weight of the epidemiological, experimental animal, and mutagenicity  
7 evidence was sufficient to conclude that EtO is carcinogenic to humans.  
8

9 The Panel concluded that the assessment would be improved by: 1) a better introduction  
10 to the hazard characterization section, including a brief description of the information  
11 that will be presented; 2) a clear articulation of the criteria by which epidemiologic  
12 studies were judged as to strengths and weaknesses; 3) addition of a more inclusive  
13 summary figure and/or table at the beginning of section 3.0; and 4) inclusion of material  
14 now provided in Appendix A within the main body of the draft assessment.  
15

16 The Panel agreed with the EPA in their reliance on “internal” study findings in the  
17 presence of “external” findings due to well recognized limitations to the latter method of  
18 analysis.  
19

20 The Draft Assessment appropriately characterizes the magnitude of the risk associated  
21 with EtO as “weak”. This finding is well substantiated by the epidemiologic evidence  
22 where a relatively small number of excess cancers are found above background even  
23 among highly exposed individuals.  
24

25  
26 ***1. b. Are there additional key published studies or publicly available scientific reports that***  
27 ***are missing from the draft document and that might be useful for the discussion of the***  
28 ***carcinogenic hazard of EtO?***  
29

30 The Panel agreed that the discussion of endogenous metabolic production of ethylene  
31 oxide and the formation of background adducts should be expanded.  
32

33 The Panel believed that the description of studies of DNA adduct formation resulting  
34 from EtO exposure appears incomplete and superficial. This discussion should be  
35 expanded – both in terms of the number of studies cited and the depth of the discussion.  
36

37 Some members recommended adding results of studies with ethylene. Most members  
38 were hesitant about adding them to the document, but if added, they cautioned that a  
39 discussion of the caveats associated with their interpretation relative to ethylene oxide  
40 should be included.  
41

42 ***1. c. Do the available data and discussion in the draft document support the mode of***  
43 ***action conclusions?***  
44

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1 The Panel agreed with the Draft Assessment conclusion of a mutagenic mode of action.  
2 However, an expanded discussion of the formation of DNA adducts and mutagenicity is  
3 warranted.  
4  
5

6 ***1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced***  
7 ***and sound description that synthesizes the human, laboratory animal, and supporting***  
8 ***(e.g., in vitro) evidence for human carcinogenic hazard?***  
9

10 While some members of the Panel found the hazard characterization section of the Draft  
11 Assessment to be satisfactory, a majority expressed concerns that this section did not  
12 achieve the necessary level of rigor and balance. A critical issue in this characterization,  
13 in particular in the face of epidemiological data that are not strongly conclusive, is  
14 whether the precursor events leading to cancer in animals are observed in humans. This  
15 issue needs to be addressed in greater detail.  
16

17 **Issue 2: Risk Estimation (Section 4 and Appendices C and D of the EPA Draft**  
18 **Assessment)**  
19

20 ***2. Do the available data and discussion in the draft document support the approaches***  
21 ***taken by EPA in its derivation of cancer risk estimates for EtO? In your response, please***  
22 ***include consideration of the following:***  
23

24 ***2.a. EPA concluded that the epidemiological evidence alone was strong but less than***  
25 ***completely conclusive (although EPA characterized the total evidence - from human,***  
26 ***laboratory animal, and in vitro studies - as supporting a conclusion that EtO as***  
27 ***"carcinogenic to humans"). Is the use of epidemiological data, in particular the***  
28 ***Steenland et al. (2003, 2004) data set, the most appropriate for estimating the magnitude of***  
29 ***the carcinogenic risk to humans from environmental EtO exposures? Are the scientific***  
30 ***justifications for using this data set transparently described? Is the basis for selecting the***  
31 ***Steenland et al. data over other available data (e.g., the Union Carbide data) for***  
32 ***quantifying risk adequately described?***  
33

34 The Panel concurred that the NIOSH cohort is the best single epidemiological data set  
35 with which to study the relationship of cancer mortality to the full range of occupational  
36 exposures to EtO. That said, the Panel encouraged the EPA to broadly consider all of the  
37 epidemiological data in developing its Draft Assessment. In particular, the Panel  
38 encourages the EPA to explore uses for the Greenberg et al. (1990) data on cancer  
39 outcomes and EtO exposures for 2174 Union Carbide workers at that firms' two Kana  
40 Valley, West Virginia facilities. (See also Teta et al. 1993; Teta et al., 1999).  
41

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

1  
2  
3 *2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use*  
4 *of a linear regression model fit to Steenland et al.'s categorical results for all*  
5 *lymphohematopoietic cancer in males in only the lower exposure groups scientifically and*  
6 *statistically appropriate for estimating potential human risk at the lower end of the*  
7 *observable range? Is the use of the grouping of all lymphohematopoietic cancer for the*  
8 *purpose of estimating risk appropriate? Are there other appropriate analytical approaches*  
9 *that should be considered for estimating potential risk in the lower end of the observable*  
10 *range? Is EPA's choice of a preferred model adequately supported and justified? In*  
11 *particular, has EPA adequately explained its reasons for not using a quadratic model*  
12 *approach such as that of Kirman et al. (2004) based? What recommendations would you*  
13 *make regarding low-dose extrapolation below the observed range?*  
14  
15

16 The Panel identified several important shortcomings in the linear regression modeling  
17 approach used to establish the point of departure for low dose extrapolation of cancer risk  
18 due to EtO. The Panel was unanimous in its recommendation that the EPA develop its  
19 risk models based on direct analysis of the individual exposure and cancer outcome data  
20 for the NIOSH cohort rather than the approach based on grouped data that is presently  
21 used.  
22

23 The Panel discussed whether low dose extrapolation of risk to environmental EtO  
24 exposure levels should be linear (following Cancer Guideline defaults for carcinogenic  
25 agents operating via a mutagenic MOA) or whether plausible biological mechanisms  
26 argued for a non-linear and possibly a threshold form for the low dose response  
27 relationship. With appropriate discussion of the statistical and biological uncertainties,  
28 several Panel members strongly advocated the consideration of both linear and nonlinear  
29 calculations in the final EtO Risk Assessment.  
30

31 In conjunction with its recommendation to use the individual NIOSH cohort data to  
32 model the relationship of cancer risk to exposures in the occupational range, the Panel  
33 recommended that the EPA analysts explore the use of the full NIOSH data set to  
34 estimate the cancer slope coefficients that will in turn be used to extrapolate risk below  
35 the established point of departure. Truncation of the high exposure data should not be  
36 the default method for estimating the dose response relationship.  
37

38 Although the analysis based on total LH cancers might have value as part of a complete  
39 risk assessment, the rationale for the groupings needs to be better justified. The Panel  
40 recommends that data be analyzed by subtype of LH cancers (e.g. lymphatic, myeloid)  
41 with biological rationale for any groupings that are formed.  
42

43 The Panel was divided in its views concerning the appropriateness of estimating the  
44 population unit risk for lymphohematopoietic cancer based only on the NIOSH data for  
45 males. Several Panel members pointed out that a standard approach in cancer

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1 epidemiology and risk analysis begins by conducting separate dose-response analyses on  
2 males and females and combining the data only if the results are similar. Conducting  
3 separate analyses for males and females is also the standard practice when analyzing data  
4 from animal carcinogenicity bioassays. A second approach to dealing with the  
5 possibility of gender differences in response is to include gender as a fixed effect in the  
6 statistical modeling of the data and determine whether gender or its interaction with other  
7 predictors (e.g. gender x exposure) are significant explanatory variables. If so, the  
8 combined model with the estimated gender effects could be used directly or separate,  
9 gender-specific dose-response analysis would be performed. If not, the gender effects  
10 could be dropped and the model re-estimated for the combined male and female data.  
11

12 ***2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit***  
13 ***risk estimate, in accordance with EPA’s Supplemental Guidance (U.S. 2005b), appropriate***  
14 ***and transparently described?***  
15

16 In accordance with EPA guidance, the draft assessment applied an Age Dependent  
17 Adjustment Factor (ADAF) to adjust the unit risk for early life exposure. While the  
18 majority of the Panel felt that the application of a default value by the Agency was  
19 appropriate, the description in the Draft Assessment was not adequate, particularly for  
20 those not familiar with the EPA Guidance.  
21

22 ***2.d Is the use of different models for estimation of potential carcinogenic risk to humans***  
23 ***from the higher exposure levels more typical of occupational exposures (versus the lower***  
24 ***exposure levels typical of environmental exposures) appropriate and transparently***  
25 ***described in Section 4.5?***  
26

27 The Panel felt that the description of the EPA’s methodology for estimating the point of  
28 departure (POD) and extrapolating from the POD to the baseline using a linear dose  
29 response assumption was transparently described.  
30

31 ***2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data***  
32 ***appropriate and transparently described? Is the use of “ppm equivalence” adequate for***  
33 ***interspecies scaling of EtO exposures from the rodent data to humans?***  
34

35 The ppm equivalence method is a reasonable method for interspecies scaling of EtO  
36 exposures from rodent data to humans. If the use of animal data becomes more important  
37 (i.e., the principal basis for the ethylene oxide unit risk value), more sophisticated  
38 approaches such as PBPK modeling should be considered.  
39  
40  
41  
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45

## INTRODUCTION

This report was prepared by the Science Advisory Board (SAB) 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.

[NOTE: further information regarding the development of this draft will be added]

### Charge Questions

The memo requesting this review along with the 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 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

1 utility of the internal (based on exposure category) versus external (e.g., SMR and SIR)  
2 comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the  
3 epidemiological evidence.  
4

5 1.b. Are there additional key published studies or publicly available scientific reports that are  
6 missing from the draft document and that might be useful for the discussion of the  
7 carcinogenic hazard of EtO?  
8

9 1.c. Do the available data and discussion in the draft document support the mode of action  
10 conclusions?  
11

12 1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced  
13 and sound description that synthesizes the human, laboratory animal, and supporting (e.g., *in*  
14 *vitro*) evidence for human carcinogenic hazard?  
15

## 16 **Issue 2: Risk Estimation (Section 4 and Appendices C and D)** 17

18 2. Do the available data and discussion in the draft document support the approaches taken  
19 by EPA in its derivation of cancer risk estimates for EtO? In your response, please include  
20 consideration of the following:  
21

22 2.a. EPA concluded that the epidemiological evidence alone was strong but less than  
23 completely conclusive (although EPA characterized the total evidence - from human,  
24 laboratory animal, and *in vitro* studies - as supporting a conclusion that EtO as "carcinogenic  
25 to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003,  
26 2004) data set, the most appropriate for estimating the magnitude of the carcinogenic risk to  
27 humans from environmental EtO exposures? Are the scientific justifications for using this  
28 data set transparently described? Is the basis for selecting the Steenland et al. data over other  
29 available data (e.g., the Union Carbide data) for quantifying risk adequately described?  
30

31 2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of  
32 a linear regression model fit to Steenland et al.'s categorical results for all  
33 lymphohematopoietic cancer in males in only the lower exposure groups scientifically and  
34 statistically appropriate for estimating potential human risk at the lower end of the  
35 observable range? Is the use of the grouping of all lymphohematopoietic cancer for the  
36 purpose of estimating risk appropriate? Are there other appropriate analytical approaches that  
37 should be considered for estimating potential risk in the lower end of the observable range?  
38 Is EPA's choice of a preferred model adequately supported and justified? In particular, has  
39 EPA adequately explained its reasons for not using a quadratic model approach such as that  
40 of Kirman et al. (2004) based? What recommendations would you make regarding low-dose  
41 extrapolation below the observed range?  
42

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

1  
2 2.d Is the use of different models for estimation of potential carcinogenic risk to humans  
3 from the higher exposure levels more typical of occupational exposures (versus the lower  
4 exposure levels typical of environmental exposures) appropriate and transparently described  
5 in Section 4.5?  
6

7 2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data  
8 appropriate and transparently described? Is the use of “ppm equivalence” adequate for  
9 interspecies scaling of EtO exposures from the rodent data to humans?  
10

11 **Issue 3: Uncertainty (Sections 3 and 4)**  
12

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

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

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1 human exposure and cancer. The differing views as to the appropriate descriptor for ethylene  
2 oxide were based on whether all of the requirements for designation as “Carcinogenic to  
3 Humans” in the absence of convincing epidemiological evidence were met. Panel members  
4 favoring a descriptor of “Carcinogenic to Humans” found the epidemiological evidence for  
5 an association between ethylene oxide exposure and cancer to be adequate, albeit not strong  
6 enough to assert causality. Other panel members found the epidemiological evidence to be  
7 weak, lacking consistency across multiple studies, and they concluded that the data were  
8 currently insufficient to conclude that key precursor events were observed in humans.  
9

10 The panel believes that the document would be improved by a better introduction to the  
11 hazard characterization section, including a brief description of the information that will be  
12 presented. EPA has provided a comprehensive review (when the Draft Assessment as a  
13 whole is considered) of the existing epidemiologic evidence relevant to ethylene oxide and a  
14 fair, transparent, and critical assessment of this evidence for purposes of classifying EtO as a  
15 human carcinogen. Presentation of the epidemiologic evidence would be strengthened by  
16 including a summary figure and/or table at the beginning of section 3.0. In particular, the  
17 authors should include the material now provided in Appendix A within the main body of the  
18 Draft Assessment. These tables should also provide clearer information on the observed  
19 endpoints, in particular any information regarding cancer type within the broad category of  
20 lymphohematopoietic cancers.  
21

22 Based on this review, their assessment that the evidence is “strong but less than  
23 completely conclusive” is well supported although a characterization of the epidemiologic  
24 evidence as “strong” is questionable. This ambiguity and the “less than completely  
25 conclusive” assessment is appropriate given the uncertainties and inconsistencies in the  
26 occupational epidemiology as is accurately summarized on page 11 of the Draft Assessment  
27 “3.1.1. Conclusions Regarding the Evidence of Cancer in Humans.” EPA has both  
28 appropriately applied the Hill criteria to assess causality and correctly interpreted their  
29 application to the existing data. EPA’s determination of EtO as a human carcinogen is robust  
30 in that this conclusion is sustained by the largest and highest quality study (i.e., the NIOSH  
31 study) under a variety of approaches to exposure classification. EPA appropriately identifies  
32 Steenland et al. as the critical study for establishing human carcinogenicity. We agree with  
33 EPA in their reliance on “internal” study findings in the presence of “external” findings due  
34 to well recognized limitations to the latter method of analysis. The Draft Assessment  
35 appropriately characterizes the magnitude of the risk associated with EtO as “weak”. This  
36 finding is well substantiated by the epidemiologic evidence where a relatively small number  
37 of excess cancers are found above background even among highly exposed individuals.  
38 Additional perspective to this discussion can be provided by comparing EtO’s risk to other  
39 similar carcinogens such as benzene, 1,3-butadiene, and/or formaldehyde.  
40

41 The EPA’s reliance on the NIOSH studies in providing a robust basis for assessment is  
42 well justified based on the sample size and available quantitative exposure data. In this  
43 study, the strongest exposure response associations were found with cumulative exposure  
44 rather than average or peak exposure. Such a basis for exposure classification is well  
45 supported for a chronic effect such as cancer. The Draft Assessment describes both the

1 internal and external cancer rates reported within the literature. This is appropriate both for  
2 providing an accurate summary and for addressing the different dimensions of EPA's  
3 evaluation, i.e. strength of evidence and unit risk estimate. There was a strong sense on the  
4 panel that the EPA's risk characterization could be improved by additional analyses of the  
5 raw NIOSH data.

### 6 7 **1.b. Relevant Additional Key Studies**

#### 8 9 **Are there additional key published studies or publicly available scientific reports that** 10 **are missing from the draft document and that might be useful of the discussion of the** 11 **carcinogenic hazard of EtO?**

12  
13 Although the Draft Assessment generally provided a clear and concise summary of the  
14 literature regarding EtO, the panel identified two areas that deserve a more expansive  
15 treatment. First, endogenous production of EtO results in some measure of background DNA  
16 adducts and this issue should be addressed more fully in the document. The presentation of  
17 data from a single reference (Bolt, 1996) giving background levels of 7-HEG in unexposed  
18 humans suggests that (i) these values are the most reliable and (ii) the potential impact of  
19 spontaneous hydroxyethylation of DNA by endogenously formed EtO has little to no  
20 importance in the estimation of human cancer risk for this chemical. However, it has been  
21 known for nearly 20 years that endogenous formation of ethylene and conversion to EtO  
22 leads to 2-hydroxyethylation of DNA yielding background levels of 7-HEG in unexposed  
23 humans and rodents (Föst et al., 1989; Walker et al., 1992b, 2000; Cushnir et al., 1993;  
24 Farmer et al., 1993; van Delft et al., 1993, 1994; Leutbecher, 1995; Bolt et al., 1997; Wu et  
25 al., 1999; Zhao et al., 1999). Table V in Walker et al. (2000) lists a series of studies of  
26 background levels of these adducts in differing tissues of unexposed humans (see references  
27 therein), showing that lower spontaneous levels of 7-HEG have been typically found using  
28 more sensitive detection methods than those used in reports cited in Bolt's commentary  
29 (1996) (see references therein). In another commentary/review, Farmer and Shuker (1999)  
30 suggest that in order to estimate the increase in cancer risk attributable to a given external  
31 exposure, it is clearly important to establish and consider background levels of corresponding  
32 DNA damage so that the scale of the incremental increase can be calculated. It is mainly for  
33 this reason that more sensitive and specific analytical methods have been developed for the  
34 measurement of background and EtO treatment-induced levels of 7-HEG than for any single  
35 other DNA adduct (supporting references available). Because the levels of background 7-  
36 HEG are fairly substantial, and there are no chemical differences in DNA damage by  
37 endogenous versus exogenous EtO, the Draft Assessment requires a section considering the  
38 potential impact of endogenous versus exogenous EtO exposure that carefully lays out (i)  
39 why the current evidence of background levels of 2-hydroxyethylation of DNA do not  
40 constitute a threshold and (ii) whether the magnitude and variability in endogenous EtO-  
41 induced damage may overwhelm any contribution from exogenous EtO exposure (other than  
42 some acute high dose exposure).

43  
44 Second, a more comprehensive discussion of the production of DNA adducts by EtO  
45 exposure would be appropriate. For the last paragraph of section 3.3.1 (page 21), a report by

1 Dan Segerbäck (1990) showed that treatment of calf thymus DNA with <sup>14</sup>C-labelled EtO  
2 resulted in the formation of N7-HEG, N3-HEA, and O<sup>6</sup>-HEG at a ratio of 200:8.8:1. The  
3 Draft Assessment suggested that this ratio of DNA adducts was found in a study of EtO-  
4 exposed rats by Zhao et al. (1997); however, N7-HEG was the only product of EtO-induced  
5 hydroxyethylation measured in this study. Instead, Walker et al. (1992b) found that the ratio  
6 of the steady-state concentrations of 7-HEG, 3-HEA, and O<sup>6</sup>-HEG was 300:1.2:1 following  
7 repeated exposures of rats to EtO, indicating that 3-HEA and O<sup>6</sup>-HEG do not accumulate in  
8 vivo to the levels predicted by the in vitro ratios of these adducts and 7-HEG. The same  
9 misquoting of Zhao et al., (1997 about the ratio of these three DNA adducts is present  
10 beginning on the last line of page 21 of the Draft Assessment.

11  
12 Finally, some panel members supported the inclusion of the cancer bioassay results  
13 for ethylene exposure and believed they were relevant and should be discussed in the Draft  
14 Assessment. However, others on the panel were less enthusiastic about this addition and felt  
15 that, were the ethylene results to be included, a careful discussion of the caveats to their  
16 interpretation relative to EtO carcinogenicity would be essential. The rationale for including  
17 the bioassay results for inhalation exposure of F344 rats to ethylene (Hamm et al., 1984) is as  
18 follows. There were no treatment- or dose-dependent increases in the induction of neoplasms  
19 following 2 years of exposure to 0, 300, 1000, or 3000 ppm ethylene, suggesting that the  
20 levels of in vivo formation of EtO during exogenous exposures to ethylene were insufficient  
21 to have carcinogenic effects. In vivo metabolism of ethylene at high exogenous exposures  
22 (>1000 ppm) is saturated and EtO is formed at the highest rate possible in the rat, with  
23 ethylene concentrations higher than 1000 ppm corresponding to exogenous exposure to  
24 approximately 6 ppm EtO based upon N7-(2-hydroxyethyl)valine values and a two-  
25 compartment model (Bolt and Filser, 1987; Czanády et al., 2000; Walker et al., 2000).  
26 Measurements of N7-(2-hydroxyethyl)guanine (7-HEG) adduct levels in rats exposed to  
27 ethylene or directly to EtO indicate that 3000 ppm ethylene exposures yield equivalent EtO  
28 levels of 6.4 to 9.5 ppm in various tissues except for liver (Walker et al., 2000). The  
29 resulting reactions with nucleic acids and proteins following in vitro or in vivo exposures to  
30 EtO are purely chemical in nature. In terms of potential differences in the nature and/or the  
31 degree of DNA damage produced by hydroxyethylcarbonium ions resulting from (i) in vivo  
32 conversion of endogenously formed ethylene to EtO, (ii) in vivo formation of EtO following  
33 exogenous exposure to ethylene, and (iii) exogenous exposures to EtO – there is no  
34 biological, chemical, or theoretical basis for believing that hydroxyethylation arising from  
35 these three different sources are different and impose more or less genetic risk. Furthermore,  
36 EtO arising from metabolism of endogenous/exogenous ethylene or from exogenous EtO  
37 exposures are rapidly and evenly distributed to all tissues (except for testis) in vivo (Wu et  
38 al., 1999; Walker et al., 2000). Thus, under standard cancer bioassay conditions using 63 to  
39 80 rats per group, the ethylene equivalent of approximately 6 ppm EtO appears to represent a  
40 threshold for neoplastic transformation over the spontaneous background in the F344 rat.

41  
42 For the first paragraph of section 3.3.2.1, increased frequencies of *Hprt* gene  
43 mutations were also observed in lymphocytes of rats at concentrations of EtO used in cancer  
44 studies with this species (Tates et al., 1999; van Sittert et al., 2000; Walker et al., 2000).  
45 Likewise, for the sentence beginning on page 24, line 27, the underlined changes are

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1 suggested: “Increases in the frequency of gene mutations in the lung, in T-lymphocytes, in  
2 bone marrow, and/or in the testes have been observed in transgenic mice and in rats exposed  
3 to EtO by inhalation.....” For the remainder of this section, it should be noted that *Hprt*  
4 refers to the rodent gene while *HPRT* is reserved for the human counterpart in discussing  
5 data about this reporter gene.  
6  
7

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

10  
11 Albertini, R.A., and Sweeney, L.M. (2006) Propylene oxide: genotoxicity profile of a rodent  
12 nasal carcinogen. *CRC Toxicology*, in press.

13  
14 Applegren, L.E., Eneroth, G., Grant, C., Lanström, L.E., and TENGHAGEN, K. (1978) Testing  
15 of ethylene oxide for mutagenicity using the micronucleus test in mice and rats. *Act*  
16 *Pharmacol. Toxicol.* 43: 69-71.  
17

18 Bastlová, T., Andersson, B., Lambert, B., and Kolman, A. (1993) Molecular analysis of  
19 ethylene oxide-induced mutations at the HPRT locus in human diploid fibroblasts.  
20 *Mutat. Res.* 287: 283-292.  
21

22 Bolt, H.M. and Filser, J.G. (1987) Kinetics and disposition in toxicology. Example:  
23 carcinogenic risk estimate for ethylene. *Arch. Toxicol.* 60: 73-76.  
24

25 Conan, R.A., Waggy, G.T., Spiegel, M.H., and Berglund, R.L. (1979) Study of the  
26 mutagenic action of ethylene oxide, ethylene glycol, and 2-chloroethanol residues in  
27 plastic material sterilized by ethylene oxide. *Ann. Falsif. Expert. Chim.* 72: 141-151.  
28

29 Eisenbrand, G., Muller, N., Denkel, E., and Sterzel, W. (1986) DNA adducts and DNA  
30 damage by antineoplastic and carcinogenic N-nitrosocompounds. *J. Cancer Res. Clin.*  
31 *Oncol.* 112: 196-204.  
32

33 Farmer, P.B., Bailey, E., Naylor, S., Anderson, D., Brooks, A., Cushnir, J., Lamb, J.H.,  
34 Sepai, O., and Tang, Y.-S. (1993) Identification of endogenous electrophiles by means  
35 of mass spectrometric determination of protein and DNA adducts. *Environ. Health*  
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37 Farmer, P.B., and Shuker, D.E.G. (1999) What is the significance of increases in background  
38 levels of carcinogen-derived protein and DNA adducts? Some considerations for  
39 incremental risk assessment. *Mutat. Res.* 424: 275-286.  
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41 Farooqi, Z., Tornqvist, M., Ehrenberg, L., and Natarajan, A.T. (1993) Genotoxic effects of  
42 ethylene oxide and propylene oxide in mouse bone marrow cells. *Mutat. Res.* 299: 223-  
43 228.  
44

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- 1 Fomenko, V.N., Strelakova, E. Ye (1973) The mutagenic effect of some industrial toxins as a  
2 function of concentration and exposure time. *Toksikol Nov Prom Khim Veshchestv* 13:  
3 51-57.  
4
- 5 Golberg, L. (1986) Hazard Assessment of Ethylene Oxide. CRC Press, Boca Raton, FL, pp  
6 3-7.  
7
- 8 Hamm, T.E. Jr., Guest, D., and Dent, J.G. (1984) Chronic toxicity and oncogenicity bioassay  
9 of inhaled ethylene in Fischer-344 rats. *Fund. Appl. Toxicol.* 4: 473-478.  
10
- 11 Hurst, D.T. (1980) An Introduction to the Chemistry and Biochemistry of Pyrimidines,  
12 Purines, and pteridines. Wiley, New York, pp 5-8.  
13
- 14 Jenssen D., and Ramel, C. (1980) The micronucleus test is part of a short-term mutagenicity  
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16 *Res.* 75: 191-202.  
17
- 18 Kligerman, A.D., Erexson, G.L., Phelps, M.E., and Wilmer, J.L. (1983) Sister-chromatid  
19 exchange induction in peripheral blood lymphocytes of rats exposed to ethylene oxide  
20 by inhalation. *Mutat. Res.* 120:37-44.  
21
- 22 Lambert, B., Andersson, B., Bastlova, T., Hou, S.-M., Hellgren, D., and Kolman, A. (1994)  
23 Mutations induced in the hypoxanthine phosphoribosyl transferase gene by three urban  
24 air pollutants: acetaldehyde, benzo[a]pyrene diolepoxide, and ethylene oxide. *Environ.*  
25 *Health Perspect* 102 (Suppl. 4): 135-138.  
26
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28 persistence of micronuclei, sister-chromatid exchanges and chromosomal aberrations in  
29 splenocytes and bone-marrow cells of rats exposed to ethylene oxide. *Mutat. Res.* 492:  
30 59-67.  
31
- 32 Marsden DA, Jones DJ, Lamb JH, Tompkins EM, Farmer PB, Brown K (2007)  
33 Determination of endogenous and exogenously derived N7-(2-hydroxyethyl)guanine  
34 adducts in ethylene oxide-treated rats. *Chem Res Toxicol* 20:290-299.  
35
- 36 Ong, T., Bi H.K., Xing, S., Stewart, J., and Moorman, W. (1993) Induction of sister  
37 chromatid exchange in spleen and bone marrow cells of rats exposed by inhalation to  
38 different dose rates of ethylene oxide. *Environ. Mol. Mutagen.* 22: 147-151.
- 39 Ribeiro, L.R., Tabetto-Gay, M.N., Salvadori, D.M., Pereira, C.A., and Becak, W. (1987)  
40 Cytogenetic effects of inhaled ethylene oxide in somatic and germ cells of mice. *Arch.*  
41 *Toxicol.* 59: 332-335.  
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- 43 Rusyn, I., Asakura, S., Li, Y., Kosyk, O., Koc, H., Nakamura, J., Upton, P.B., and Swenberg,  
44 J.A. (2005) Effects of ethylene oxide and ethylene inhalation on DNA adducts,

1 apurinic/aprimidinic sites and expression of base excision DNA repair gene in rat brain,  
2 spleen, and liver. DNA Repair (Amst.) 4: 1099-1110.

3  
4 Segerbäck, D. (1990) Reaction products in hemoglobin and DNA after in vitro treatment  
5 with ethylene oxidized and *N*-(2-hydroxyethyl)-*N*-nitrosourea. *Carcinogenesis* 11:307-  
6 312.

7  
8 Strelkova, E. Ye, Chirkova, E.M., and Golubovich, E. (1975) Mutagenic action of ethylene  
9 oxide on sex and somatic cells in male white rats. *Toksikol Nov Prom Khim*  
10 *Veshchestv* 14: 11-16.

11  
12 Van Delft J.H.M., van Winden M.J.M., van den Ende, A.M.C., and Baan R.A. (1993)  
13 Determining N7-alkylguanine adducts by immunochemical methods and HPLC with  
14 electrochemical detection: application in animal studies and in monitoring human  
15 exposure to alkylating agents. *Environ. Health Perspect.* 99: 25-32.

16  
17 Walker, V.E., Wu, K.-Y., Upton, P.B., Ranasinghe, A., Scheller, N., Cho, M.-H., Vergnes,  
18 J.S., Skopek, T.R., and Swenberg, J.A. (2000) Biomarkers of exposure and effect as  
19 indicators of potential carcinogenic risk arising from *in vivo* metabolism of ethylene to  
20 ethylene oxide. *Carcinogenesis*, 21: 1661-1669.

### 21 22 23 **1.c. Mode of Action**

#### 24 25 **Do the available data and discussion in the draft document support the mode of action** 26 **conclusions?**

27  
28 The panel agrees with the conclusion in the draft assessment that the available data  
29 strongly support the action of EtO as a genotoxic agent producing DNA adducts as well as  
30 cytogenetic and small-scale mutagenic effects. However, a more careful discussion of the  
31 sequence of events that are presumed to lead to EtO-induced mutagenesis is warranted. In  
32 the Draft Assessment, the description of the events leading to gene mutations and  
33 chromosome damage presume that 7-HEG and *N*-alkylated bases are indirectly responsible,  
34 or primarily responsible, for genetic changes. The section on the mode of action does not  
35 consider any other possibilities to explain the genotoxicity of EtO, which include (but are not  
36 limited to) the potential consequences of (i) formation of minor promutagenic adducts, (ii)  
37 hydroxyethylation of the DNA backbone, and (iii) the formation of secondary reactive  
38 oxygen species. The sentence beginning on line 4 of page 22 states that “HEG adducts  
39 results in various types of cytogenetic damage, including gene mutations, which have been  
40 observed in mice and rats”. However, there is currently limited evidence to directly support  
41 this statement.

42  
43 As discussed in a recent review by Albertini and Sweeney (2006), N7-alkylguanine  
44 adducts formed from small epoxides such as EtO and propylene oxide do not cause distortion  
45 of the double helix and do not interfere with hydrogen bonding; rather, they are hypothesized

1 to result in mutation via loss of N7-alkylguanine via depurination or the action of DNA  
2 glycosylases, leaving an apurinic site in the DNA. The action of apurinic endonuclease  
3 indeed creates a DNA single-strand break which, if unresolved, can lead to DNA double-  
4 strand breaks. Furthermore, depurination of N7-alkylguanine can result in preferential  
5 insertion of an adenine (according to the A-rule) or another base leading to  
6 mispairing/mutations. Based upon the initial mutational spectra data for EtO in mice  
7 (Walker and Skopek, 1993), it was hypothesized that formation of apurinic (AP) sites might  
8 be involved in the mutagenesis of EtO. In order for these mutagenic events to occur at a rate  
9 sufficient to result in an EtO-induced changes in mutational spectra (including increases in  
10 double-strand breaks and changes in mutant fractions for point mutations), then accumulation  
11 of AP sites arising from high levels of 7-HEG would be expected to occur over time. A  
12 study was recently completed to test the hypothesis that EtO exposure results in the  
13 accumulation of AP sites and induces changes in the expression of genes for base excision  
14 DNA repair, predisposing to point mutations and chromosomal aberrations in F344 rats  
15 exposed by inhalation for 4 weeks to 0 or 100 ppm EtO, or 0 to 3000 ppm ethylene, (Rusyn  
16 et al., 2005). The resulting data demonstrated that DNA damage induced by exposure to EtO  
17 is repaired without accumulation of AP sites, and that the mechanisms proposed above play a  
18 minor role in the mutagenicity of EtO. The same conclusions would apply to the  
19 accumulation of 3-HEA formed in minor amounts in EtO-exposed rats (Walker et al.,  
20 1992b), and the induction of strand breaks or point mutations at A:T base pairs. Rusyn et al.  
21 (2005) have suggested that the mutagenic effects of EtO were likely to be the result of minor  
22 promutagenic adducts, such as O<sup>6</sup>-HEG, N1-HEAdenine, or possibly ring-opened 7-HEG.  
23

24 Drs. Lars Ehrenberg and Timothy Fennell have independently proposed that EtO may  
25 induce strand breaks and chromosomal alterations via 2-hydroxyethylation of the DNA  
26 backbone. 2-Hydroxyethylation of phosphate groups introduces extreme instability into the  
27 sugar-phosphate backbone since the resulting phosphotriester breaks down through a  
28 dioxaphospholane ring intermediate (Eisenbrand et al., 1986). This alternative mechanism  
29 for EtO-induced strand breaks and chromosomal damage is not mentioned in the Draft  
30 Assessment.  
31

32 In summary, the overall genetic toxicology data strongly support the consistent action  
33 of EtO as a relatively weak mutagen and clastogen, but the underlying mechanisms for its  
34 mode of action as a genotoxin are not known with a high degree of certainty. The paucity of  
35 knowledge about the fundamental ways in which EtO acts to induce large- and small-scale  
36 mutations is not reflected in the mode-of-action section; rather this section is presented as if  
37 there is a good basic understanding (which does not currently exist).  
38

#### 39 **1.d. Hazard Characterization**

40  
41 **Does the hazard characterization discussion for EtO provide a scientifically-balanced**  
42 **and sound description that synthesizes the human, laboratory animal, and supporting**  
43 **(e.g., *in vitro*) evidence for human carcinogenic hazard?**  
44

1 While some members of the panel found the hazard characterization section of the  
2 Draft Assessment to be satisfactory, a majority expressed concerns that this section did not  
3 achieve the necessary level of rigor and balance. As discussed above, a majority of panel  
4 members agreed with the overall characterization of EtO as a human carcinogen. However, a  
5 critical issue in this characterization, in particular in the face of epidemiological data that are  
6 not strongly conclusive, is whether the precursor events leading to cancer in animals are  
7 observed in humans at the levels to which they are exposed to EtO.  
8

9 The mode of action for EtO carcinogenicity involves the key events of DNA  
10 alkylation and the induction of point mutations and/or chromosomal changes. Evidence for  
11 genotoxicity of EtO in humans is largely based on cytogenetic analyses. The frequency of  
12 cells with chromosomal aberrations and micronuclei in peripheral blood cells are two of the  
13 most accepted cytogenetic biomarkers used in human population studies because they were  
14 the first indicators of effect shown to be early predictors of cancer risk. However, the  
15 micronucleus data in EtO-exposed humans are weak with very small increases reported and  
16 the abundant data on chromosomal aberrations in EtO-exposed people have not  
17 demonstrated, with confidence, the occurrence of stable chromosome changes leading to  
18 mutations. As indicated at the bottom of page 20 of the Draft Assessment, chromosome  
19 painting/FISH are needed to detect and quantify stable chromosomal aberrations which  
20 would provide more conclusive evidence for classifying EtO as a human carcinogen. A  
21 problem in the hazard characterization in the Draft Assessment is the lack of an adequate  
22 review of the cytogenetic data for EtO in exposed rodents and head-to-head comparisons to  
23 corresponding data in humans. The sections concerning SCEs (3.3.2.2) and chromosomal  
24 aberrations (3.3.2.3) in the Supporting Evidence present only data from human studies and  
25 overlook contradictory or equivocal findings from studies of EtO-exposed rodents.  
26 Furthermore, there is no discussion of findings related to micronuclei in humans or rodents in  
27 the Supporting Evidence section. In brief, several studies have shown that repeated  
28 exposures of rats to high concentrations of EtO induces dose-related increases in SCEs  
29 (Kligerman et al., 1982; Ong et al., 1993; van Sittert et al., 2000; Lorenti Garcia et al., 2001).  
30 Treatment of rats and mice with high acute doses of EtO by i.p./i.v. injection or oral dosing  
31 (i.e., routes of exposure not relevant to humans) also caused increases in the frequencies of  
32 micronuclei or chromosomal aberrations (Strekalova et al., 1971; Applegren et al., 1978;  
33 Conan et al., 1979; Jensen and Ramel, 1980; Farooqui et al., 1993). In contrast, following  
34 inhalation exposures (i.e., a route of exposure relevant to humans), no increases in the  
35 frequencies of micronuclei or chromosomal aberrations were found in peripheral  
36 blood/splenic lymphocytes from rats exposed at concentrations of 50 to 450 ppm EtO for 1  
37 or 3 days (Kligerman et al., 1982) or 50 to 200 ppm EtO for 4 weeks (5 days/week, 6 h/day)  
38 (van Sittert et al., 2000; Lorenti Garcia et al., 2001). Furthermore, two studies showed that 4  
39 weeks of exposure of rats to 200 ppm EtO failed to cause an increase in translocations (van  
40 Sittert et al., 2000; Lorenti Garcia et al., 2001) (e.g., the % translocation in controls and 200  
41 ppm rats were 0.1% and 0.09%, respectively, in the latter study). In the study by van Sittert  
42 et al. (2000), the authors concluded that "The absence of effects on reciprocal translocations  
43 (assessed by FISH) demonstrates that 4 weeks of inhalation exposure to EO at high levels  
44 does not produce genetically transmissible chromosome aberrations in the rat". A single  
45 study reported that repeated exposures of mice at 200 to 600 ppm EtO for two weeks induced

1 chromosomal aberrations in bone marrow cells (Ribeiro et al., 1987), but no studies have  
2 been performed to assess whether this chemical causes transmissible chromosome  
3 aberrations in somatic cells in this species.

4  
5 In contrast to lack of data supporting induction of CAs and reciprocal translocations  
6 at EtO concentrations used in rodent carcinogenicity studies of this chemical, there are  
7 unequivocal data from three research groups (cited reports by Les Recio, Ad Tate, and  
8 Vernon Walker) showing that EtO causes dose-related increases in point mutations in  
9 multiple tissues of mice and rats exposed by inhalation to 50, 100, or 200 ppm EtO, or  
10 concentrations used in the cancer bioassays of EtO. In these rodent studies using the *Hprt*  
11 and/or *lacI* reporter genes, EtO was consistently a weak point mutagen. However, as noted in  
12 the Draft Assessment, studies of the induction of *Hprt* mutations in EtO-exposed humans  
13 have been inconclusive.

14  
15 Thus, studies of both humans and rodents exposed to EtO have yielded evidence  
16 consistent with the genotoxic mode of action of EtO, but different types of genetic alterations  
17 are demonstrated in the two species.

## 18 19 20 **Charge Question 2- Dose-Response Analysis**

21  
22 The Agency's assessment describes the derivation of inhalation unit risk estimates for  
23 cancer mortality and incidence based on the human data. An  $EC_{01}$  of  $44 \mu\text{g}/\text{m}^3$  (0.024 ppm)  
24 was calculated using a life-table analysis and linear modeling of the categorical Cox  
25 regression analysis results for excess lymphohematopoietic cancer mortality in males  
26 reported in a high-quality occupational epidemiologic study (Steenland et al., 2004). Linear  
27 low-dose extrapolation from the  $LEC_{01}$  yielded a lifetime extra cancer mortality unit risk  
28 estimate of  $5.0 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  (0.92 per ppm) of continuous EtO exposure. Applying the  
29 same linear regression coefficient and life-table analysis to background male  
30 lymphohematopoietic cancer *incidence* rates yielded an  $EC_{01}$  of  $24 \mu\text{g}/\text{m}^3$  (0.013 ppm) and a  
31 preferred lifetime extra cancer unit risk estimate of  $9.0 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  (1.6 per ppm). The  
32 preferred estimate is greater than the estimate of  $5.0 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  (0.91 per ppm;  $EC_{01} =$   
33  $44 \mu\text{g}/\text{m}^3$ ) calculated, using the same approach, from the results of a breast cancer incidence  
34 study of the same worker cohort (Steenland et al., 2003), and is recommended as the potency  
35 estimate for Agency use.

36  
37 According to the Agency's assessment, the weight of evidence supports a mutagenic  
38 mode of action for EtO carcinogenicity, and in the absence of chemical-specific data on  
39 early-life susceptibility, the authors conclude that increased early-life susceptibility should be  
40 assumed and the age-dependent adjustment factors (ADAFs) should be applied, in  
41 accordance with EPA's *Supplemental Guidance for Assessing Susceptibility From Early-Life*  
42 *Exposure to Carcinogens*, hereinafter referred to as "EPA's Supplemental Guidance" (U.S.  
43 EPA, 2005b). Applying the ADAFs to the unit risk estimate of  $9.0 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  yields  
44 an adjusted full lifetime unit risk estimate of  $1.5 \times 10^{-3}$  per  $\mu\text{g}/\text{m}^3$ , and the commensurate  
45 lifetime chronic exposure level of EtO corresponding to an increased cancer risk of  $10^{-6}$  is

1 0.0007 $\mu\text{g}/\text{m}^3$ . [Note that for less-than-lifetime exposure scenarios (or for exposures that vary  
2 with age), the unadjusted (adult-based) potency estimate of  $9.0 \times 10^{-4}$  per  $\mu\text{g}/\text{m}^3$  should be  
3 used, in conjunction with the ADAFs as appropriate, in accordance with EPA's  
4 Supplemental Guidance.]  
5

6 Unit risk estimates were also derived from the three chronic rodent bioassays for EtO  
7 reported in the literature. These estimates, ranging from  $2.2 \times 10^{-5}$  per  $\mu\text{g}/\text{m}^3$  to  $4.6 \times 10^{-5}$   
8 per  $\mu\text{g}/\text{m}^3$ , are about an order of magnitude lower than the estimates based on human data  
9 [unadjusted for early-life susceptibility]. The Agency takes the position that human data, if  
10 adequate data are available, provide a more appropriate basis than rodent data for estimating  
11 population risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating  
12 quantitative risks from rodents to humans are avoided. Although there is a fairly sizable  
13 difference between the rodent- and human-based estimates, the assessment infers that the  
14 similarity between the unit risk estimates based on the male lymphohematopoietic cancer and  
15 the female breast cancer results increases confidence in the use of the unit risk estimate based  
16 on the male lymphohematopoietic cancer results.  
17

18 The unit risk estimates were developed for environmental exposure levels and are not  
19 necessarily applicable to higher-level occupational exposures, which appear to be subject to a  
20 different exposure-response relationship. However, occupational exposure levels are of  
21 concern to EPA when EtO is used as a pesticide (e.g., fumigant for spices). Therefore, this  
22 document also presents extra risk estimates for cancer for a number of occupational exposure  
23 scenarios.  
24

25 **2. Do the available data and discussion in the draft document support the approaches**  
26 **taken by EPA in its derivation of cancer risk estimates for EtO? In your response,**  
27 **please include consideration of the following:**  
28

### 29 **2.a. Selection of Epidemiology Studies**

30

31 **EPA concluded that the epidemiological evidence alone was strong but less than**  
32 **completely conclusive (although EPA characterized the total evidence - from human,**  
33 **laboratory animal, and in vitro studies - as supporting a conclusion that EtO as**  
34 **"carcinogenic to humans"). Is the use of epidemiological data, in particular the**  
35 **Steenland et al. (2003, 2004) data set, the most appropriate for estimating the**  
36 **magnitude of the carcinogenic risk to humans from environmental EtO exposures? Are**  
37 **the scientific justifications for using this data set transparently described? Is the basis**  
38 **for selecting the Steenland et al. data over other available data (e.g., the Union Carbide**  
39 **data) for quantifying risk adequately described?**  
40

41 The Panel agreed that the epidemiological evidence is strong but less than completely  
42 conclusive. It is not unusual for epidemiological evidence to be strong but in and of itself not  
43 provide conclusive evidence of causation. It is appropriate in light of conclusive evidence in  
44 animals to use sound human epidemiological studies to determine the dose response even  
45 though in and of themselves these studies may not provide conclusive evidence of

1 carcinogenicity.

2  
3 The Panel agreed that the NIOSH retrospective cohort study with observations on in  
4 excess of 18,000 workers from 13 sterilizing facilities is the best single source of data for  
5 determining the dose-response relationship for evaluating the risk of low level EtO exposure  
6 in human populations (Steenland et al, 2004).

7  
8 As a single source, the epidemiological data for the NIOSH cohort has the following distinct  
9 advantages:

- 10 1) A large (18,000+) sample of workers with long periods of exposure to EtO;
- 11 12
- 13 2) A roughly 55%/45% female to male gender ratio;
- 14
- 15 3) 13 distinct facilities with worker exposure estimates. (Facility intra-class correlation is  
16 never considered in any of the models applied to the NIOSH data);
- 17
- 18 4) Limited coincidental exposure in the occupational cohort to other compounds, e.g.  
19 ethylene dichloride, that might confound the interpretation of the relationship of EtO  
20 exposure to cancer outcomes.
- 21
- 22 5) Careful mortality follow-up using multiple sources, and finally
- 23
- 24 6) Continuity of the investigators who have been building and analyzing the data set.
- 25

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

38  
39 The worker exposure observations used to fit the model were not a random sample  
40 (effect unknown) of workers or work environments but were designed to represent the  
41 typical range of exposure conditions that occur in the contemporary work place. A total of  
42 2350 full-shift charcoal tube measurements were collected from workers in twelve plants.  
43 By design, the observations were distributed across workers involved in eight exposure  
44 activity types (e.g. sterilizer area, production, maintenance) and five product types (e.g. spore  
45 strips, plastics, etc.). In addition to these two main effects, the multivariate regression model

1 for predicting exposures for the NIOSH cohort workers includes additional covariates for age  
2 of product, year (of exposure), and size and ventilation characteristics of the work area. A  
3 random sub-sample of observations of the worker measurements was set aside as a cross-  
4 validation sample for purposes of evaluating the predictive potential of the fitted model.  
5 The final model  $R^2$  produced an  $R^2$  to the cross-validation exposure measurements (cross  
6 validation sample) of  $\sim .85$ . There was consensus among the Panel that the exposure model  
7 development for the NIOSH data was conducted in a rigorous fashion and it would be  
8 difficult to improve on the exposure estimates generated by the NIOSH exposure  
9 measurement study ( Griefe et al., 1988, Steenland et al. 1987).

10  
11 In its discussion of the predicted exposure measures in NIOSH cohort data, the Panel  
12 focused in some detail on the role of chronological time in the prediction of annual exposures  
13 for the individual cohort members. From Table VI of Hornung et al. (1994), the year in  
14 which the exposure occurred is highly predictive of the exposure concentration. Quoting  
15 from the paper, “We had hoped that some combination of engineering controls would  
16 eliminate the need for including calendar year in the model, .... However, no combination of  
17 variables could be found to allow removal of calendar year from the model. We attributed  
18 this finding to calendar year acting as a surrogate for improvement in work practices due to  
19 increased awareness of the potential health effects of ETO”. The effect of chronological  
20 time is highly significant and quadratic--- $-0.446 \times (\text{year}-82)$  and  $0.062 \times (\text{year}-82)^2$ . The  
21 model-based assignment of exposures to individuals in the NIOSH cohort truncates this  
22 highly significant time effect on exposure (quadratic) at 1978. That is, all exposures for  
23 work years prior to 1978 receive the same contribution to modeled exposure as in 1978—the  
24 year at which the trend in exposures is at a maximum for the quadratic time effect. The  
25 reason for assigning all pre-1978 exposures to the 1978 level is that prior to that time data on  
26 general work place exposures was scarce (7 activity x product combination mean exposures  
27 based on 23 individual samples collected in 1976-1978).

28  
29 Regarding the handling of time in modeling annual exposures for individual NIOSH  
30 cohort members, the Panel’s concern is less on the modeling decision but rather on how this  
31 estimation decision may interact with subsequent dose response model fitting in which the  
32 exposure metric is itself a function of time. For example, the 1978 truncation point is very  
33 close to the analysis censoring exposure point of t-15, or t-20 for the Steenland et al. (2004)  
34 dose response models that use time lagged exposure as the independent variable. Does  
35 introducing the lag into the cumulative exposure measurement alter the quality of the  
36 effective exposure model since the parameters for non-time factors (e.g. activity type,  
37 product type, engineering controls, size of workplace) have been estimated in the process of  
38 a strong and dominant quadratic time trend?. Without access to the full NIOSH data set, the  
39 Panel could not answer this question directly. The Panel encourages the EPA to investigate  
40 potential instability that may result from interaction between the chosen time metric for the  
41 dose response model and the treatment of time in the estimated exposure (e.g. log  
42 cumulative, exposure with 15 year lag) that is the independent variable in that dose-response  
43 model.

44  
45 While the advantages of the Steenland dataset are described, the Draft Assessment

1 contains no list of the criteria that were utilized to select studies for inclusion in the risk  
2 assessment process. For example, a description of what constituted adequate sample size,  
3 exposure assessment, minimum length of employment, length of follow-up, lag time for  
4 selected outcomes, etc., would be helpful. It is certainly appropriate to critique all available  
5 datasets and provide justification for excluding those who did not meet these criteria. While  
6 a review of most studies conducted between 1985 (when the last EtO assessment was  
7 conducted) and 2004 was included, it was not always clear why some studies were not  
8 considered in the process. For example, Steenland's dataset was deemed most appropriate  
9 because of the larger sample size (n=18,254), gender diversity (45% male, 55% female), lack  
10 of potentially confounding co-exposures, and more developed measures of individual worker  
11 exposures. There were disadvantages, e.g., lack of information on age of the cohort  
12 members. The authors state that earlier exposures decreased markedly by the mid-1980's.  
13 Did the risk of cancer decrease in later time intervals, i.e., what is the risk for workers  
14 initiating employment in the 1980's when levels are lower over a similar time period (20  
15 years)? Also, age at exposure, an increasingly critical factor in environmental/occupational  
16 exposures and adverse health effects, was not able to be evaluated. (Younger workers with  
17 similar cumulative exposures may be at different risk than older workers).  
18

19 Other cohorts consisted of smaller sample sizes, less precise measures of individual  
20 worker's exposure levels, and concurrent chemical exposures. Some of these studies were  
21 justifiably omitted from the risk assessment because of sufficient weaknesses in design  
22 and/or analysis. However, it seems it would be of value to examine some of these studies to  
23 determine the potential for interaction between EtO and other common workplace exposures.  
24

25 To summarize, the Panel concurred that the NIOSH cohort is the best single  
26 epidemiological data set with which to study the relationship of cancer mortality to the full  
27 range of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly  
28 consider all of the epidemiological data in developing its Draft Assessment. In particular, the  
29 Panel encourages the EPA to consider the Greenberg et al. (1990) data on cancer outcomes  
30 and EtO exposures for 2174 Union Carbide workers at that firms' two Kana Valley, West  
31 Virginia facilities (See Teta et al., 1993; Teta et al., 1999).  
32

33 The Panel did not believe that it was necessary to use only one study to arrive at a  
34 single potency estimate or to limit the assessment to a single modeling approach. Panel  
35 members emphasized that the EPA's own cancer risk assessment guidelines support the  
36 consideration of the full range of available data as well as alternatives to the default exposure  
37 models. Quoting from the EPA's Guidelines for Cancer Risk Assessment,  
38

39 Section 1.3, p. 1-8, "[T]hese cancer guidelines view a critical analysis of all of the available  
40 information that is relevant to assessing the carcinogenic risk as the starting point from  
41 which a default option may be invoked if needed to address uncertainty or the absence of  
42 critical information".  
43  
44

## 45 2.b. Methods of Analysis

1  
2 **Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use**  
3 **of a linear regression model fit to Steenland et al.'s categorical results for all**  
4 **lymphohematopoietic cancer in males in only the lower exposure groups scientifically**  
5 **and statistically appropriate for estimating potential human risk at the lower end of the**  
6 **observable range? Is the use of the grouping of all lymphohematopoietic cancer for the**  
7 **purpose of estimating risk appropriate? Are there other appropriate analytical**  
8 **approaches that should be considered for estimating potential risk in the lower end of**  
9 **the observable range? Is EPA's choice of a preferred model adequately supported and**  
10 **justified? In particular, has EPA adequately explained its reasons for not using a**  
11 **quadratic model approach such as that of Kirman et al. (2004) based? What**  
12 **recommendations would you make regarding low-dose extrapolation below the**  
13 **observed range?**

14  
15 The Panel's discussion of this multi-part question was extensive. To simplify the  
16 presentation here, the written discussion is divided into seven segments: 1) linearity vs.  
17 nonlinearity in dose response modeling and extrapolation; 2) the linear regression  
18 methodology of grouped risk ratios employed in the EPA Draft Assessment; 3) exclusion of  
19 high exposure data points; 4) grouping lymphohematopoietic cancers in analysis; 5) using  
20 only male data; 6) justification of approach and alternatives; and 7) statistical and  
21 computational issues.

#### 22 23 1. Linearity vs. non-linearity of low dose extrapolation

24  
25 The Panel was “philosophically” and scientifically divided on the whether low dose  
26 extrapolation of risk to environmental EtO exposure levels should be linear (following  
27 Cancer Guideline defaults for mutagenic MOA) or whether plausible biological mechanisms  
28 argued for a non-linear and possibly a threshold form for the low dose response relationship.  
29 Some panel members thought that the data on ethylene oxide imply a non-linear response  
30 despite a mutagenic mode of action. They encouraged the exploration of the use of non-  
31 linear models for low-dose extrapolation, such as the quadratic and linear quadratic. Others  
32 thought that non-linear extrapolation was inappropriate given the mutagenic mode of action.  
33 After considerable debate, the Panel was unable to arrive at consensus. Therefore the two  
34 distinct lines of argument are presented below.

35  
36 The Linear Extrapolation Argument: In general a linear no-threshold interpolation to zero  
37 for ethylene oxide external exposure is consistent with available information about the  
38 mutagenic mode of action in this case. General arguments are that DNA repair and other  
39 genomic defense processes (e.g. apoptosis) are not likely to be perfect in the sense of  
40 repairing all incremental damage before the next cycle of DNA copying which would  
41 otherwise lead to miscoding errors or more extensive chromosome level changes including  
42 breakage, recombination, and nondisjunction events. Genomic defense processes have costs  
43 to the cell, and some are also known to create their own baseline damage, so that it is highly  
44 likely that the extent of the effectiveness of such processes has been subject to an  
45 evolutionary optimization that falls short of perfection. Thus, even with background and

1 endogenous exposures there should be some expectation of ongoing equilibrium damage on a  
2 cellular stochastic basis. Such equilibrium damage is likely to contribute to the appreciable  
3 “background” of cancers of all types that humans suffer. A detailed review of the argument  
4 for "low dose linearity" in cancer risk assessment involving a mutagenic mode of action is  
5 given by Hattis (2007, Appendix A).  
6

7 The Non-linear Low Dose Response Model Argument: Linear extrapolation of risk below  
8 the chosen point of departure (POD) to a zero baseline is a conservative assumption, given  
9 EtO's reactivity (which will diminish the amount reaching the nucleus), mutagenic mode of  
10 action, and that it is generated endogenously. Some repair seems likely and some threshold  
11 probably exists. Thus, the human risk estimates at the lower end of the observable range are  
12 likely to be exaggerated under a linear extrapolation. Furthermore, a linear model through  
13 zero (linear model per se at low doses is acceptable) assumes that other effects of EtO on the  
14 development of cancer are insignificant. This seems unlikely given the reactive nature of this  
15 compound and thus its ability to affect signaling pathways that may positively and negatively  
16 influence the development of cancer. Measuring such effects is problematic, but they must  
17 exist and impact the incidence of cancer. Linear regression is for "extra" risk; but this still  
18 seems problematic given the endogenous level of EtO and base levels of damage and repair.  
19 In other words, is it justified to assume linear above baseline levels? At low doses, a reactive  
20 compound like EtO will react with cellular constituents before it ever gets to DNA. Linear  
21 defaults are not supported when a framework analysis is done of genotoxicity and this is  
22 even more strongly so for clastogenic agents, which are quadratic in dose response (Preston,  
23 1999). Swenberg (2007, Appendix B) provides a framework analysis of Genotoxicity and  
24 Risk Assessment in support of an argument for a nonlinear low dose response mechanism for  
25 EtO.  
26

27 At the conclusion of its discussion, the Panel was not in agreement on the linearity vs.  
28 non-linearity of the cancer response to EtO exposure levels in: 1) the occupational exposure  
29 data used to estimate the point of departure for the low dose extrapolation; and 2) in the form  
30 of the model used to extrapolate cancer risk below the POD to a zero or baseline exposure  
31 level. With appropriate discussion of the statistical and biological uncertainties, several  
32 panel members strongly advocated the consideration of both linear and nonlinear functional  
33 forms in the final EtO Risk Assessment. These panel members pointed out that such an  
34 approach was consistent with the latest guidance in the EPA Guidelines for Cancer Risk  
35 Assessment. Quoting Section 1.3 p. 1-9, “*Significant risk management decisions will often*  
36 *benefit from a more comprehensive assessment, including alternative risk models having*  
37 *significant biological support.*”  
38

## 39 2. Linear regression model for categorical data 40

41 The Panel identified several important shortcomings in the linear regression  
42 modeling approach used to establish the point of departure for low dose extrapolation of  
43 cancer risk due to EtO. Based on its review of the methods and results presented at the  
44 January 17,18, 2007 meeting, the Panel was unanimous in its recommendation that the EPA  
45 develop its risk models based on direct analysis of the individual exposure and cancer

1 outcome data for the NIOSH cohort. The Panel understands that these data are available to  
2 EPA analysts upon request to the CDC/NIOSH. The Panel recognizes the burden that a  
3 reanalysis of the individual data places on the EPA ORD staff but given the importance of a  
4 best scientific and statistical treatment of all the available epidemiological data, it sees no  
5 alternative.

6  
7 The following paragraphs present the statistical basis for the Panel's assessment of the  
8 linear regression model approach and the use of categorized exposure and outcome data.  
9

10 The approach described in the Draft Assessment uses a model based on categories  
11 defined by cumulative exposure ranges for male subjects in the NIOSH cohort. Steenland et  
12 al. identified several models that provide a significant ( $p < .05$ ) fit to the exposure data;  
13 however, the EPA has elected to use model-based relative rate parameter estimates for  
14 categories of 15 year lagged, cumulative exposure. In Steenland, et al. (2004) this model was  
15 not one that provided a significant fit to the NIOSH data ( $p = .15$  for the likelihood ratio test  
16 of  $\underline{\beta} = \{\beta_1, \beta_2, \beta_3, \beta_4\} = 0$ ). The use of the weighted least squares regression fit of a linear  
17 regression line through the three data points defined by the estimated rate ratios and mean  
18 cumulative exposures for the first three exposure categories of the Steenland, et al. 15 year  
19 lag, cumulative exposure category model is not a robust application of this technique. The  
20 Panel identified four weaknesses in the approach.  
21

22 a) Model-based dependent variable: The dependent variables are model-based  
23 estimates of rate ratios for exposure categories. The rate ratio values used in the weighted  
24 least squares regression are derived from a cumulative exposure model (15 year lag) in which  
25 the estimated regression parameters in the proportional hazards regression model are not  
26 significantly different from 0 at  $\alpha = .05$  ( $p = .15$ ). In Steenland et al. (2004), the only  
27 individually based (proportional hazards) model that fits the data for males in the NIOSH  
28 cohort is a model for log of individual exposure through t-15 years.  
29

30 b) Grouped data regression: The weighted least squares fit applies estimates of  
31 variance for the individual rate ratios under that assumption that these inverse weighting  
32 corrections correctly adjust for heteroscedasticity of residuals in the underlying regression  
33 model. Historically, models for grouped proportions applied adjustments of this type but it is  
34 by no means a preferred technique when the underlying individual data are available. The  
35 "ecological regression" model per Rothman (1998, Second edition) is subject to bias due to  
36 within group heterogeneity of predictors and unmeasured confounders. The heterogeneity in  
37 the grouped model involves the range of exposures within the collapsed categories. The  
38 unmeasured confounders include variables (other than gender) that affect the potency of  
39 exposure or may have produced gross misclassification based on the original exposure model  
40 estimation for the individual (Hornung, et al., 1994).  
41

42  
43 c) The model fitting does not conform to the Rothman (xxxx) procedure: The target  
44 model for the risk of EtO exposure at low exposure levels is:

1  
2 
$$Expected(Rate | Exposure) = h_0(0) + B \cdot h(Exposure) + \varepsilon$$

3  
4 The 1998 (Second edition) of Rothman describes the technique for estimating this  
5 risk from grouped data in Chapter 23. In that updated version of the original monograph the  
6 model that is fitted is:

7  
8 
$$E(Rate | Exposure) = B_0 + B_1 \cdot Mean(Exposure)$$

9  
10 The objective is to estimate the risk ratio (for exposure 0=no, 1=yes). That estimator is then:

11  
12 
$$rr = 1 + \hat{B}_1 / \hat{B}_0$$

13  
14 The model estimated by the EPA method is:

15  
16 
$$Expected(rr | Exposure) = B_0^* \cdot Mean(Exposure)$$

17  
18 In the former, the expected error of estimation of the relative risk is a function of the error of  
19 the slope and the baseline hazard, represented by the estimated intercept. This error is  
20 present in the estimation of the baseline hazard in the Steenland, et al. (2004) estimation of  
21 the rate ratios but is not propagated as an “errors” in variable in the EPA adaptation to the  
22 linear risk ratio model. The EPA approach permits no intercept (>0) for the background  
23 exposure or any allowance for an effect of true non-zero exposures in the internal control  
24 group (exposures less than 15 years).

25  
26 If this approach (categories of exposure) must be used, the crude rates should be  
27 computed for a large number of equally spaced exposure ranges and the Rothman and  
28 Greenland (1998) model fitted to these multiple points.

29  
30 d) n=3 – the degrees of freedom (1 for error) are not adequate to fit a robust weighted  
31 least squares regression line and to estimate the 95% UCL, LCL for the estimated linear  
32 regression coefficient. By definition, the linear regression forced through mean rates that  
33 exhibit a non-linear pattern will violate the conventional assumptions of the regression  
34 method. In a statistical/arithmetic sense, this a line fitting exercise, not an estimation of the  
35 underlying population regression model.

36  
37 In general, the use of categorical exposure ranges is not the optimal strategy for using  
38 epidemiologic data. When continuous data are categorized and then used in dose response  
39 modeling, it amounts to starting with a full range of exposures, collapsing that range into  
40 somewhat arbitrary boundaries, and then deriving a continuous dose response model for an  
41 even larger range of exposures.

42  
43 Categorizing continuous variables results in a host of issues:

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

8  
9 A preferred strategy is to use continuous exposure estimates as they were originally  
10 developed for individual cohort members.

### 11 3. Exclusion of high exposure groups

12  
13  
14 In conjunction with its recommendation to use the individual NIOSH cohort data to  
15 model the relationship of cancer risk to exposures in the occupational range (see 2.B.1  
16 above), the Panel recommends that the EPA analysts explore the use of the full NIOSH data  
17 set to estimate the cancer slope coefficients that will in turn be used to extrapolate risk below  
18 the established point of departure. Truncation of the high exposure data should not be the  
19 default method for estimating the dose response relationship.

20  
21 In the Draft Assessment, EPA analysts have faithfully adhered to the paradigm of  
22 cancer dose response analysis usually used for animal data in analyzing the human  
23 epidemiological data for this case. This is a useful step toward harmonizing the treatment of  
24 animal and human-derived information in carcinogenic risk estimation. However, while  
25 achieving operational consistency, the Agency's current analysis does not yet take into  
26 account some important differences between animal and human carcinogenic dose response  
27 data. These differences need to be factored in for designing a modern set of analytical  
28 procedures for human data to achieve more comparable types of risk inferences and a better  
29 analysis of uncertainties.

30  
31 The most important differences in human vs. animal data that may require differences in  
32 analytical approach are:

- 33 • Animal exposures are the result of intentional and consistent administration of the
- 34 test material at specific target levels, often reinforced with frequent empirical
- 35 measurements of differences between target exposures and actual delivered doses.
- 36 Human exposures, by contrast, are unintended, often variable over time, and at
- 37 best estimated from occasional measurements of exposures of the subjects
- 38 themselves or subjects considered to have similar exposures. Uncertainty in
- 39 exposures is thus nearly always much greater for human than for animal data.
- 40 Such uncertainties in human data lead to distortions in both central estimates and
- 41 uncertainties in potency estimates that require at least discussion and preferably
- 42 adjustment of ordinary dose response model fitting and "slope factor" estimation
- 43 procedures. Procedures for adjusting traditional regression analyses for such
- 44 effects are relatively well known in biostatistics under the general heading of

1 “errors in variables” models,\* but have rarely been applied to occupational cancer  
2 data in part because, unlike this case, exemplary quantitative analyses of likely  
3 errors in exposure estimates have not often been available. There are some  
4 examples of the use of errors in variables models in epidemiological studies of  
5 other effects (Stayner et al. 2003; Richardson et al. 2004; Brown et al. 2004;  
6 Choi, 2000; Carrothers and Evans, 2000; Kulathinal et al. 2002; Siebert et al.  
7 2001). The current case, where very extensive efforts have been devoted to  
8 development of exposure estimates and quantitative errors in exposure estimates  
9 (Hornung et al. 1994), represents an invaluable opportunity to analyze and offset  
10 the distortion in dose response estimates from this type of problem. The analysis  
11 presented in Appendix C illustrates a relatively simple analytical approach to  
12 gauging the extent of the modification in the low-dose cancer risk for male  
13 lymphoid cancers that could be indicated for this case.

- 14 • The subjects of human epidemiological studies are subject to a variety of  
15 selection biases, including the “healthy worker” effect, and the “healthy worker  
16 survivor” effect. The former complicates comparisons with general population  
17 mortality data, but the internal comparisons used for analyses of the Steenland et  
18 al. (2004) avoid these. However the “healthy worker survivor” effect is a known  
19 phenomenon that produces established distortions in relationships between  
20 measured risks and measures of cumulative exposure, as shorter term workers  
21 suffer greater mortality than workers who work at exposure-producing jobs for  
22 longer periods of time (Steenland et al., 1996; Kolstad and Olsen, 1999; Garshick  
23 et al. 2004; Siebert et al. 2001; Steenland and Stayner 1991). Interestingly, in the  
24 light of the gender differences in the current analysis, in at least one study the  
25 healthy worker effect was found to be greater for women workers than for men  
26 (Lea et al. 1999). Adjustments for this effect are at the cutting edge of current  
27 practice for the treatment of human epidemiological data, but they are vital for  
28 achieving the best possible analysis of those data. The authors of some of the  
29 leading studies documenting the healthy worker survivor effect include authors  
30 involved in the Steenland et al. (2004) ethylene oxide mortality study (e.g.,  
31 Steenland et al., 1996; Stayner et al. 2003). It might be useful for EPA to consult  
32 with Steenland and coworkers to judge whether analytical adjustments for this  
33 effect are possible in this case. Even if the data will not support the more  
34 complex analyses [and analyses of this sort are notoriously complex (Robins,  
35 1986; Arrighi and Hertz-Picciotto, 1996; Hertz-Picciotto, personal  
36 communication) EPA could provide at least some discussion of how large the  
37 distortions might be by citing previous cases such as the cancer risks from diesel  
38 particles (Garshick et al. 2004) and the approach that California risk assessors  
39 (and possibly others) have taken to risk analysis where the healthy worker  
40 survivor effect is even more prominent than it may be in this case. (For diesel

---

\* 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.

1           particulates, the relative risk vs. cumulative dose curve even had a negative,  
2           rather than a positive slope.)  
3  
4

5           Another source of the dose estimation problem that is more distinctive for this case is the  
6           presence of a background of ethylene oxide exposure from endogenous generation.  
7           Conceivably this could be substantial enough to limit the potency of ethylene oxide that  
8           would be compatible with observations of “background” rates of lymphatic cancers in people  
9           without occupational exposures. Should the EPA analysts accommodate this possibility by  
10          adding estimates of exposure from this source (perhaps including variability and uncertainty)  
11          to the estimates of occupational exposures for all the groups in the Steenland et al. (2004)  
12          study? If so, how should such estimates be derived? In preliminary work, Hattis (see  
13          Appendix C) attempted to estimate this effect using the model parameters and data from  
14          Csanady et al. (2000). Generally, the exploratory work by Hattis (Appendix C) finds the  
15          effect to be small enough (about 1.8 ppb occupational equivalent ethylene oxide exposure,  
16          amounting to about 26 occupational equivalent ppm-days of cumulative exposure over a 60  
17          year period) as not to be likely to appreciably distort the EPA analysis.  
18

#### 19           4. Cancer groupings 20

21           Although the analysis based on total LH cancers might have value as part of a complete  
22           risk assessment, the rationale for the groupings needs to be better justified. Certainly, a  
23           rational grouping of cancer types with a similar pathophysiology can lead to improved power  
24           to detect significant effects of EtO exposure. By the same turn, grouping cancers that affect  
25           a single organ system (e.g. blood) but with very different cancer etiology could produce a  
26           spurious and therefore misleading result. The Panel therefore recommends that data be  
27           analyzed by subtype of LH cancers with biological rationale for any groupings that are  
28           formed.  
29

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

1 because of their critical function. Steenland et al's category of lymphoid tumors is more  
 2 consistent with modern lympho-haematopoietic (LH) classification techniques (WHO 2001)  
 3 and should be used as the preferred disease outcome.  
 4

#### 5. Males Only

6  
 7 The Panel diverged in its views concerning the appropriateness of estimating the  
 8 population unit risk based only on the NIOSH data for males.  
 9

10 Several panel members pointed out that a standard approach in cancer epidemiology  
 11 and risk analysis begins by conducting separate dose-response analyses on males and females  
 12 and combining the data only if the results are similar. This approach assumes the possibility  
 13 of known and unknown gender specific differences in the cancer etiology. From a risk  
 14 assessment perspective, it is protective against a gap in our biological knowledge of an  
 15 underlying mode of action that is truly gender dependent. By the same turn, it is not a  
 16 statistically conservative approach if in fact no gender difference exists. In the case of no  
 17 gender effect, a sex-specific analysis will have reduced power to detect any common effect  
 18 for women and men.  
 19

20 A second approach to dealing with the possibility of gender differences in response is  
 21 to include gender as a fixed effect in the statistical modeling of the data and determine  
 22 whether gender or its interaction with other predictors (e.g. gender x exposure) are  
 23 significant explanatory variables. If so, the combined model with the estimated gender  
 24 effects could be used directly or separate, gender-specific dose-response analysis would be  
 25 performed. If not, the gender effects could be dropped and the model re-estimated for the  
 26 combined male and female data.  
 27

28 Members of the panel who argued for the second approach were concerned that there  
 29 appears to be no genetic or other physiological basis for the observed differences in the  
 30 trends observed for males and females (see overall SMRs). Women comprise 55% of the  
 31 NIOSH cohort and in general have lower average levels of cumulative exposures to EtO than  
 32 the males in the original cohort. From Steenland, et al. (2004), Table 4 the SMRs and  
 33 observed deaths for males and females in 5 categories of lagged (10 year) cumulative  
 34 exposure are:  
 35

36 Table 1: NIOSH Cohort. SMRs and observed lymphohematopoietic cancer deaths.

37 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)

1 \* 8 of 13 are NHL  
2  
3

4 Cox proportional hazards models with a random set of  $m=100$  controls matched by  
5 race, birth year and gender found no significant effect of the cumulative exposure level on  
6 the all haematopoeitic cancer hazard for a combined analysis ( $p=.20$ ) or separately for males  
7 ( $p=.12$ ) or females ( $p=.34$ ). Steenland et al. (2004) refitted the models to indicator variables  
8 representing four quartiles of categorical exposure and again found no significant  
9 relationship between the cumulative exposure category and the lymphohematopoietic cancer  
10 hazard. Introducing time lags of 10, 15 and 20 years in cumulative exposure, Steenland et al.  
11 (2004) re-estimated the proportional hazards models using both actual estimated exposure  
12 values and the natural log transformation. For males only, the best fitting model (log  
13 cumulative exposure, 15 year lag) achieved a marginal significance level ( $p=.02$ ). In the  
14 broad class of models considered for females, the significance of the modeled relationship  
15 between lymphohematopoietic cancer hazard and the various exposure metrics did not  
16 approach significance under a nominal  $\alpha =.05$  level.  
17

18 Steenland, et al (2004) defend their model exploration steps against criticism of  
19 possible “data dredging” (and thus consideration of multiple testing criteria) by pointing out  
20 that prior work has shown latency (a lag in exposure measures) to be important in studies of  
21 cancer for occupational exposures and that empirically the log of cumulative exposure has  
22 provided a better fit to these types of time integrated data. To summarize, Steenland et al’s  
23 work hints at a relationship between EtO exposure and all haematopoeitic cancer hazards, but  
24 only for men and not for women. In a statistical sense, this evidence for an exposure  
25 relationship in males is at best marginally significant and its estimated strength is influenced  
26 by the chosen exposure metric—the best metric being the log of estimated cumulative  
27 exposure (lagged to t-15 years). Similar sensitivity to the transformation of the exposure  
28 metric is seen in the Cox regression results relating breast cancer hazard to EtO exposure.  
29

30 Given these results, the EPA should carefully consider the scientific justification for a  
31 “men only” model for its assessment of the risk of lymphohematopoietic cancer hazards.  
32 There should be a strong scientific argument for excluding the female data. Presently, the  
33 draft document identifies no basis for excluding the female data. In the data set, women on  
34 average have lower average levels of estimated exposure to EtO (possibly more relevant to  
35 the exposures of interest in the risk assessment). By the same turn, the results of the  
36 extensive modeling effort suggest that significance of the model fit is influenced by the  
37 chosen exposure metric and the best fitting models are nonlinear with respect to exposure.  
38 Panel members are not challenging the statistical analyses presented in the Steenland et al  
39 (2004) paper. However, the Panel encourages the EPA to narrow its modeling efforts to  
40 functional forms and exposure metrics that make sense from a biological and risk assessment  
41 perspective. The process of model building should also include a challenge to any model  
42 (e.g. log cumulative exposure, 15 year lag) which yields results that differ substantially from  
43 a second model that only changes the scale of the exposure metric (e.g. cumulative exposure,  
44 15 year lag).  
45

1 6. Preferred model justified, alternative analytical approaches.  
2

3 As discussed in 2.b.1-2.b.4 above, the Panel recommended exploration of use of a  
4 number of models, including non-linear models, to fit data within the observation range and  
5 calculate a point of departure (POD). Preference for biologically-based models was  
6 indicated.  
7

8  
9 7. Statistical issues and errors  
10

11 Pages 29-49 of the draft Evaluation outline the EPA's proposed approach to estimation  
12 of the Inhalation Unit Risk for EtO. In addition to the general issues of estimation and  
13 model-based extrapolation described above, there are a number of statistical assumptions and  
14 methods used in this approach that deserve mention.  
15

16 a) BEIR IV for Estimating  $LEC_{.01}$  for Cancer Mortality and Incidence  
17

18 Conditional on the cancer slope factor results from the weighted least squares  
19 regression analysis, the life table (BEIR IV) approach to the determination of the  $EC_{.01}$  is  
20 programmed correctly. The computation of the  $EC_{.01}$  is influenced by the choice of the  
21 terminal age category. As noted in the response to 2.C below, the Panel recommends that the  
22 terminal age point should be consistent between the  $LEC_{.01}$  derivation and the unit risk  
23 computation (e.g., 70 years).  
24

25 The life table methodology that is the basis for the BEIR IV algorithm is designed to  
26 estimate excess mortality and is not readily adapted to modeling excess risk for events  
27 (incidence) that do not censor observation on the individual in population under study. The  
28 methodology for substituting the mortality slope to an excess risk computation for HL cancer  
29 incidence requires the assumption of a proportional rate of incidence/mortality across the  
30 cancer types that are included in the grouped analysis. This is generally not a viable  
31 assumption. The Panel therefore discourages the use of the BEIR IV algorithm for  
32 extrapolation of the cancer mortality algorithm to estimation of excess cancer incidence.  
33

34 Several panel members commented on the use of the upper confidence limit for the  
35 estimated slope coefficient as the basis for estimating an  $LEC_{.01}$ . The Panel encourages the  
36 EPA to consider presenting unit risk estimates based on the range of  $EC_{.01}$  values  
37 corresponding to the lower 95% confidence limit, point estimate, and upper 95% confidence  
38 limit for the estimated cancer slope coefficients from the final dose-response models.  
39

40 b) Estimating the Rothman RR Risk model for Grouped Data  
41

42 The estimates of the regression parameters and standard errors computed in the  
43 "Rothman" spreadsheet do not match an independent fit of the weighted least squares  
44 regression lines conducted in SAS V9.1.3]. If the existing computation tools are used again,  
45 the EPA spreadsheet computations should be checked for accuracy.

1  
2 One panel member noted a logical error in the computation of the “inverse variance”  
3 weights for the weight least squares fit of the linear regression model. See ACC (Sielkin)  
4 public comments for a description of an error in the computation of the estimation standard  
5 errors for the rate ratios.  
6

## 7 **2.c. Age-dependent Adjustment**

### 8 9 **Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk** 10 **estimate, in accordance with EPA’s Supplemental Guidance (U.S. 2005b), appropriate** 11 **and transparently described?** 12

13 The majority of the Panel felt that the application of ADAF by the agency was  
14 appropriate, but that the description in the Draft Assessment was not adequate, particularly  
15 for those not familiar with the EPA Guidance. A clear description of the ADAFs is  
16 important for the Draft Assessment. For example, on page 57 line 15 the lifetime unit risk  
17 calculation with ADAFs is presented. Unless the reader knows the binnings and associated  
18 uncertainty factors, this would not be understandable to the average reader. There was also  
19 discussion of the type of information that would be needed to address the issue of potential  
20 increased sensitivity of children.  
21

22 The Panel noted that the computation of unit risk uses a 70 year life time. This should  
23 probably be made consistent with the 85 year life span used with the LEC<sub>01</sub> estimation.  
24 Given the more complex patterns of mortality post age 70 and the increasing life expectancy  
25 over cohorts included in the data set, it seems reasonable to use age 70 as the terminal point.  
26

27 While the panel recognized the role of the childhood exposure uncertainty factors in  
28 the broader risk assessment process, it did not simply accept the defaults without first  
29 attempting to establish the biological arguments for their application in the case of EtO.  
30

31 The Draft Assessment notes that because of the immaturity of detoxifying enzymes, a  
32 child's susceptibility may be increased. This should, however, be extended to include the  
33 same comment about DNA repair enzymes being immature, and the presence of more DNA  
34 synthesis due to growth, and thus a further increased risk if exposure occurs during  
35 development. The EPA also needs to recognize that if these metabolism factors increase  
36 cancer risk of EtO in children, they must also decrease risk in adults. Thus, the ability to  
37 detoxify EtO and repair damaged DNA implies a threshold for carcinogenicity - something  
38 that is rejected in the risk assessment assumptions. It is inconsistent to conclude that these  
39 enzyme systems have important effects that affect risk and not also conclude there is a  
40 threshold.  
41

42 Developmental stage effects have been shown for vinyl chloride-- the mechanism  
43 associated with p450 2E1 activity in young rats. That mechanism is not involved with EtO.  
44 Increased replication associated with growth is also not likely to drive such differences at  
45 environmental exposures, as shown by the fact that Drosophila shows a threshold for

1 mutagenesis under conditions where cell replication is ~100 times faster than in the human  
2 embryo. A panel member pointed out that the age-dependent adjustment factors are  
3 inconsistent with observed population cancer incidence. Cancer would be prevalent by even  
4 younger ages if children were uniquely susceptible. The blood forming system, when  
5 measured in terms of cell turnover, is approximately 20% more active in children than adults.  
6 An adjustment of this magnitude might be more reasonable and biologically consistent than  
7 the factors used in the EtO risk assessment.

8  
9 *[EDITOR Note: Address error in computation of age adjusted exposure].*

## 10 11 12 **2.d Low-dose Extrapolation**

13  
14 **Is the use of different models for estimation of potential carcinogenic risk to humans**  
15 **from the higher exposure levels more typical of occupational exposures (versus the**  
16 **lower exposure levels typical of environmental exposures) appropriate and**  
17 **transparently described in Section 4.5?**

18  
19 The Panel felt that the description of the EPA's methodology for estimating the point  
20 of departure (POD) and extrapolating from the POD to the baseline using a linear dose  
21 response assumption was transparently described. As covered in previous responses, there  
22 were differences of opinion regarding both: 1) the form of the model to be used in estimating  
23 the POD; and 2) the model for low-dose extrapolation below the established POD.

24  
25 The case for simply removing the high dose point from the assessment of low dose  
26 risks should be more fully discussed. If possible, the biases producing downward departures  
27 from linear expected risks at the higher doses should be modeled and their likely influence  
28 removed, rather than exclude the high dose point. Alternative analyses should be shown for  
29 the consequences of various analytical interpretations of the convexity in the overall  
30 relationships. Simply excluding the high dose point because it doesn't fit a line determined  
31 from the lower dose points, as is traditionally done for animal data, should be a last resort.

## 32 33 **2.e. Extrapolation from animal studies**

34  
35 **Are the methodologies used to estimate the carcinogenic risk based on rodent data**  
36 **appropriate and transparently described? Is the use of "ppm equivalence" adequate**  
37 **for interspecies scaling of EtO exposures from the rodent data to humans?**

38  
39 The ppm equivalence method is a reasonable method for interspecies scaling of EtO  
40 exposures from rodent data to humans. If the use of animal data becomes more important  
41 (i.e., the principal basis for the ethylene oxide unit risk value), more sophisticated approaches  
42 such as PBPK modeling should be considered. The PBPK models that Filser's group (cite)  
43 and Fennell and Brown (cite) have developed are appropriate. One panel member conducted  
44 a PBPK model some time ago and found that it gave very similar results to the ppm

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1 equivalence approach, although this should be revisited [in the event that animal data assume  
2 a greater role in the ethylene oxide unit risk].  
3

4 All of the animal cancer data need to be presented as survival adjusted data.  
5

6 *[EDITOR NOTE: Address error in computation of UCL (upper confidence limit) for rodent*  
7 *data? ]*  
8  
9

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30  
31  
32  
33  
34  
35

**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

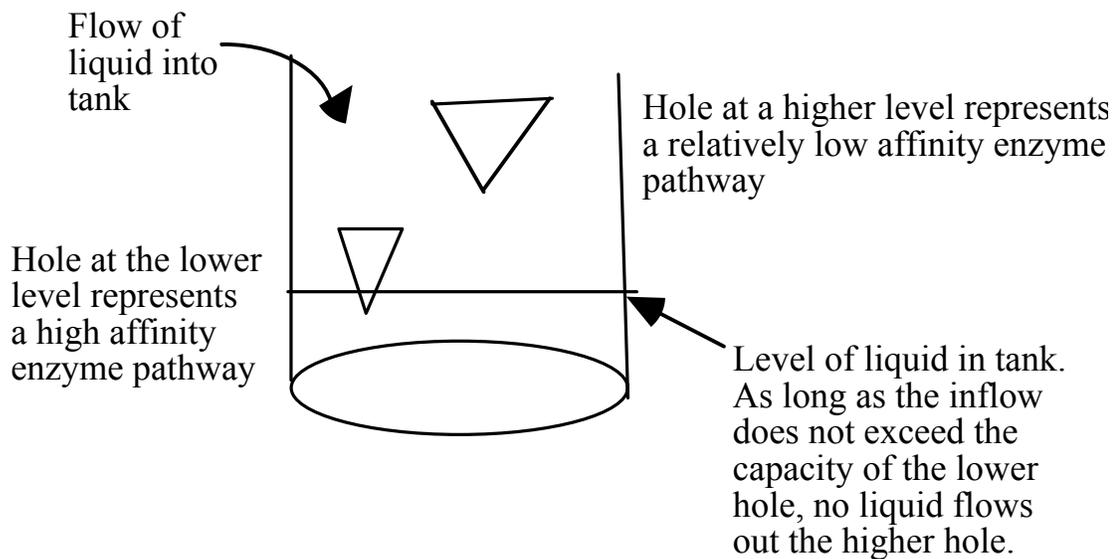
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1 (representing a continuous dosage of a toxicant) flows into a tank with two triangular holes.  
 2 The level of liquid rises in the tank until some begins to flow out of the lower of the two  
 3 holes (representing a high-affinity metabolic pathway producing a “safe” metabolite). A  
 4 further rise occurs until the amount of liquid flowing out of the tank equals the amount  
 5 flowing in. If the inflow is small enough that it can be completely balanced by flow out of  
 6 the lower hole, then the liquid will not rise to the level of the higher hole (representing the  
 7 lower affinity enzyme producing the dangerous metabolite). Thus the analogy predicts a  
 8 threshold of inflow into the tank, below which all of the metabolism is via the “safe” high  
 9 affinity pathway.

10 Unfortunately, this representation of the competition between higher and lower  
 11 affinity metabolic pathways is not compatible with conventional Michaelis-Menten enzyme  
 12 kinetics (Hattis, 1990; Slikker et al. 2004). Using the basic Michaelis/Menten equation, the  
 13 rate of the activating reaction (producing the dangerous metabolite, D) is:  
 14  
 15

**Figure 1**

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



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

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 Km'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

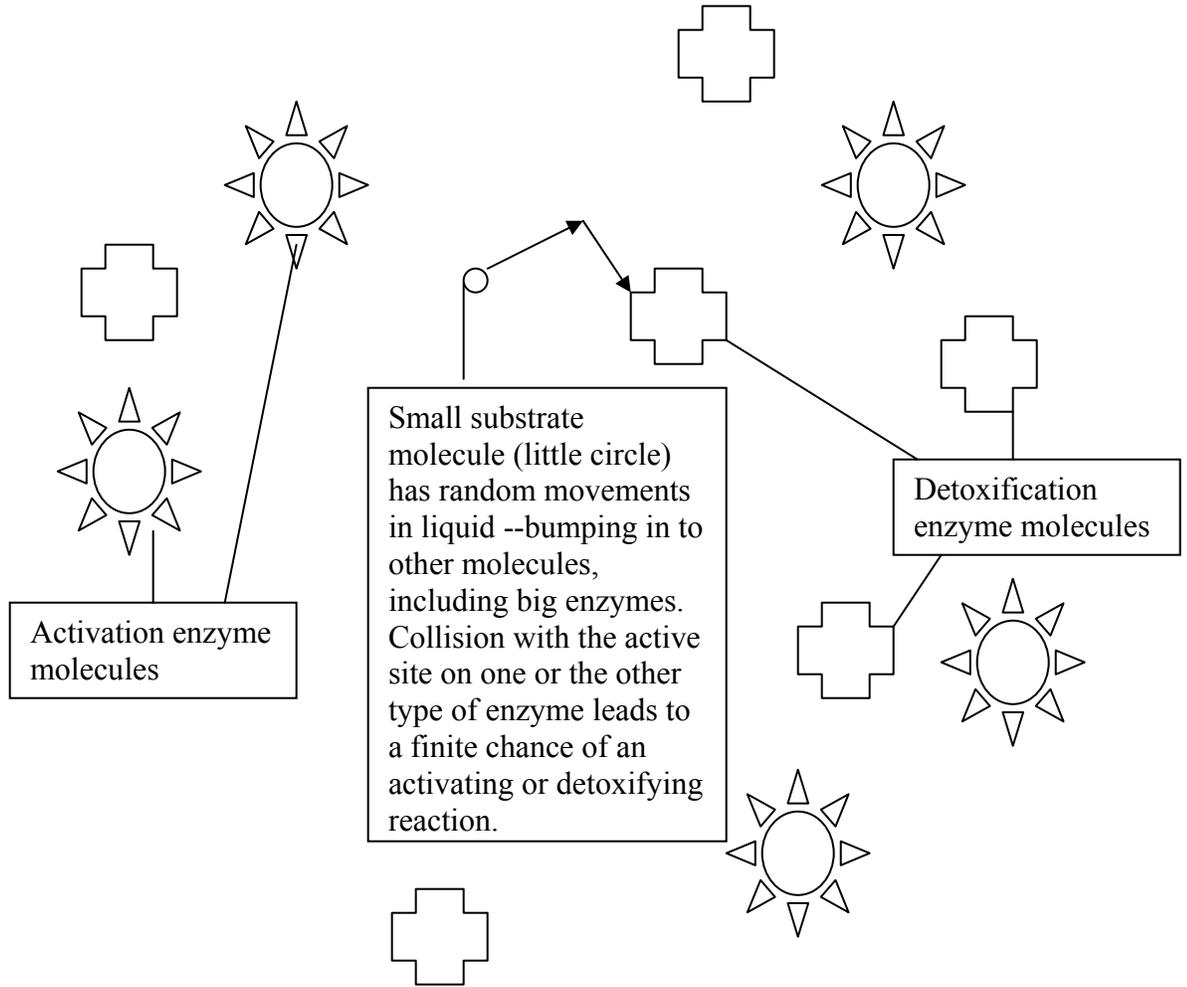
1 for some “albeit negligible” carcinogenic risk from genetically acting chemicals at low  
2 doses.

3 It is well to emphasize that the basic Michaelis-Menten equation applied above is not  
4 simply an empirical formula. It is well grounded in fundamental mechanistic considerations  
5 of receptor association and dissociation kinetics with reasonably wide applicability (Hoel,  
6 1985). The maximal velocity,  $V_{max}$ , arises because there are a limited number of enzyme  
7 molecules available to catalyze the reaction, and each enzyme molecule is necessarily  
8 constrained to operate at a finite rate in converting its substrate into its product. The fact that  
9 the reaction proceeds linearly at low doses (with a rate constant of  $V_{max}/K_m$ ) arises from  
10 the fact that the reaction is limited by the rate of diffusion of the substrate molecules into the  
11 active site of the enzyme—a rate that must be linear with substrate concentration at the limit  
12 of low doses. In the light of this Figure 2 offers a more accurate molecular-scale vision of the  
13 competition between enzyme-mediated activating and detoxifying processes. Each small  
14 substrate molecule has a “random walk” through a cellular compartment as it rebounds from  
15 collisions with other molecules. At the limit of low dosage, when there are few or no other  
16 similar substrate molecules around, the substrate molecule must have a finite chance of  
17 encountering the active site of each type of enzyme (or, similarly, a transport molecule taking  
18 it to a different compartment). Therefore each type of enzyme or macromolecular transporter  
19 must have finite opportunity to process the substrate molecule at the limit of low dosage.

20 The basic Michaelis-Menten equation form applies with equal force to active  
21 transport processes (in which specialized molecules utilize energy to pump specific  
22 molecules or ions into our out of cells), and to DNA repair processes. Thus the fundamental  
23 expectation for low dose linearity applies similarly to these other components of the causal  
24 chain between external exposure and the generation of somatic mutations that are  
25 components of carcinogenesis. At the limit of low dose the Michaelis Menten  
26 enzyme/transport reaction rates are limited by the rate of diffusion of substrate molecules  
27 into the active sites of the enzymes/transport molecules; and those diffusion processes, given  
28 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



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1 With this as background, we can now examine the bases for some more recent claims  
2 that thresholds should be expected at low doses for genetically acting agents. A convenient  
3 starting point for this examination is a special issue of Mutation Research published in 2000  
4 by participants at a conference on threshold mechanisms of carcinogenesis sponsored by the  
5 European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Without  
6 going through the threshold claims from each of the papers in this collection individually  
7 (Kirshch-Volders et al. 2000; Schulte-Hermann et al. 2000; Muller and Kasper 2000;  
8 Moustacchi 2000; Parry et al. 2000; Swenberg et al. 2000; Lowell 2000; Madle et al. 2000;  
9 Henderson et al. 2000; Crebelli 2000; Kirkland and Muller 2000; Speit et al. 2000; Parry  
10 2000), three main types of arguments stand out:

- 11 • Multiple targets. Some specific modes of genotoxicity are reported to depend  
12 on multiple interactions between chemicals and target macromolecules (rather  
13 than the single-DNA-reactant-molecule DNA adduct formation mechanism  
14 discussed above). If the number of target interactions required to produce an  
15 effect is large, the resulting low dose dose-response relationship can be  
16 expected to be highly upward-turning, and well approximated by a threshold.
- 17 • Multiple barriers. A molecule of a chemically reactive agent must pass  
18 multiple transport, potential detoxification, alternative targets for reaction  
19 other than DNA, and DNA repair hurdles in order to cause a permanent  
20 change in DNA sequence or chromosomal damage. The multiplicity of these  
21 hurdles makes it unlikely that any single molecule could cause an actual  
22 mutation along the pathway to carcinogenesis. If these multiple barriers do  
23 not produce an “absolute threshold” they can at least be expected to lead to a  
24 “pragmatic” or “practical” threshold below which exposure is of no real  
25 biological consequence.
- 26 • Inducible detoxification, apoptosis, and/or DNA repair processes. One result  
27 of exposure to a toxicant may be the induction of the levels of a variety of  
28 cellular and genomic defense processes. If this induction is effective enough,  
29 and occurs at low enough doses, it is possible that the prevention “good” that  
30 results from avoidance or repair of mutagenic damage from background  
31 processes may even be great enough to exceed the direct mutagenic harm  
32 done by the toxicant itself. This gives rise to “hormetic” dose response  
33 relationships in which the net mutagenesis and carcinogenesis is even reduced  
34 by some range of exposures to the toxicant compared to background (zero  
35 dose) levels.

### 36 *Modes of Genetic Action Requiring Multiple Interactions with Macromolecular* 37 *Target Molecules or Structures*

38 The first paper in the Mutation Research special issue (Kirsch-Volders et al. 2000)  
39 gives a reasonable theoretical mathematical account of the dependence of the shape of the

1 dose response curve on the number of macromolecular targets that must be “hit” in order to  
 2 produce an effect. Essentially, if a single hit on DNA, an alpha or beta tubulin structure, or  
 3 topoisomerase is sufficient to cause an effect (assuming imperfect repair) then the  
 4 fundamental math calls for a single hit Poisson process:

$$5 \quad \text{Probability of Effect/Target} = 1 - e^{-m} \quad (1)$$

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

$$11 \quad \text{Probability of Effect/Target} = 1 - e^{-m} \frac{m^n}{n!} \quad (2)$$

12 (where the notation  $n!$ , translated to English as “ $n$  factorial” means  $n \times n-1 \times \dots$  All the way  
 13 down to 1.)

14 The larger the  $n$ , the more steeply upward-turning the resulting curve will be—increasingly  
 15 resembling, but not the same as a curve with a true threshold of zero probability of effect for  
 16 a finite dose.  
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20 Later on, Kirsch-Volders et al (2000). add:

21 “To be able to clearly assess a threshold, the spontaneous frequency of the analysed  
 22 endpoint should be very low, ideally equal to zero; indeed a too high spontaneous  
 23 background will lead to additive effects and a difficult estimation of small increases  
 24 at low dose level.”  
 25

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

34 The essential low-dose linearity of agents that act in concert with background  
 35 processes was discussed in some of the foundational papers that derived the methods for  
 36 inferring low dose cancer risks in the 1970s (e.g. Crump et al. 1976). This general  
 37 expectation can be illustrated with a simple example of a two-stage mutation process in  
 38 which there is a background of 1 arbitrary unit and an expectation for 1 additional induced

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1 unit per 1 mg/kg continuous dose of a mutagenic agent (Table 2, adapted from Hattis and  
2 Smith, 1986). It can be seen that at high doses, the dose response relationship between  
3 excess tumors over background vs dose of the inducing agent appears almost perfectly  
4 proportional to (dose)<sup>2</sup>. This is because at high doses, far above the background rate of the  
5 tumors, the agent predominantly acts by causing both mutations in the two-step process. As  
6 the dose is reduced to regions where it causes mutations that are a small fraction of  
7 background rates, the induced mutations predominantly cooperate with mutations that result  
8 from the background processes—leading to an increment in tumors over background that  
9 approaches linearity with dose of the added inducing mutagen.

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

16

1 **Table 2**  
 2 **Effect of Background Mutation Rates on the Carcinogenesis Dose-Response Curve at**  
 3 **Low Doses, Assuming a Hypothetical Two-Stage Carcinogenic Process**

Dose	Rate of 1 <sup>st</sup> Transition (1 extra unit per unit of dose)	Rate of 2 <sup>nd</sup> 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

4 Source: Adapted from Hattis and Smith, 1986.

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

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\* 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).

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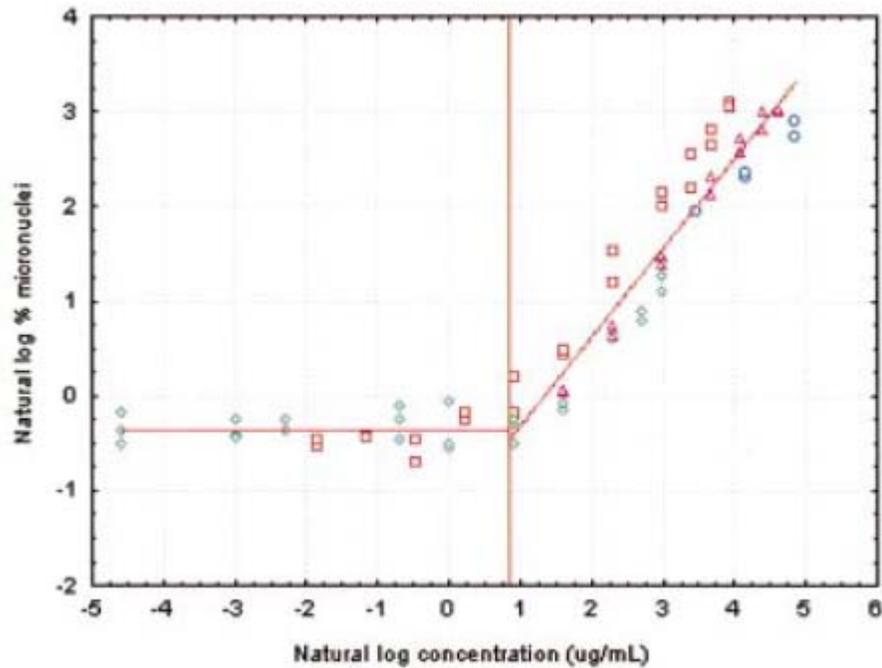
Figure 3

2

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\*

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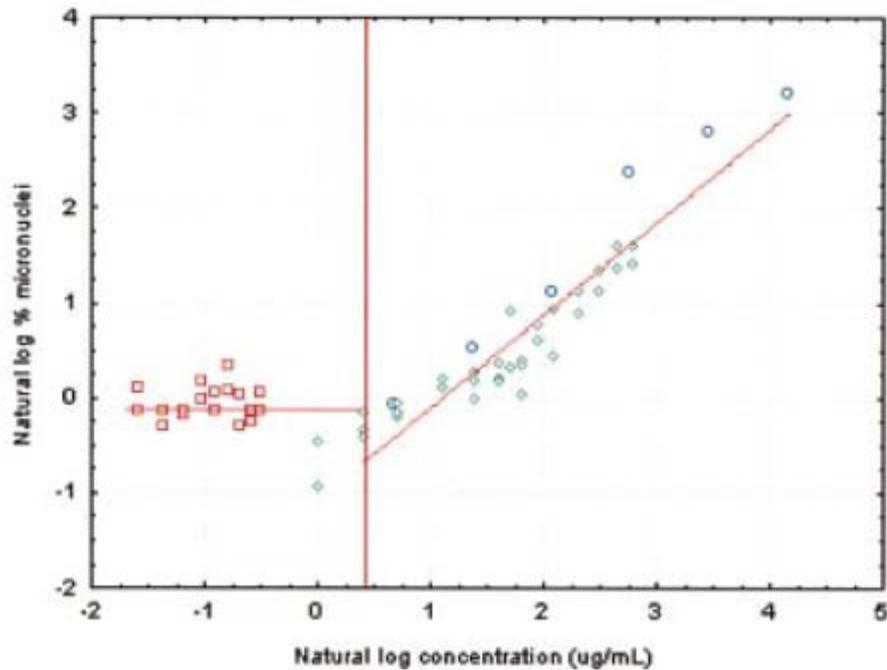
\* Source: Lynch et al. 2003. The different colored points represent different assays. The caption to this graph is “Broken stick model. The breakpoint was identified at  $\ln(\text{conc.}) = -6.049$  or  $0.00236 \mu\text{g/ml}$  on the original concentration scale with  $\ln(\%MN) = -0.364$  fitted before the breakpoint and  $\ln(\%MN) = -5.234 + .94 \times \ln(\text{conc})$  fitted afterwards. The goodness of fit ( $R^2$ ) was 93.4%.”

1

Figure 4

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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\*

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\* 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  $\ln(\text{conc.}) = -6.495$  or  $0.00151 \mu\text{g/ml}$  on the original concentration scale with  $\ln(\%MN) = -0.135$  before the breakpoint and  $\ln(\%MN) = -5.674 + 0.979 \times \ln(\text{conc})$  fitted afterwards. The goodness of fit ( $R^2$ ) was 89.2%.”

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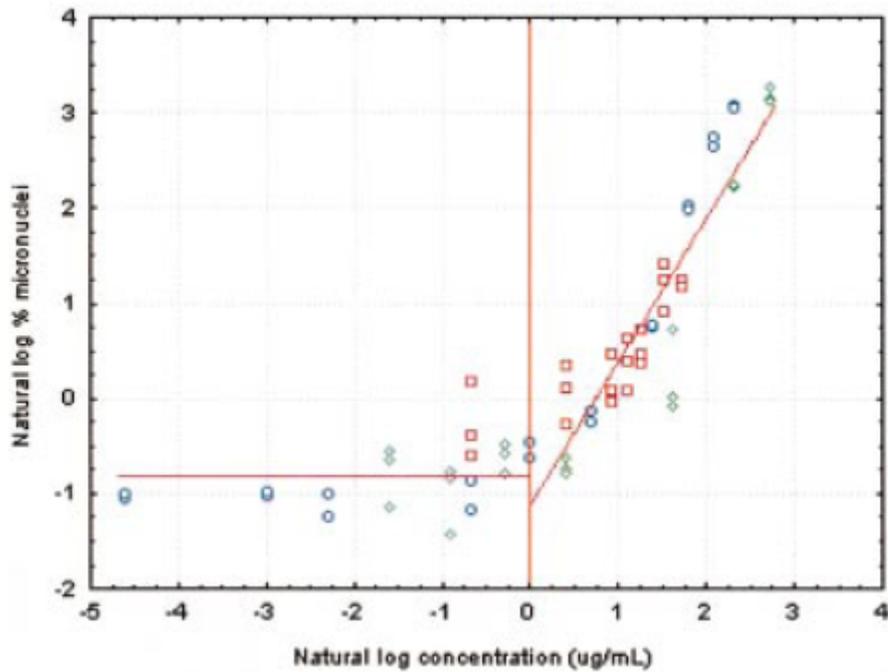
Figure 5

2

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\*

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\* 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  $\ln(\text{conc.}) = 0$  or 1  $\mu\text{g}/\text{ml}$  on the original concentration scale with  $\ln(\% \text{MN}) = -0.817$  before the breakpoint and  $\ln(\% \text{MN}) = -1.117 + 1.508 \times \ln(\text{conc})$  fitted afterwards. The goodness of fit ( $R^2$ ) was 88%.”

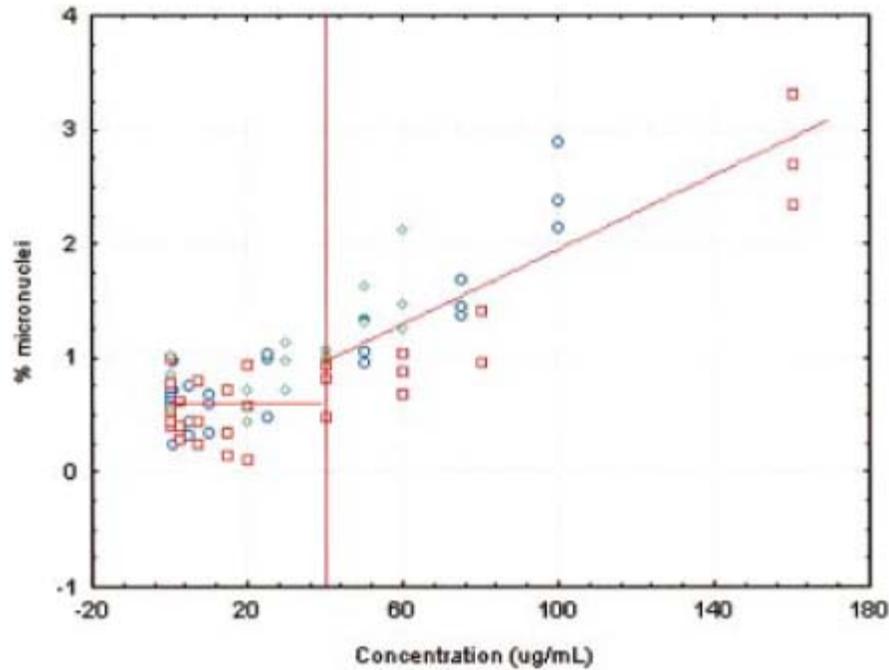
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Figure 6

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



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\* 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  $\mu\text{g/ml}$  with % micronuclei = 0.636 fitted before the breakpoint and % MN =  $0.392 + 0.016 \times \text{conc.}$  Fitted after the breakpoint. The goodness of fit ( $R^2$ ) was 77.1%.”

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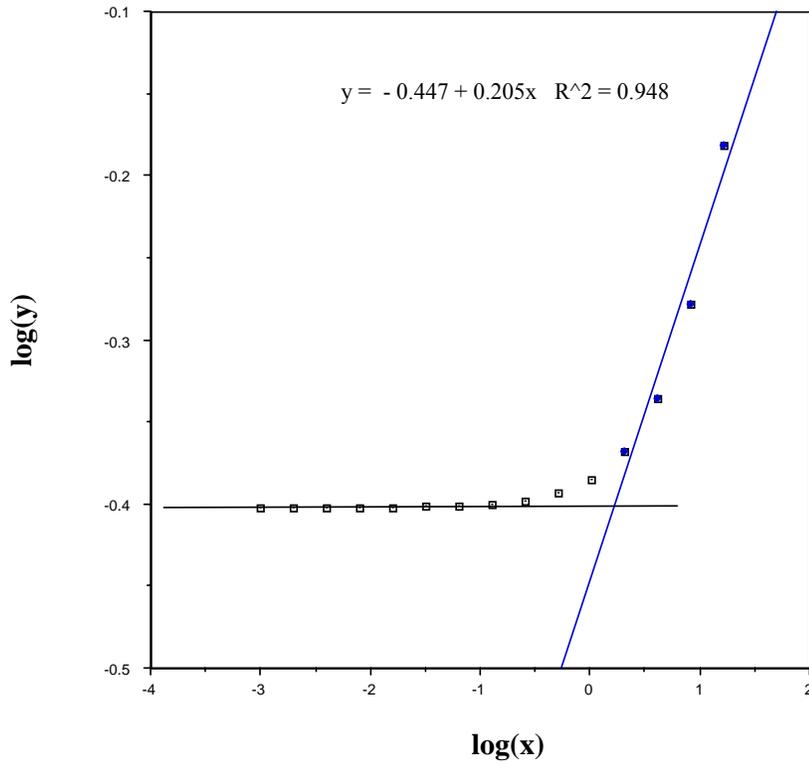
2           The log log plots of Figures 3-5 are an example of how disciplinary perspectives can  
3 shape the presentation and interpretation of evidence. From a toxicological perspective,  
4 there are two realms of concern – the realm of homeostasis and the realm in which  
5 homeostasis has broken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

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

1

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



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1 *Arguments for Absolute Thresholds or “Practical Thresholds” from the Presence of*  
2 *Multiple Transport, Detoxification, and Repair Processes*

3  
4 The “multiple barriers” argument appears to be mostly a rhetorical device since its  
5 mathematical implications reinforce linearity. By reciting the series of opportunities that a  
6 molecule of a DNA-reactive agent has to go astray rather than react with DNA and then have  
7 the adduct cause a mutation in a gene that matters in a cell that matters for carcinogenesis, a  
8 speaker can make it appear very unlikely that such a chain of events could occur (Parry et al.  
9 2000). And indeed, from the standpoint of a single molecule, the probability is necessarily  
10 very small. However each physical barrier has some probability of being surmounted by  
11 each molecule; each alternate reaction opportunity or detoxification enzyme will divert a  
12 finite fraction of the molecules, each DNA repair process will repair the molecules/adducts at  
13 a finite rate and therefore a finite fraction of the DNA lesions will persist to the time of the  
14 next passage of the polymerase enzyme responsible for copying the DNA. Similarly not all  
15 cells with damaged DNA will be removed by apoptosis, and cell cycle checkpoint functions  
16 will not perfectly prevent the copying of damaged DNA. If there is a finite rate of DNA  
17 lesion generation and a finite rate of DNA repair or removal by apoptosis, then there must be  
18 a finite rate of mutation that, at the limit of low dosage where saturation effects are  
19 negligible, must be a linear function of the number of DNA reactive molecules (or their  
20 precursors) that enter the system. Moreover once an initial tumor cell is generated there must  
21 be a finite probability that it will escape repression by its normal neighbors through gap  
22 junction communication and by other immune-based defense processes. The presumption of  
23 some of the discussion in the Mutation Research special issue (Herman et al. 2000) seems to  
24 be that at low doses some or many of these processes can be assumed to be perfect; but this  
25 is just not possible. The dose response relationship for the combined process is a simple  
26 multiplicative combination of the component processes. If all of these are linear at low  
27 doses, then the combined dose response relationship must also be linear at low doses.

28 A final refuge of this set of arguments is to distinguish between an “absolute”  
29 threshold (a true zero response at a finite dose rate)\* and a “pragmatic” or “practical”  
30 threshold. Lowell (2000) argues:

31 “A ‘pragmatic’ threshold can be considered as a concentration below which any  
32 effect is considered biologically unimportant (Figure 2)\*\* (Lutz, 1998). This term is  
33 used in a somewhat similar way to how ECETOC defines a biological threshold  
34 except that it implies that there may be effects occurring because of treatment or  
35 exposure but these are considered below what might be considered biologically

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\* 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”.

1 important. An example might be increases that did not exceed the range of responses  
2 seen in negative control material in a well-conducted series of experiments. Such a  
3 threshold may be defined, in part, with the help of statistical tests. The distinction  
4 between the various classifications of thresholds can initiate a philosophical  
5 discussion but is not relevant to regulatory risk assessment.”

6 It appears, therefore, that this line of argument reverts to treating an effect that may  
7 be present but which cannot be clearly demonstrated as above “the range of responses seen in  
8 negative control material” (with some undefined sample size and sensitivity/detection noise)  
9 as if it weren’t there. It seems to us that risk assessment methods have been created precisely  
10 to make fair assessments of the likely magnitude of effects that cannot be directly measured  
11 but are still potentially substantial enough that decision-makers and the public may  
12 reasonably care about reducing them. Or, put another way, “practical” for a biologist to  
13 detect, may not mean the same as of “practical” concern because someone might get hurt.  
14 Our view is that terms such as “practical threshold” are inherently evasive and do a  
15 disservice to transparent public analysis and discussion of likely underlying realities.  
16

### 17 ***Arguments for Thresholds or Hormetic Dose Response Relationships from Possible*** 18 ***Inducible Detoxification or DNA Repair Processes***

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

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

- 38 • “Background damage” (e.g. from the DNA damaging free radicals produced  
39 as a byproduct of metabolism, other endogenous DNA reactive agents such as  
40 ethylene oxide and possibly formaldehyde, and other exogenous DNA reactive

1 agents) must occur at sufficient rates that offsetting prevention benefits could  
2 occur,

- 3 • The usual “baseline” state of expression of the detoxification, DNA repair,  
4 apoptosis, or cell cycle check point mediators needs to be sub-optimal. Normally,  
5 one would expect that if it were net beneficial to have higher standing levels of a  
6 particular enzyme, then that would have been of selective advantage during  
7 evolution. Consequently people’s normal constitutive should have been adjusted  
8 to at least approximate optimality by natural selection. However, the types and  
9 levels of present-day exposures to mutagenic agents could conceivably be  
10 different enough from those present during the recent evolution of modern  
11 humans that prevailing constitutive levels of defensive enzymes are not perfectly  
12 tuned to current exposures. (For example cancer rates in wealthier industrialized  
13 countries tend to be very substantially higher than in poorer, less-developed  
14 countries. (Harris et al.1985)) In evaluating such possibilities, it is important to  
15 bear in mind that both detoxification enzymes and DNA repair enzymes can have  
16 adverse biological side-effects themselves. For example the same P450  
17 “detoxification” enzyme that is induced by ethyl alcohol (Daiker et al. 2000;  
18 Feierman et al. 2003; Sato 1993) also is involved in the transformation of vinyl  
19 chloride to the activated form that reacts with DNA to induce the characteristic  
20 liver cancers produced by that compound. Epidemiologic data now exist  
21 indicating that high consumers of alcohol are much more susceptible to the  
22 carcinogenic effects of vinyl chloride (Mastrangelo et. al 2004). P450s also affect  
23 estrogen metabolism including some to genetically active agents. DNA excision  
24 repair enzymes, which repair DNA by cutting out small sections of DNA that has  
25 been damaged, also do damage themselves by making cuts at some finite rate in  
26 sections of DNA that do not contain pre-existing damage (Branum et al. 2001).  
27 Thus it is likely to be beneficial, biologically, to induce these enzymes above their  
28 baseline levels only when there is sufficient damage in a particular cell that the  
29 biological “costs” of the excision repair enzyme itself are outweighed by the need  
30 to repair an unusual amount of damage by a relatively rare exposure episode.

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

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**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”

---

\* 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.

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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.

**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

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

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

1 exposure values). Where CV is the coefficient of variation, an estimate of the geometric  
 2 standard deviation is:  
 3

$$4 \quad \text{Geometric Standard Deviation} = e^{\{\ln[CV^2 + 1]\}^{0.5}}$$

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

10 In fact, however, there is reason to expect that the actual uncertainty may be larger  
 11 than this. All of the exposure measurements used to derive and test the model were from the  
 12 mid 1970s and later, whereas some of the exposures that were estimated appear to go back to  
 13 the first regular use of ethylene oxide for sterilization in 1938 (Stayner et al. 1973). In the  
 14 use of their exposure model, Hornung et al. assumed that exposures prior to 1978 were equal  
 15 to the values that would apply to 1978. This creates some additional uncertainty in the  
 16 exposure analysis that is not captured in the analysis of precision of exposure estimates from  
 17 the 1970s and 1980s. Therefore the analysis below will include a hypothetical case assuming  
 18 a much larger random error (3 times the estimated variance, corresponding to a geometric  
 19 standard deviation of about 3.2) than that directly derived from the estimated imprecision in  
 20 1970s-1980s exposures.

21  
 22 • **Estimating the Cumulative Exposure Distribution for the Reference Group (Non-  
 23 Lymphoid Cancer Cases)**

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

31  
 32 **Table 2**  
 33 **Inputs for the Estimation of the Distribution of Exposures in**  
 34 **Referent Workers (Non-Lymphohematopoietic Cancer Cases)**

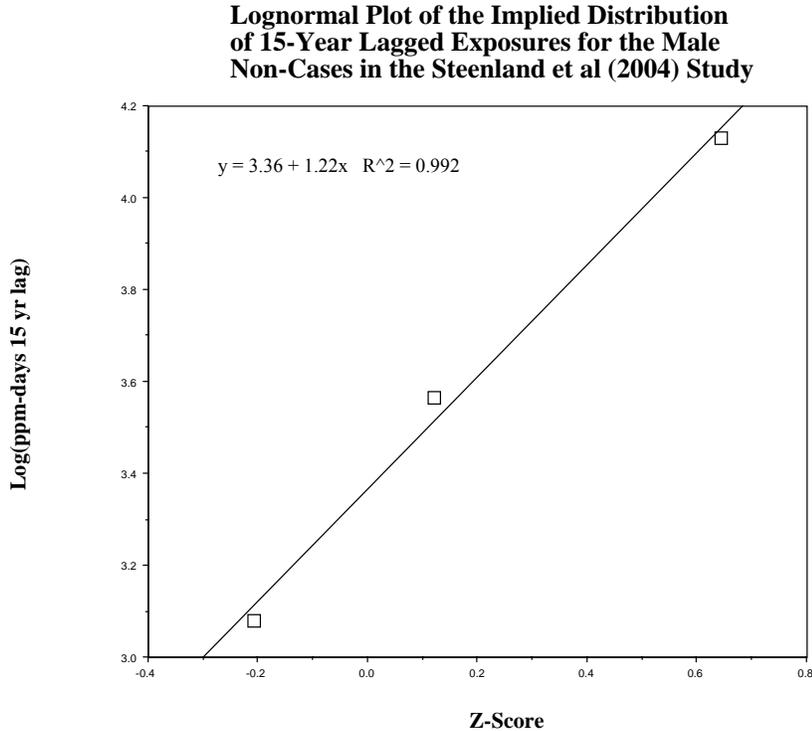
Exposure Group	median exp (ppm-days)	mean exp (ppm-days)	RR
0 (lagged out)	0	0	1
<1200 ppm-days	360	442	1.23
1200-3679	2093	2191	2.52

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3680-13499	6230	7105	3.13
≥13500	43212	60269	3.42

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**Figure 1**  
**Initial Lognormal Probability Plot of the Exposure Distribution for Referent Workers**  
**Based on the Estimated Numbers within Each Group**



6  
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8

9 A more extensive optimization analysis using the additional information on the  
10 within-group means and medians for the exposure groups from Table 2 led to an adjustment  
11 of the basic parameters for the lognormal distribution (geometric mean = 2910 ppm and  
12 geometric standard deviation = 9.86) and a conclusion that the exposure estimates were  
13 likely to be truncated at about 337,500 ppm-days (corresponding to 45 years of 250 day/year  
14 8 hr/day occupational exposure at an average of 30 ppm, which would otherwise correspond  
15 to about the 98<sup>th</sup> percentile of a full lognormal distribution). This truncation point was  
16 chosen to avoid having key model parameters such as the mean of the highest exposure  
17 group being importantly dependent on cumulative exposures that are not likely to be present  
18 in the actual data. The resulting fit to the referent within-group mean and median cumulative  
19 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**

Exposure Group (ppm-days)	Observations		Lognormal Model "Predictions" <sup>a</sup>		Ratio: Model "Predictions"/Observations	
	median exp (ppm-days)	mean exp (ppm-days)	median exp (ppm-days)	mean exp (ppm-days)	median exp (ppm-days)	mean exp (ppm-days)
0 (lagged out)	0	0				
<1200	360	442	342	426	0.949	0.964
1200-3679	2093	2191	2121	2226	1.013	1.016
3680-13499	6230	7105	6805	7376	1.092	1.038
≥13500 <sup>a</sup>	43212	60269	36277 <sup>a</sup>	62371 <sup>a</sup>	0.840 <sup>a</sup>	1.035 <sup>a</sup>

<sup>a</sup>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:

$$\begin{aligned} \text{Real lognormal variance [the square of the real log(geometric standard deviation)]} &= \\ \text{Observed lognormal variance} - \text{lognormal variance from measurement/estimation error} &= \\ = [\log(9.86)]^2 - [\log(1.95)]^2 &= 0.903 \end{aligned}$$

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.

1 Similar variance subtraction techniques have been used previously to quantify likely  
2 underlying real variability in a wide variety of parameters including survival and fecundity  
3 rates in ecological analyses and coal miner breathing rates (Akcakaya 2002; Hattis and Silver  
4 1994). For the more general case where the distributional forms of the real variation and  
5 measurement variation one needs “errors in variables” regression models (Stayner et al.  
6 2003; Richardson et al. 2004; Brown et al. 2004; Choi, 2000; Carrothers and Evans, 2000;  
7 Kulathinal et al. 2002; Siebert et al. 2001 or more complex “deconvolution” procedures that  
8 are not yet in widespread use.

9  
10 • **Derivation of Adjusted Estimates of the Real Distribution of Cumulative Exposures**  
11 **in Each of the Four Categorical Dose Groups**

12 Using the adjusted estimates of the geometric standard deviation and the same  
13 geometric mean and upper percentile truncation point derived earlier, it is straightforward to  
14 calculate a large number of percentiles of the indicated lognormal distributions and the mean  
15 values for exposures in each of the worker groups. The results in Table 3 are derived from  
16 dividing the original and error-variance corrected lognormal distributions into 3700 equal  
17 parts corresponding to the number of referent workers at a 100:1 ratio to cases (the exact  
18 number is not critical).

19 **Table 3**  
20 **Changes in Estimates of Mean Cumulative Exposures in Previously Defined Groups**  
21 **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

22  
23 It can be seen in Table 3 that the narrowing of the distributions by subtraction of  
24 estimation error causes a reversion toward the mid point of the exposure distribution. The  
25 estimates of the mean cumulative exposures for the lower two groups are raised; and the  
26 estimated means for the higher two groups are lowered, with the greatest effects seen on the  
27 group with the largest exposures.

28 In addition to the regression to the mean effect shown in Table 3, there is an  
29 additional effect that results from what is classically known as “classification error”. The  
30 error in estimating individual workers’ exposures causes some estimated exposures to be  
31 ‘scrambled’—that is, misclassified from their real exposure ranges to adjacent ranges. To  
32 model this, ten replicate Monte Carlo simulations were done of 3700 trials each for both  
33 assumed error level. On each trial, a random draw was made from the estimated underlying

1 lognormal distribution of real exposures (after subtracting the variance attributed to  
 2 measurement error) and then a random perturbation was added back corresponding to the  
 3 lognormal distribution of measurement errors. The final two columns of Table 4 show the  
 4 effects of this on the true mean exposures within each group for workers classified into  
 5 exposure groups using their “observed”/estimated exposure levels. The calculation also  
 6 took into account the censoring of both the “real” and estimated exposures at 337,500 ppm-  
 7 days. Comparing Table 4 with Table 3, it can be seen that that undoing the effects of this  
 8 scrambling misclassification leads to a much larger set of changes in the estimated real  
 9 exposures within each of the groups of workers classified by observed exposures.

10 **Table 4**  
 11 **Changes in Estimates of Mean Cumulative Exposures (ppm-days) in Previously**  
 12 **Defined Groups after Including the Scrambling of Individual Exposures among**  
 13 **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

14  
 15 These shifts have predictable effects on the estimates of the linear cancer potencies  
 16 (Tables 5-6), using the same estimation methods (based on the same spreadsheet formulas) as  
 17 were used by the EPA analysts. In the case of the 3 point calculation (Table 5), the overall  
 18 effects are modest—slope factor estimates even decrease somewhat in the calculations with  
 19 the scramble effect because of the increased estimated real mean exposure for workers with  
 20 in the observed 3,680—13,499 ppm-day group. The full implications of the different  
 21 amounts of estimation error are more apparent for the full analysis of all four points (Table  
 22 6). It can be seen that in this case the effect of the GSD 1.95 estimate of measurement error  
 23 is to increase the slope factor by about 25%; whereas the effect of the larger GSD 3.2  
 24 assumption for estimation error is to slightly more than double the estimate of low dose risk.  
 25 In the latter case, the ratio of the low-dose risks projected using the 3- vs the 4-point analysis  
 26 is reduced to about 3-fold, compared with about 7.5-fold using the group means of the  
 27 original observations. Thus, errors-in-variables distortion of the high dose point in particular  
 28 seems to be a reasonable candidate explanation for some, but not all, of the convex  
 29 nonlinearity seen in the data.

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**Table 5**  
**Changes in Estimates of the Linear Slope Coefficient, ED10, and LED 10 for Dose Response Analyses Based on the Lowest 3 Exposure Groups**

Risk Parameter	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

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**Table 6**  
**Changes in Estimates of the Linear Slope Coefficient, ED10, and LED 10 for Dose Response Analyses Based on All 4 Exposure Groups**

Risk Parameter	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

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## Appendix C

### Framework Analysis of Genotoxicity and Risk Assessment James Swenberg, PhD

Slide 1

**Framework Analysis of Genotoxicity  
and Risk Assessment**

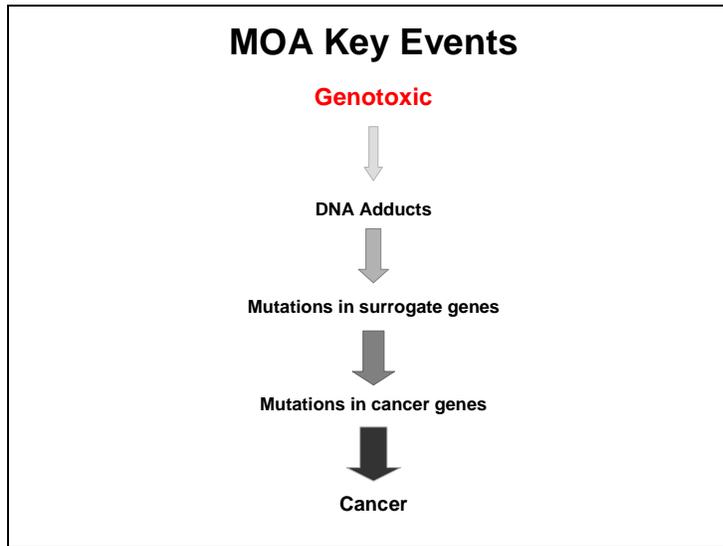
**James Swenberg  
University of North Carolina**

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

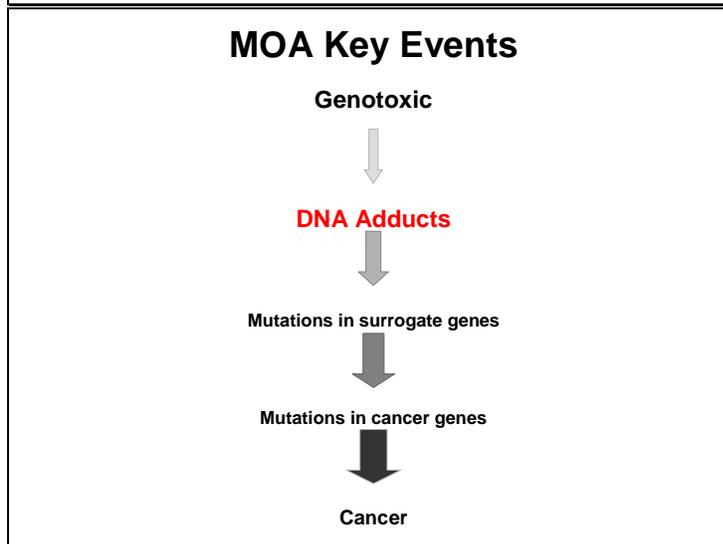


Slide 4

**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



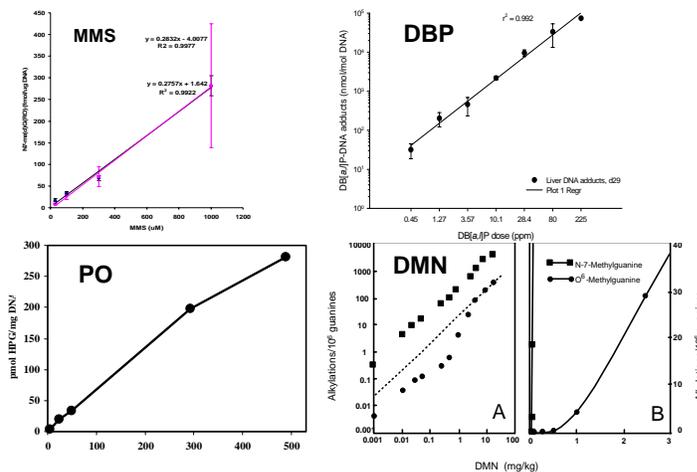
Slide 6

## 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.

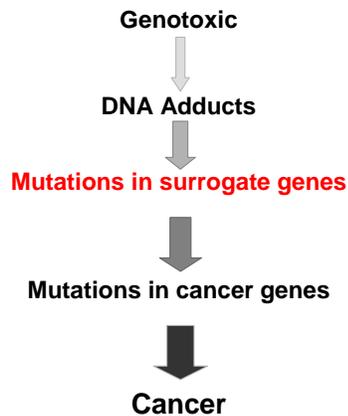
Slide 7

## Linear DNA Adducts at Low Doses



Slide 8

## MOA Key Events



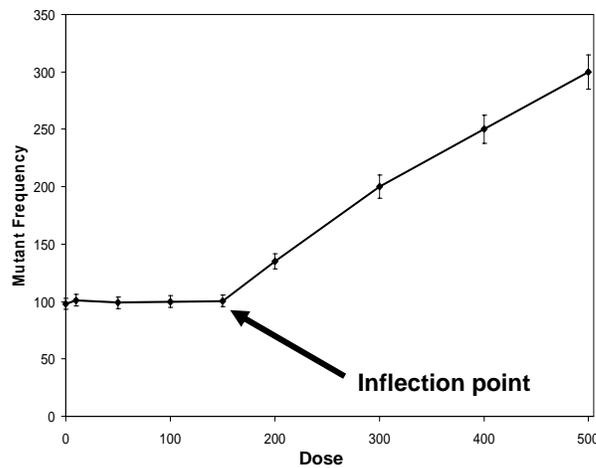
Slide 9

### 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.

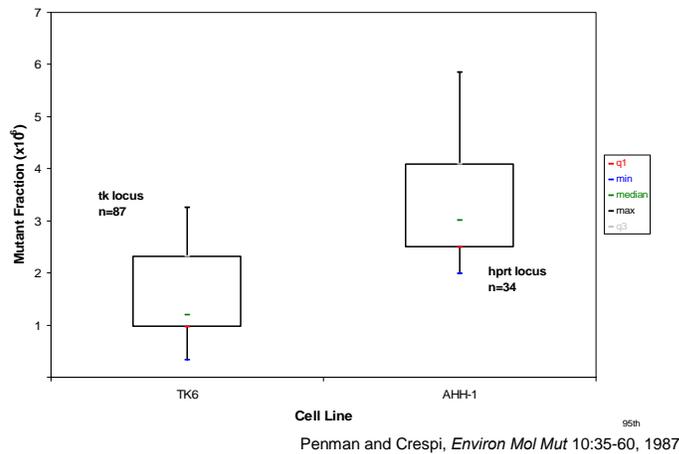
Slide 10

### Typical Mutation Dose Response

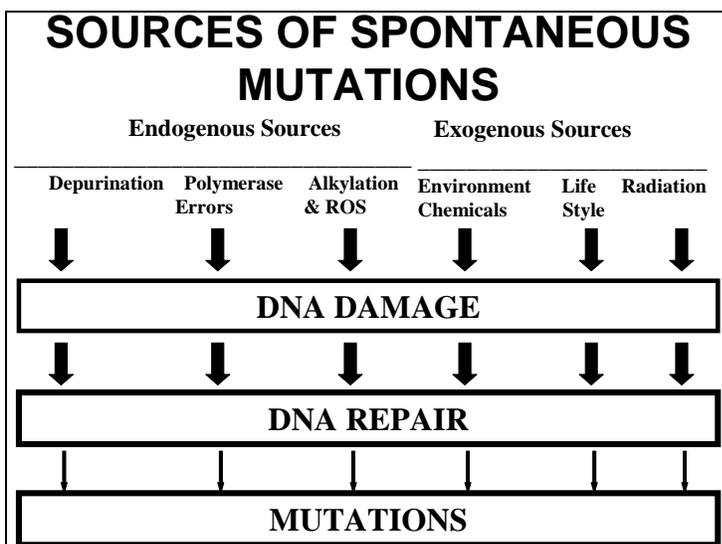


Slide 11

### Historical Control Data for *HPRT* and TK Mutations *in vitro*

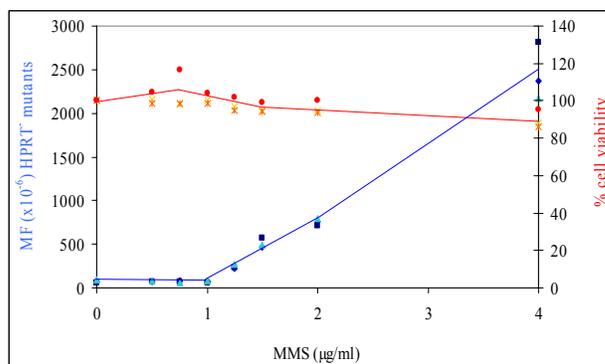


Slide 12



Slide 13

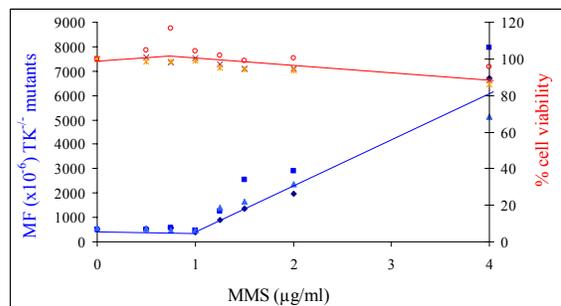
### HPRT Mutation in AHH-1 cells with MMS



- 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

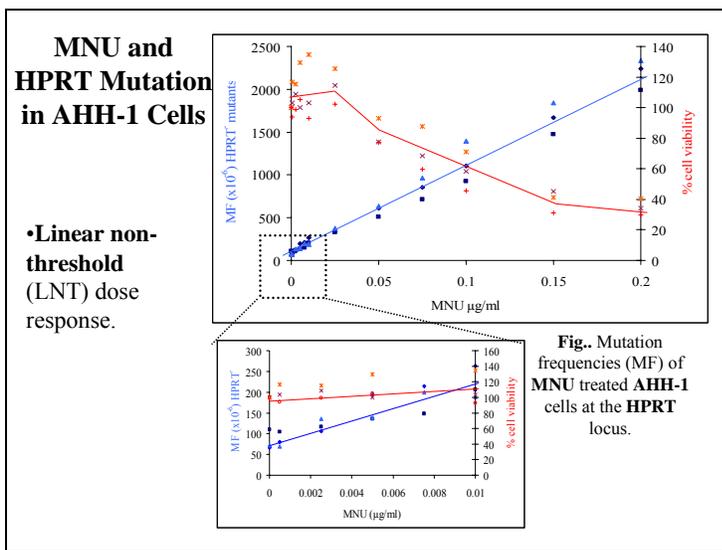
Slide 14

### MMS-induced TK<sup>-/-</sup> Mutants

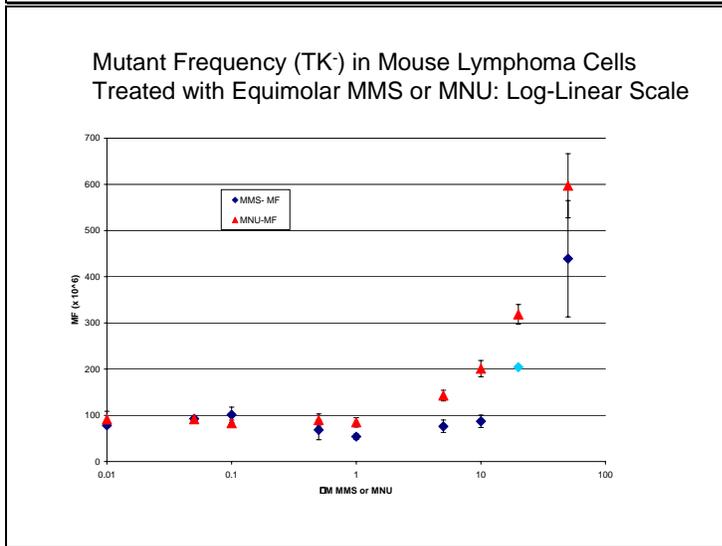


- The NOEL for mutation at the TK locus is located at 1µg/ml MMS - same as seen with HPRT locus.

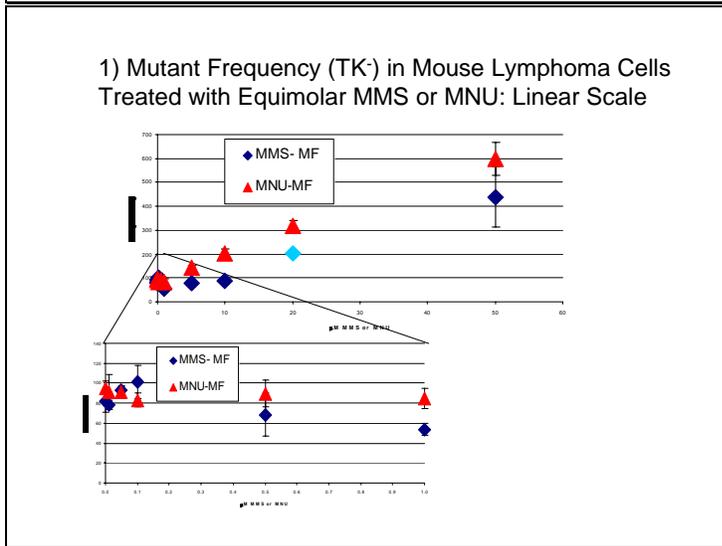
Slide 15



Slide 16

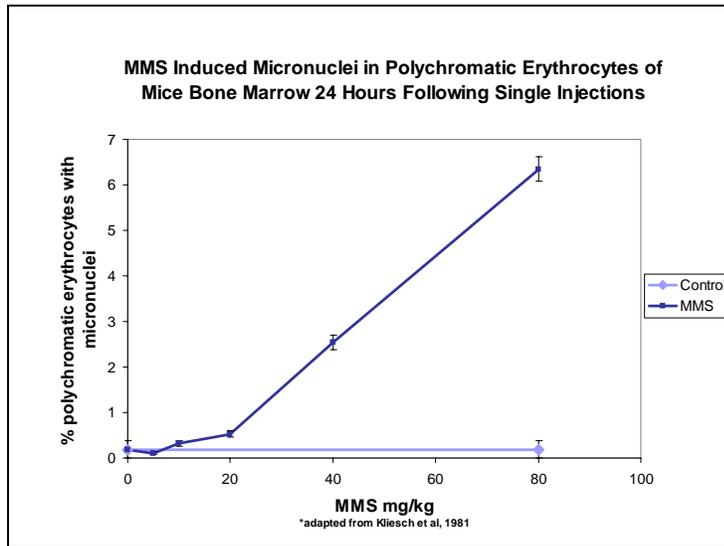


Slide 17

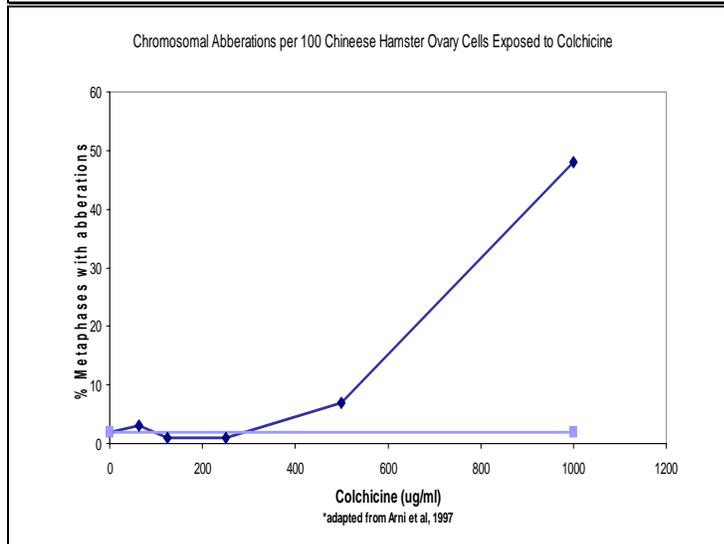


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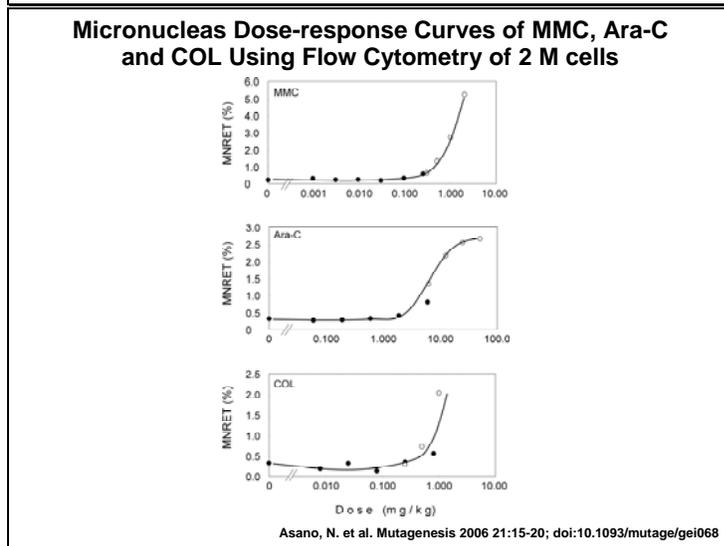
Slide 18



Slide 19



Slide 20



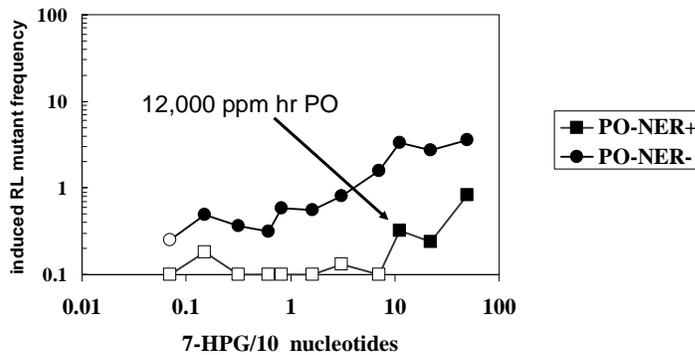
Slide 21

## 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



<sup>6</sup> 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 toxicology.
- Formed endogenously in humans and animals from metabolism of ethylene.
- HEG is present in all human and animal cells.

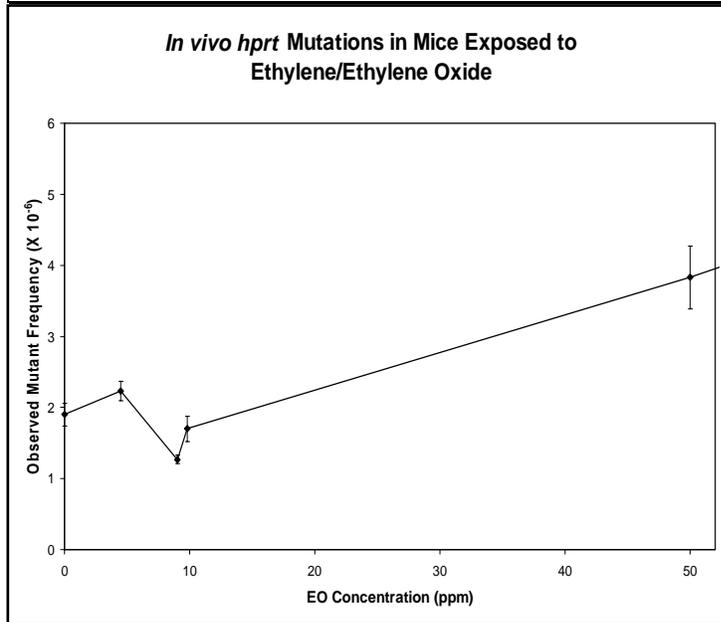
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Slide 24

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

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

Slide 25

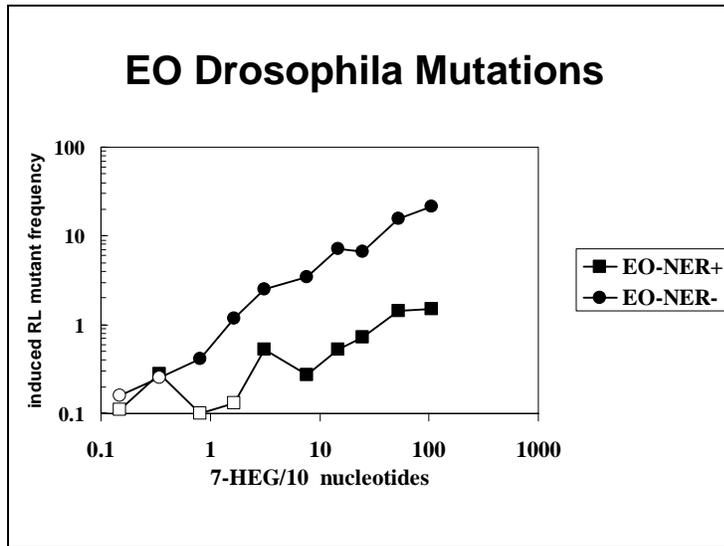


Slide 26

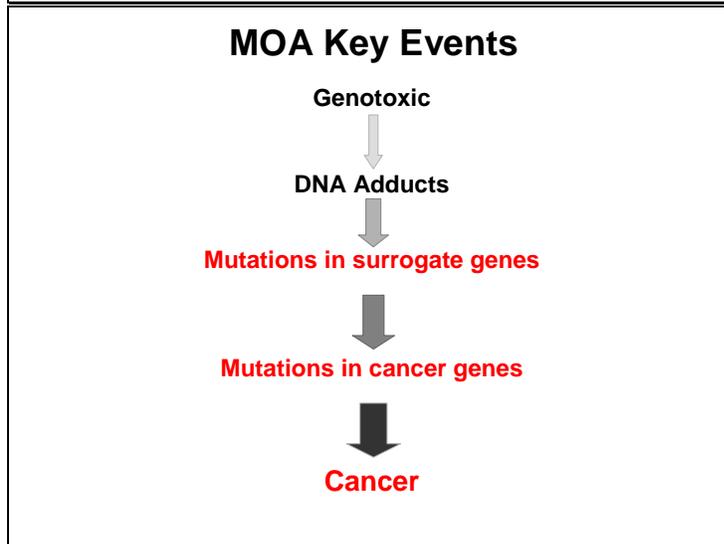
**EO *hprt* Mutations in Mice**

Ethylene Concentration (ppm)	EO Concentration (or Estimated EO Concentration) (ppm)	Observed Mutant Frequency (x 10 <sup>-6</sup> ) 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



Slide 28



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.

Slide 31

## 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]**

- 1  
2  
3
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1 **ATTACHMENT 1 MEMO AND CHARGE QUESTIONS**



2  
3 UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
4 OFFICE OF RESEARCH AND DEVELOPMENT  
National Center for Environmental Assessment  
Washington, DC 20460

October 27, 2006

NCEA Washington Office (8623D)

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12  
13 **MEMORANDUM**

14  
15  
16 **SUBJECT:** Request for SAB review of the Draft Ethylene Oxide (EtO) Carcinogenicity  
17 Assessment

18  
19 *David A Bussard*

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

23  
24 **TO:** Sue Shallal, Ph.D.  
25 Designated Federal Officer  
26 EPA Science Advisory Board Staff Office (1400F)

27  
28 This is to request a review by the Science Advisory Board of the draft document  
29 entitled "Evaluation of the Carcinogenicity of Ethylene Oxide". This document is an  
30 assessment of the carcinogenicity of ethylene oxide (EtO). The assessment was prepared by  
31 the National Center for Environmental Assessment (NCEA), which is the health risk  
32 assessment program in the Office of Research and Development. The document has been  
33 made available for public comment on the Agency's NCEA web site at the following URL:  
34 <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157664>. The assessment broadly  
35 supports activities authorized in the 1990 Clean Air Act and is of particular interest to EPA's  
36 Office of Air and Radiation. However, the assessment should also be applicable to the needs  
37 of all program Offices and Regions in evaluating the carcinogenicity of EtO.

38  
39 EPA last published an assessment of the potential carcinogenicity of EtO in 1985.  
40 The current assessment reviews the more recent database on the carcinogenicity of EtO. The  
41 scientific literature search for this assessment is generally current through June 2004,  
42 although a few later publications are included. This assessment focuses on lifetime cancer  
43 risk from inhalation exposure.  
44

**DO NOT CITE OR QUOTE** This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy

1 EtO is a gas at room temperature. It is manufactured from ethylene and used  
2 primarily as a chemical intermediate in the manufacture of ethylene glycol. It is also used as  
3 a sterilizing agent for medical equipment and as a fumigating agent for spices. The largest  
4 sources of human exposure are in occupations involving contact with the gas in plants  
5 (facilities) and in hospitals that sterilize medical equipment. EtO can also be inhaled by  
6 residents living near production or sterilizing/fumigating facilities. This document describes  
7 the derivation of inhalation unit risk estimates for cancer mortality and incidence based on  
8 human epidemiological data.  
9

10 Attached is a draft of a charge to the Science Advisory Board that identifies the  
11 questions and issues we want the Science Advisory Board to address in reviewing the  
12 document.  
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2 **CHARGE QUESTIONS FOR EPA'S SCIENCE ADVISORY BOARD (SAB) REVIEW**  
3 **OF THE ETHYLENE OXIDE (EtO) CARCINOGENICITY ASSESSMENT**  
4  
5

6 EPA's Office of Research and Development (ORD) has requested that the Science  
7 Advisory Board (SAB) review its document entitled "Evaluation of the Carcinogenicity of  
8 Ethylene Oxide". This document is EPA's draft of the evaluation of the carcinogenicity of  
9 ethylene oxide (EtO). The assessment was prepared by the National Center for  
10 Environmental Assessment which is the health risk assessment program in the Office of  
11 Research and Development. The assessment broadly supports activities authorized in the  
12 1990 Clean Air Act and is of particular interest to EPA's Office of Air and Radiation.  
13 However, this review also should be applicable to the needs of all program Offices and  
14 Regions in evaluating the carcinogenicity of EtO.  
15

16 EPA last published a health assessment of the potential carcinogenicity of EtO in  
17 1985 (U.S. EPA, 1985). The current assessment reviews the more recent database on the  
18 carcinogenicity of EtO. The scientific literature search for this assessment is generally  
19 current through June 2004, although a few later publications are included. This assessment  
20 focuses on lifetime cancer risk from inhalation exposure.  
21

22 EtO is a gas at room temperature. It is manufactured from ethylene and used  
23 primarily as a chemical intermediate in the manufacture of ethylene glycol. It is also used as  
24 a sterilizing agent for medical equipment and as a fumigating agent for spices. The largest  
25 sources of human exposure are in occupations involving contact with the gas in plants  
26 (facilities) and in hospitals that sterilize medical equipment. EtO can also be inhaled by  
27 residents living near production or sterilizing/fumigating facilities.  
28

29 The DNA-damaging properties of EtO have been studied since the 1940s. EtO is  
30 known to be mutagenic in a large number of living organisms, ranging from bacteriophage to  
31 mammals, and it also induces chromosome damage. It is carcinogenic in mice and rats,  
32 inducing tumors of the lymphohematopoietic system, brain, lung, connective tissue, uterus,  
33 and mammary gland. In humans employed in EtO-manufacturing facilities and in sterilizing  
34 facilities, the greatest evidence of a cancer risk from exposure is for cancer of the  
35 lymphohematopoietic system. Increases in the risk of lymphohematopoietic cancer have  
36 been seen in several studies, manifested as an increase either in leukemia and/or in cancer of  
37 the lymphoid tissue. In one large epidemiologic study of sterilizer workers that had a well-  
38 defined exposure assessment for individuals, positive exposure-response trends for  
39 lymphohematopoietic cancer mortality in males and for breast cancer mortality in females  
40 were reported (Steenland et al., 2004). The positive exposure-response trend for female  
41 breast cancer was confirmed in an incidence study based on the same worker cohort  
42 (Steenland et al., 2003).  
43

## FOR DISCUSSION AND DELIBERATION ONLY

**DO NOT CITE OR QUOTE** This draft is a work in progress, does not reflect consensus advice or recommendations, has not been reviewed or approved by the chartered SAB and does not represent EPA policy

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

4  
5 This evidence, as assessed by EPA, included:

- 6  
7 a) strong, though less than completely conclusive, evidence of carcinogenicity from  
8 human studies  
9 b) sufficient evidence of carcinogenicity in laboratory animals  
10 c) EtO is a direct-acting alkylating agent with clear evidence of  
11 mutagenicity/genotoxicity, and there is sufficient evidence that DNA adduct formation  
12 and the resulting mutagenic/genotoxic effects are key events in the mode of action of EtO  
13 carcinogenicity  
14 d) evidence of chromosome damage in humans exposed to EtO, supporting the inference  
15 that the same mode of action for EtO carcinogenicity is operative in humans  
16

17 This document describes the derivation of inhalation unit risk estimates for cancer  
18 mortality and incidence based on the human data. An EC<sub>01</sub> of 44 µg/m<sup>3</sup> (0.024 ppm) was  
19 calculated using a life-table analysis and linear modeling of the categorical Cox regression  
20 analysis results for excess lymphohematopoietic cancer mortality in males reported in a high-  
21 quality occupational epidemiologic study (Steenland et al., 2004). Linear low-dose  
22 extrapolation from the LEC<sub>01</sub> yielded a lifetime extra cancer mortality unit risk estimate of  
23  $5.0 \times 10^{-4}$  per µg/m<sup>3</sup> (0.92 per ppm) of continuous EtO exposure. Applying the same linear  
24 regression coefficient and life-table analysis to background male lymphohematopoietic  
25 cancer *incidence* rates yielded an EC<sub>01</sub> of 24 µg/m<sup>3</sup> (0.013 ppm) and a preferred lifetime  
26 extra cancer unit risk estimate of  $9.0 \times 10^{-4}$  per µg/m<sup>3</sup> (1.6 per ppm). The preferred estimate  
27 is greater than the estimate of  $5.0 \times 10^{-4}$  per µg/m<sup>3</sup> (0.91 per ppm; EC<sub>01</sub> = 44 µg/m<sup>3</sup>)  
28 calculated, using the same approach, from the results of a breast cancer incidence study of  
29 the same worker cohort (Steenland et al., 2003), and is recommended as the potency estimate  
30 for Agency use.  
31

32 Because the weight of evidence supports a mutagenic mode of action for EtO  
33 carcinogenicity, and in the absence of chemical-specific data on early-life susceptibility, this  
34 assessment finds that increased early-life susceptibility should be assumed and the age-  
35 dependent adjustment factors (ADAFs) should be applied, in accordance with EPA's  
36 *Supplemental Guidance for Assessing Susceptibility From Early-Life Exposure to*  
37 *Carcinogens*, hereinafter referred to as "EPA's Supplemental Guidance" (U.S. EPA, 2005b).  
38 Applying the ADAFs to the unit risk estimate of  $9.0 \times 10^{-4}$  per µg/m<sup>3</sup> yields an adjusted full  
39 lifetime unit risk estimate of  $1.5 \times 10^{-3}$  per µg/m<sup>3</sup>, and the commensurate lifetime chronic  
40 exposure level of EtO corresponding to an increased cancer risk of  $10^{-6}$  is  $0.0007 \mu\text{g}/\text{m}^3$ .  
41 [Note that for less-than-lifetime exposure scenarios (or for exposures that vary with age), the  
42 unadjusted (adult-based) potency estimate of  $9.0 \times 10^{-4}$  per µg/m<sup>3</sup> should be used, in  
43 conjunction with the ADAFs as appropriate, in accordance with EPA's Supplemental  
44 Guidance.]  
45

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

12  
13 The unit risk estimates were developed for environmental exposure levels and are not  
14 necessarily applicable to higher-level occupational exposures, which appear to be subject to a  
15 different exposure-response relationship. However, occupational exposure levels are of  
16 concern to EPA when EtO is used as a pesticide (e.g., fumigant for spices). Therefore, this  
17 document also presents extra risk estimates for cancer for a number of occupational exposure  
18 scenarios.

19  
20 The SAB Ethylene Oxide Review Panel is being asked to comment on the scientific  
21 soundness of this carcinogenicity assessment. The specific charge questions to the Panel are  
22 as follows:

23  
24 **Issue 1: Carcinogenic Hazard (Section 3 and Appendix A of the Draft)**

25  
26 1. Do the available data and discussion in the draft document support the hazard conclusion  
27 that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's  
28 2005 *Guidelines for Carcinogen Risk Assessment*? In your response, please include  
29 consideration of the following:

30  
31 1.a EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but  
32 less than completely conclusive. Does the draft document provide sufficient description of  
33 the studies, balanced treatment of positive and negative results, and a rigorous and  
34 transparent analysis of the data used to assess the carcinogenic hazard of ethylene oxide  
35 (EtO) to humans? Please comment on the EPA's characterization of the body of  
36 epidemiological data reviewed. Considerations include: a) the consistency of the findings,  
37 including the significance of differences in results using different exposure metrics, b) the  
38 utility of the internal (based on exposure category) versus external (e.g., SMR and SIR)  
39 comparisons of cancer rates, c) the magnitude of the risks, and d) the strength of the  
40 epidemiological evidence.

41  
42 1.b. Are there additional key published studies or publicly available scientific reports that  
43 are missing from the draft document and that might be useful for the discussion of the  
44 carcinogenic hazard of EtO?  
45

1 1.c. Do the available data and discussion in the draft document support the mode of action  
2 conclusions?  
3

4 1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced  
5 and sound description that synthesizes the human, laboratory animal, and supporting (e.g., *in*  
6 *vitro*) evidence for human carcinogenic hazard?  
7  
8

9 **Issue 2: Risk Estimation (Section 4 and Appendices C and D)**  
10

11 2. Do the available data and discussion in the draft document support the approaches taken  
12 by EPA in its derivation of cancer risk estimates for EtO? In your response, please include  
13 consideration of the following:  
14

15 2.a. EPA concluded that the epidemiological evidence alone was strong but less than  
16 completely conclusive (although EPA characterized the total evidence - from human,  
17 laboratory animal, and *in vitro* studies - as supporting a conclusion that EtO as "carcinogenic  
18 to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003,  
19 2004) data set, the most appropriate for estimating the magnitude of the carcinogenic risk to  
20 humans from environmental EtO exposures? Are the scientific justifications for using this  
21 data set transparently described? Is the basis for selecting the Steenland et al. data over other  
22 available data (e.g., the Union Carbide data) for quantifying risk adequately described?  
23

24 2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use  
25 of a linear regression model fit to Steenland et al.'s categorical results for all  
26 lymphohematopoietic cancer in males in only the lower exposure groups scientifically and  
27 statistically appropriate for estimating potential human risk at the lower end of the  
28 observable range? Is the use of the grouping of all lymphohematopoietic cancer for the  
29 purpose of estimating risk appropriate? Are there other appropriate analytical approaches  
30 that should be considered for estimating potential risk in the lower end of the observable  
31 range? Is EPA's choice of a preferred model adequately supported and justified? In  
32 particular, has EPA adequately explained its reasons for not using a quadratic model  
33 approach such as that of Kirman et al. (2004) based? What recommendations would you  
34 make regarding low-dose extrapolation below the observed range?  
35

36 2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk  
37 estimate, in accordance with EPA's Supplemental Guidance ( U.S. 2005b), appropriate and  
38 transparently described?  
39

40 2.d. Is the use of different models for estimation of potential carcinogenic risk to humans  
41 from the higher exposure levels more typical of occupational exposures (versus the lower  
42 exposure levels typical of environmental exposures) appropriate and transparently described  
43 in Section 4.5?  
44

1 2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data  
2 appropriate and transparently described? Is the use of “ppm equivalence” adequate for  
3 interspecies scaling of EtO exposures from the rodent data to humans?  
4

5 **Issue 3: Uncertainty (Sections 3 and 4)**  
6

7 1. EPA’s *Risk Characterization Handbook* requires that assessments address in a transparent  
8 manner a number of important factors. Please comment on how well this assessment clearly  
9 describes, characterizes and communicates the following:  
10

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