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18 **Evaluation of the Inhalation Carcinogenicity of Ethylene**
19 **Oxide**

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27 **In Support of Summary Information on the**
28 **Integrated Risk Information System (IRIS)**

29

30 *July 2011*

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LIST OF ABBREVIATIONS

ADAF	age-dependent adjustment factor	
AIDS	acquired immune deficiency syndrome	
AML	acute myeloid leukemia	
AUC	areas under the curve	
BEIR	Committee on the Biological Effects of Ionizing Radiation	
CI	confidence interval	
DSB	double-strand breaks	
EC	effective concentration	
EOIC	Ethylene Oxide Industry Council	
EPA	U.S. Environmental Protection Agency	
EtO	ethylene oxide	
FRG	Federal Republic of Germany	
GST	glutathione S-transferase	
HAP	hazardous air pollutants	
N7-HEG	N7-(2-hydroxyethyl)guanine	
IARC	International Agency for Research on Cancer	
ICD	International Classification of Diseases	
IRIS	Integrated Risk Information System	
LEC	lower confidence limit	
MLE	maximum likelihood estimates	
NCEA	National Center for Environmental Assessment	
NHL	non-Hodgkin lymphoma	
NIOSH	National Institute for Occupational Safety and Health	
NTP	National Toxicology Program	
OBS	observed number	
OR	odds ratios	
PBPK	physiologically based pharmacokinetic	
POD	point of departure	
RR	relative rate, i.e., rate ratio	
SCE	sister chromatid exchanges	
SE	standard error	
SEER	Surveillance, Epidemiology, and End Results	
SIR	standardized incidence ratio	
SMR	standard mortality ratios	
TWA	time-weighted average	
UCC	Union Carbide Corporation	
UCL	upper confidence limit	
WHO	World Health Organization	

1 **AUTHORS, CONTRIBUTORS, AND REVIEWERS**
2
3 **ASSESSMENT AUTHORS**
4
5 Henry D. Kahn, Chemical Manager
6 National Center for Environmental Assessment
7 U.S. Environmental Protection Agency
8 Washington, DC
9
10 David Bayliss (retired)
11 National Center for Environmental Assessment
12 U.S. Environmental Protection Agency
13 Washington, DC
14
15 Jennifer Jinot
16 National Center for Environmental Assessment
17 U.S. Environmental Protection Agency
18 Washington, DC
19
20 Nagu Keshava
21 National Center for Environmental Assessment
22 U.S. Environmental Protection Agency
23 Washington, DC
24
25 Robert McGaughy (retired)
26 National Center for Environmental Assessment
27 U.S. Environmental Protection Agency
28 Washington, DC
29
30 Ravi Subramaniam
31 National Center for Environmental Assessment
32 U.S. Environmental Protection Agency
33 Washington, DC
34
35 Larry Valcovic (retired)
36 National Center for Environmental Assessment
37 U.S. Environmental Protection Agency
38 Washington, DC
39
40 Suryanarayana Vulimiri
41 National Center for Environmental Assessment
42 U.S. Environmental Protection Agency
43 Washington, DC
44
45

1

REVIEWERS

This document has been provided for review to EPA scientists, interagency reviewers
from other federal agencies and White House offices, and the public, and peer reviewed by
independent scientists external to EPA. A summary and EPA's disposition of the comments
received from the independent external peer reviewers and from the public is included in
Appendix H.

INTERNAL EPA REVIEWERS

Michele Burgess, OSWER
Deborah Burgin, OPEI
Kerry Dearfield, ORD/OSP
Joyce Donahue, OW
Michael Firestone, AO/OCHP
Karen Hogan, ORD/NCEA–IO
Aparna Koppikar, ORD/NCEA–W
Deirdre Murphy, OAR/ESD
Steve Nesnow, ORD/NHEERL
Marian Olsen, Region 2
Brenda Perkovich-Foos, AO/OCHP
Julian Preston, ORD/NHEERL
Santhini Ramasamy, OPP/HED
Nancy Rios-Jafolla, Region 3
Tracey Woodruff, AO/NCEE

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EXTERNAL PEER REVIEWERS

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University of Florida

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41
42 Dr. Vernon Walker
43 Lovelace Respiratory Research Institute
44
45

1. EXECUTIVE SUMMARY

Ethylene oxide (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 DNA-damaging properties of EtO have been studied since the 1940s. EtO is known to be mutagenic in a large number of living organisms, ranging from bacteriophage to mammals, and it also induces chromosome damage. It is carcinogenic in mice and rats, inducing tumors of the lymphohematopoietic system, brain, lung, connective tissue, uterus, and mammary gland. In humans employed in EtO-manufacturing facilities and in sterilizing facilities, the greatest evidence of a cancer risk from exposure is for cancer of the lymphohematopoietic system. Increases in the risk of lymphohematopoietic cancer have been seen in several (but not all) studies, manifested as an increase either in leukemia or in cancer of the lymphoid tissue. Of note, in one large epidemiologic study conducted by the National Institute for Occupational Safety and Health (NIOSH) of sterilizer workers that had a well-defined exposure assessment for individuals, positive exposure-response trends for lymphohematopoietic cancer mortality in males, in particular for lymphoid cancer (i.e., non-Hodgkin lymphoma, myeloma, and lymphocytic leukemia), and for breast cancer mortality in females were reported (Steenland et al., 2004). The positive exposure-response trend for female breast cancer was confirmed in an incidence study based on the same worker cohort (Steenland et al., 2003).

Although the evidence of carcinogenicity from human studies was deemed short of conclusive on its own, EtO is characterized as carcinogenic to humans by the inhalation route of exposure based on the total weight of evidence, in accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Supporting information includes: (1) strong, but less than conclusive, evidence of lymphohematopoietic cancers and some evidence of breast cancer in EtO-exposed workers, (2) extensive evidence of carcinogenicity in laboratory animals, including lymphohematopoietic cancers in rats and mice and mammary carcinomas in mice following inhalation exposure, (3) clear evidence that EtO is genotoxic and sufficient weight of evidence to support a mutagenic mode of action for EtO carcinogenicity, and (4) strong evidence that the key precursor events are anticipated to occur in humans and progress to tumors, including evidence of chromosome damage in humans exposed to EtO.

This document describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on the human data from the large NIOSH study (Steenland et al., 2003, 2004). This study was selected for the derivation of risk estimates because it was the

1   largest of the available studies and it had exposure estimates for the individual workers from a
2   high-quality exposure assessment. Multiple modeling approaches were evaluated for the
3   exposure-response data, including modeling the cancer response as a function of either
4   categorical exposures or continuous individual exposure levels. Preferred approaches were
5   defined for each cancer endpoint in consideration of both the statistical properties and biological
6   reasonableness of the resulting model forms.
7   Under the common assumption that relative risk is independent of age, an EC_{01}
8   (estimated effective concentration associated with 1% extra risk) of 103 μg/m^3 (56.4 ppb) was
9   calculated using a life-table analysis and linear modeling of the categorical Cox regression
10 analysis results for excess lymphoid cancer mortality (Steenland et al., 2004; additional results
11 for both sexes combined provided by Dr. Steenland in Appendix D), excluding the highest
12 exposure group to mitigate the supralinearity of the exposure-response data. Linear low-dose
13 extrapolation below the range of observations is supported by the conclusion that a mutagenic
14 mode of action is operative in EtO carcinogenicity. Linear low-dose extrapolation from the
15 LEC_{01} (lower 95% confidence limit on the EC_{01}) for lymphoid cancer mortality yielded a
16 lifetime extra cancer unit risk estimate of 2.2×10^{-4} per μg/m^3 (4.0×10^{-4} per ppb) of continuous
17 EtO exposure. Applying the same linear regression coefficient and life-table analysis to
18 background lymphoid cancer *incidence* rates yielded an EC_{01} of 46 μg/m^3 (25 ppb), and applying
19 linear low-dose extrapolation resulted in a preferred lifetime extra lymphoid cancer unit risk
20 estimate of 4.8×10^{-4} per μg/m^3 (8.8×10^{-4} per ppm), as cancer incidence estimates are generally
21 preferred over mortality estimates.
22   Using the same approach, an EC_{01} of 71 μg/m^3 (39 ppb) and a unit risk estimate of $2.8 \times$
23 10^{-4} per μg/m^3 (5.1×10^{-4} per ppb) were derived from the breast cancer mortality results of the
24 same epidemiology study (Steenland et al., 2004). Breast cancer incidence risk estimates, on the
25 other hand, were calculated from the data from a breast cancer incidence study of the same
26 occupational cohort (Steenland et al., 2003), and, for these data, a two-piece linear spline model
27 was used for the exposure-response modeling. Using the same life-table approach and linear
28 low-dose extrapolation, an EC_{01} of 20 μg/m^3 (11 ppb) and a unit risk estimate of 9.5×10^{-4} per
29 μg/m^3 (1.7×10^{-3} per ppb) were obtained for breast cancer incidence. Again, the incidence
30 estimate is preferred over the mortality estimate. Combining the incidence risk estimates for the
31 two cancer types resulted in a total cancer unit risk estimate of 1.2×10^{-3} per μg/m^3 (2.3×10^{-3}
32 per ppb).
33   Unit risk estimates were also derived from the three chronic rodent bioassays for EtO
34 reported in the literature, without considering early-life susceptibility. These estimates, ranging
35 from 2.2×10^{-5} per μg/m^3 to 4.6×10^{-5} per μg/m^3, are about an order of magnitude lower than the

1   estimates based on human data. The Agency takes the position that human data, if adequate data
2   are available, provide a more appropriate basis than rodent data for estimating population risks
3   (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from
4   rodents to humans are avoided. Although there is a sizeable difference between the rodent-based
5   and the human-based estimates, the human data are from a large, high-quality study, with EtO
6   exposure estimates for the individual workers and little reported exposure to chemicals other
7   than EtO. Therefore, the estimates based on the human data are the preferred estimates for this
8   assessment.
9   Because the weight of evidence supports a mutagenic mode of action for EtO
10   carcinogenicity, and as there are no chemical-specific data from which to assess early-life
11   susceptibility, increased early-life susceptibility should be assumed, according to EPA's
12   *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens,*
13   hereinafter referred to as "EPA's *Supplemental Guidance*" (U.S. EPA, 2005b). This assumption
14   of increased early-life susceptibility supersedes the assumption of age independence under which
15   the human-data-based estimates presented above were derived. Thus, using the same approach
16   as for the estimates discussed above but initiating exposure in the life-table analysis at age 16
17   instead of at birth, adult-only-exposure unit risk estimates were calculated from the human data
18   under an alternate assumption that realtive risk is independent of age for adults, which represent
19   the life-stage for which the data upon which the exposure-response modeling was conducted
20   pertain. These adult-only-exposure unit risk estimates were then re-scaled to a 70-year basis for
21   use in the standard ADAF calculations and risk estimate calculations involving less-than-lifetime
22   exposure scenarios. The resulting adult-based unit risk estimates were 4.35×10^{-4} per μg/m^3
23   (7.95×10^{-4} per ppb) for lymphoid cancer incidence, 8.21×10^{-4} per μg/m^3 (1.50×10^{-3} per ppb)
24   for breast cancer incidence in females, and 1.08×10^{-3} per μg/m^3 (1.98×10^{-3} per ppb) for both
25   cancer types combined. For exposure scenarios involving early-life exposure, the age-dependent
26   adjustment factors (ADAFs) should be applied, in accordance with EPA's *Supplemental*
27   *Guidance* (U.S. EPA, 2005b). Applying the ADAFs to obtain a full lifetime total cancer unit risk
28   estimate yields 1.8×10^{-3} per μg/m^3 (3.3×10^{-3} per ppb), and the commensurate lifetime chronic
29   exposure level of EtO corresponding to an increased cancer risk of 10^{-6} is 0.0006 μg/m^3.
30   The major sources of uncertainty in the unit risk estimates derived from the human data
31   include the low-dose extrapolation, the retrospective exposure assessment conducted for the
32   epidemiology study, and the exposure-response modeling of the epidemiological data.
33   The unit risk estimate is intended to provide a reasonable upper bound on cancer risk.
34   The estimate was developed for environmental exposure levels (it is considered valid for
35   exposures up to 110 μg/m^3 [60 ppb]) and is not applicable to higher-level exposures, such as may

1   occur occupationally, which appear to have a different exposure-response relationship.

2   Therefore, this document also presents extra risk estimates for the two cancer types for a number

3   of occupational exposure scenarios.

2. INTRODUCTION

The purpose of this document is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to carcinogenicity from chronic inhalation exposure to ethylene oxide (EtO). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of EtO. In general, this IRIS Carcinogenicity Assessment provides information on the carcinogenic hazard potential of EtO and quantitative estimates of risk from inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates for inhalation exposure (inhalation unit risks) are derived. The definition of an inhalation unit risk is a plausible upper bound on the estimate of risk per μg/m3 air breathed.

Development of the hazard identification and dose-response assessments for EtO has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that were used in the development of this assessment include the following: *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)*, Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), and *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant scientific literature for this Carcinogenicity Assessment was reviewed through January 2010. It should be noted that references have been added after the External Peer Review in response to the reviewers' and public comments. References have also been added for completeness. These references have not changed the overall qualitative or quantitative conclusions. See Appendix I for a list of these references.

1   For general information about this assessment or other questions relating to IRIS, the

2 reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or

3 hotline.iris@epa.gov (email address).

4

5

3. HAZARD IDENTIFICATION

4   This chapter presents the evidence considered in the hazard identification of EtO

5 carcinogenicity and the hazard characterization resulting from the weight-of-evidence evaluation.

6 Section 3.1 summarizes the human evidence (a more detailed discussion of the human cancer

7 studies is presented in Appendix A). Section 3.2 describes the evidence from experimental

8 animal studies. Section 3.3 discusses supporting evidence, in particular evidence regarding the

9 genotoxicity of EtO. Section 3.4 provides the mode-of-action analysis for EtO carcinogenicity.

10 To conclude the chapter, Section 3.5 presents the hazard characterization for EtO carcinogenicity

11 and a discussion of life-stages and populations with potentially increased susceptibility.

12

3.1. EVIDENCE OF CANCER IN HUMANS

14   The literature from 1988 to present contains numerous epidemiological studies of the

15 carcinogenic effects of EtO in occupational cohorts; some of these cohorts were the subject of

16 multiple reports. The conclusions about the human evidence of carcinogenicity in this

17 assessment are based on the following summary of those studies, which are discussed in more

18 detail and critically reviewed in Appendix A. Table A-4 in Appendix A provides a tabular

19 summary of the epidemiological studies, including some study details, results, and limitations.

20 The strengths and weaknesses of these studies were evaluated individually using standard

21 considerations in evaluating epidemiological studies. The major areas of concern are study

22 design, exposure assessment, and data analysis. General features of study design considered

23 include sample size and assessment of the health endpoint. For case-control studies, design

24 considerations include representativeness of cases, selection of controls, use of proxy

25 respondents, and interview approach (e.g., blinding). For cohort studies, design considerations

26 include selection of referent population (e.g., internal comparisons are generally preferred to

27 comparisons with an external population), loss to follow-up, and length of follow-up. Exposure

28 assessment issues include specificity of exposure (exposure misclassification), characterization

29 of exposure (e.g., ever exposed or quantitative estimate of exposure level), and potential

30 confounders. Analysis considerations include adjustment for potential confounders or effect

31 modifiers and modeling of exposure-response relationships.

32   Two primary sources of exposures to EtO are production facilities and sterilization

33 operations. There are two types of production facilities (IARC, 1994b):

34   1. those using the older chlorohydrin process, where ethylene is reacted with hypochlorous

35   acid and then with calcium oxide to make EtO (this method produces unwanted

36   byproducts, the most toxic of which is ethylene dichloride), and

2. those producing EtO via direct oxidation of ethylene in a pressurized vessel, which involves less EtO exposure and eliminates the chemical byproducts of the chlorohydrin process.

Exposure in the sterilization of medical equipment and in the direct oxidation process is predominantly to EtO, whereas exposure in the chlorohydrin process is to EtO mixed with other chemicals.

Hogstedt et al. (1986) and Hogstedt (1988) summarized findings of three Swedish occupational cohorts (539 men and 170 women) exposed in a plant where hospital equipment is sterilized, in a chlorohydrin production facility, and in a direct oxidation production facility. The incidence of leukemia was elevated in all cohorts, although the risk was not statistically significant in the cohort from the direct oxidation facility. For the three cohorts combined there were statistically significantly elevated standard mortality ratios (SMRs) for leukemia (SMR = 9.2; 95% confidence interval [CI] = 3.7–19), based on 7 deaths, and for stomach cancer (SMR = 5.5; 95% CI = 2.6–10), based on 10 deaths. Although this study produced high SMRs for leukemia, stomach cancer, and total cancer, there are some limitations, such as multiple exposures to numerous other chemicals, lack of personal exposure information, and lack of latency analysis. No gender differences were separately analyzed. No dose-response calculations were possible. This study provides suggestive evidence of the carcinogenicity of EtO.

Coggon et al. (2004) reported the results of a follow-up study of a cohort originally studied by Gardner et al. (1989). The cohort included workers in three EtO production facilities (two using both chlorohydrin and direct oxidation processes and the third using direct oxidation only); in a fourth facility that used EtO in the manufacture of other chemicals; and in eight hospitals that used EtO in sterilizing units. The total cohort comprised 1,864 men and 1,012 women. No statistically significant excesses were observed for any cancer site. Slight increases, based on small numbers, were observed for the various lymphohematopoietic cancers: Hodgkin lymphoma (2 vs. 1 expected), non-Hodgkin lymphoma (NHL) (7 vs. 4.8), multiple myeloma (3 vs. 2.5), and leukemia (5 vs. 4.6). The increases were concentrated in the 1,471 chemical-manufacturing workers, of whom all but 1 were male. In the chemical-manufacturing workers with "definite" exposure, 4 leukemias were observed (1.7 expected) and 9 lymphohematopoietic cancers were observed (4.9 expected). A slight deficit in the risk of breast cancer deaths (11 vs. 13.2) was observed in the cohort. No individual exposure measurements were obtained from cohort members, and no exposure measurements were available before 1977. Multiple exposures to other chemicals, small numbers of deaths, and lack of individual EtO measurements make this study only suggestive of a higher risk of leukemia from exposure to EtO.

1   A series of retrospective mortality studies of about 2,000 male workers who were
2 assigned to operations that used or produced EtO in either of two Union Carbide Corporation
3 (UCC) chemical production facilities in West Virginia (Greenberg et al., 1990; Teta et al., 1993,
4 1999; Benson and Teta, 1993; Swaen et al., 2009; Valdez-Flores et al., 2010) has been published.
5 EtO was produced at these facilities until 1971, after which it was imported to the facilities. For
6 EtO production, the chlorohydrin process was used from 1925 to 1957, and the direct oxidation
7 process was used from 1937 to 1971 (during overlapping years, both processes were in use). The
8 cohort was observed from 1940 through 1978 in the original study (Greenberg et al., 1990),
9 through 1988 in the Teta et al. (1993, 1999) and Benson and Teta (1993) studies, and through
10 2003 in the latter two studies. A large-scale industrial hygiene survey and monitoring of EtO
11 concentrations was carried out in 1976, at which time EtO was in use at the facilities but no
12 longer in production.
13   Greenberg et al. (1990) found elevated but not statistically significant risks of pancreatic
14 cancer (SMR = 1.7) and leukemia (SMR = 2.3) (each based on seven cases) in the entire cohort;
15 most of the cases occurred in the chlorohydrin production unit (note that the chlorohydrin
16 production unit produced primarily ethylene chlorohydrin, which is used in chlorohydrin-based
17 EtO production, but this unit is not where chlorohydrin-based EtO production took place).
18 Limitations to this study included multiple exposures to many different chemicals in the facility
19 through the years and lack of EtO exposure measurements prior to 1976. Three categories of
20 exposure were established for analysis—low, intermediate, and high—based on a qualitative
21 characterization of the potential for EtO exposure. The number of workers in each exposure
22 category was not reported. No significant findings of a dose-response relationship were
23 discernable. No quantitative estimates of individual exposure were made in this study, and no
24 latency analysis was conducted (average follow-up was 20 years). Furthermore, EtO is not the
25 only chemical to which the observed excesses in cancer mortality could be attributed.
26   A follow-up study (Teta et al., 1993) that extended the observation of this cohort
27 (excluding the 278 chlorohydrin production unit workers, who reportedly had low EtO
28 exposures) for an additional 10 years to 1988 found no significant risk of total cancer; there was
29 a slight trend in the risk of leukemia with increasing duration of assignment to departments using
30 or processing EtO, but it was not significant ($p = 0.28$) and was based on only five cases. The
31 average follow-up was 27 years, and at least 10 years had elapsed since first exposure for all
32 workers. The same problems of exposure ascertainment exist for this study as for that of
33 Greenberg et al. (1990), and, furthermore, the follow-up did not update work histories for the
34 workers after 1978. EtO production at the plants was discontinued before 1978, as noted by Teta
35 et al. (1993); however, according to Greenberg et al. (1990), certain non-production areas had
36 "intermediate" potential for EtO exposure, although estimates of exposure levels suggest that the

1   levels would also be lower during the update period (<1 ppm 8-hour TWA, according to Teta et
2   al. [1993]). It appears from the Greenberg et al. (1990) publication that the high potential
3   exposure group was reserved for EtO production workers, and, according to Teta et al. (1993),
4   there were only 425 EtO production workers in the cohort. Of these, only 118 worked in the
5   chlorohydrin-based production process, where exposures were reportedly highest. Essentially,
6   the study did not support the earlier studies of cancer in EtO workers; however, it was limited by
7   low statistical power and a crude exposure assessment and, thus, is not very informative
8   regarding whether exposure to EtO is causally related to cancer.
9   In a parallel follow-up study through 1988 of only the chlorohydrin production
10 employees, Benson and Teta (1993) found that pancreatic cancer and lymphohematopoietic
11 cancer cases continued to accumulate and that the SMRs were statistically significant for
12 pancreatic cancer (SMR = 4.9; Obs = 8, $p < 0.05$) and for lymphohematopoietic cancer (SMR =
13 2.9; Obs = 8, $p < 0.05$). These investigators interpreted these excesses as possibly due to
14 ethylene dichloride, a byproduct in the chlorohydrin process. Again, this small study of only 278
15 workers was limited by the same problems as the Greenberg et al. (1990) study and the Teta et
16 al. (1993) study. No individual estimates of exposure are available and the workers were
17 potentially exposed to many different chemicals (Table A-4). Furthermore, the chlorohydrin
18 production unit was reportedly considered a low potential EtO exposure department. Hence this
19 study has little weight in determining the carcinogenicity of EtO.
20   In a later analysis, Teta et al. (1999) fitted Poisson regression dose-response models to
21 the UCC data (followed through 1988 and excluding the chlorohydrin production workers) and
22 to data (followed through 1987) from a study by the National Institute for Occupational Safety
23 and Health (NIOSH) (described below). Because Teta et al. (1999) did not present risk ratios for
24 the cumulative exposure categories used to model the dose-response relationships, the only
25 comparison that can be made between the UCC and NIOSH data is based on the fitted models.
26 These models are almost identical for leukemia, but, for the lymphoid category, the risk—
27 according to the fitted model for the UCC data—decreased as a function of exposure, whereas
28 the risk for the modeled NIOSH data increased as a function of exposure. However, the models
29 are based on small numbers of cases (16 [5 UCC, 11 NIOSH] for leukemia; 22 [3 UCC, 19
30 NIOSH] for lymphoid cancers), and no statistics are provided to assess model goodness of fit or
31 to compare across models. In any event, this analysis is superseded by the more recent analysis
32 by the same authors (Valdez-Flores et al.,) of the results of more recent follow-up studies of
33 these cohorts (see below).
34   Swaen et al. (2009) studied the same UCC cohort identified by Teta et al. (1993), i.e.,
35 without the chlorohydrin production workers, but extended the cohort enumeration period from
36 the end of 1978 to the end of 1988, identifying 167 additional workers, and conducted mortality

1 follow-up of the resulting cohort of 2063 male workers through 2003. Work histories were also
2 extended through 1988 (exposures after 1988 were considered negligible compared to earlier
3 exposure levels). Swaen et al. (2009) used an exposure assessment based on the qualitative
4 categorizations of potential EtO exposure in the different departments developed by Greenberg et
5 al. (1990) and time-period exposure estimates from Teta et al. (1993). This exposure assessment
6 was relatively crude, based on just a small number of department-specific and time-period-
7 specific categories, and with exposure estimates for only a few of the categories derived from
8 actual measurements (see Appendix A.3.20 for details).
9   At the end of the 2003 follow-up, 1,048 of the 2,063 workers had died (Swaen et al.,
10 2009). The all-cause mortality SMR was 0.85 (95% CI = 0.80, 0.90) and the cancer SMR was
11 0.95 (95% CI = 0.84, 1.06). None of the SMRs for specific cancer types showed any statistically
12 significant increases. In analyses stratified by hire date (pre- [inclusive] or post-1956), the SMR
13 for leukemia was elevated but not statistically significant (1.51; 95% CI 0.69, 2.87) in the early-
14 hire group, based on 9 deaths. In analyses stratified by duration of employment, no trends were
15 apparent for any of the lymphohematopoietic cancers, although in the 9+ years of employment
16 subgroup, the SMR for NHL was nonsignificantly increased (1.49; 95% CI 0.48, 3.48), based on
17 5 deaths. In SMR analyses stratified by cumulative exposure, no trends were apparent for any of
18 the lymphohematopoietic cancers and there were no notable elevations for the highest
19 cumulative exposure category. Note that only 27 lymphohematopoietic cancer deaths (including
20 12 leukemias and 11 NHLs) were observed in the cohort.
21   Swaen et al. (2009) also did internal Cox proportional hazards modeling for some disease
22 categories (all-cause mortality, leukemia mortality, and lymphoid cancer [NHL, lymphocytic
23 leukemia, and myeloma] mortality [17 deaths]), using cumulative exposure as the exposure
24 metric. These analyses showed no evidence of an exposure-response relationship. Alternate
25 Cox proportional hazard analyses and categorical exposure-response analyses of the UCC data
26 conducted by Valdez-Flores et al. (2010) for a larger set of cancer endpoints similarly reported
27 an absence of any exposure-response relationships. Each of these cancer analyses, however,
28 relies on small numbers of cases and a crude exposure assessment, where there is a high potential
29 for exposure misclassification.
30   In a study of 2,658 male workers at eight chemical plants where EtO is produced
31 (manufacturing process not stated), Kiesselbach et al. (1990) found slightly increased SMRs for
32 cancers of the stomach, esophagus, and lung. A latency analysis was done only for stomach
33 cancer and total mortality. The investigators considered 71.6% of the cohort to be "weakly"
34 exposed; only 2.6% were "strongly exposed." No data were provided to explain how these
35 exposure categories were derived. The workers were followed for a median 15.5 years. Without

1   additional information on exposure to EtO, this study is of little help at this time in evaluating the

2   carcinogenicity of EtO.

3   NIOSH conducted an industry-wide study of 18,254 workers (45% male and 55%

4   female) in 14 plants where EtO was used (Steenland et al., 1991; Stayner et al., 1993; Steenland

5   et al., 2004). Most of the workers were exposed while sterilizing medical supplies and treating

6   spices and in the manufacture and testing of medical sterilizers. Individual exposure estimates

7   were derived for workers from 13 of the 14 plants. The procedures for selecting the facilities and

8   defining the cohort are described in Steenland et al. (1991), and the exposure model and

9   verification procedures are described in Greife et al. (1988) and Hornung et al. (1994). Results

10   of the original follow-up study through 1987 are presented in Steenland et al. (1991) and Stayner

11   et al. (1993). The cohort averaged 26.8 years of follow-up in the extended follow-up study

12   through 1998, and 16% of the cohort had died (Steenland et al., 2004).

13   The overall SMR for cancer was 0.98, based on 860 deaths (Steenland et al., 2004). The

14   SMR for (lympho)hematopoietic cancer was 1.00, based on 79 cases. Exposure-response

15   analyses, however, revealed exposure-related increases in hematopoietic cancer mortality risk,

16   although the effect was limited to males. In categorical life-table analysis, men with >13,500

17   ppm-days of cumulative exposure had an SMR of 1.46 (Obs = 13). In internal Cox regression

18   analyses (i.e., analyses in which the referent population is within the cohort) with exposure as a

19   continuous variable, statistically significant trends in males for all hematopoietic cancer

20   ($p = 0.02$) and for "lymphoid" cancers (NHL, lymphocytic leukemia, and myeloma; $p = 0.02$)

21   were observed using log cumulative exposure (ppm-days) with a 15-year lag. In internal

22   categorical analyses, statistically significant odds ratios (ORs) were observed in the highest

23   cumulative exposure quartile (with a 15-year lag) in males for all hematopoietic cancer (OR =

24   3.42; 95% CI = 1.09–10.73) and "lymphoid" cancer (OR = 3.76; 95% CI = 1.03–13.64). The

25   exposure metrics of duration of exposure, average concentration, and maximum (8-hour time-

26   weighted average [TWA]) concentration did not predict the hematopoietic cancer results as well

27   as did the cumulative exposure metric.

28   Although the overall SMR for female breast cancer was 0.99, based on 102 deaths, the

29   NIOSH mortality follow-up study reported a significant excess of breast cancer mortality in the

30   highest cumulative exposure quartile using a 20-year lag period compared to the U.S. population

31   (SMR = 2.07; 95% CI = 1.10–3.54; Obs = 13). Internal exposure-response analyses also noted a

32   significant positive trend for breast cancer mortality using the log of cumulative exposure and a

33   20-year lag time ($p = 0.01$). In internal categorical analyses, a statistically significant OR for

34   breast cancer mortality was observed in the highest cumulative exposure quartile with a 20-year

35   lag (OR = 3.13; 95% CI = 1.42–6.92).

1   In summary, although the overall external comparisons did not demonstrate increased
2   risks, the NIOSH investigators found significant internal exposure-response relationships
3   between exposure to EtO and cancers of the hematopoietic system, as well as breast cancer
4   mortality. (Internal comparisons are considered superior to external comparisons in occupational
5   epidemiology studies because internal comparisons help control for the healthy worker effect and
6   other factors that might be more comparable within a study's worker population than between
7   the workers and the general population.) Exposures to other chemicals in the workplace were
8   believed to be minimal or nonexistent. This study is the most useful of the epidemiologic studies
9   in terms of carrying out a quantitative dose-response assessment. It possesses more attributes
10   than the others for performing risk analysis (e.g., good-quality estimates of individual exposure,
11   lack of exposure to other chemicals, and a large and diverse cohort of workers).
12   It should be noted that Steenland et al. (2004) used Cox regression models, which are
13   log-linear relative rate models, thus providing some low-dose sublinear curvature for doses
14   expressed in terms of cumulative exposure. However, the best-fitting dose-response model for
15   both male lymphoid and male all hematopoietic cancers was for dose expressed in terms of log
16   cumulative exposure, indicating supralinearity of the low-dose data. Supralinearity of the dose-
17   response data was also indicated by the categorical exposure results. This is in contrast to the
18   reported results of Kirman et al. (2004) based on the Teta et al. (1999) analysis combining the
19   1993 UCC leukemia data with the 1993 NIOSH leukemia data, which are claimed by the authors
20   to provide empirical evidence supporting a quadratic dose-response relationship. The 2004
21   NIOSH dose-response data for hematopoietic cancers clearly do not provide empirical evidence
22   in support of a quadratic dose-response relationship. On the contrary, the NIOSH data suggest a
23   supralinear dose-response relationship in the observable range.
24   Wong and Trent (1993) investigated the same cohort as Steenland et al. (1991) but added
25   474 new unexplained subjects and increased the follow-up period by one year. They
26   incremented the total number of deaths by 176 and added 392.2 more expected deaths. The only
27   positive finding was a statistically significantly increased risk of NHL among men (SMR = 2.5;
28   Obs = 16; $p < 0.05$). However, there was a deficit risk of NHL among women. For breast
29   cancer, there was no trend of increasing risk by duration of employment or by latency. This
30   study has major limitations, not the least of which is a lack of detailed employment histories,
31   making it impossible to quantify individual exposures and develop dose-response relationships.
32   Furthermore, the addition of more than twice as many expected deaths as observed deaths makes
33   the analysis by the authors questionable.
34   Valdez-Flores et al. (2010) conducted alternative Cox proportional hazards modeling and
35   categorical exposure-response analyses using data from the UCC cohort (Swaen et al., 2009), the
36   NIOSH cohort (Steenland et al., 2004) and the two cohorts combined, analyzing the sexes both

1   separately and together. These investigators reported that they found no evidence of exposure-
2   response relationships for cumulative exposure with either the Cox model or categorical analyses
3   for all of the cohort/endpoint datasets examined (endpoints included all lymphohematopoietic
4   cancers, lymphoid cancers, and female breast cancer, the latter in the NIOSH cohort only).
5   Valdez-Flores et al. (2010) did observe statistically significant increases in response rates in the
6   highest exposure quintile relative to the lowest exposure quintile for lymphohematopoietic and
7   lymphoid cancers in males in the NIOSH cohort, consistent with the categorical results of
8   Steenland et al. (2004), as well as a statistically significant increase in the highest exposure
9   quintile for lymphoid cancers in males and females combined in the NIOSH cohort, consistent
10 with the results in Appendix D. Because the exposure assessment conducted for the UCC cohort
11 is much cruder (see above and Appendix A.3.20), especially for the highest exposures, than the
12 NIOSH exposure assessment (which was based on a validated regression model; see A.3.8), EPA
13 considers the results of exposure-response analyses of the combined cohort data to have greater
14 uncertainty than those from analyses of the NIOSH cohort alone, despite the additional cases
15 contributed by the UCC cohort (e.g., the UCC cohort contributes 17 cases of lymphoid cancer to
16 the 53 from the NIOSH cohort). Furthermore, Valdez-Flores et al. (2010) did not use any log
17 cumulative exposure models, and these were the models that were statistically significant in the
18 Steenland et al. (2004) analyses, consistent with the apparent supralinearity of the NIOSH
19 exposure-response data. See Appendix A.3.20 for a more detailed discussion of the Valdez-
20 Flores analyses and how they compared with the Steenland et al. (2004) analyses.
21   In a mortality study of 1,971 male chemical workers in Italy, 637 of whom were licensed
22 to handle EtO but not other toxic gases, Bisanti et al. (1993) reported statistically significant
23 excesses of hematopoietic cancers (SMR = 7.1, Obs = 5, $p < 0.05$). The study was limited by the
24 lack of exposure measurements and by the young age of the cohort. Although this study
25 suggests that exposure to EtO leads to a significant excess of hematopoietic cancer, the lack of
26 personal exposure measurements and the fact that members were potentially exposed to other
27 chemicals in the workplace lessen its usefulness for establishing the carcinogenicity of EtO.
28   Hagmar et al. (1991, 1995) studied cancer incidence in 2,170 Swedish workers (861 male
29 and 1,309 female) in two medical sterilizing plants. They determined concentrations in six job
30 categories and estimated exposure (ppm-years) for each worker. They found hematopoietic
31 cancers in 6 individuals versus 3.4 expected (SMR = 1.8) and a nonsignificant doubling in the
32 risk when a 10-year latency period was considered. Even though the cohort was young, the
33 follow-up time was short, and only a small fraction of the workers was highly exposed, the report
34 is suggestive. The risk of breast cancer was less than expected (standardized incidence ratio
35 [SIR] = 0.5, Obs = 5). In the latent category of 10 years or more, the risk was even lower (SIR =
36 0.4, Obs = 2).

1   In a large chemical manufacturing plant in Belgium (number of employees not stated),
2   Swaen et al. (1996) performed a nested case-control study of Hodgkin lymphoma to determine
3   whether a cluster of 10 cases in the active male work force was associated with any particular
4   chemical. They found a significant association for benzene and EtO. This study is limited by
5   the exclusion of inactive workers and the potential confounding effect of other chemicals besides
6   EtO, and it is not useful for quantitative dose-response assessment.
7   Olsen et al. (1997) studied 1,361 male employees working in the ethylene and propylene
8   chlorohydrin production and processing areas located within the EtO and propylene oxide
9   production plants at four Dow Chemical Company sites in the United States. Although these
10   investigators found a nonsignificant positive trend between duration of employment as
11   chlorohydrin workers and lymphohematopoietic cancer (Obs = 10), they concluded that there
12   was no appreciable risk in these workers, in contrast to the findings of Benson and Teta (1993).
13   The small cohort size and the lack of data on EtO exposures limit the usefulness of this study in
14   inferring risks due to EtO.
15   Norman et al. (1995) studied 1,132 workers (204 male and 928 female) in a medical
16   sterilizing plant in the United States. In the women, there was a significant excess incidence of
17   breast cancer (SIR = 2.6, Obs = 12, $p < 0.05$); no other cancer sites were elevated. The risk of
18   breast cancer was not noted to be excessive in the few previous studies where adequate numbers
19   of females were included and analyzed for breast cancer; however, only one of these was also an
20   incidence study. The follow-up time was too short to draw meaningful conclusions at this time.
21   This study lacks the power to determine whether risks for cancers other than breast cancer are
22   statistically significantly elevated. It has no information regarding historical exposure and some
23   breast cancer victims had worked for less than one month.
24   Tompa et al. (1999) reported a cluster of 8 breast cancers and 8 other cancers in 98 nurses
25   exposed to EtO in a hospital in Hungary; however, the expected number of cases cannot be
26   identified.
27   The NIOSH investigators used the NIOSH cohort to conduct a study of breast cancer
28   incidence and exposure to EtO (Steenland et al., 2003). The researchers identified 7,576 women
29   from the initial cohort who had been employed in the commercial sterilization facilities for at
30   least 1 year (76% of the original cohort). Breast cancer incidence was determined from
31   interviews (questionnaires), death certificates, and cancer registries. Interviews were obtained
32   for 5,139 women (68% of the study cohort). The main reason for non-response was inability to
33   locate the study subject (22% of cohort). The average duration of exposure for the cohort was
34   10.7 years. For the full study cohort, 319 incident breast cancer cases were identified, including
35   20 cases of carcinoma in situ. Overall, the SIR was 0.87 (0.94 excluding the in situ cases) using
36   Surveillance, Epidemiology, and End Results (SEER) reference rates for comparison. Results

1   with the full cohort are expected to be underestimated, however, because of case

2   underascertainment in the women without interviews. A significant exposure-response trend was

3   observed for SIR across cumulative exposure quintiles, using a 15-year lag time ($p = 0.002$). In

4   internal Cox regression analyses, with exposure as a continuous variable, a significant trend for

5   breast cancer incidence was obtained for log cumulative exposure with a 15-year lag ($p = 0.05$),

6   taking age, race, and year of birth into account. Using duration of exposure, lagged 15 years,

7   provided a slightly better fit ($p = 0.02$), while models with cumulative (non-transformed),

8   maximum or average exposure did not fit as well. In the Cox regression analysis with

9   categorical exposures and a 15-year lag, the top cumulative exposure quintile had a statistically

10   significant OR for breast cancer incidence of 1.74 (95% CI = 1.16–2.65).

11   In the subcohort with interviews, 233 incident breast cancer cases were identified.

12   Information on various risk factors for breast cancer was also collected in the interviews, but

13   only parity and breast cancer in a first-degree relative turned out to be important predictors of

14   breast cancer incidence. In internal analyses with continuous exposure variables, the model with

15   duration of exposure (lagged 15 years) again provided the best fit ($p = 0.006$). Both the

16   cumulative exposure and log cumulative exposure models also yielded significant regression

17   coefficients with a 15-year lag ($p = 0.02$ and $p = 0.03$, respectively), taking age, race, year of

18   birth, parity, and breast cancer in a first-degree relative into account. In the Cox regression

19   analysis with categorical exposures and a 15-year lag, the top cumulative exposure quintile had a

20   statistically significant OR of 1.87 (95% CI = 1.12–3.10).

21   Steenland et al. (2003) suggest that their findings are not conclusive of a causal

22   association between EtO exposure and breast cancer incidence because of inconsistencies in

23   exposure-response trends, possible biases due to non-response, and an incomplete cancer

24   ascertainment. Although that conclusion seems appropriate, those concerns do not appear to be

25   major limitations. As noted by the authors, it is not uncommon for positive exposure-response

26   trends not to be strictly monotonically increasing, conceivably due to random fluctuations or

27   imprecision in exposure estimates. Furthermore, the consistency of results between the full

28   study cohort, which is less subject to non-response bias, and the subcohort with interviews,

29   which should have full case ascertainment, alleviates some of the concerns about those potential

30   biases.

31   In a study of 299 female workers employed in a hospital in Hungary where gas sterilizers

32   were used, Kardos et al. (2003) observed 11 cancer deaths, including 3 breast cancer deaths,

33   compared with slightly more than 4 expected total cancer deaths. Site-specific expected deaths

34   are not available in this study, so it cannot be determined whether there is an excess risk of any

35   site-specific cancer.

36

3.1.1. Conclusions Regarding the Evidence of Cancer in Humans

Most of the human studies suggest a possible increased risk of lymphohematopoietic cancers, but the total weight of the epidemiological evidence does not provide conclusive proof of causality. Of the seven criteria of causality envisioned by Hill (1965), temporality, coherence, and biological plausibility are clearly satisfied. There is also evidence of consistency in the response, of a dose-response relationship (biological gradient), and of specificity when the loosely defined blood malignancies are combined under the rubric "cancer of the hematopoietic system." On the other hand, most of the relative risk estimates are not large (strong) in magnitude.

The large NIOSH study (Steenland et al., 1991, 2004; Stayner et al., 1993) of workers at 14 chemical plants around the country provides the strongest evidence of carcinogenicity. A statistically significant positive trend was observed in the risk of lymphohematopoietic neoplasms with increasing (log) cumulative exposure to EtO, although reportedly only in males (the sex difference is not statistically significant, however, and the trend for both sexes combined is statistically significant; see Appendix D). Despite limitations in the data, most other epidemiologic studies have also found elevated risks of lymphohematopoietic cancer from exposure to EtO. Furthermore, when the exposure is relatively pure, such as in sterilization workers, there is an elevated risk of lymphohematopoietic cancer that cannot be attributed to the presence of confounders such as those that could potentially appear in the chlorohydrin process. Moreover, the studies that do not report a significant lymphohematopoietic cancer effect from exposure to EtO have major limitations, such as small numbers of cases and inadequate exposure information (see Table A-4).

In addition, there is evidence of an increase in the risk of both breast cancer mortality and incidence in women who are exposed to EtO. Studies have reported increases in the risk of breast cancer in women employees of commercial sterilization plants (Steenland et al., 2003, 2004; Norman et al., 1995) as well as in Hungarian hospital workers exposed to EtO (Kardos et al., 2003). In several other studies where exposure to EtO would be expected to have occurred among female employees, no elevated risks were seen (Hagmar et al., 1991; Hogstedt, 1988; Hogstedt et al., 1986; Coggon et al., 2004). However, these studies had far fewer cases to analyze than the NIOSH studies, did not have individual exposure estimates, and relied on external comparisons. The Steenland et al. (2003, 2004) studies, on the other hand, used the largest cohort of women potentially exposed to EtO and clearly show significantly increased risks of breast cancer incidence and mortality based upon internal exposure-response analyses.

In summary, the most compelling evidence of a cancer risk from human exposure to EtO is for cancer of the lymphohematopoietic system. Increases in the risk of lymphohematopoietic cancer are present in most of the studies, manifested as an increase in either leukemia and/or

1 cancer of the lymphoid tissue. The evidence of lymphohematopoietic cancer is strongest in the
2 one study (the NIOSH study) that appears to possess the fewest limitations. In this large study, a
3 significant dose-response relationship was evident with cumulative exposure to EtO. However,
4 this effect was observed only in males and the magnitude of the effect was not large. Similarly,
5 in most of the other studies, the increased risks are not great, and other chemicals in some of the
6 workplaces cannot be ruled out as possible confounders. Thus, the findings of increased risks of
7 lymphohematopoietic cancer in the NIOSH and other studies cannot conclusively be attributed to
8 exposure to EtO. The few studies that fail to demonstrate any increased risks of cancer do not
9 have those strengths of study design that give confidence to the reported lack of an exposure-
10 related effect.
11   There is also evidence of an elevated risk of breast cancer from exposure to EtO in a few
12 studies. The strongest evidence again comes from the NIOSH studies, which found positive
13 exposure-response relationships for both breast cancer incidence and mortality. Hopefully,
14 future studies will shed more light on this more recent finding.
15
16 **3.2.   EVIDENCE OF CANCER IN LABORATORY ANIMALS**
17   The International Agency for Research on Cancer (IARC) monograph (IARC, 1994b) has
18 summarized the rodent studies of carcinogenicity, and Health Canada (2001) has used this
19 information to derive the levels of concern for human exposure. EPA concludes that the IARC
20 summary of the key studies is valid and is not aware of any animal cancer bioassays that have
21 been published since 1994. The Ethylene Oxide Industry Council (EOIC, 2001) also reviewed
22 the same studies and did not cite additional studies. The qualitative results are described here
23 and the incidence data are tabulated in the unit risk derivation section of this document.
24   One study of oral administration in rats has been published; there are no oral studies in
25 mice. Dunkelberg (1982) administered EtO in vegetable oil to groups of 50 female Sprague-
26 Dawley rats by gastric intubation twice weekly for 150 weeks. There were two control groups
27 (untreated and oil gavage) and two treated groups (7.5 and 30 mg/kg-day). A dose-dependent
28 increase in the incidence of malignant tumors in the forestomach was observed in the treated
29 groups (8/50 and 31/50 in the low- and high-dose groups, respectively). Of the 39 tumors, 37
30 were squamous cell carcinomas, and metastases to other organs were common in these animals.
31 This study was not evaluated quantitatively because oral risk estimates are beyond the scope of
32 this document.
33   One inhalation assay was reported in mice (NTP, 1987) and two inhalation assays were
34 reported in rats (Lynch et al., 1982, 1984a, in males; Snellings et al., 1984; Garman et al., 1985,
35 1986, in both males and females). In the National Toxicology Program (NTP) mouse bioassay
36 (NTP, 1987), groups of 50 male and 50 female B6C3F$_1$ mice were exposed to EtO via inhalation

1   at concentrations of 0, 50, and 100 ppm for 6 hours per day, 5 days per week, for 102 weeks.

2   Mean body weights were similar for treated and control animals, and there was no decrease in

3   survival associated with treatment. A concentration-dependent increase in the incidence of

4   tumors at several sites was induced in both sexes. These data are summarized in Table 3-1.

5   Males had carcinomas and adenomas in the lung. Females had carcinomas and adenomas in the

6   lung, malignant lymphomas, adenocarcinomas in the uterus, and adenocarcinomas in the

7   mammary glands. The NTP also reports that both sexes had dose-related increased incidences of

8   cystadenomas of the Harderian glands, but these are benign lesions and are not considered

9   further here.

10   In the Lynch et al. (1982, 1984a) bioassay in male Fischer 344 (F344) rats, groups of 80

11   animals were exposed to EtO via inhalation at concentrations of 0, 50, and 100 ppm for 7 hours

12   per day, 5 days per week, for 2 years. Mean body weights were statistically significantly

13   decreased in both treated groups compared with controls ($p < 0.05$). Increased mortality was

14   observed in the treated groups, and the increase was statistically significant in the 100-ppm

15   exposure group ($p < 0.01$). Lynch et al. (1984a) suggest that survival was affected by a

16   pulmonary infection alone and in combination with EtO exposure. Concentration-dependent

17   increases in the incidence of mononuclear cell leukemia in the spleen, peritoneal mesothelioma

18   in the testes, and glioma in the brain were observed (see Table 3-2). The fact that the increased

19   incidence of mononuclear cell leukemia was statistically significant in the low-exposure group

20   but not in the high-exposure group is probably attributable to the increased mortality in the high-

21   exposure group. The increased incidence in just the terminal kill rats in the 100-ppm group was

22   statistically significant compared with controls.

23   In the bioassay conducted by Snellings et al. (1984), 120 male and 120 female F344 rats

24   in each sex and dose group were exposed to EtO via inhalation at concentrations of 0 (2 control

25   groups of 120 rats of each sex were used), 10, 33, and 100 ppm for 6 hours per day, 5 days per

26   week, for 2 years, with scheduled kills at 6 (10 rats per group), 12 (10 rats per group), and 18 (20

27   rats per group) months. Significant decreases in mean body weight were observed in the 100-

28   ppm exposure group in males and in the 100-ppm and 33-ppm exposure groups in females.

29   During the 15th month of exposure, an outbreak of viral sialodacryoadenitis occurred, resulting

30

Table 3-1. Tumor incidence data in National Toxicology Program Study of B6C3F$_1$ mice (NTP, 1987)[a]

Gender/tumor type	EtO concentration (time-weighted average)[b]			EC$_{10}$ (LEC$_{10}$)[c], (mg/m^3)	Unit risk (0.1/LEC$_{10}$) (per mg/m^3)
	0 ppm	50 ppm (16.3 mg/m^3)	100 ppm (32.7 mg/m^3)		
Males					
Lung adenomas plus Carcinomas	11/49	19/49	26/49[f]	6.94 (4.51)	2.22×10^{-2}
Females					
Lung adenomas plus Carcinomas	2/44	5/44	22/49[g]	14.8 (9.12)	1.1×10^{-2}
Malignant Lymphoma	9/44	6/44	22/49[e]	21.1 (13.9)	7.18×10^{-3}
Uterine Carcinoma	0/44	1/44	5/49[h]	32.8 (23.1)	4.33×10^{-3}
Mammary carcinoma[d]	1/44	8/44[e]	6/49	9.69 (5.35)	1.87×10^{-2}

[a]Incidence data were adjusted by eliminating the animals that died prior to the occurrence of the first tumor or prior to 52 weeks, whichever was earlier.
[b]Adjusted to continuous exposure from experimental exposure conditions of 6 hours/day, 5 days/week; 1 ppm = 1.83 mg/m^3.
[c]Calculated using Tox_Risk program.
[d]Highest dose was deleted while fitting the dose-response data.
[e]$p < 0.05$ (pairwise Fisher's exact test).
[f]$p < 0.01$ (pairwise Fisher's exact test).
[g]$p < 0.001$ (pairwise Fisher's exact test).
[h]$p = 0.058$ by pairwise Fisher's exact test compared to concurrent controls; however, uterine carcinomas are rare tumors in female B6C3F$_1$ mice, and $p < 0.0001$ by pairwise Fisher's exact test compared to the NTP historical control incidence of 1/1077 for inhalation (air) female B6C3F$_1$ mice fed the NIH-07 diet.

Table 3-2. Tumor incidence data in Lynch et al. (1982, 1984a) study of male F344 rats

| Tumor type | Concentration (time-weighted average)[a] | | | EC_{10} (LEC_{10})[b], (mg/m^3) | Unit risk $(0.1/LEC_{10})$ (per mg/m^3) |
	0 ppm	50 ppm $(19.1\ mg/m^3)$	100 ppm $(38.1\ mg/m^3)$		
Splenic mononuclear cell leukemia[c]	24/77	38/79[d]	30/76	7.11 (3.94)	2.54×10^{-2}
Testicular peritoneal mesothelioma	3/78	9/79	21/79[e]	16.7 (11.8)	8.5×10^{-3}
Brain mixed-cell glioma	0/76	2/77	5/79[e]	65.7 (37.4)	2.68×10^{-3}

[a]Adjusted to continuous exposure from experimental exposure conditions of 7 hours/day, 5 days/week; 1 ppm = 1.83 mg/m^3.
[b]Calculated using Tox_Risk program.
[c]Highest dose deleted while fitting the dose-response data.
[d]$p < 0.05$ (pairwise Fisher's exact test).
[e]$p < 0.01$ (pairwise Fisher's exact test).

in the deaths of 1–5 animals per group. Snellings et al. claim that it is unlikely that the viral outbreak contributed to the EtO-associated tumor findings. After the outbreak, mortality rates returned to pre-outbreak levels and were similar for all groups until the 20th or 21st month, when cumulative mortality in the 33-ppm and 100-ppm exposure groups of each sex remained above control values. By the 22nd or 23rd months, mortality was statistically significantly increased in the 100-ppm exposure groups of both sexes.

In males, concentration-dependent increases in the incidence of mononuclear cell leukemia in the spleen and peritoneal mesothelioma in the testes were observed, and in females an increase in mononuclear cell leukemia in the spleen was seen. These data are summarized in Table 3-3. Note that these investigators observed the same types of tumors (splenic leukemia and peritoneal mesothelioma) seen by Lynch et al. (1982, 1984a). Snellings et al. (1984) only report incidences (of incidental and nonincidental primary tumors for all exposure groups) for the 24-month (terminal) kill. However, in their paper they state that significant findings for the mononuclear cell leukemias were also obtained when all rats were included and that a mortality-adjusted trend analysis yielded positive findings for the EtO-exposed females ($p < 0.005$) and males ($p < 0.05$). Similarly, Snellings et al. report that when male rats with unscheduled deaths were included in the analysis of peritoneal mesotheliomas, it appeared that EtO exposure was

1   associated with earlier tumor occurrence, and a mortality-adjusted trend analysis yielded a

2   significant positive trend ($p < 0.005$). In later publications describing brain tumors (Garman et

3   al., 1985, 1986), both males and females had a concentration-dependent increased incidence of

4   brain tumors (see Table 3-3). Garman et al. report incidences including all rats from the 18- and

5   24-month kills and found dead or killed moribund. The earliest brain tumors were observed in

6   rats killed at 18 months.

7

8   **3.2.1. Conclusions Regarding the Evidence of Cancer in Laboratory Animals**

9   In conclusion, EtO causes cancer in laboratory animals. After inhalation exposure to

10   EtO, statistically significant increased incidences of cancer have been observed in both rats and

11   mice, in both males and females, and in multiple tissues (lung, mammary gland, uterus,

12   lymphoid cells, brain, tunica vaginalis testis). In addition, one oral study in rats has been

13   conducted, and a significant dose-dependent increase in carcinomas of the forestomach was

14   reported.

15

16   **3.3.   SUPPORTING EVIDENCE**

17   **3.3.1. Metabolism and Kinetics**

18   Information on the kinetics and metabolism of EtO has been derived primarily from

19   studies conducted with laboratory animals exposed via inhalation, although some limited data

20   from humans have been identified. Details are available in several reviews (Brown et al., 1996,

21   1998; Csanády et al., 2000; Fennell and Brown, 2001).

22   Following inhalation, EtO is absorbed efficiently into the blood and rapidly distributed to

23   all organs and tissues. EtO is metabolized primarily by two pathways (see Figure 3-1): (1)

24   hydrolysis to ethylene glycol (1,2-ethanediol), with subsequent conversion to oxalic acid, formic

25   acid, and carbon dioxide; and (2) glutathione conjugation and the formation of

26   S-(2-hydroxyethyl)cysteine and N-acetylated derivatives (WHO, 2003). From the available data,

27   the route involving conjugation with glutathione appears to predominate in mice; in larger

28   species (including humans), the conversion of EtO is primarily via hydrolysis through ethylene

29   glycol. Because EtO is an epoxide capable of reacting directly with cellular macromolecules,

30   both pathways are considered to be detoxifying.

31   Among rodent species, there are clear quantitative differences in metabolic rates. The

32   rate of clearance of EtO from the blood, brain, muscle, and testes was measured by Brown et al.

33   (1996, 1998). Clearance rates were nearly identical across blood and other tissues. Following a

34   4-hour inhalation exposure to 100 ppm EtO in mice and rats, the average blood elimination

Table 3-3. Tumor incidence data in Snellings et al. (1984) and Garman et al. (1985) reports on F344 rats[a]

| Gender/tumor type | Concentration (time-weighted average)[b] | | | | EC_{10} $(LEC_{10})^d$ (mg/m^3) | Unit risk $(0.1/LEC_{10})$ $(per\ mg/m^3)$ |
	0 ppm[c]	10 ppm $(3.27\ mg/m^3)$	33 ppm $(10.8\ mg/m^3)$	100 ppm $(32.7\ mg/m^3)$		
Males						
Splenic mononuclear cell leukemia	13/97 (13%)[e]	9/51 (18%)	12/39[f] (32%)	9/30[f] (30%)	12.3 (6.43)	1.56×10^{-2}
Testicular peritoneal mesothelioma	2/97 (2.1%)	2/51 (3.9%)	4/39 (10%)	4/30[f] (13%)	22.3 (11.6)	8.66×10^{-3}
Primary brain tumors	1/181 (0.55%)	1/92 (1.1%)	5/85[f] (5.9%)	7/87[g] (8.1%)	36.1 (22.3)	4.5×10^{-3}
Females						
Splenic mononuclear cell leukemia	11/116 (9.5%)	11/54[f] (21%)	14/48[g] (30%)	15/26[h] (58%)	4.46 (3.1)	3.23×10^{-2}
Primary brain tumors	1/188 (0.53%)	1/94 (1.1%)	3/92 (3.3%)	4/80[f] (5%)	63.8 (32.6)	3.07×10^{-3}

[a]Denominators refer to the number of animals for which histopathological diagnosis was performed. For brain tumors Garman et al. (1985) included animals in the 18-month and the 24-month sacrifice and found dead or euthanized moribund of those alive at the time of the first brain tumor, whereas for the other sites Snellings et al. (1984) included animals only at the 24-month sacrifice.
[b]Adjusted to continuous exposure from experimental exposure conditions of 6 hours/day, 5 days/week; 1 ppm = 1.83 mg/m^3.
[c]Results for both control groups combined.
[d]Using Tox_Risk program.
[e]Numbers in parentheses indicate percent incidence values.
[f]$p < 0.05$ (pairwise Fisher's exact test).
[g]$p < 0.01$ (pairwise Fisher's exact test).
[h]$p < 0.001$ (pairwise Fisher's exact test).

$$CH_2 \overset{\displaystyle O}{\text{—}} CH_2$$

Ethylene oxide

Glutathione transferase   *Epoxide hydrolase?*

GSCH$_2$CH$_2$OH
S-2-(hydroxyethyl-glutathione)

HOCH$_2$CH$_2$OH
1,2-ethanediol

HOCH$_2$CHO
hydroxyacetaldehyde

HOCH$_2$CO$_2$H
Glycolic acid

CYS-CH$_2$CH$_2$OH
S-2-(hydroxyethyl)cysteine

OHCCO$_2$H
Glyoxylic acid

N-acethyl-S-(2-hydroxyethyl)
cysteine

HCO$_2$H
Formic acid

CO$_2$HCO$_2$H
Oxalic acid

+

CO$_2$
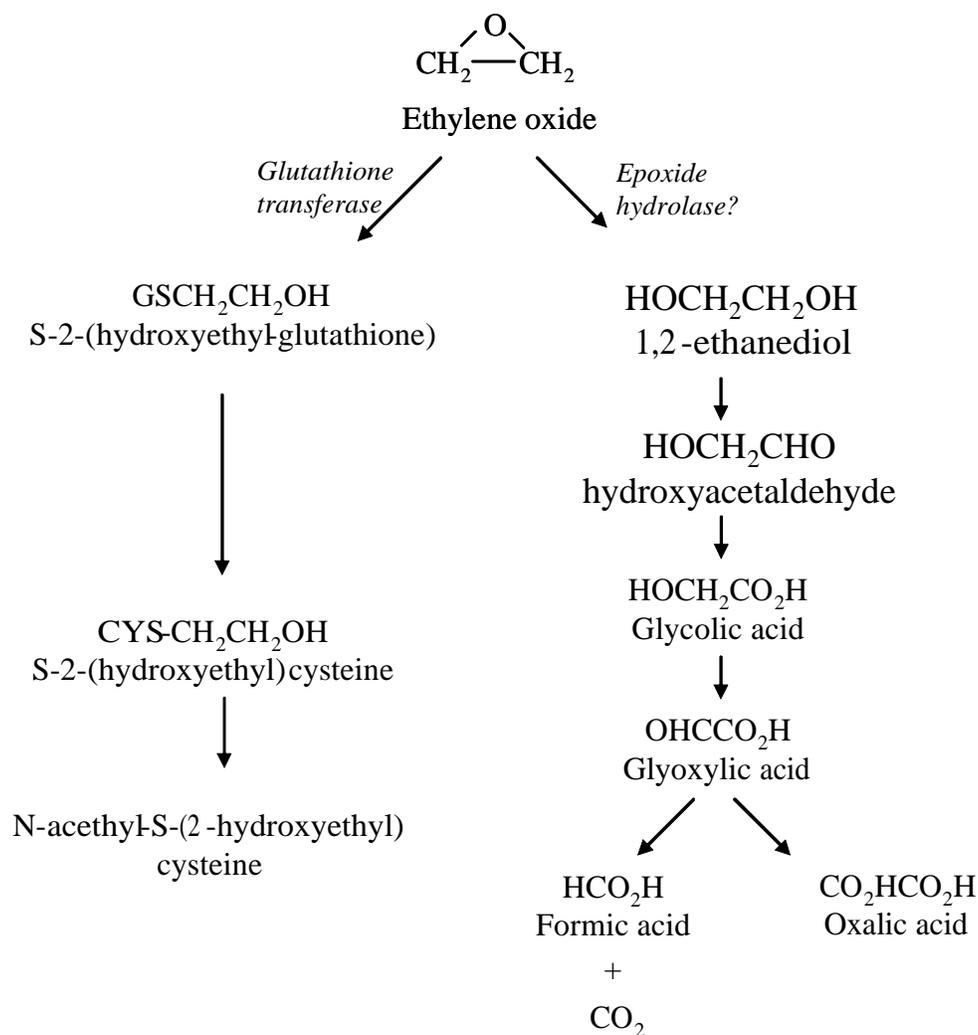
1
2   **Figure 3-1. Metabolism of ethylene oxide.**
3
4
5   half-lives ranged from 2.4 to 3.2 minutes in mice and 11 to 14 minutes in rats. The elimination
6   half-life in humans is 42 minutes (Filser et al., 1992), and the half-life in salt water is 4 days
7   (IARC, 1994b).
8   In a more detailed study in mice, Brown et al. (1998) measured EtO concentrations in
9   mice after 4-hour inhalation exposures at 0, 50, 100, 200, 300, or 400 ppm. They found that
10 blood EtO concentration increased linearly with inhaled concentrations of less than 200 ppm, but
11 above 200 ppm the blood concentration increased more rapidly than linearly. In addition,
12 glutathione levels in liver, lung, kidney, and testes decreased as exposures increased above 200
13 ppm. The investigators interpreted this, along with other information, to mean that at low
14 concentrations the metabolism and disappearance of EtO is primarily a result of glutathione

1 conjugation, but at higher concentrations, when tissue glutathione begins to be depleted, the
2 elimination occurs via a slower non-enzymatic hydrolysis process, leading to a greater-than-
3 linear increase in blood EtO concentration.

4 Fennell and Brown (2001) constructed physiologically based pharmacokinetic (PBPK)
5 models of uptake and metabolism in mice, rats, and humans, based on previous studies. They
6 reported that the models adequately predicted blood and tissue EtO concentrations in rats and
7 mice, with the exception of the testes, and blood EtO concentrations in humans. Modeling
8 6-hour inhalation exposures yielded simulated blood peak concentrations and areas under the
9 curve (AUCs) that are similar for mice, rats, and humans (human levels are within about 15% of
10 rat and mouse levels; see Figure 3-2). In other words, exposure to a given EtO concentration in
11 air results in similar predicted blood EtO AUCs for mice, rats, and humans.

12 These studies show that tissue concentrations in mice, rats, and humans exposed to a
13 particular air concentration of EtO are approximately equal and that they are linearly related to
14 inhalation concentration, at least in the range of exposures used in the rodent cancer bioassays
15 (i.e., 100 ppm and below).

16

17 **3.3.2. Protein Adducts**

18 EtO forms DNA (see Section 3.3.3.1) and hemoglobin adducts within tissues throughout
19 the body (Walker et al., 1992a, b). Formation of hemoglobin adducts has been used as a measure
20 of exposure to EtO. The main sites of alkylation are cysteine, histidine, and the *N*-terminal
21 valine; however, for analytical reasons, the *N*-(2-hydroxyethyl)valine adduct is generally
22 preferred for measurements (Walker et al., 1990). Walker et al. (1992a) reported measurements
23 of this hemoglobin adduct and showed how the concentration of the adducts changes according
24 to the dynamics of red blood cell turnover. Walker et al. (1992a) measured hemoglobin adduct
25 formation in mice and rats exposed to 0, 3, 10, 33, 100, and 300 (rats only) ppm of EtO (6 h/day,
26 5 days/wk, for 4 weeks). Response was linear in both species up to 33 ppm, after which the
27 slope significantly increased. The exposure-related decrease in glutathione concentration in
28 liver, lung, and other tissues observed by Brown et al. (1998) in mice is a plausible explanation
29 for the increasing rate of hemoglobin adduct formation at higher exposures.

30 In humans, hemoglobin adducts can be used as biomarkers of recent exposure to EtO
31 (IARC, 1994b, 2008; Boogaard, 2002), and several studies have reported exposure-response
32 relationships between hemoglobin adduct levels and EtO exposure levels (e.g., Schulte et al.,
33 1992; van Sittert et al., 1993). Hemoglobin adducts are good general indicators of exposure
34 because they are stable (DNA adducts, on the other hand, may be repaired or fixed as mutations
35

1
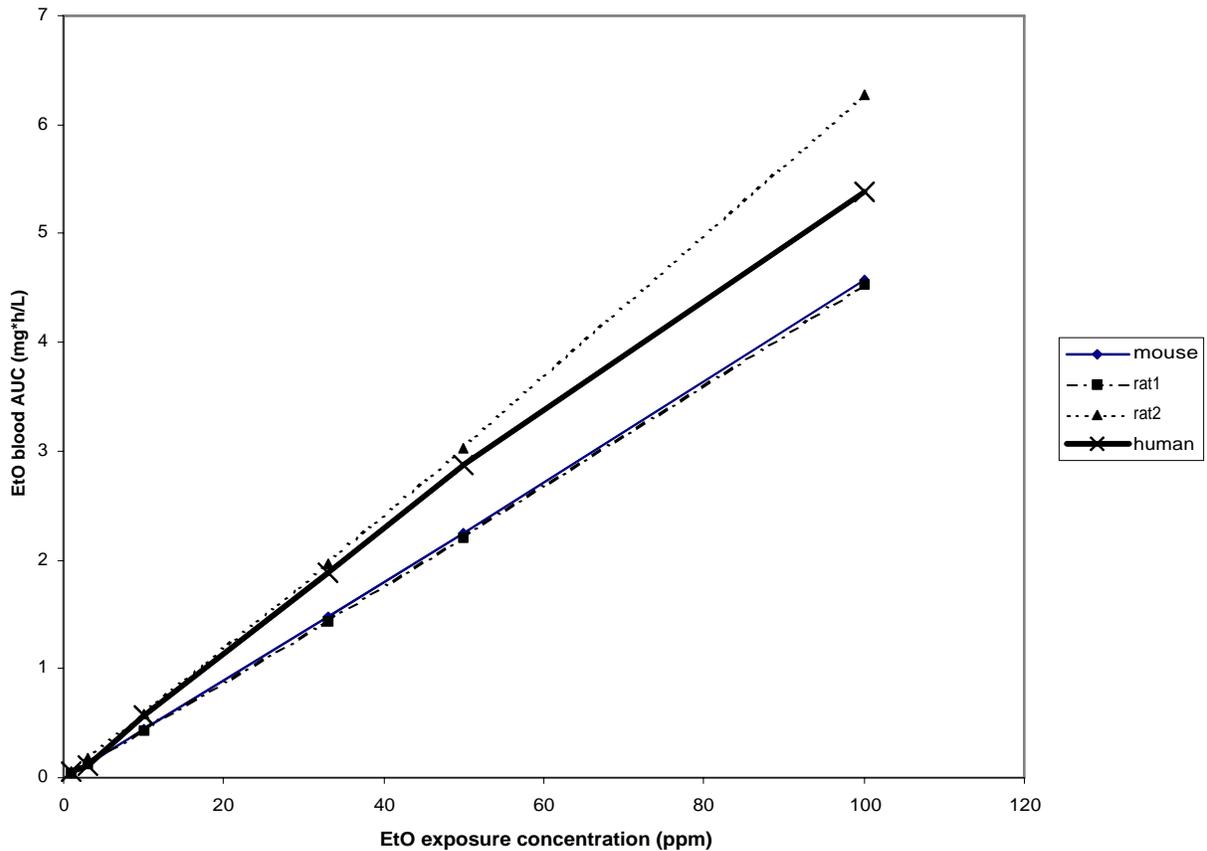
2
3   **Figure 3-2. Simulated blood AUCs for EtO following a 6-hour exposure to**
4   **EtO from the rat, mouse, and human PBPK models of Fennell and Brown**
5   **(2001); based on data presented in Fennell and Brown (2001).** (Rat1 and rat2
6   results use different values for pulmonary uptake.)
7
8
9   and hence are less reliable measures of exposure). However, Föst et al. (1991) noted that human

10 erythrocytes showed marked inter-individual differences in the amounts of EtO bound to

11 hemoglobin, and Yong et al. (2001) reported that levels of *N*-(2-hydroxyethyl)valine were

12 approximately twofold greater in persons with a *GSTT1*-null genotype than in those with positive

13 genotypes. Endogenous ethylene oxide (see Section 3.3.3.1) also contributes to hemoglobin

14 adduct levels, making it more difficult to detect the impacts of low levels of exogenous EtO

15 exposure.   In addition, Walker et al. (1993) reported that hemoglobin adducts in mice and rats

16 were lost at a greater rate than would be predicted by the erythrocyte life span.

3.3.3. Genotoxicity

Since the first report of EtO induction of sex-linked recessive lethals in Drosophila (Rapoport, 1948), numerous papers have been published on the positive genotoxic activity in biological systems, spanning the whole range of assay systems, from bacteriophage to higher plants and animals. Figure 3-3 shows the 203 test entries in the EPA Genetic Activity Profile database in 2001. In prokaryotes and lower eukaryotes, EtO induced DNA damage and gene mutations in bacteria, yeast, and fungi and gene conversions in yeast. In mammalian cells (from in vitro and/or in vivo exposures), EtO-induced effects include unscheduled DNA synthesis, gene mutations, sister chromatid exchanges (SCEs), micronuclei, and chromosomal aberrations. Genotoxicity, in particular increased levels of SCEs and chromosomal aberrations, has also been observed in blood cells of workers occupationally exposed to EtO. Several publications contain details of earlier genetic toxicity studies (e.g., Ehrenberg and Hussain, 1981; Dellarco et al., 1990; Natarajan et al., 1995; Preston et al., 1995; Thier and Bolt, 2000; Kolman et al., 2002; IARC, 1994b, 2008). This review briefly summarizes the evidence of the genotoxic potential of EtO, focusing primarily on recently published studies that provide information on the mode of action of EtO (see Appendix C for more details from some individual studies).

3.3.3.1. *DNA Adducts*

EtO is a direct-acting S_N2 (substitution-nucleophilic-bimolecular)-type monofunctional alkylating agent that forms adducts with cellular macromolecules such as proteins (e.g., hemoglobin, see Section 3.3.2) and DNA (Pauwels and Veulemans, 1998). Alkylating agents may produce a variety of different DNA alkylation products (Beranek, 1990) in varying proportions, depending primarily on the electrophilic properties of the agent. Reactivity of an alkylating agent is estimated by its Swain Scott substrate constant (*s*-value), which ranges from 0 to 1, and EtO has a high *s*-value of 0.96 (Warwick, 1963; Golberg, 1986; Beranek, 1990). Acting by the S_N2 mechanism and having a high substrate constant both favor alkylation at the N7 position of guanine in the DNA (Walker et al., 1990). The predominant DNA adduct formed by EtO and other S_N2-type alkylating agents is N7-(2-hydroxyethyl)guanine (N7-HEG). After in vitro treatment of DNA with EtO, Segerbäck (1990) identified three adducts, N7-HEG, N3-hydroxyethyladenine, and O-6 hydroxyethylguanine, in the ratios 200:8.8:1; two other peaks, suspected of representing other adenine adducts, were also observed at levels well below that of N7-HEG.

Ethylene, an endogenous precursor of EtO, is produced during normal physiological processes. Such processes reportedly include oxidation of methionine and hemoglobin, lipid peroxidation of fatty acids, and metabolism of intestinal bacteria (reviewed in IARC 1994a;

ETHYLENE OXIDE
75-21-8

GENETIC ACTIVITY PROFILE

NCEA-01
2001

LED

0.0001
0.001
0.01
0.1
1.0
10
100
1000
10000

10
100
1000
10000
100000
1000000

HID

PROKARY | LOW EUK | PLNT | INS | MAMM VITRO | HUMAN | F | MAMM VIVO | HU

IARC human carcinogen (group 1: human - limited, animal - sufficient)

1
2 **Figure 3-3**. Display of 203 data sets, including bacteria, fungi, plants, insects,
3 and mammals (in vitro and in vivo), measuring the full range of genotoxic
4 endpoints. **(This is an updated version of the figure in IARC, 1994b.)**
5
6 See Appendix B for list of references.
7
8
9 Thier and Bolt, 2000). EtO is then endogenously produced through the cytochrome P450-
10 mediated conversion of ethylene (Tornqvist, 1996). This endogenous production of EtO
11 contributes significantly to background levels of DNA adducts, making it difficult to detect the
12 impacts of low levels of exogenous EtO exposure on DNA adduct levels. For example, in DNA
13 extracted from the lymphocytes of unexposed individuals, mean background levels of N7-HEG
14 ranged from 2 to 8.5 pmol/mg DNA (Bolt, 1996). Using sensitive detection techniques and an
15 approach designed to separately quantify both endogenous N7-HEG adducts and "exogenous"
16 N7-HEG adducts induced by EtO treatment in rats, Marsden et al. (2009) reported increases in
17 exogenous adducts in DNA of spleen and liver consistent with a linear dose-response
18 relationship (p < 0.05), down to the lowest dose administered (0.0001 mg/kg injected i.p. daily

1   for 3 days). Note that the whole range of doses studied by Marsden et al. (2009) lies well below

2   the dose corresponding to the lowest LOAEL from an EtO cancer bioassay (see Appendix C).

3   Marsden et al. (2009) also observed increases in endogenous N7-HEG adduct formation at the 2

4   highest doses (0.05 and 0.1 mg/kg), suggesting that, in addition to direct adduct formation via

5   alkylation, EtO can induce N7-HEG adduct production indirectly. Marsden et al. (2009)

6   hypothesized that this indirect adduct formation by EtO results from the induction of ethylene

7   generation under conditions of oxidative stress.

8   In experiments with rats and mice exposed to EtO at concentrations of 0, 3, 10, 33, 100,

9   or 300 (rats only) ppm for 6 hours per day, 5 days per week, for 4 weeks, Walker et al. (1992b)

10   measured N7-HEG adducts in the DNA of lung, brain, kidney, spleen, liver, and testes. At 100

11   ppm, the adduct levels for all tissues except testis were similar (within a factor of 3), despite the

12   fact that not all of these tissues are targets for toxicity. The study's data on the persistence of the

13   DNA adducts indicate that DNA repair rates differ in different tissues. Although Walker et al.

14   (1992b) suggested that N7-HEG adducts are likely to be removed by depurination forming

15   apurinic/apyrimidinic (AP) sites in DNA, a later study from the same group showed that EtO-

16   induced DNA damage is repaired without accumulation of AP sites or involving base excision

17   repair (Rusyn et al., 2005). Rats exposed to high doses of EtO (300 ppm) by inhalation showed

18   steady-state levels of O^6-HEG adducts that are ~250-300 times lower than the N7-HEG levels

19   (Walker et al., 1992b). Even though low levels of O^6-HEG adducts were detected, they are more

20   mutagenic in nature and may contribute to the tumors observed in target organs.

21   Two studies provide evidence of N7-HEG DNA adduct formation in human populations

22   occupationally exposed to EtO, one reporting a modest increase in white blood cells (van Delft et

23   al., 1994) and the other a four- to five-fold increase in granulocytes (Yong et al., 2007) compared

24   to unexposed controls. However, these differences were not statistically significant due to high

25   inter-individual variation in adduct levels.

26

27   **3.3.3.2.** *Point Mutations*

28   EtO has consistently yielded positive results in in vitro mutation assays from

29   bacteriophage, bacteria, fungi, yeast, insects, plants, and mammalian cell cultures (including

30   human cells). For example, EtO induces single base pair deletions and base substitutions in the

31   *HPRT* gene in human diploid fibroblasts (Bastlova et al., 1993; Lambert et al., 1994; Kolman

32   and Chovanec, 2000) in vitro. The results of in vivo studies on the mutagenicity of EtO have

33   also been consistently positive following ingestion, inhalation, or injection (e.g., Tates et al.,

34   1999). Increases in the frequency of gene mutations in T-lymphocytes (*Hprt* locus) (Walker et

35   al., 1997) and in bone marrow and testes (*LacI* locus) (Recio et al., 2004) have been observed in

36   transgenic mice exposed to EtO via inhalation at concentrations similar to those in

1   carcinogenesis bioassays with this species (NTP, 1987). At somewhat higher concentrations

2   than those used in the carcinogenesis bioassays (200 ppm, but for only 4 weeks), increases in the

3   frequency of gene mutations have also been observed in the lung of transgenic mice (*LacI* locus)

4   (Sisk et al., 1997) and in T-lymphocytes of rats (*Hprt* locus) (Tates et al., 1999; van Sittert et al.,

5   2000). In in vivo studies with male mice, EtO also causes heritable mutations and other effects

6   in germ cells (Lewis et al., 1986; Generoso et al., 1990).

7   In a study of mammary gland carcinomas in EtO-exposed B6C3F$_1$ mice from the 1987

8   NTP bioassay (NTP, 1987) and 19 mammary gland carcinomas from concurrent controls in the

9   1987 NTP EtO bioassay and a 1986 NTP benzene bioassay, Houle et al. (2006) measured

10   mutation frequencies in exons 5-8 of the *p53* tumor suppressor gene and in codon 61 of the *Hras*

11   oncogene. Mutation frequencies in the mammary carcinomas of EtO-exposed mice were only

12   slightly increased over frequencies in spontaneous mammary carcinomas (33% of the

13   carcinomas in the EtO-exposed mice had *Hras* mutations versus 26% of spontaneous tumors;

14   67% of the carcinomas in the EtO-exposed mice had *p53* mutations versus 58% of spontaneous

15   tumors); however, the EtO-induced tumors exhibited a distinct shift in the mutational spectra of

16   the *p53* and *Hras* genes and more commonly displayed concurrent mutations of the two genes

17   (Houle et al., 2006). Furthermore, Houle et al. (2006) detected about six-fold higher levels of

18   p53 protein expression in the mammary carcinomas of EtO-exposed mice than in spontaneous

19   mammary carcinomas, and there was an apparent dose-response relationship between EtO

20   exposure level and both p53 protein expression and *p53* gene mutation (3 of the 7 tumors in the

21   50-ppm exposure group and all 5 tumors in the 100-ppm group had increased protein expression;

22   also, three *p53* gene mutations were found in the 7 tumors in the 50-ppm exposure group and 9

23   were found in the 5 tumors in the 100-ppm group). Some of the same investigators conducted a

24   similar study of *Kras* mutations in lung, Harderian gland, and uterine tumors (Hong et al., 2007).

25   Substantial increases were observed in *Kras* mutation frequencies in the tumors from the EtO-

26   exposed mice. *Kras* mutations were reported in 100% of the lung tumors from EtO-exposed

27   mice versus 25% of spontaneous lung tumors (108 NTP control animal tumors, including 8 from

28   the EtO bioassay), in 86% of Harderian gland tumors from EtO-exposed mice versus 7% of

29   spontaneous Harderian gland tumors (27 NTP control animal tumors, including 2 from the EtO

30   bioassay), and in 83% of uterine tumors from EtO-exposed mice (there were no uterine tumors in

31   control mice in the 1986 NTP bioassay and none were examined from other control animals).

32   Furthermore, a specific *Kras* mutation, a G → T transversion in codon 12, was nearly universal

33   in lung tumors from EtO-exposed mice (21/23) but rare in lung tumors from control animals

34   (1/108). Other specific mutations were also predominant in the Harderian gland and uterine

35   tumors, but too few *Kras* mutations were available in spontaneous Harderian gland tumors, and

36   no spontaneous uterine tumors were examined; thus, meaningful comparisons could not be made

1   for these sites. Overall, these data strongly suggest that EtO-induced mutations in oncogenes and

2   tumor-supressor genes play a role in EtO-induced carcinogenesis in multiple tissues.

3   Only a few studies have investigated gene mutations in people occupationally exposed to

4   EtO. In one study, *HPRT* mutant frequency in peripheral blood lymphocytes was measured in a

5   group of 9 EtO-exposed hospital workers, a group of 15 EtO-exposed factory workers, and their

6   respective controls (Tates et al., 1991). EtO exposure scenarios suggest higher exposures in the

7   factory workers, and this is supported by the measurement of higher hemoglobin adduct levels in

8   those workers. *HPRT* mutant frequencies were 55% increased in the hospital workers, but the

9   increase was not statistically significant. In the factory workers, a statistically significant

10   increase of 60% was reported. In a study of workers in an EtO production facility (Tates et al.,

11   1995), *HPRT* mutations were measured in three exposed groups and one unexposed group (seven

12   workers per group). No significant differences in mutant frequencies were observed between the

13   groups; however, the authors stated that about 50 subjects per group would have been needed to

14   detect a 50% increase.

15   Major et al. (2001) measured *HPRT* mutations in female nurses employed in hospitals in

16   Eger and Budapest, Hungary. This study and an earlier study measuring effects on chromosomes

17   (see Table 3-4) were conducted to examine a possible causal relationship between EtO exposure

18   and a cluster of cancers (mostly breast) in nurses exposed to EtO in the Eger hospital. The

19   Budapest hospital was chosen because there was no apparent increase in cancer among nurses

20   exposed to EtO. Controls were female hospital workers in the respective cities, and nurses in

21   Eger with known cancers were excluded. Mean peak levels of EtO were 5 mg/m^3 (2.7 ppm) in

22   Budapest and 10 mg/m^3 (5.4 ppm) in Eger. *HPRT* variant frequencies in both controls and

23   EtO-exposed workers in the Eger hospital were higher than either group in the Budapest hospital,

24   but there was no significant increase among the EtO-exposed workers in either hospital when

25   compared with the respective controls. The authors noted that the *HPRT* variant frequencies

26   among smoking EtO-exposed nurses in Eger were significantly higher than among smokers in

27   the Eger controls; however, the fact that the *HPRT* variant frequency was almost three times

28   higher in nonsmokers than in smokers in the Eger hospital control group raises questions about

29   the basis of the claimed EtO effect.

30

31   **3.3.3.3. *Chromosomal Effects***

32   As discussed by Preston (1999) in an extensive review of the cytogenetic effects of EtO,

33   a variety of cytogenetic assays can be used to measure induced chromosome damage. However,

Table 3-4. Cytogenetic effects in humans

Number exposed (number of controls)	Exposure time (years) Range	Mean	Ethylene oxide level in air (ppm)[a] Range	Mean (TWA)	Cytogenetic observations CA	SCE	MN	Reference
33 (0)	1–14		$\pm$0.05–8	$\pm$0.01[b]	(+)			Clare et al., 1985
Site I: 13 Site II: 22 Site III: 25–26 (171 total)			0.5[c] 5–10[c] 5–20[c]		− − +	− + +		Stolley et al., 1984; Galloway et al., 1986
12 (12)			$\pm$36			+		Garry et al., 1979
14 (14)			<0.07–4.3[c]			−		Hansen et al., 1984
Factory I: 18 Factory II: l0 (20 total)	0.5–8 0.5–8	3.2 1.7		<1 <1	+ +	− −	+[d]	Hogstedt et al., 1983
15 smokers (7) 10 nonsmokers (15)	0.5–10 0.5–10	5.7 4.5	20–123 20–123			+ +		Laurent et al., 1984
10 (10)		3	60–69[c]		+	+		Lerda and Rizzi, 1992
Low dose: 9 (48) High dose: 27 (10)		4 15	2.7–10.9 2.7–82	2.7 5.5	+ +	− +		Major et al., 1996
34 (23)		8[e]	<0.1–2.4[c]	<0.3	−	+		Mayer et al., 1991
11 smokers 14 nonsmokers (10 total)			0.5–4l7[f] 0.5–208[f]			− −		Popp et al., 1994
75 (22)	3–14	7	2–5[c]		+		+	Ribeiro et al., 1994
56 (141)	1–10		1–40[c]		+	+		Richmond et al., 1985

Table 3-4. Cytogenetic effects in humans (continued)

Number exposed (number of controls)	Exposure time (years) Range	Mean	Ethylene oxide level in air (ppm)[a] Range	Mean (TWA)	CA	SCE	MN	Reference
22 (22)	0.6–4	3	0.2–0.5[c]	0.35	(+)	+		Sarto et al., 1984a
19 (19)	1.5–15	6.8	3.7–20[c]	10.7	+	+		
10 (10)			0–9.3[c]	1.84		+		Sarto et al., 1987
9	0.5–12	5	0.025–0.38[c]				–	Sarto et al., 1990
3			>0.38[g]				+[h]	
(27 total)								
5	0.1–4	2		0.025		–	–[i]	Sarto et al., 1991
5	4–12	8.6	<1–4.4	0.38		+	–[i]	
(10 total)								
32		5.1	0–0.3[c]	0.04		+	–	Schulte et al., 1992
11		9.5	0.1 3–0.3[c]	0.16		+	–	
(8 total)								
9 hospital workers (8)	2–6	4	20–25		+	+	–	Tates et al., 1991
15 factory workers (15)	3–27	12	17–33		+	+	+	
7	Accidental		28–429[c]			–	–	Tates et al., 1995
7	<5		<0.005–0.02			–	–	
7	>15		<0.005–0.01			–	–	
(7 total)								
Low exposure: 9				13[j]		–		Yager et al., 1983
High exposure: 5				501[j]		+		
(13 total)								

Table 3-4. Cytogenetic effects in humans (continued)

Number exposed (number of controls)	Exposure time (years)		Ethylene oxide level in air (ppm)[a]		Cytogenetic observations			Reference
	Range	Mean	Range	Mean (TWA)	CA	SCE	MN	
19 17 (35 total)	1–5 6–14		<0.05–8 <0.05–8	<0.05 <0.05	– –			van Sittert et al., 1985

[a]1 ppm = 1.83 mg ethylene oxide/m^3.
[b]Calculated by linear extrapolation.
[c]TWA (8-hour).
[d]Positive for erythroblasts and polychromatic erythrocytes (negative for lymphocytes).
[e]Maximum years exposed.
[f]Peak concentrations.
[g]Exposed acutely from sterilizer leakage in addition to chronic exposure.
[h]Nasal mucosa.
[i]Buccal cells.
[j]Average 6-month cumulative exposure (mg).

CA = chromosomal aberrations
MN = micronucleus
SCE = sister chromatid exchange
TWA = time-weighted average

1   most of the assays commonly employed measure events that are detectable only in the first (or in
2   some cases the second) metaphase after exposure and require DNA synthesis to convert DNA
3   damage into a chromosomal aberration. In addition, DNA repair is operating in peripheral
4   lymphocytes to repair induced DNA damage. Thus, for acute exposures, the timing of sampling
5   is of great importance. For chronic studies, the endpoints measure only the most recent
6   exposures, and if the time between last exposure and sampling is long, any induced DNA
7   damage not converted to a stable genotoxic alteration is certain to be missed. The events
8   measured include all types of chromosomal aberrations, micronuclei, SCE, and numerical
9   chromosomal changes. Stable chromosomal aberrations include reciprocal translocations,
10   inversions, and some fraction of insertions and deletions as well as some numerical changes.
11   However, until the development of fluorescent in situ hybridization (FISH), chromosome
12   banding techniques were needed to detect these types of aberrations.
13   In in vitro assays, EtO has consistently tested positive in studies for multiple types of
14   chromosomal effects, including DNA strand breaks, SCEs, micronuclei, and chromosomal
15   aberrations (see, e.g., Table 11 of IARC, 2008). Of note, Ádám et al. (2005) measured the
16   sensitivity of different human cell types to EtO-induced DNA damage using the comet assay,
17   which measures direct strand breaks and/or DNA damage converted to strand breaks during
18   alkaline treatment. Ádám at al. reported dose-dependent increases in DNA damage in the
19   concentration range 0 – 100 μM in each of the cell types examined with no notable cytotoxicity.
20   At the lowest concentration reported (20 μM), significant increases in DNA damage were
21   observed in lymphoblasts, lymphocytes, and breast epithelial cells, but not in keratinocytes or
22   cervical epithelial cells, suggesting that breast epithelial cells may have increased sensitivity to
23   EtO-induced genotoxicity compared to other non-lymphohematopoietic cell types. In addition,
24   Godderis et al. (2006) investigated the effects of genetic polymorphisms on DNA damage
25   induced by EtO in peripheral blood lymphocytes of 20 nonsmoking university students. No
26   significant increases in micronuclei were observed following EtO treatment; however, dose-
27   related increases in DNA strand breaks were seen in the comet assay. GST polymorphisms did
28   not have a significant impact on the EtO-induced effects; however, significant increases in DNA
29   strand breaks were associated with low-activity alleles of two DNA repair enzymes compared to
30   wild type alleles.
31   In vivo, several inhalation studies in laboratory animals have demonstrated that EtO
32   exposure levels in the range of those used in the rodent bioassays induce SCEs (see Table 11 of
33   IARC, 2008); however, evidence for micronuclei and chromosomal aberrations from these same
34   exposure levels is less consistent. In particular, studies by van Sittert et al. (2000) and Lorenti
35   Garcia et al. (2001) observed increases in micronuclei and chromosomal aberrations in splenic
36   lymphocytes of rats exposed to 50, 100, or 200 ppm EtO for 6 hours/day, 5 days/week, for 4

1 weeks compared to levels from control rats, but the increases were not statistically significant.

2 IARC (2008) noted, however, that "strong conclusions cannot be drawn" from these two studies

3 because the cytogenetic analyses "were initiated 5 days after the final day of exposure, a

4 suboptimal time, and the power of the (FISH) studies were limited by analysis of only a single

5 chromosome and the small numbers of rats per group examined", which was 3 per exposure

6 group in both of the studies, although numerous cells/rat were examined. Moreover, a recent

7 study by Donner et al. (2010) showed clear, statistically significant increases in chromosomal

8 aberrations with longer durations of exposure ($\geq$ 12 weeks) to the concentration levels used in

9 the rodent bioassays.

10   In humans, various studies of occupationally exposed workers have reported SCEs and

11 other chromosomal effects associated with EtO exposure, including micronuclei and

12 chromosomal aberrations. The genotoxicity of EtO was demonstrated in humans as early as

13 1979. Table 3-4 summarizes the cytogenetic effects of EtO on human exposures (see also

14 Appendix C for more details on some of the studies).

15   As illustrated in Table 3-4, numerous studies observed increased SCEs in occupationally

16 exposed workers, especially for workers with the highest exposures (e.g., Sarto et al., 1987,

17 1991; Tates et al., 1991; Major et al., 1996). Several studies of occupationally exposed workers

18 have also reported increased micronucleus formation in lymphocytes (Tates et al., 1991; Ribeiro

19 et al., 1994), in nasal mucosal cells (Sarto et al., 1990), and in bone marrow cells (Hogstedt et al.,

20 1983), although this endpoint seems to be less sensitive than SCEs. An association between

21 increased micronucleus frequency and cancer risk has been reported in at least one large

22 prospective general population study (Bonassi et al., 2007). In addition, chromosomal

23 aberrations have been reported in multiple studies of workers occupationally exposed to EtO

24 (Sarto et al., 1987; Tates et al., 1991; Ribeiro et al., 1994). Chromosomal aberrations have been

25 linked to an increased risk of cancer in several large prospective general population studies (e.g.,

26 Liou et al., 1999; Hagmar et al., 2004; Rossner et al., 2005; Boffetta et al., 2007).

27

28 **3.3.3.4.** *Summary*

29   The available data from in vitro studies, laboratory animal models, and epidemiological

30 studies establish that EtO is a mutagenic and genotoxic agent that causes a variety of types of

31 genetic damage.

32

33 **3.4.   MODE OF ACTION**

34   EtO is an alkylating agent that has consistently been found to produce numerous

35 genotoxic effects in a variety of biological systems ranging from bacteriophage to occupationally

36 exposed humans. It is carcinogenic in mice and rats, inducing tumors of the

1 lymphohematopoietic system, brain, lung, connective tissues, uterus, and mammary gland. In

2 addition, epidemiological studies have shown an increased risk of various types of human

3 cancers (Table A-4), in particular lymphohematopoietic and breast cancers. Target tissues for

4 EtO carcinogenicity in laboratory animals are varied, and the cancers are not clearly attributable

5 to any specific type of genetic alteration. Although the precise mechanisms by which the multi-

6 site carcinogenicity in mice, rats, and humans occurs are unknown, EtO is clearly a mutagenic

7 and genotoxic agent, as discussed in Section 3.3.3, and mutagenicity and genotoxicity are well

8 established to play a key role in carcinogenicity.

9   Exposure of cells to DNA-reactive agents results in the formation of carcinogen-DNA

10 adducts. The formation of DNA adducts results from a sequence of events involving absorption

11 of the agent, distribution to different tissues, and accessibility of the molecular target (Swenberg

12 et al., 1990). Alkylating agents may induce several different DNA alkylation products (Beranek,

13 1990) with varying proportions, depending primarily on the electrophilic properties of the agent.

14 The predominant DNA adduct formed by EtO is N7-HEG, although other adducts, such as N3-

15 hydroxyethyladenine and O-6 hydroxyethylguanine, have also been observed, in much lesser

16 amounts (Zhao et al., 1997). In addition to direct DNA adduct formation via alkylation, Marsden

17 et al. (2009) observed an indirect effect of EtO exposure on endogenous N7-HEG adduct

18 formation and hypothesized that EtO could also indirectly cause adduct formation via oxidative

19 stress (see also Section 3.3.3.1 and Appendix C).   The various adducts are processed by different

20 repair pathways, and the subsequent genotoxic responses elicited by unrepaired DNA adducts are

21 dependent on a wide range of variables. The specific adduct(s) responsible for EtO-induced

22 genotoxicity and the mechanism(s) by which this adduct(s) induces the genotoxic damage are

23 unknown.

24   It had been postulated that the predominant EtO-DNA adduct, N7-HEG, although

25 unlikely to be directly promutagenic, could be subject to depurination, resulting in an apurinic

26 site which could be vulnerable to miscoding during cell replication (e.g., Walker and Skopek,

27 1993). However, in a study designed to test this hypothesis, Rusyn et al. (2005) failed to detect

28 an accumulation of abasic sites in brain, spleen, and liver tissues of rats exposed to EtO. Rusyn

29 et al. (2005) conclude that the accumulation of abasic sites is unlikely to be a primary

30 mechanism for EtO mutagenicity, although they note that it is also possible that their assay was

31 not sufficiently sensitive to detect small increases in abasic sites or that abasic sites are only

32 mutagenic under conditions of rapid cell turnover, when cell replication may occur before repair

33 of the abasic site (the tissues examined in their study were relatively quiescent). Another

34 potential mechanism for EtO-induced mutagenicity is the direct mutagenicity of the

35 promutagenic adducts such as O-6 hydroxyethylguanine, although these adducts are generally

36 considered to occur at levels too low to explain all of the observed mutagenicity (IARC, 2008).

1   The events involved in the formation of chromosomal damage by EtO are similarly
2   unknown. N–alklylated bases are removed from DNA by base excision repair pathways. A
3   review by Memisoglu and Samson (2000) notes that the action of DNA glycosylase and apurinic
4   endonuclease creates a DNA single-strand break, which can in turn lead to DNA double-strand
5   breaks (DSBs). DSBs can also be produced by normal cellular functions, such as during V(D)J
6   recombination in the development of lymphoid cells or topoisomerase II-mediated cleavage at
7   defined sites. A review of mechanisms of DSB repair indicates that the molecular mechanisms
8   are not fully understood (Pfeiffer et al., 2000). This review provides a thorough discussion of
9   both sources (endogenous and exogenous) of DSBs and the variety of repair pathways that have
10 evolved to process the breaks. Although homology-directed repair generally restores the original
11 sequence, during nonhomologous end-joining, the ends of the breaks are frequently modified by
12 addition or deletion of nucleotides. The lack of accumulation of abasic sites observed in the
13 Rusyn et al. (2005) study discussed above argues against a mechanism involving abasic sites as
14 hot spots for strand breaks, although it is possible that abasic sites accumulate more readily in
15 replicating lymphocytes, which were not examined in the study of Rusyn et al. (2005). Another
16 postulated mechanism for EtO-induced strand breaks is via the formation of hydroxyethyl
17 adducts on the phosphate backbone of the DNA, but this mechanism requires further study
18 (IARC, 2008).
19   Lymphohematopoietic malignancies, like all other cancers, are considered to be a
20 consequence of an accumulation of genetic and epigenetic changes involving multiple genes and
21 chromosomal alterations. Although it is clear that chromosome translocations are common
22 features of some hematopoietic cancers, there is evidence that mutations in *p53* or *NRAS* are
23 involved in certain types of leukemia (U.S. EPA, 1997). It should also be noted that therapy-
24 related leukemias exhibiting reciprocal translocations are generally only seen in patients who
25 have previously been treated with chemotherapeutic agents that act as topoisomerase II inhibitors
26 (U.S. EPA, 1997). In NHL, the *BCL6* gene is frequently activated by translocations (Chaganti et
27 al., 1998) as well as by mutations within the gene coding sequence (Lossos and Levy, 2000).
28 Preudhomme et al. (2000) observed point mutations in the *AML1* gene in 9 of 22 patients with
29 the M0 type (minimally differentiated acute myeloblastic leukemia) of acute myeloid leukemia
30 (AML), and Harada et al. (2003) identified *AML1* point mutations in cases of radiation-
31 associated and therapy-related myelodysplastic syndrome (MDS)/AML. In both reports, point
32 mutations within the coding sequence were found in patients with normal karyotypes as well as
33 some with translocations or other chromosomal abnormalities. Zharlyganova et al. (2008)
34 identified *AML1* mutations in 7 of 18 radiation-exposed MDS/AML patients but in none of 13
35 unexposed MDS/AML cases. Other point mutations have also been identified in therapy-related
36 MDS/AML patients, including *p53* gene mutations after exposure to alkylating agents

1 (Christiansen et al., 2001) and mutations in *RAS* and other genes in the receptor tyrosine kinase

2 signal transduction pathway (Christiansen et al., 2005). Several models have been developed to

3 integrate these various types of genetic alterations. One recent model suggests that the

4 pathogenesis of MDS/AML can be subdivided into at least eight genetic pathways that have

5 different etiologies and different biologic characteristics (Pedersen-Bjergaard et al., 2006).

6 A mode-of-action-motivated modeling approach based solely on chromosome

7 translocations has been proposed by Kirman et al. (2004). The authors suggested a nonlinear

8 dose-response for EtO and leukemia, based on a consideration that "chromosomal aberrations are

9 the characteristic initiating events in chemically induced acute leukemia and gene mutations are

10 not characteristic initiating events." They proposed that EtO must be responsible for two nearly

11 simultaneous DNA adducts, yielding a dose-squared (quadratic) relationship between EtO

12 exposure and leukemia risk. However, as discussed above, there is evidence that does not

13 support the assumption that chromosomal aberrations represent the sole initiating event. In fact,

14 these aberrations or translocations could be a downstream event resulting from genomic

15 instability. In addition, it is not clear that acute leukemia is the lymphohematopoietic cancer

16 subtype associated with EtO exposure; in the large NIOSH study, increases in

17 lymphohematopoietic cancer risk were driven by increases in lymphoid cancer subtypes.

18 Furthermore, even if two reactions with DNA resulting in chromosomal aberrations or

19 translocations are early-occurring events in some EtO-induced lymphohematopoietic cancers, it

20 is not necessary that both events be associated with EtO exposure (e.g., background error repair

21 rates or exposure to other alkylating agents may be the cause). Moreover, EtO could also

22 produce translocations indirectly by forming DNA or protein adducts that affect the normally-

23 occurring recombination activities of lymphocytes or the repair of spontaneous double-strand

24 breaks. Thus, broader mode-of-action considerations were not regarded as supportive of the

25 hypothesis that the exposure-response relationship is purely quadratic.

26 Breast cancer is similarly considered to be a consequence of an accumulation of genetic

27 and epigenetic changes involving multiple genes and chromosomal alterations (Ingvarsson,

28 1999). Again, the precise mechanisms by which EtO induces breast cancer are unknown. As

29 discussed in Section 3.3.3.2, in a study of mammary gland carcinomas in EtO-exposed mice,

30 Houle et al. (2006) noted that the EtO-induced tumors exhibited a distinct shift in the mutational

31 spectra of the *p53* and *Hras* genes and more commonly displayed concurrent mutations of the

32 two genes.

33 In summary, EtO induces a variety of types of genetic damage. It directly interacts with

34 DNA, resulting in DNA adducts, gene mutations, and chromosome damage. Depending on a

35 number of variables, EtO-induced DNA adducts (1) may be repaired, (2) may result in a base-

36 pair mutation during replication, or (3) may be converted to a DSB, which also may be repaired

1 or result in unstable (micronuclei) or stable (translocation) cytogenetic damage. All of the

2 available data are strongly supportive of a mutagenic mode of action involving gene mutations

3 and chromosomal aberrations (translocations, deletions, or inversions) that critically alter the

4 function of oncogenes or tumor suppressor genes. Although it is clear that chromosome

5 translocations are common features of many hematopoietic cancers, there is evidence that

6 mutations in *p53*, *AML1*, or *Nras* are also involved in some leukemias. The current scientific

7 consensus is that there is very good correspondence between ability of an agent to cause

8 mutations, as does EtO, and carcinogenicity. All of the above scientific evidence provides

9 support for a mutagenic mode of action.

10

3.4.1. Analysis of the Mode of Action for Ethylene Oxide Carcinogenicity Under EPA's Mode of Action Framework

13   In this section, the mode of action evidence for EtO carcinogenicity is analyzed under the

14 mode of action framework in EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S.

15 EPA, 2005a, Section 2.4.3).

16   The *hypothesis* is that EtO carcinogenicity has a mutagenic mode of action. This

17 hypothesized mode of action is presumed to apply to all of the tumor types.

18   The *key events* in the hypothesized mutagenic mode of action are DNA adduct formation

19 by EtO, which is a direct-acting alkylating agent, and the resulting genetic damage, including the

20 formation of point mutations as well as chromosomal alterations. Mutagenicity is a well

21 established cause of carcinogenicity.

22

23 1. *Is the hypothesized mode of action sufficiently supported in the test animals?*

24   Numerous studies have demonstrated that EtO forms protein and DNA adducts, in mice

25 and rats (see Sections 3.3.1 and 3.4 and Figure 3-2). For example, Walker et al. (1992a, b)

26 demonstrated that EtO forms protein adducts with hemoglobin in the blood and DNA adducts

27 with tissues throughout the body, including in the lung, brain, kidney, spleen, liver, and testes.

28   In addition, there is incontrovertible evidence that EtO is mutagenic (see Section 3.3.3).

29 The evidence is *strong* and *consistent*; EtO has invariably yielded positive results in in vitro

30 mutation assays from bacteriophage, bacteria, fungi, yeast, insects, plants, and mammalian cell

31 cultures. The results of in vivo studies on the mutagenicity and genotoxicity of EtO have also

32 been consistently positive following ingestion, inhalation, or injection. Increases in the

33 frequency of gene mutations in the lung, in T-lymphocytes, in bone marrow, and in testes have

34 been observed in transgenic mice exposed to EtO via inhalation at concentrations similar to those

35 in the mouse carcinogenesis bioassays. Furthermore, in a study of *p53* (tumor supressor gene)

36 and *Hras* (oncogene) mutations in mammary gland carcinomas of EtO-exposed and control

1   mice, Houle et al. (2006) noted that the EtO-induced tumors exhibited a distinct shift in the

2   mutational spectra of the *p53* and *Hras* genes and more commonly displayed concurrent

3   mutations of the two genes, and, in a similar study of *Kras* (oncogene) mutations in lung,

4   Harderian gland, and uterine tumors, substantial increases were observed in *Kras* mutation

5   frequencies in the tumors from the EtO-exposed mice (Hong et al., 2007).

6   Ethylene oxide induces a variety of mutagenic and genotoxic effects, including

7   chromosome breaks, micronuclei, SCEs, and gene mutations; however, the more general effect

8   of mutagenicity/genotoxicity is *specific* and occurs in the absence of cytotoxicity or other overt

9   toxicity. A *temporal relationship* is also clearly evident, with adducts and mutagenicity

10   observed in subchronic assays.

11   *Dose-response relationships* have been observed between EtO exposure in vivo and

12   hemoglobin adducts (e.g., Walker et al., 1992a), as well as DNA adducts, SCEs, and *Hprt*

13   mutations (e.g., van Sittert et al., 2000) (see also Sections 3.3 and 3.4). A mutagenic mode of

14   action for EtO carcinogenicity also clearly comports with notions of *biological plausibility* and

15   *coherence* because EtO is a direct-acting alkylating agent. Such agents are generally capable of

16   forming DNA adducts, which in turn have the potential to cause genetic damage, including

17   mutations; and mutagenicity, in its turn, is a well-established cause of carcinogenicity. This

18   chain of key events is consistent with current understanding of the biology of cancer.

19   In addition to the clear evidence supporting a mutagenic mode of action in test animals,

20   there are no compelling alternative or additional hypothesized modes of action for EtO

21   carcinogenicity.

22

23   2. *Is the hypothesized mode of action relevant to humans?*

24   The evidence discussed above demonstrates that EtO is a systemic mutagen in test

25   animals; thus, there is the presumption that it would also be a mutagen in humans. Moreover,

26   there is human evidence directly supporting a mutagenic mode of action for EtO carcinogenicity.

27   Several studies of humans have reported exposure-response relationships between hemoglobin

28   adduct levels and EtO exposure levels (e.g., Schulte et al., 1992; van Sittert et al., 1993; see

29   Section 3.3.2), demonstrating the ability of EtO to bind covalently in systemic human cells, as it

30   does in rodent cells. DNA adducts in EtO-exposed humans have not been well studied, and the

31   evidence of increased DNA adducts is limited.

32   In addition, EtO has yielded positive results in in vitro mutagenicity studies of human

33   cells (see Figure 3-3). Although the studies of point mutations in EtO-exposed humans are few

34   and insensitive and the evidence for mutations is limited, there is clear evidence from a number

35   of human studies that EtO causes chromosomal aberrations, SCEs, and micronucleus formation

36   in peripheral blood lymphocytes (see Section 3.3.3.3 and Table 3-4). At least one study

1 suggested an exposure-response relationship for the formation of SCEs in peripheral blood
2 lymphocytes (Major et al., 1996). Another study reported a statistically significant increase in
3 micronuclei in bone marrow cells in EtO-exposed workers (Hogstedt et al., 1983).
4   Finally, there is strong evidence that EtO causes cancer in humans, including cancer
5 types observed in rodent studies (i.e., lymphohematopoietic cancers and breast cancer),
6 providing further weight to the relevance of the aforementioned events to the development of
7 cancer in humans (see Sections 3.1 and 3.5.1).
8   *In conclusion, the weight of evidence supports a mutagenic mode of action for EtO*
9 *carcinogenicity.*
10
11 3. *Which populations or lifestages can be particularly susceptible to the hypothesized mode of*
12   *action?*
13   The mutagenic mode of action is considered relevant to all populations and lifestages.
14 According to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life*
15 *Exposure to Carcinogens,* hereinafter referred to as "EPA's *Supplemental Guidance*" (U.S. EPA,
16 2005b), there may be increased susceptibility to early-life exposures to carcinogens with a
17 mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic
18 mode of action for EtO carcinogenicity, and in the absence of chemical-specific data to evaluate
19 differences in susceptibility, increased early-life susceptibility should be assumed and, if there is
20 early-life exposure, the age-dependent adjustment factors should be applied, in accordance with
21 the *Supplemental Guidance* (see Section 4.4).
22   In addition, as discussed in Section 3.5.2, people with DNA repair deficiencies or genetic
23 polymorphisms conveying a decreased efficiency in detoxifying enzymes may have increased
24 susceptibility to EtO-induced carcinogenicity.
25
26 **3.5.   HAZARD CHARACTERIZATION**
27 **3.5.1. Characterization of Cancer Hazard**
28   In humans there is substantial evidence that EtO exposure is causally associated with
29 lymphohematopoietic cancer, but the evidence is not strong enough to be conclusive. The
30 strongest evidence comes from a high-quality study of a large NIOSH cohort. Of the seven
31 relevant Hill "criteria" (or considerations) for causality (Hill, 1965), *temporality*, *coherence*, and
32 *biological plausibility* are largely satisfied. There is evidence of *consistency* between studies
33 with respect to cancer of the lymphohematopoietic system as a whole. There is some evidence
34 of a dose-response relationship (*biological gradient*), particularly in males. There is little
35 *strength* in the magnitude of most of the risk estimates.

1   Most of the relevant studies focus on examining risks of cancer associated with

2   subcategories of the lymphohematopoietic organ system. These cancers include leukemia and its

3   various forms (i.e., myeloid or lymphocytic) and also Hodgkin lymphoma, NHL,

4   reticulosarcoma, and myeloma. One study has focused on "lymphoid cancer," which is a

5   combination of lymphocytic leukemia, NHL, and myeloma. No other study has examined the

6   risk of this particular combination. In this study, risk of cancer of the lymphoid tissue was

7   significantly elevated in subgroups of the workforce likely to have received the highest

8   exposures to EtO. Elevated risks of other subcategories of the hematopoietic system—either

9   singly or in combination—have sometimes, but not always, appeared in other studies.

10   In most of these studies, when all the subcategories are combined, an enhanced risk of

11   cancer of the lymphohematopoietic system is evident, and in some studies, it is significant.

12   Hence there is some *specificity* with respect to the lymphohematopoietic system. Moreover, the

13   *specificity* criterion is not expected to be satisfied by agents, such as EtO, that are not only

14   widely distributed in all tissues but are also directly acting chemicals.

15   There is also recent evidence of an increased breast cancer risk in females from exposure

16   to EtO. This evidence comes predominantly from high-quality studies of the large NIOSH

17   cohort, in which positive exposure-response relationships for both breast cancer incidence and

18   mortality were observed. The criteria of temporality, coherence, and biological plausibility are

19   also satisfied. On the other hand, the magnitudes of the risk were not large, and none of the other

20   studies had enough breast cancer cases to be very informative.

21   Stomach cancer was noted in the earlier Hogstedt studies but is not found in recent

22   studies. Pancreatic cancer was observed in some studies and not others, and some studies

23   observed no EtO-related cancer risks.

24   The experimental animal evidence for carcinogenicity is concluded to be "sufficient"

25   based on findings of tumors at multiple sites, by both oral and inhalation routes of exposure, and

26   in both sexes of both rats and mice. Tumor types resulting from inhalation exposure included

27   mononuclear cell leukemia in male and female rats and malignant lymphoma and mammary

28   carcinoma in female mice, suggesting some site concordance with the lymphohematopoietic and

29   breast cancers observed in humans, also exposed by inhalation.

30   The evidence of EtO genotoxicity and mutagenicity is unequivocal. EtO is a direct-

31   acting alkylating agent and has invariably tested positive in in vitro mutation assays from

32   bacteriophage, bacteria, fungi, yeast, insects, plants, and mammalian cell cultures (including

33   human cells). In mammalian cells (including human cells), EtO-induced genotoxic effects

34   include unscheduled DNA synthesis, gene mutations, SCEs, and chromosomal aberrations. The

35   results of in vivo genotoxicity studies of EtO have also been largely positive, following

36   ingestion, inhalation, or injection. Increases in frequencies of gene mutations have been reported

1   in the lung, T-lymphocytes, bone marrow, and testes of EtO-exposed mice. In particular,
2   increases in frequencies of oncogene mutations have been observed in several tumor types from
3   EtO-exposed mice compared to spontaneous mouse tumors of the same types. Several inhalation
4   studies in laboratory animals have demonstrated that EtO exposure levels in the range of those
5   used in the rodent bioassays (i.e., 10-100 ppm, 6-7 h/day, 5 days/week) induce SCEs. Evidence
6   for micronuclei and chromosomal aberrations from these same exposure levels in short-term
7   studies (4 weeks or less) is less consistent, although concerns have been raised about some of the
8   negative studies. A recent study showed clear, statistically significant increases in chromosomal
9   aberrations with longer durations of exposure ($\geq$ 12 weeks) to the concentration levels used in
10 the rodent bioassays. The studies of point mutations in EtO-exposed humans are few and
11 insensitive and the evidence for mutations is limited; however, there is clear evidence from a
12 number of human studies that EtO causes chromosomal aberrations, SCEs, and micronucleus
13 formation in peripheral blood lymphocytes, and one study has reported increased levels of
14 micronuclei in bone marrow cells in EtO-exposed workers.
15   In the framework of EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,
16 2005a), the conclusion can be made that EtO is "carcinogenic to humans." In general, the
17 descriptor "carcinogenic to humans" is appropriate when there is convincing epidemiologic
18 evidence of a causal association between human exposure and cancer. This descriptor is also
19 appropriate when there is a lesser weight of epidemiologic evidence that is strengthened by
20 specific lines of evidence set forth in the *Guidelines*, which are satisfied for EtO and include the
21 following: (1) there is evidence, although less than conclusive, of cancer in humans associated
22 with EtO exposure via inhalation—strong evidence for lymphohematopoietic cancers and some
23 evidence for breast cancer in EtO-exposed workers; (2) there is extensive evidence of EtO-
24 induced carcinogenicity in laboratory animals, including lymphohematopoietic cancers in rats
25 and mice and mammary carcinomas in mice following inhalation exposure; (3) EtO is a direct-
26 acting alkylating agent whose mutagenic and genotoxic capabilities have been well established in
27 a variety of experimental systems, and a mutagenic mode of carcinogenic action has been
28 identified in animals involving the key precursor events of DNA adduct formation and
29 subsequent DNA damage, including point mutations and chromosomal effects; and (4) there is
30 strong evidence that the key precursor events are anticipated to occur in humans and progress to
31 tumors, including evidence of chromosome damage, such as chromosomal aberrations, SCEs,
32 and micronuclei in EtO-exposed workers.
33
34 **3.5.2. Susceptible Lifestages and Subpopulations**
35   There are no data on the relative susceptibility of children and the elderly when compared
36 with adult workers, in whom the evidence of hazard has been gathered, but because EtO does not

1 have to be metabolized before binding to DNA and proteins, the maturing of enzyme systems in
2 very young children is thought not to be a predominant factor in its hazard, at least for activation.
3 However, the immaturity of *detoxifying* enzymes in very young children may increase children's
4 susceptibility because they may clear EtO at a slower rate than adults. As discussed in Section
5 3.3.1, EtO is metabolized (i.e., detoxified) primarily by hydrolysis in humans but also by
6 glutathione conjugation. Both hydrolytic activity and glutathione-S-transferase activity
7 apparently develop after birth (Clewell et al., 2002); thus, very young children might have a
8 decreased capacity to detoxify EtO compared to adults. In the absence of data on the relative
9 susceptibility associated with EtO exposure in early life, increased early-life susceptibility is
10 assumed, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), because the
11 weight of evidence supports the conclusion of a mutagenic mode of action for EtO
12 carcinogenicity (Section 3.4).
13   People with DNA repair deficiencies such as xeroderma pigmentosum, Bloom's
14 syndrome, Fanconi anemia, and ataxia telangiectasia (Gelehrter and Collins, 1990) are expected
15 to be especially sensitive to the damaging effects of EtO exposure. Paz-y-Mino et al. (2002)
16 have recently identified a specific polymorphism in the excision repair pathway gene *hMSH2*.
17 The polymorphism was present in 7.5% of normal individuals and in 22.7% of NHL patients,
18 suggesting that this polymorphism may be associated with an increased risk of developing NHL.
19 In addition, Yong et al. (2001) measured approximately twofold greater EtO-hemoglobin adduct
20 levels in occupationally exposed persons with a null GSTT1 genotype than in those with positive
21 genotypes.
22

4. CANCER DOSE-RESPONSE ASSESSMENT FOR INHALATION EXPOSURE

This chapter presents the derivation of cancer unit risk estimates from human and rodent data. Section 4.1 discusses the derivation of unit risk estimates for lymphohematopoietic cancers, breast cancer, and total cancer from human data, as well as sources of uncertainty in these estimates. Section 4.2 presents the derivation of unit risk estimates from rodent data. Section 4.3 summarizes the unit risk estimates derived from the different datasets. Section 4.4 discusses adjustments for assumed increased early-life susceptibility, based on recommendations from EPA's *Supplemental Guidance* (U.S. EPA, 2005b), because the weight of evidence supports the conclusion of a mutagenic mode of action for EtO carcinogenicity (Section 3.4). Section 4.5 presents conclusions about the unit risk estimates. Section 4.6 compares the unit risk estimates derived in this U.S. EPA assessment to those derived in other assessments. Finally, Section 4.7 provides risk estimates derived for some general occupational exposure scenarios.

4.1. INHALATION UNIT RISK ESTIMATES DERIVED FROM HUMAN DATA

The NIOSH retrospective cohort study of more than 18,000 workers in 13 sterilizing facilities (most recent update by Steenland et al., 2003, 2004) provides the most appropriate data sets for deriving quantitative cancer risk estimates in humans for several reasons: (1) exposure estimates were derived for the individual workers using a comprehensive exposure assessment, (2) the cohort was large and diverse (e.g., 55% female), and (3) there was little reported exposure to chemicals other than EtO. The early exposures for which no measurements were available were determined by consultations with plant industrial hygienists and the use of regression modeling to estimate exposures to each individual as a function of facility, exposure category, and time period. The investigators were then able to estimate the cumulative exposure (ppm $\times$ days) for each individual worker by multiplying the estimated exposure for each job (exposure category) held by the worker by the number of days spent in that job and summing over all the jobs held by the worker. Steenland et al. (2004) present follow-up results for the cohort mortality study previously discussed by Steenland et al. (1991) and Stayner et al. (1993). Positive findings in the current follow-up include increased rates of (lympho)hematopoietic cancer mortality and of breast cancer mortality in females. Steenland et al. (2003) present results of a breast cancer incidence study of a subcohort of 7,576 women from the NIOSH cohort.

The other major occupational study (most recent update by Swaen et al., 2009) described risks to Union Carbide workers exposed to ethylene oxide at two chemical plants in West Virginia, but this study is less useful for estimating quantitative cancer risks for a number of reasons. First, the exposure assessment is much less extensive than that used for the NIOSH cohort, with greater likelihood for exposure misclassification, especially in the earlier time

1 periods when no measurements were available (1925-1973). Exposure estimation for the

2 individual workers was based on a relatively crude exposure matrix which cross-classified 3

3 levels of exposure intensity with 4 time periods. The exposure estimates for 1974-1988 were

4 based on measurements from air sampling at the West Virginia plants since 1976. The exposure

5 estimates for 1957-1973 were based on measurements in a similar plant in Texas. The exposure

6 estimates for 1940-1956 were based loosely on "rough" estimates reported for chlorohydrin-

7 based EtO production in a Swedish facility in the 1940s. The exposure estimates for 1925-1939

8 were essentially guesses. Thus, for the two earliest time periods (1925-1939 and 1940-1956) at

9 least, the exposure estimates are highly uncertain. This is in contrast to the NIOSH exposure

10 assessment in which exposure estimates were based on extensive sampling data and regression

11 modeling. In addition, the sterilization processes used by the NIOSH cohort workers were fairly

12 constant back in time, unlike chemical production processes, which likely involved much higher

13 and more variable exposure levels in the past. Furthermore, the Union Carbide cohort is of much

14 smaller size and has far fewer deaths than the NIOSH cohort, it is restricted to males and so

15 cannot be used to investigate breast cancer risk in females, and there are co-exposures to other

16 chemicals.

17   The derivation of unit risk estimates, defined as the lifetime risk of cancer from chronic

18 inhalation of EtO per unit of air concentration, for lymphohematopoietic cancer mortality and

19 incidence and for breast cancer mortality and incidence in females, based on results of the recent

20 analyses of the NIOSH cohort, is presented in the following subsections.

21

22 **4.1.1. Risk Estimates for Lymphohematopoietic Cancer**

23 **4.1.1.1. *Lymphohematopoietic Cancer Results From the NIOSH Study***

24   Steenland et al. (2004) investigated the relationship between (any) EtO exposure and

25 mortality from cancer at a number of sites using life-table analyses with the U.S. population as

26 the comparison population. Categorical SMR analyses were also done by quartiles of cumulative

27 exposure. Then, to further investigate apparent exposure-response relationships observed for

28 (lympho)hematopoietic cancer and breast cancer, internal exposure-response analyses were

29 conducted using Cox proportional hazards models, which have the form

30

31   Relative rate (RR) $= e^{\beta X}$,   (4-1)

32

33 where β represents the regression coefficient and X is the exposure (or some function of

34 exposure, e.g., the natural log of exposure). Internal analyses were done two ways — with

35 exposure as a categorical variable and with exposure as a continuous variable. A nested case-

36 control approach was used, with age as the time variable used to form the risk sets. Risk sets

1   were constructed with 100 controls randomly selected for each case from the pool of those

2   surviving to at least the age of the index case. According to the authors, use of 100 controls per

3   case has been shown to result in ORs virtually identical to the RR estimates obtained with full

4   cohorts. Cases and controls were matched on race (white/nonwhite), sex, and date of birth

5   (within 5 years). Exposure was the only covariate in the model, so the *p* value for the model also

6   serves as a *p* value for the regression coefficient, β, as well as for a test of exposure-response

7   trend.

8   For lymphohematopoietic cancer mortality, Steenland et al. (2004) analyzed both all

9   lymphohematopoietic cancers combined and a subcategory of lymphohematopoietic cancers that

10   they called "lymphoid" cancers; these included NHL, myeloma, and lymphocytic leukemia.

11   Their exposure-response analyses focused on cumulative exposure and (natural) log cumulative

12   exposure, with various lag periods. Other EtO exposure metrics (duration of exposure, average

13   exposure, and peak exposure) were also examined, but models using these metrics did not

14   generally predict lymphohematopoietic cancer as well as models using cumulative exposure. A

15   lag period defines an interval before death, or end of follow-up, during which any exposure is

16   disregarded because it is not considered relevant to the outcome under investigation. For

17   lymphohematopoietic (and lymphoid) cancer mortality, a 15-year lag provided the best fit to the

18   data, based on the likelihood ratio test. One ppm $\times$ day was added to cumulative exposures in

19   lagged analyses to avoid taking the log of 0. For both all lymphohematopoietic and lymphoid

20   cancers, Steenland et al. found stronger positive exposure-response trends in males and so

21   presented the results for some of the regression models separately by sex. The apparent sex

22   difference was not statistically significant (Appendix D), however, and results for both sexes

23   combined were subsequently obtained from Dr. Steenland (Appendix D; Section 3 for lymphoid

24   cancer, Section 4 for all lymphohematopoietic cancer). These results are presented in Table 4-1.

25   For additional details and discussion of the Steenland et al. (2004) study, see Appendix A.

26

27   **4.1.1.2.** *Prediction of Lifetime Extra Risk of Lymphohematopoietic Cancer Mortality*

28   The exposure-response trends for lymphohematopoietic cancers observed by Steenland et

29   al. (2004) appear to be driven largely by the lymphoid cancers; therefore, the primary risk

30   analyses for lymphohematopoietic cancer are based on the lymphoid cancer results.

31   Lymphohematopoietic cancers are a diverse group of diseases with diverse etiologies, and

32   myeloid and lymphoid cells develop from different progenitor cells; thus, there is stronger

33   support for an etiologic role of EtO in the development of lymphoid cancers than in the

34

1 **Table 4-1**. Cox regression results for all lymphohematopoietic cancer and
2 lymphoid cancer mortality in both sexes in the NIOSH cohort

3

Exposure variable[a]	*p* value	Coefficient (SE)	ORs by category[b] (95% CI)
All lymphohematopoietic cancer[c]			
Cumulative exposure, 15-year lag	0.35	0.00000326 (0.00000349)	
Log cumulative exposure, 15-year lag	0.01	0.107 (0.0418)	
Categorical cumulative exposure, 15-year lag	0.10		1.00, 2.33 (0.93–5.86), 3.46 (1.33–8.95), 3.02 (1.16–7.89), 2.96 (1.12–7.81)
Lymphoid cancer[d]			
Cumulative exposure, 15-year lag	0.16	0.00000474 (0.00000335)	
Log cumulative exposure, 15-year lag	0.02	0.112 (0.0486)	
Categorical cumulative exposure, 15-year lag	0.21		1.00, 1.75 (0.59–5.25), 3.15 (1.04–9.49), 2.44 (0.80–7.50), 3.00 (1.02–8.45)

4
5 [a]Cumulative exposure is in ppm $\times$ days.
6 [b]Exposure categories are 0, >0–1,199, 1,200–3,679, 3,680–13,499, $\geq$13,500 ppm $\times$ days.
7 [c]9th revision ICD codes 200–208; results based on 74 cases.
8 [d]NHL, myeloma, and lymphocytic leukemia (9th revision ICD codes 200, 202, 203, 204); results based on 53 cases.
9
10 Source: Additional analyses performed by Dr. Steenland (Appendix D).
11

12 development of the cancers in the aggregate all lymphohematopoietic cancer category.

13 Nonetheless, for comprehensiveness and for the reasons listed below, risk estimates based on the

14 all lymphohematopoietic cancer results are presented for comparison. Judging roughly from the

15 *p* values, the model fits do not appear notably better for lymphoid cancers than for all

16 lymphohematopoietic cancers (see Table 4-1, *p* values for log cumulative exposure models), and

17 the "lymphoid" category did not include Hodgkin lymphoma, which also exhibited evidence of

18 exposure-response trends, although based on few cases (Steenland et al., 2004). In addition,

19 misclassification or nonclassification of tumor type is more likely to occur for subcategories of

20 lymphohematopoietic cancer (e.g., 4 of the 25 leukemias in the analyses were classified as "not

21 specified" and so could not be considered for the lymphoid cancer analysis).

1   The results of internal exposure-response analyses of lymphoid cancer in the NIOSH
2   cohort (Cox regression analyses, summarized in Table 4-1) were used for predicting the extra
3   risks of lymphoid cancer mortality from continuous environmental exposure to EtO. Extra risk
4   is defined as
5
6   Extra risk = (Rx − Ro)/(1 − Ro),   (4-2)
7
8   where Rx is the lifetime risk in the exposed population and Ro is the lifetime risk in an
9   unexposed population (i.e., the background risk). These risk estimates were calculated using the
10   β regression coefficients and an actuarial program (life-table analysis) that accounts for
11   competing causes of death.[1] An inherent assumption in the Cox regression model and its
12   application in the life-table analyses is that RR is independent of age. (An alternate assumption
13   of increased susceptibility from early-life exposure to EtO, as recommended in EPA's
14   *Supplemental Guidance* [U.S. EPA, 2005b] for chemicals, such as EtO [see Section 3.4], with a
15   mutagenic mode of action, is considered in Section 4.4. This alternate assumption is the
16   prevailing assumption in this assessment, based on the recommendations in the *Supplemental*
17   *Guidance.* Risk estimates are first developed under the assumption of age independence,
18   however, because that is the standard approach in the absence of evidence to the contrary or of
19   sufficient evidence of a mutagenic mode of action to invoke the divergent assumption of
20   increased early-life susceptibility.)
21   United States age-specific all-cause mortality rates for 2004 for both sexes of all race
22   groups combined (NCHS, 2007) were used to specify the all-cause background mortality rates in
23   the actuarial program. For the cause-specific background mortality rates for lymphoid cancers,
24   age-specific mortality rates for the relevant subcategories of lymphohematopoietic cancer (NHL
25   [C82-C85 of 10th revision of the International Classification of Diseases (ICD)], multiple
26   myeloma [C88, C90], and lymphoid leukemia [C91]) for the year 2004 were obtained from the
27   National Center for Health Statistics Data Warehouse website
28   (http://www.cdc.gov/nchs/datawh/statab/unpubd/mortabs.htm). The risks were computed up to
29   age 85 for continuous exposures to EtO beginning at birth.[2] Conversions between occupational
30   EtO exposures and continuous environmental exposures were made to account for differences in

---

[1] This program is an adaptation of the approach previously used by the Committee on the Biological Effects of Ionizing Radiation (BEIR, 1988). A spreadsheet illustrating the extra risk calculation for the derivation of the LEC_{01} for lymphoid cancer incidence (see Section 4.1.1.3) is presented in Appendix E.

[2] Rates above age 85 years are not included because cause-specific disease rates are less stable for those ages. Note that 85 years is not employed here as an average lifespan but, rather, as a cut-off point for the life-table analysis, which uses actual age-specific mortality rates. The average lifespan for males and females combined in a lifetable analysis truncated at age 85 years is about 75 years.

1 the number of days exposed per year (240 vs. 365 days) and in the amount of EtO-contaminated
2 air inhaled per day (10 vs. 20 m^3; U.S. EPA, 1994). An adjustment was also made for the lag
3 period. The reported standard errors for the regression coefficients from Table 4-1 were used to
4 compute the 95% upper confidence limits (UCLs) for the relative rates, based on a normal
5 approximation.
6   The only statistically significant Cox regression model presented by Steenland et al.
7 (2004) for lymphoid cancer mortality in males was for log cumulative exposure with a 15-year
8 lag ($p = 0.02$). This was similarly true for the analyses of lymphoid cancer using the data for
9 both sexes (Table 4-1). However, using the log cumulative exposure model to estimate the risks
10 from low environmental exposures is problematic because this model, which is intended to fit the
11 full range of occupational exposures in the study, is inherently supralinear (i.e., risk increases
12 steeply with increasing exposures in the low exposure range and then plateaus), and results are
13 unstable for low exposures (i.e., small changes in exposure correspond to large changes in risk;
14 see Figure 4-1). Consideration was thus given to the cumulative exposure model, which is
15 typically used and which is stable at low exposures, although the fit to these data was not
16 statistically significant ($p = 0.16$). However, the Cox regression model with cumulative exposure
17 is inherently sublinear (i.e., risk increases gradually in the low exposure range and then with
18 increasing steepness as exposure increases) and does not reflect the apparent supralinearity of the
19 data exhibited by the categorical results and the superior fit of the log cumulative exposure
20 model.
21   In a 2006 External Review Draft of this assessment (U.S. EPA, 2006), which relied on
22 the original published results of Steenland et al. (2004), EPA proposed that the best way to
23 represent the exposure-response relationship in the lower exposure region, which is the region of
24 interest for low-exposure extrapolation, was through the use of a weighted linear regression of
25 the results from the Cox regression model with categorical cumulative exposure and a 15-year
26 lag (for males only, as this was the significant finding in the published paper). In addition, the
27 highest exposure group was not included in the regression to alleviate some of the "plateauing"
28 in the exposure-response relationship at higher exposure levels and to provide a better fit to the
29 lower exposure data. Linear modeling of categorical (i.e., grouped) epidemiologic data and
30 elimination of the highest exposure group(s) under certain circumstances to obtain a better fit of
31 low-exposure data are both standard techniques used in EPA dose-response assessments (U.S.
32 EPA, 2005a; 2000a). An established methodology was employed for the weighted linear
33 regression of the categorical epidemiologic data, as described by Rothman (1986) and used by
34 others (e.g., van Wijngaarden and Hertz-Picciotto, 2004).
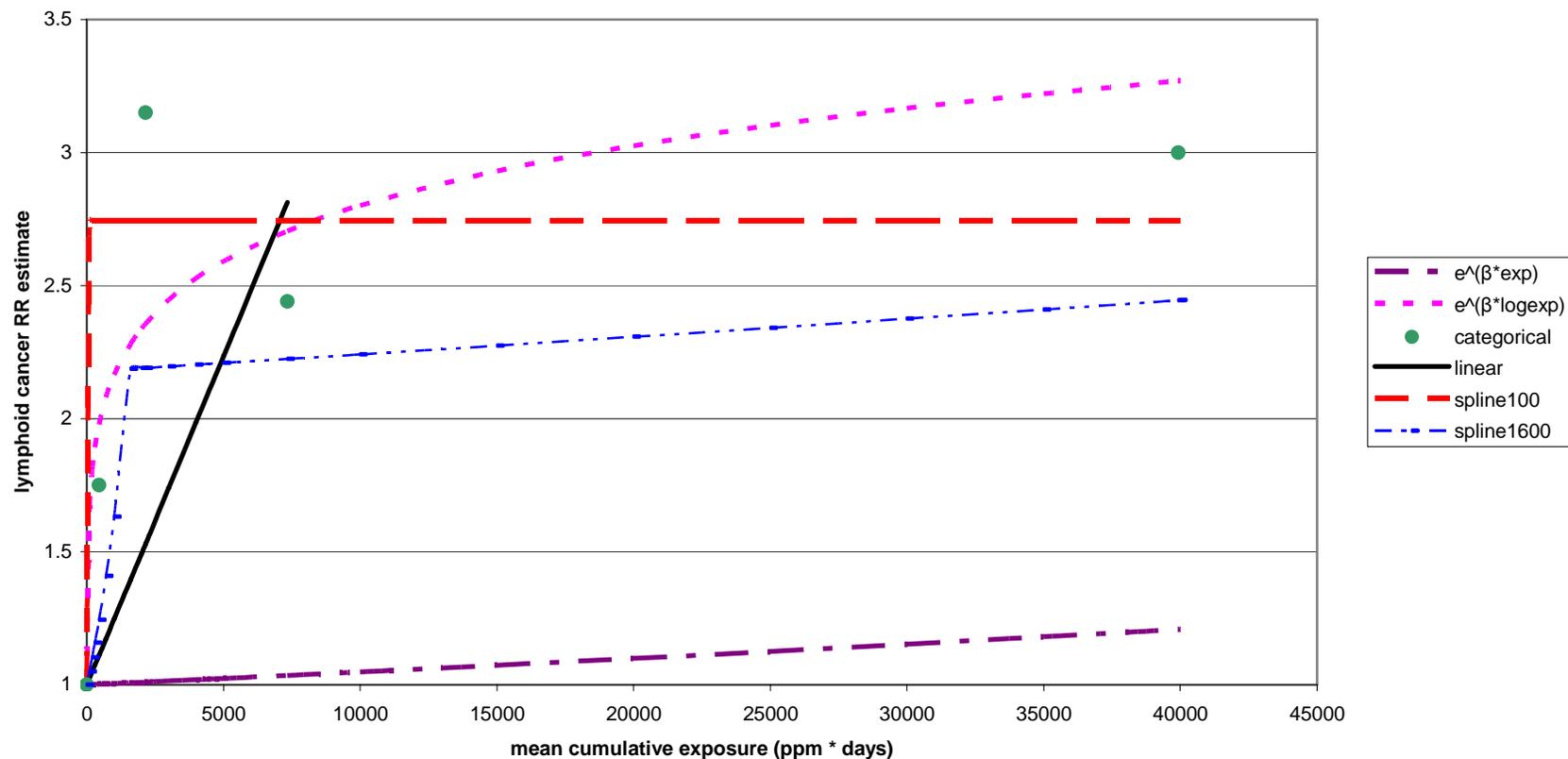
Figure 4-1. RR estimate for lymphoid cancer vs. mean exposure (with 15-year lag, unadjusted for continuous exposure).

e^(β*exp): Cox regression results for RR = e^{(β*exposure)}; e^(β*logexp): Cox regression results for RR = e^{(β*ln(exposure))}; categorical: Cox regression results for RR = e^{(β*exposure)} with categorical exposures; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); spline100(1600): 2-piece log-linear spline model with knot at 100 (1600) ppm*days (see text).

Source: Steenland re-analyses for male and female combined; see Appendix D (except for linear regression, which was done by EPA).

1   However, the Science Advisory Board panel that reviewed the draft assessment recommended
2   that EPA employ models using the individual exposure data as an alternative to modeling the
3   published grouped data. The SAB also recommended that both males and females be included in
4   the modeling of lymphohematopoietic cancer mortality (SAB, 2007).
5   In response to these recommendations and in consultation with Dr. Steenland, one of the
6   investigators from the NIOSH cohort studies, EPA determined that, using the full dataset, an
7   alternative way to address the supralinearity of the data (while avoiding the extreme low-
8   exposure curvature obtained with the log cumulative exposure model) might be to use a two-
9   piece log-linear spline model. Spline models have been used previously for exposure-response
10   analyses of epidemiological data (Steenland and Deddens, 2004; Steenland et al., 2001). These
11   models are particularly useful for exposure-response data such as the EtO lymphoid cancer data,
12   for which RR initially increases with increasing exposure but then tends to plateau, or level off,
13   at higher exposures. Such plateauing exposure-response relationships have been seen with other
14   occupational carcinogens and may occur for various reasons, including the depletion of
15   susceptible sub-populations at high exposures, mismeasurement of high exposures, or a healthy
16   worker survivor effect (Stayner et al., 2002). No other traditional exposure-response models for
17   continuous data which might suitably fit the observed exposure-response pattern were apparent.
18   Dr. Steenland was commissioned to do the spline analyses using the full dataset with cumulative
19   exposure as a continuous variable, and his findings are included in Appendix D (Section 3 for
20   lymphoid cancer, Section 4 for all lymphohematopoietic cancer). The results of the spline
21   analyses are presented below.
22   For the two-piece log-linear spline modeling approach, the Cox regression model
23   (equation 4-1) was the underlying basis for the splines which were fit to the lymphoid cancer
24   exposure-response data.[3] Taking the log of both sides of Equation 4-1, log RR is a linear
25   function of exposure (cumulative exposure is used here), and, with the two-piece log-linear
26   spline approach, log RR is a function of two lines which join at a single point of inflection, called
27   a "knot". The shape of the two-piece log-linear spline model, in particular the slope in the low-
28   exposure region, depends on the location of the knot. For this assessment, the knot was
29   generally selected by trying different knots in increments of 1000 ppm $\times$ days, starting at 1000
30   ppm $\times$ days, and choosing the one that resulted in the largest model likelihood. In some cases,
31   increments of 100 ppm $\times$ days were used between the increments of 1000 ppm $\times$ days to fine-
32   tune the knot selection. The model likelihood did not change much across the different trial
33   knots (see Figure 3a of Appendix D), but it did change slightly; therefore, the largest calculated

---

[3] As parameterized in Appendix D, for cumulative exposures less than the value of the knot, $RR = e^{\beta 1 * exposure}$; for cumulative exposures greater than the value of the knot, $RR = e^{(\beta 1 * exposure + \beta 2 * (exposure - knot))}$.

1 likelihood was used as a basis for knot selection. For more discussion of the two-piece spline

2 approach, see Appendix D.

3 Using this approach, the largest likelihood was observed with the knot at 1600 ppm $\times$

4 days. However, the graphical results for the two-piece log-linear spline model with a knot at

5 1600 ppm $\times$ days suggested that the model was underestimating RR in the region where the data

6 were plateauing (Figure 4-1).[4] Therefore, knots below 1000 ppm $\times$ days were also evaluated in

7 increments of 100 ppm $\times$ days, and a likelihood was observed with the knot at 100 ppm $\times$ days

8 that exceeded the likelihood with the knot at 1600 ppm $\times$ days, although, again, the model

9 likelihood did not actually change much across the different trial knots. The graphical results for

10 the two-piece spline model with a knot at 100 ppm $\times$ days suggested that this model provided a

11 better fit to the region where the data were plateauing (Figure 4-1). Furthermore, the overall fit

12 of this two-piece spline model was statistically significant ($p = 0.048$), whereas the p value for

13 the two-piece spline model with the knot at 1600 ppm $\times$ days exceeded 0.05, although minimally

14 ($p = 0.072$). Thus, for the lymphoid cancer mortality data, the optimal two-piece log-linear

15 spline model appeared to be the one with the knot at 100 ppm $\times$ days. This model provided the

16 largest calculated likelihood, was statistically significant, and presented the best apparent

17 graphical fit to the majority of the range of the data. Using this optimal two-piece log-linear

18 spline model with the knot at 100 ppm $\times$ days, a regression coefficient of 0.01010 per ppm $\times$ day

19 (SE = 0.00493 per ppm $\times$ day) was obtained for the low-exposure spline segment ($p = 0.040$;

20 Appendix D). However, this model yielded a very steep slope in the low-exposure region

21 (Figure 4-1), and, as such, there was low confidence in the slope given that it is based on a

22 relatively small number of cases in that exposure range. Thus, after examining the new

23 modeling analyses, it was determined that the the weighted linear regression of the categorical

24 data still provided the best available approach for risk estimates for lymphohematopoietic

25 cancer.[5]

26 For the weighted linear regression, the Cox regression results from the model with

27 categorical cumulative exposure and a 15-year lag (see Table 4-1) was used, excluding the

28 highest exposure group, as discussed above.[6] The weights used for the ORs were the inverses of

---

[4] The loglinear spline segments appear fairly linear in the plotted range; however, they are not strictly linear.
[5] When this assessment was near completion, a two-piece linear spline model (with a linear model, i.e., RR = 1 + β $\times$ exposure, as the underlying basis for the spline pieces) was attempted, using the just-published approach of Langholz and Richardson (2010); however, this model did not alleviate the problem of the excessively steep low-exposure spline segment (see Figure 3c in Appendix D) and was not pursued further for the lymphoid cancer data.
[6] Concerns have been raised that this approach of dropping high-dose data appears arbitrary. It should be noted, however, that only the highest exposure group was omitted from the linear regression, and the exposure groupings were derived *a priori* by the NIOSH investigators and not by US EPA in the course of its analyses.

1 the variances, which were calculated from the confidence intervals.[7] Mean and median
2 exposures for the cumulative exposure groups were provided by Dr. Steenland (Table 5 of
3 Appendix D).[8] The mean values were used for the weighted regression analysis because the
4 cancer response is presumed to be a function of cumulative exposure, which is expected to be
5 best represented by mean exposures. If the median values had been used, a slightly larger
6 regression coefficient would have been obtained, resulting in slightly larger risk estimates.
7 Using this approach, a regression coefficient of 0.000247 per ppm × day (standard error [SE] =
8 0.000185 per ppm × day) was obtained for the weighted linear regression of the categorical
9 results and mean exposures (see Figure 4-1 for a depiction of the resulting linear regression
10 model).
11   The linear regression of the categorical results for males and females combined and the
12 actuarial program (life-table analysis) were used to estimate the exposure level (EC_x; "effective
13 concentration") and the associated 95% lower confidence limit (LEC_x) corresponding to an extra
14 risk of 1% ($x = 0.01$). A 1% extra risk level is commonly used for the determination of the point
15 of departure (POD) for low-exposure extrapolation from epidemiological data; higher extra risk
16 levels, such as 10%, would be an upward extrapolation for these data. Thus, 1% extra risk was
17 selected for determination of the POD, and, consistent with EPA's *Guidelines for Carcinogen*
18 *Risk Assessment* (U.S. EPA, 2005a), the LEC value corresponding to that risk level was used as
19 the POD to derive the cancer unit risk estimates.
20   Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3),
21 which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,
22 2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was
23 performed. The EC_{01}, LEC_{01}, and inhalation unit risk estimate calculated for lymphoid cancer
24 mortality from the linear regression model are presented in Table 4-2 (the incidence results also
25 presented in Table 4-2 are discussed in Section 4.1.1.3 below). The resulting unit risk estimate
26 for lymphoid cancer mortality based on the linear regression of the categorical results for both
27 sexes using cumulative exposure with a 15-year lag is 0.397 per ppm. EC_{01} and LEC_{01} estimates
28 from the other models considered are presented for comparison only, to illustrate the differences
29 in model behavior at the low end of the exposure-response range. Unit risk estimates are not
30 presented for these other models because, as discussed above, these models were deemed
31 unsuitable for the derivation of risks from (low) environmental exposure levels. The standard
32 Cox regression cumulative exposure model, with its extreme sublinearity in the lower exposure

---

[7] Equations for this weighted linear regression approach are presented in Rothman (1986) and summarized in Appendix F.

[8] Mean exposures for both sexes combined with a 15-year lag for the categorical exposure quartiles in Table 4-1 were 446; 2,143; 7,335; and 39,927 ppm × days. Median values were 374; 1,985; 6,755; and 26,373 ppm × days. These values are for the full cohort, not just the risk sets.

1 region, yields a substantially higher EC_{01} estimate (2.09 ppm) than the EC_{01} estimate of 0.0564

2 ppm from the linear regression, while the log cumulative exposure model, with its extreme

3 supralinearity in the lower exposure region, and the optimal two-piece log-linear spline model,

4 with its very steep low-exposure slope, yield substantially lower EC_{01} estimates (0.00441 ppm

5 and 0.000982 ppm, respectively). Converting the units, the resulting unit risk estimate of 0.397

6 per ppm from the linear regression model corresponds to a unit risk estimate of 2.17×10^{-4} per

7 $\mu g/m^3$ for lymphoid cancer mortality.

8

9 **Table 4-2. EC_{01}, LEC_{01}, and unit risk estimates for lymphoid cancer[a]**

10

Model[b]	Incidence			Mortality		
	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)
Cumulative exposure, 15-year lag	1.12	0.517	--[c]	2.09	0.967	--[c]
Log cumulative exposure, 15-year lag	0.000288	0.0000898	--[c]	0.00441	0.000428	--[c]
Optimal low-exposure log-linear spline (knot at 100 ppm $\times$ days)[d] cumulative exposure, 15-year lag	0.000525	0.000291	--[c]	0.000982	0.000545	--[c]
Alternate low-exposure log-linear spline (knot at 1600 ppm $\times$ days);[e] cumulative exposure, 15-year lag	0.0108	0.00583	--[e]	0.0203	0.0109	--[e]
Linear regression of categorical results, cumulative exposure, 15-year lag	0.0254	0.0114	**0.877**	0.0564[f]	0.0252	0.397

11
12 [a]From lifetime continuous exposure. Unit risk = $0.01/LEC_{01}$.
13 [b]From Dr. Steenland's analyses for males and females combined (Appendix D), Cox regression models. Note that
14 the EC_{01} and LEC_{01} results presented here will not exactly match those presented in Appendix D because, although
15 EPA used the regression coefficients reported by Dr. Steenland in Appendix D, the life-table analyses using 2004
16 all-cause mortality rates were re-done to be more up-to-date and consistent with the cause-specific mortality rates;
17 the results presented in Appendix D were based on life-table analyses using 2000 all-cause mortality rates.

1 [c]Unit risk estimates are not presented for these models because these models were deemed unsuitable for the
2 derivation of risks from (low) environmental exposure levels (see text).
3 [d]Using regression coefficient from low-exposure segment of optimal two-piece log-linear spline model (largest
4 likelihood) with knot at 100 ppm × days; see text and Appendix D. Each of the EC_{01} values is appropriately below
5 the value of 0.0013 ppm roughly corresponding to the knot of 100 ppm × days and, thus, in the range of the low-
6 exposure segment.
7 [e]Using regression coefficient from low-exposure segment of alternate two-piece log-linear spline model (local
8 largest likelihood) with a knot at 1600 ppm × days. Each of these EC_{01} values is appropriately below the value of
9 0.021 ppm roughly corresponding to the knot of 1600 ppm × days and, thus, in the range of the low-exposure
10 segment. Unit risk estimates were not calculated from this model because the fit was inferior to that of the optimal
11 model (see text).
12 [f]Because this value was close to the value of 0.06 ppm which loosely equates to the occupational exposure of
13 roughly 5000 ppm × days above which the linear regression model does not apply, a POD of 0.1% extra risk was
14 also used for lymphoid mortality with this model. With a POD of 0.1%, the resulting EC_{01}, LEC_{01}, and unit risk
15 estimates were 0.00560 ppm, 0.00251 ppm, and 0.398 per ppm, respectively. This alternate unit risk estimate is
16 essentially the same because these estimates are based on a linear model.
17
18
19 As discussed above, risk estimates based on the all lymphohematopoietic cancer results
20 are also derived, for comparison. The same methodology presented above for the lymphoid
21 cancer results was used for the all lymphohematopoietic cancer risk estimates. Age-specific
22 background mortality rates for all lymphohematopoietic cancers for the year 2004 were obtained
23 from the NCHS Data Warehouse website
24 (http://www.cdc.gov/nchs/datawh/statab/unpubd/mortabs.htm). The results of Dr. Steenland's
25 re-analyses using the Cox regression models presented in the Steenland et al. (2004) paper with
26 data for males and females combined are presented in Table 4-1. As for lymphoid cancer and for
27 all hematopoietic cancer in males presented in the 2004 paper, the only statistically significant
28 Cox regression model was for log cumulative exposure with a 15-year lag ($p = 0.01$). The
29 cumulative exposure model did not provide an adequate fit to the data and is not considered
30 further here ($p = 0.35$).
31 Because of the problems with the supralinear log cumulative exposure model which are
32 discussed for the lymphoid cancers above, EPA again investigated the use of a two-piece log-
33 linear spline model to attempt to address the supralinearity of the data while avoiding the
34 extreme low-exposure curvature obtained with the log cumulative exposure model. For the all
35 lymphohematopoietic cancer mortality data, the largest calculated likelihood was obtained with a
36 knot of 500 ppm × days ($p = 0.018$; Figure 4a of Appendix D). Using this optimal two-piece
37 log-linear spline model with the knot at 500 ppm × days, a regression coefficient of 0.00201 per
38 ppm × day (SE = 0.000773 per ppm × day) was obtained for the low-exposure spline segment (p
39 = 0.009; Appendix D). As with the lymphoid cancer mortality results, however, this model

1   resulted in an apparently excessively steep low-exposure spline (Figure 4-2), so, again, the linear

2   regression model was used to derive the cancer unit risk estimate for this data set.[9]

3   For the weighted linear regression, the results from the Cox regression model with

4   categorical cumulative exposure and a 15-year lag (see Table 4-1) were used, excluding the

5   highest exposure group, and the approach discussed above for lymphoid cancer mortality. A

6   regression coefficient of 0.0003459 per ppm $\times$ day (SE = 0.0001944 per ppm $\times$ day) was

7   obtained for the weighted linear regression of the categorical results and mean exposures (see

8   Figure 4-2 for a graphical presentation of the resulting linear regression model). As discussed

9   above, this linear regression model was used to derive the unit risk estimates for all

10   lymphohematopoietic cancer.

11   The EC_{01}, LEC_{01}, and inhalation unit risk estimate calculated for all

12   lymphohematopoietic cancer mortality from the linear regression model are presented in Table

13   4-3 (the incidence results also presented in Table 4-3 are discussed in Section 4.1.1.3 below).

14   The resulting unit risk estimate for all lymphohematopoietic cancer mortality based on the linear

15   regression of the categorical results for both sexes using cumulative exposure with a 15-year lag

16   is 0.680 per ppm. EC_{01} and LEC_{01} estimates from the other models considered are presented for

17   comparison only, to illustrate the differences in model behavior at the low end of the exposure-

18   response range. Unit risk estimates are not presented for these other models because, as

19   discussed above, these models were deemed unsuitable for the derivation of risks from (low)

20   environmental exposure levels. The resulting unit risk estimate for all lymphohematopoietic

21   cancer mortality from the linear regression model is similar to that for lymphoid cancer mortality

22   (70% higher; see Table 4-2). Converting the units, the resulting unit risk estimate of 0.680 per

23   ppm corresponds to a unit risk estimate of 3.72×10^{-4} per μg/m^3 for all lymphohematopoietic

24   cancer mortality.

25

26   **4.1.1.3.** *Prediction of Lifetime Extra Risk of Lymphohematopoietic Cancer Incidence*

27   EPA cancer risk estimates are typically derived to represent an upper bound on increased

28   risk of cancer *incidence*, as from experimental animal incidence data. Cancer data from

29   epidemiologic studies are more generally mortality data, as is the case in the Steenland et al.

30   (2004) study. For tumor sites with low survival rates, mortality-based estimates are reasonable

31   approximations of cancer incidence risk; however, for many lymphohematopoietic cancers, the

---

[9] When this assessment was near completion, a two-piece linear spline model (with a linear model, i.e., RR = 1 + β $\times$ exposure, as the underlying basis for the spline pieces) was attempted, using the just-published approach of Langholz and Richardson (2010); however, this model did not alleviate the problem of the excessively steep low-exposure spline segment (see Figure 4c in Appendix D) and was not pursued further for the all lymphohematopoietic cancer data.

1   survival rate is substantial, and incidence-based risks are preferred because EPA endeavors to

2   protect against cancer occurrence, not just mortality (U.S. EPA, 2005a).
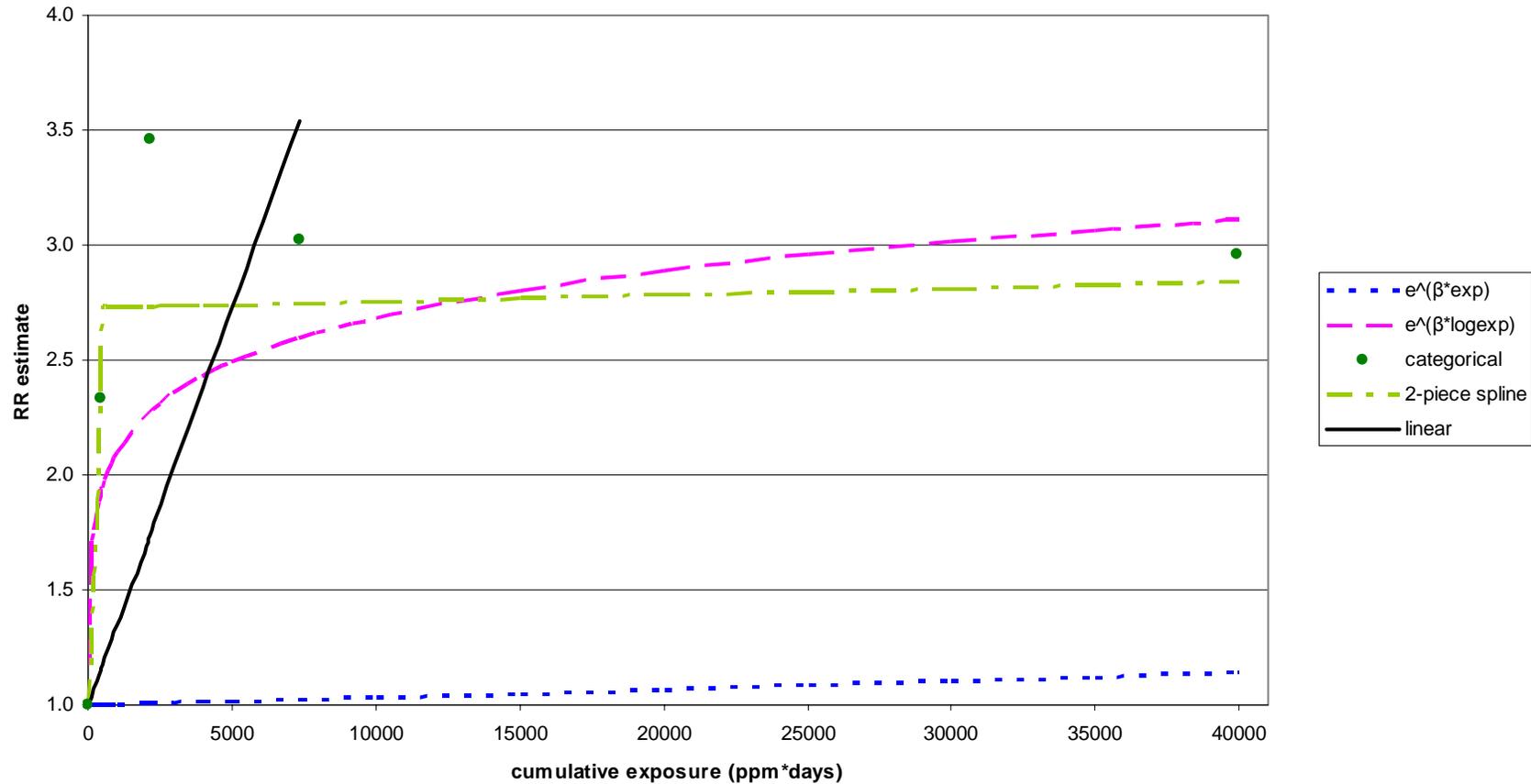
Figure 4-2. **RR estimate for all lymphohematopoietic cancer vs. mean exposure (with 15-year lag, unadjusted for continuous exposure).**

e^(β*exp): Cox regression results for RR = $e^{(\beta*exposure)}$; e^(β*logexp): Cox regression results for RR = $e^{(\beta*\ln(exposure))}$; categorical: Cox regression results for RR = $e^{(\beta*exposure)}$ with categorical exposures; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); 2-piece spline: 2-piece log-linear spline model with knot at 500 ppm*days (see text)

Source: Steenland re-analyses for male and female combined; see Appendix D (except for linear regression, which was done by EPA).

Table 4-3. EC_{01}, LEC_{01}, and unit risk estimates for all lymphohematopoietic cancer[a]

Model[b]	Incidence			Mortality		
	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)
Log cumulative exposure, 15-year lag	0.000190	0.0000753	--[d]	0.00140	0.000245	--[d]
Low-exposure log-linear spline;[c] cumulative exposure, 15-year lag	0.00216	0.00132	--[d]	0.00377	0.00231	--[d]
Linear regression of categorical results, cumulative exposure, 15-year lag	0.0144	0.00746	**1.34[e]**	0.0283	0.0147	0.680

4
5 [a]From lifetime continuous exposure. Unit risk = $0.01/LEC_{01}$.
6 [b]From Dr. Steenland's analyses for males and females combined (Appendix D), Cox regression models. Note that
7 the EC_{01} and LEC_{01} results presented here will not exactly match those presented in Appendix D because, although
8 EPA used the regression coefficients reported by Dr. Steenland in Appendix D, the life-table analyses using 2004
9 all-cause mortality rates were re-done to be more up-to-date and consistent with the cause-specific mortality rates;
10 the results presented in Appendix D were based on life-table analyses using 2000 all-cause mortality rates.
11 [c]Using regression coefficient from low-exposure segment of two-piece log-linear spline model with knot at 500 ppm
12 $\times$ days; see text and Appendix D. Each of the EC_{01} values is appropriately below the value of 0.0067 ppm roughly
13 corresponding to the knot of 500 ppm $\times$ days and, thus, in the range of the low-exposure segment.
14 [d]Unit risk estimates are not presented for these models because these models were deemed unsuitable for the
15 derivation of risks from (low) environmental exposure levels (see text).
16 [e]For unit risk estimates below 1, convert to risk per ppb. e.g., 1.34 per ppm = 1.34×10^{-3} per ppb.
17
18

19   Therefore, another calculation was done using the same regression coefficients presented

20 above (Section 4.1.1.2), but with age-specific lymphoid cancer incidence rates for the relevant

21 subcategories of lymphohematopoietic cancer (NHL, myeloma, and lymphocytic leukemia) for

22 2000–2004 from SEER (NCI, 2007; Tables XIX, XVIII, XIII: both sexes, all races) in place of

23 the lymphoid cancer mortality rates in the actuarial program. SEER collects good-quality cancer

24 incidence data from a variety of geographical areas in the United States. The incidence data used

1   here are from "SEER 17," a registry of seventeen states, regions, and cities covering about 26%

2   of the U.S. population.

3   The incidence-based calculation assumes that lymphoid cancer incidence and mortality

4   have the same exposure-response relationship for the relative rate of effect from EtO exposure

5   and that the incidence data are for first occurrences of primary lymphoid cancer or that relapses

6   and secondary lymphoid cancers provide a negligible contribution. (The latter assumption is

7   probably sound; the former assumption is more potentially problematic. Because various

8   lymphoid subtypes with different survival rates are included in the categorization of lymphoid

9   cancers, if the relative rates of the subtypes differ and if the relative rate-weighted survival rates

10 for the lymphoid cancers are different from those for the combined subtypes, a bias could occur,

11 resulting in either an underestimation or overestimation of the extra risk for lymphoid cancer

12 incidence.)[10] The incidence-based calculation also relies on the fact that the lymphoid cancer

13 incidence rates are small when compared with the all-cause mortality rates.[11] The resulting EC_{01}

14 and LEC_{01} estimates for lymphoid cancer incidence from the various models examined are

15 presented in Table 4-2. The unit risk estimate for lymphoid cancer incidence from the selected

16 linear regression model is 0.877 per ppm.

17   The EC_{01} estimates for cancer incidence range from about 6.5% (log cumulative exposure

18 Cox regression model) to 54% (cumulative exposure Cox regression model) of the corresponding

19 mortality-based estimates. The difference between incidence and mortality rates cannot explain

20 the large discrepancy in EC_{01} estimates for the log cumulative exposure model. Instead, the

21 discrepancy probably reflects the very different results that can occur from a small shift along the

22 dose-response curve for the log cumulative exposure model, illustrating the low-dose instability

23 of the results from this model. The incidence unit risk estimate from the linear regression model

24 is about 120% higher than (i.e., 2.2 times) the mortality-based estimate.

25   Overall, as discussed above, the preferred estimate for the unit risk for lymphoid cancer is

26 the estimate of **0.877 per ppm** (4.79×10^{-4} per $\mu g/m^3$) derived, using incidence rates for the

---

[10] Sielken and Valdez-Flores (2009a) reject the assumption that lymphohematopoietic cancer incidence and mortality have the same exposure-response relationship, reporting that, except at high exposure levels, the exposure-response data in the male workers in the NIOSH cohort are consistent with a decreased survival time and suggesting that this could explain the observed increases in mortality. However, they do not establish that this is what is occurring, and the mechanistic data support an exposure-related increase in incident cancers. See Appendix A.3.20 for a more detailed discussion of this issue.

[11] Sielken and Valdez-Flores (2009a) suggest that the methods used by EPA to calculate incidence risk estimates in the life-table analysis are inappropriate; however, as explained in more detail in Appendix A.3.20, we disagree. For the situation where the cause-specific incidence rates are small compared to the all-cause mortality rates, as with lymphoid cancer, there is no problem, as Sielken and Valdez-Flores (2009a) themselves demonstrate, and, for the situation where the cause-specific incidence rates are not negligible compared to the all-cause mortality rates, as with breast cancer, an adjustment was made in the analysis to remove those with incident cases from the population at risk, i.e., "surviving" each interval (Section 4.1.2.3). See Appendix A.3.20 for a more detailed discussion of this issue.

1 cause-specific background rates, from the weighted linear regression of the categorical results,

2 dropping the highest exposure group.

3 As discussed in Section 4.1.1.2, risk estimates based on the results of Dr. Steenland's re-

4 analyses of the all lymphohematopoietic cancer data (Appendix D and Table 4-1) are also

5 derived, for comparison. The same methodology presented above for the lymphoid cancer

6 incidence results was used for the all lymphohematopoietic cancer incidence risk estimates, and

7 the same assumptions apply. Age-specific SEER incidence rates for all lymphohematopoietic

8 cancer for the years 2000–2004 were used (NCI, 2007; Tables XIX, IX, XVIII, and XIII: both

9 sexes, all races). The EC_{01} and LEC_{01} estimates for all lymphohematopoietic cancer incidence

10 from the different all lymphohematopoietic cancer mortality models examined are presented in

11 Table 4-3. The resulting unit risk estimate for all lymphohematopoietic cancer incidence from

12 the linear regression of the categorical results is about 2.0-times the mortality-based estimate and

13 about 1.5-times the lymphoid cancer incidence estimate (see Table 4-2).

14

15 **4.1.2.   Risk Estimates for Breast Cancer**

16 **4.1.2.1.   *Breast Cancer Results From the NIOSH Study***

17 The Steenland et al. (2004) study discussed above in Section 4.1.1.1 also presents results

18 from exposure-response analyses for breast cancer mortality in female workers. Steenland et al.

19 (2003) present results of a breast cancer incidence study of a subcohort of the female workers

20 from the NIOSH cohort. In addition to the results presented in the 2003 and 2004 Steenland et

21 al. papers, Dr. Steenland did subsequent analyses of the breast cancer mortality and incidence

22 datasets for U.S. EPA; these are discussed below and reported in Sections 1 and 2 of

23 Appendix D.

24

25 **4.1.2.2.   *Prediction of Lifetime Extra Risk of Breast Cancer Mortality***

26 The Cox regression modeling results presented by Steenland et al. (2004) or reported by

27 Dr. Steenland in Appendix D (Section 2) and summarized in Table 4-4 were used for predicting

28 the unit risk estimates for breast cancer mortality in females from continuous environmental

29 exposure to EtO, applying the methodologies described in Section 4.1.1.2.

30 United States age-specific all-cause mortality rates for 2000 for females of all race groups

31 combined (NCHS, 2002) were used to specify the all-cause background mortality rates in the

32 actuarial program (life-table analysis). The National Center for Health Statistics 1997–2001

33 cause-specific background mortality rates for invasive breast cancers in females were obtained

34

Table 4-4. Cox regression results for breast cancer mortality in females[a]

Exposure variable[b]	*p* value	Coefficient (SE)	ORs by category[c] (95% CI)
Cumulative exposure, 20-year lag[d]	0.06	0.0000122 (0.00000641)	
Log cumulative exposure, 20-year lag[e]	0.01	0.084 (0.035)	
Categorical cumulative exposure, 20-year lag[e]	0.07		1.00, 1.76 (0.91–3.43), 1.77 (0.88–3.56), 1.97 (0.94–4.06), 3.13 (1.42–6.92)

[a]Based on 103 cases of breast cancer (ICD-9 174,175).
[b]Cumulative exposure is in ppm × days.
[c]Exposure categories are 0, >0–646, 647–2,779, 2,780–12,321, $\geq$12,322 ppm × days.
[d]From re-analyses in Appendix D; Steenland et al. (2004) reported the Cox regression results for cumulative exposure with no lag.
[e]From Table 8 of Steenland et al. (2004).

from a SEER report (NCI, 2004a). The risks were computed up to age 85 for continuous exposures to EtO, conversions were made between occupational EtO exposures and continuous environmental exposures, and 95% UCLs were calculated for the relative rates, as described above.

The only statistically significant Cox regression model presented by Steenland et al. (2004) for breast cancer mortality in females was for log cumulative exposure with a 20-year lag ($p = 0.01$). The re-analysis by Dr. Steenland of the cumulative exposure model with a 20-year lag provided an apparently better fit to the data ($p = 0.06$; Appendix D) than the cumulative exposure model with no lag ($p = 0.34$; Steenland et al., 2004), but this model was still inferior to the log cumulative exposure model in terms of statistical significance. However, as for the lymphohematopoietic cancers in Section 4.1.1, using the log cumulative exposure model to estimate the risks from low environmental exposures is problematic because this model is highly supralinear and results are unstable for low exposures (see Figure 4-3). The cumulative exposure model, which is typically used and which is stable at low exposures, was nearly statistically significant ($p = 0.06$ with a 20-year lag; Appendix D) in terms of the global fit to the data; however, at low exposures, the Cox regression model with cumulative exposure is sublinear and does not reflect the apparent supralinearity of the breast cancer mortality data (see Figure 4-3).

In a 2006 External Review Draft of this assessment (U.S. EPA, 2006b), which relied on the original published results of Steenland et al. (2004), EPA proposed that the best way to

1   reflect the exposure-response relationship in the lower exposure region, which is the region of

2   interest for
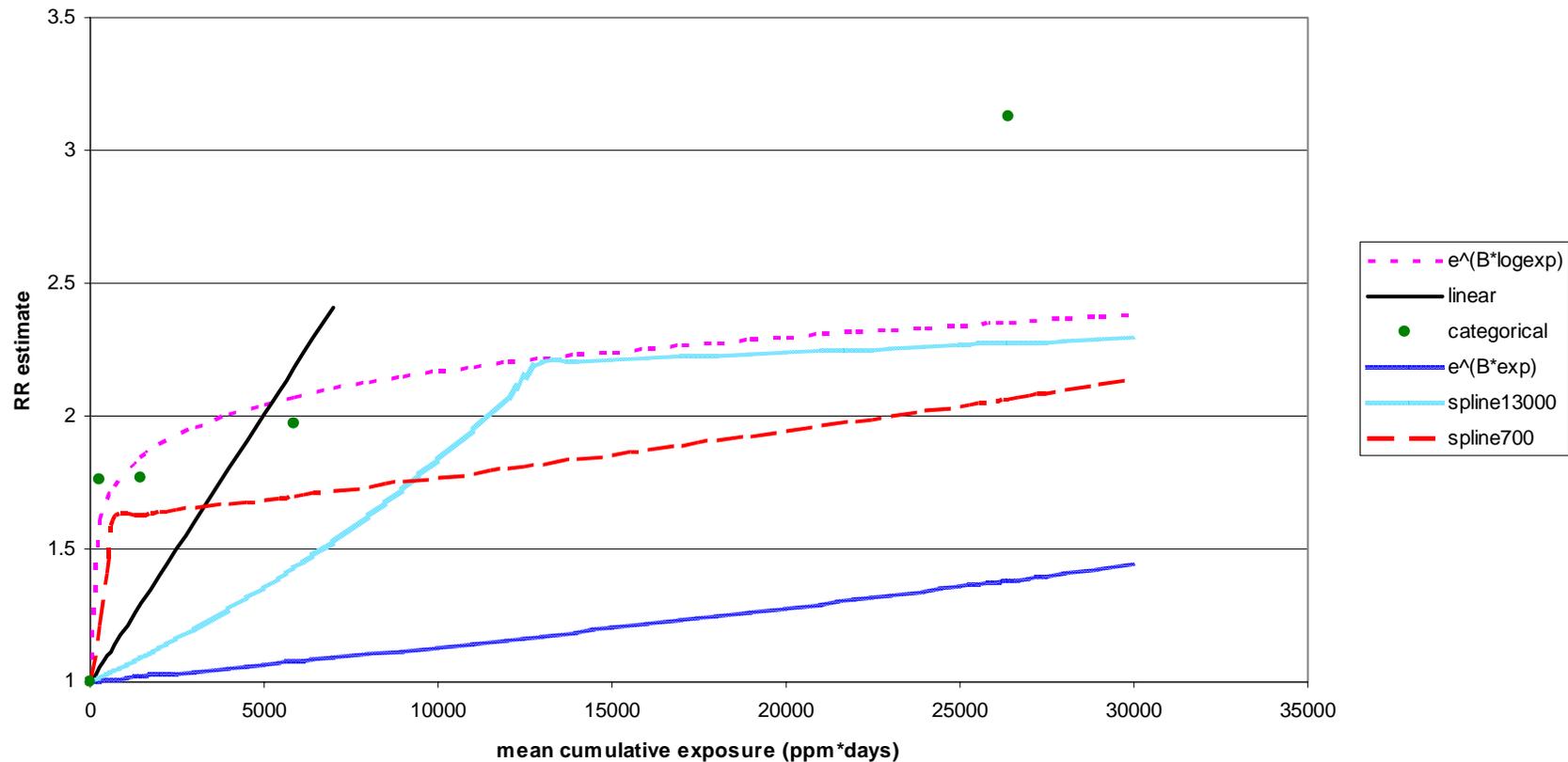
Figure 4-3. **RR estimate for breast cancer mortality vs. mean exposure (with 20-year lag, unadjusted for continuous exposure).**

e^(B*exp): Cox regression results for RR = $e^{(\beta*exposure)}$; e^(B*logexp): Cox regression results for RR = $e^{(\beta*ln(exposure))}$; categorical: Cox regression results for RR = $e^{(\beta*exposure)}$ with categorical exposures; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); spline700(13000): 2-piece log-linear spline model with knot at 700 (13000) ppm*days (see text).

Source: Steenland re-analyses with 20-year lag; see Appendix D (except for linear regression, which was done by EPA).

1   low-exposure extrapolation, was to do a weighted linear regression of the results from the Cox

2   regression model with categorical cumulative exposure and a 20-year lag. In addition, the

3   highest exposure group was not included in the regression to alleviate some of the "plateauing"

4   in the exposure-response relationship at higher exposure levels and to provide a better fit to the

5   lower exposure data. Linear modeling of categorical epidemiologic data and elimination of the

6   highest exposure group(s) in certain circumstances to obtain a better fit of low-exposure data are

7   both standard techniques used in EPA dose-response assessments (U.S. EPA, 2005a). However,

8   as discussed in Section 4.1.1.2 for the similarly supralinear lymphohematopoietic cancer data,

9   the Science Advisory Board panel that reviewed the draft assessment recommended that EPA

10   employ models using the individual exposure data as an alternative to modeling the published

11   grouped data (SAB, 2007). Consequently, it was determined that, using the full dataset, an

12   alternative way to address the supralinearity of the data (while avoiding the extreme low-

13   exposure curvature obtained with the log cumulative exposure model) might be to use a two-

14   piece spline model, and Dr. Steenland was commissioned to do the spline analyses using the full

15   dataset with cumulative exposure as a continuous variable. His findings are reported in

16   Appendix D, and the results for the breast cancer mortality analyses are summarized below.

17   For the two-piece log-linear spline modeling approach, as described in Section 4.1.1.2

18   and discussed more fully in Appendix D, the Cox regression model was the underlying basis for

19   the splines which were fit to the breast cancer mortality exposure-response data (cumulative

20   exposure is used here, with a 20-year lag), and, thus, log RR is a function of two lines which join

21   at a single point of inflection, called a "knot". The shape of the two-piece log-linear spline

22   model, in particular the slope in the low-exposure region, depends on the location of the knot.

23   For this assessment, knot selection was first attempted by trying different knots in increments of

24   1000 ppm × days, starting at 1000 ppm × days, and choosing the one that resulted in the largest

25   model likelihood. The model likelihood did not actually change much across the different trial

26   knots (see Figure 2a of Appendix D), but it did change slightly, and this approach indicated that

27   a knot of 13,000 ppm × days for the breast cancer mortality data yielded the largest likelihood.[12]

28   However, a visual inspection of the model fit suggested that the two-piece log-linear spline

29   model with a knot at 13,000 ppm × days underestimates the low-exposure results (see Figure 4-

30   3). Thus, knots below 1000 ppm × days in increments of 100 ppm × days were investigated, and

31   it was revealed that a knot at 700 ppm × days yielded a model with a likelihood that exceeded

---

[12] Using the log-linear spline model with the knot at 13,000 ppm × days, a regression coefficient of 0.0000607 per ppm × day (SE = 0.0000309 per ppm × day) was obtained for the low-exposure spline segment (Appendix D).

1   that for the model with the knot at 13,000 ppm × days (see Figures 2a and 2a' of Appendix D).[13]

2   The model with the knot at 700 ppm × days, however, has a seemingly implausibly steep low-

3   exposure slope, as was the case with the largest likelihood models for the lymphohematopoietic

4   cancers above. Moreover, neither the model with the knot at 700 ppm × days nor the one with

5   the knot at 13,000 ppm × days was statistically significant overall, although both were nearly so

6   ($p = 0.067$ and 0.074, respectively), and only the latter model had a statistically significant low-

7   exposure spline segment ($p = 0.099$ and 0.0496, respectively). Because there was low

8   confidence in the steep low-exposure slope from the two-piece spline model with the largest

9   likelihood, which is based on a relatively small number of cases in that exposure range, and

10   because the model with the knot at 13,000 ppm × days, which had a local largest likelihood,

11   appeared to have a poor fit to the low-exposure data, it was determined that the weighted linear

12   regression approach was more appropriate as the basis for the unit risk estimates. For more

13   discussion of the breast cancer mortality exposure-response modeling using the continuous data,

14   see Section 2 of Appendix D.

15   For the weighted linear regression, the results from the Cox regression model with

16   categorical cumulative exposure (and a 20-year lag) presented in Table 4-4 were used, excluding

17   the highest exposure group, and the approach discussed above for the lymphoid cancers (Section

18   4.1.1.2). Mean and median exposures for the cumulative exposure groups were provided by Dr.

19   Steenland (Appendix D).[14] Using this approach, a regression coefficient of 0.000201 per ppm ×

20   day (SE = 0.000120 per ppm × day) was obtained from the weighted linear regression of the

21   categorical results and mean exposures (see Figure 4-3 for a depiction of the resulting linear

22   regression model).

23   The linear regression of the categorical results and the actuarial program (life-table

24   analysis) were used to estimate the exposure level (EC_x) and the associated 95% lower

25   confidence limit (LEC_x) corresponding to an extra risk of 1% ($x = 0.01$). As discussed in Section

26   4.1.1.2, a 1% extra risk level is a more reasonable response level for defining the POD for these

27   epidemiologic data than 10%.

28   Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3),

29   which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,

30   2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was

31   performed. The EC_{01}, LEC_{01}, and inhalation unit risk estimate calculated for breast cancer

[13] Using the optimal two-piece log-linear spline model with the knot at 700 ppm × days, a regression coefficient of 0.0006877 per ppm × day (SE = 0.0004171 per ppm × day) was obtained for the low-exposure spline segment (Appendix D).

[14] Mean exposures for females with a 20-year lag for the categorical exposure quartiles in Table 8 of Steenland et al. (2004) were 276; 1,453; 5,869; and 26,391 ppm × days. Median values were 250; 1,340; 5,300; and 26,676 ppm × days. These values are for the risk sets but should provide a good approximation to the full cohort values.

1 mortality from the linear regression model are presented in Table 4-5. The resulting unit risk
2 estimate for breast cancer mortality based on the linear regression of the categorical results using
3 cumulative exposure with a 20-year lag is 0.513 per ppm. EC_{01} and LEC_{01} estimates from the
4 other models considered are presented for comparison only, to illustrate the differences in model
5 behavior at the low end of the exposure-response range. Unit risk estimates are not presented for
6 these other models because, as discussed above, these models were deemed unsuitable for the
7 derivation of risks from (low) environmental exposure levels. As one can see, the standard Cox
8 regression cumulative exposure model, with its extreme sublinearity in the lower exposure
9 region, yields a substantially higher EC_{01} estimate (0.530 ppm) than the EC_{01} estimate of 0.0387
10 ppm from the linear regression, while the log cumulative exposure Cox regression model, with
11 its extreme supralinearity in the lower exposure region, yields a substantially lower EC_{01}
12 estimates (0.00112 ppm). The estimates from the two-piece log-linear spline models flank the
13 result from the linear regression more closely. The steep low-exposure segment of the two-piece
14 log-linear spline model with the optimal knot at 700 ppm $\times$ days yields an EC_{01} estimate of
15 0.00941 ppm, whereas the shallower low-exposure slope from the two-piece log-linear spline
16 model with the local maximum likelihood suggesting a knot at 13,000 ppm $\times$ days yields an EC_{01}
17 estimate of 0.107 ppm. Converting the units, the unit risk estimate of 0.513 per ppm for breast
18 cancer mortality from the linear regression model corresponds to a unit risk estimate of $2.80 \times$
19 10^{-4} per $\mu g/m^3$.
20
21 **4.1.2.3.** *Prediction of Lifetime Extra Risk of Breast Cancer Incidence*
22   As discussed in Section 4.1.1.3, risk estimates for cancer incidence are preferred to
23 estimates for cancer mortality, especially for cancer types with good survival rates, such as
24 breast cancer. In the case of female breast cancer in the NIOSH cohort, there is a corresponding
25 incidence study (Steenland et al., 2003) with exposure-response results for breast cancer
26 incidence, so one can estimate cancer incidence risks directly rather than estimate them from
27 mortality data. The incidence study used a subcohort of 7,576 (76%) of the female workers from
28 the original cohort. Subcohort eligibility was restricted to the female workers who had been
29 employed at 1 of the 14 plants for at least 1 year, owing to cost considerations and the greater
30 difficulties in locating workers with short-term employment. Completed questionnaires were
31 received for 5,139 (68%) of the 7,576 women in the subcohort. The investigators also attempted
32

Table 4-5. EC_{01}, LEC_{01}, and unit risk estimates for breast cancer mortality
in females[a]

Model	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)
Log cumulative exposure, 20-year lag[b]	0.00112	0.000219	--[c]
Cumulative exposure, 20-year lag[d]	0.530	0.285	--[c]
Low-exposure log-linear spline, cumulative exposure with knot at 700 ppm × days, 20-year lag[e]	0.00941	0.00471	--[c]
Low-exposure log-linear spline, cumulative exposure with knot at 13,000 ppm × days, 20-year lag[f]	0.107	0.0580	--[c]
Categorical; cumulative exposure, 20-year lag[g]	0.0387	0.0195	**0.513**

[a]From lifetime continuous exposure. Unit risk = $0.01/LEC_{01}$.
[b]From Table 8 of Steenland et al. (2004), Cox regression model.
[c]Unit risk estimates are not presented for these models because these models were deemed unsuitable for the derivation of risks from (low) environmental exposure levels (see text).
[d]From Dr. Steenland's re-analyses (Table 4c of Appendix D), Cox regression model.
[e]From low-exposure segment of two-piece log-linear spline model with largest model likelihood and a knot at 700 ppm × days; see text and Table 4b of Appendix D. The EC_{01} value is appropriately below the value of 0.010 ppm roughly corresponding to the knot of 700 ppm × days and, thus, in the range of the low-exposure segment.
[f]From low-exposure segment of two-piece log-linear spline model with a local largest likelihood for knot at 13,000 ppm × days; see text and Table 4e of Appendix D. The EC_{01} value is appropriately below the value of 0.19 ppm roughly corresponding to the knot of 13,000 ppm × days and, thus, in the range of the low-exposure segment.
[g]Regression coefficient derived from linear regression of categorical Cox regression results from Table 8 of Steenland et al. (2004), as described in Section 4.1.2.2.

to acquire breast cancer incidence data for the entire subcohort from cancer registries (available for 9 of the 11 states in which the plants were located) and death certificates; thus, results are presented for both the full (sub)cohort ($n = 7,576$) and the subcohort of women with completed questionnaires ($n = 5,139$). For additional details and discussion of the Steenland et al. (2003) study, see Appendix A.

Steenland et al. (2003) identified 319 incident cases of breast cancer in the cohort through 1998. Interview (questionnaire) data were available for 73% (233 cases). Six percent were

carcinoma in situ (20 cases). Steenland et al. (2003) performed internal exposure-response analyses similar to those described in their 2004 paper and in Section 4.1.1.1 above. Controls for each case were selected from the cohort members without breast cancer at the age of diagnosis of the case. Cases and controls were matched on race. Of the potential confounders evaluated for those with interviews, only parity and breast cancer in a first-degree relative were important predictors of breast cancer, and only these variables were included in the final models for the subcohort analyses. In situ cases were included with invasive breast cancer cases in the analyses; however, the in situ cases represent just 6% of the total, and excluding them reportedly did not greatly affect the results.

From the Steenland et al. (2003) internal analyses (Cox regression) using the full cohort, the best-fitting model with exposure as a continuous variable was for (natural) log cumulative exposure, lagged 15 years ($p = 0.05$). Duration of exposure, lagged 15 years, provided a slightly better fitting model. Models using maximum or average exposure did not fit as well. In addition, use of a threshold model did not provide a statistically significant improvement in fit. For internal analyses using the subcohort with interviews, the cumulative exposure and log cumulative exposure models, both lagged 15 years, and the log cumulative exposure model with no lag all fit almost equally well, and the duration of exposure (also lagged 15 years) model fit slightly better. Results of the Cox regression analyses for the cumulative and log cumulative exposure models, with 15-year lags, are shown in Table 4-6, and these are the results considered for the unit risk calculations. The models using duration of exposure are less useful for estimating exposure-related risks, duration of exposure and cumulative exposure are correlated, and the fits for these models are only marginally better than those with cumulative exposure. The log cumulative exposure model with no lag was considered less biologically realistic than the corresponding model with a 15-year lag because some lag period would be expected for the development of breast cancer. Furthermore, although initial risk estimates based on the full cohort results are calculated for comparison, the preferred estimates are those based on the subcohort with interviews because the subcohort should have more complete case ascertainment and has additional information available on potential breast cancer confounders.

For the actuarial program (life-table analysis), U.S. age-specific all-cause mortality rates for 2004 for females of all race groups combined (NCHS, 2007) were used to specify the all-cause background mortality rates. Because breast cancer incidence rates are not negligible compared to all-cause mortality rates, the all-cause mortality rates in the life-table analysis were adjusted to reflect women dying *or* being diagnosed with breast cancer in a given age interval.

Table 4-6. Cox regression results for breast cancer incidence in females[a,b]

Cohort	Exposure variable[c]	Coefficient (SE), p value	ORs by category[d] (95% CI)
Full incidence study cohort $n = 7,576$ 319 cases	Cumulative exposure, 15-year lag	0.0000054 (0.0000035), $p = 0.12$	
	Log cumulative exposure, 15-year lag	0.037 (0.019), $p = 0.05$	
	Categorical cumulative exposure, 15-year lag		1.00, 1.07 (0.72–1.59), 1.00 (0.67–1.50), 1.24 (0.85–1.90), 1.17 (0.78–1.78), 1.74 (1.16–2.65)
Subcohort with interviews $n = 5,139$ 233 cases	Cumulative exposure, 15-year lag	0.0000095 (0.0000041), $p = 0.02$	
	Log cumulative exposure, 15-year lag	0.050 (0.023), $p = 0.03$	
	Categorical cumulative exposure, 15-year lag	--[e]	1.00, 1.06 (0.66–1.71), 0.99 (0.61–1.60), 1.24 (0.76–2.00), 1.42 (0.88–2.29), 1.87 (1.12–3.10)

[a]Invasive breast cancer (ICD-9 174) and carcinoma in situ (ICD-9 233.0).
[b]Cases and controls matched on age and race (white/nonwhite). Full cohort models include cumulative exposure and categorical variable for year of birth (quartiles). Subcohort models include cumulative exposure, categorical variables for year of birth (quartiles), breast cancer in first-degree relative, and parity.
[c]Cumulative exposure is in ppm × days.
[d]Exposure categories are 0, >0–647, 647–2,026, 2,026–4,919, 4,919–14,620, >14,620 ppm × days.
[e]p value for the addition of the exposure variables = 0.11 (e-mail dated 5 March 2010 from Kyle Steenland, Emory University, to Jennifer Jinot, U.S. EPA)

Source: Tables 4 and 5 of Steenland et al. (2003).

All-cause mortality rates and breast cancer incidence rates were summed, and breast cancer mortality rates were subtracted so that those dying of breast cancer were not counted twice (i.e., as deaths and as incident cases of breast cancer). The National Center for Health Statistics 2002–2006 mortality rates for invasive breast cancer in females were obtained from a SEER report (NCI, 2009). The SEER report also provided SEER–17 incidence rates for invasive and in situ breast cancer. The Cox regression results reported by Steenland et al. (2003) are for invasive and in situ breast cancers combined. It is consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S.EPA, 2005a) to combine these two tumor types because the in situ tumors can

1   progress to invasive tumors. Thus, the primary risk calculations in this assessment use the sum

2   of invasive and in situ breast cancer incidence rates for the cause-specific background rates.

3   Comparison calculations were performed using just the invasive breast cancer incidence rates for

4   the cause-specific rates; this issue is further discussed in Section 4.1.3 on sources of uncertainty.

5   The risks were computed up to age 85 for continuous exposures to EtO, conversions were made

6   between occupational EtO exposures and continuous environmental exposures, and 95% UCLs

7   were calculated for the relative rates, as described in Section 4.1.1.2 above.

8   For breast cancer incidence in both the full cohort (Figure 4-4) and the subcohort with

9   interviews (Figure 4-5), the categorical results suggest a more linear exposure-response

10   relationship than that obtained with either the continuous variable log cumulative exposure

11   (supralinear) or cumulative exposure (sublinear) Cox regression models, the two of which lie on

12   opposite sides of the low-exposure categorical results. Thus, as with the lymphohematopoietic

13   cancer and the breast cancer mortality results above, EPA proposed in the 2006 Draft

14   Assessment (U.S. EPA, 2006b), which relied on the original published results of Steenland et al.

15   (2003), that the best way to reflect the data in the lower exposure region, which is the region of

16   interest for low-exposure extrapolation, was to do a weighted linear regression of the results

17   from the model with categorical cumulative exposure (with a 15-year lag). In addition, the

18   highest exposure group was not included in the regression to provide a better fit to the lower

19   exposure data. However, as discussed in Section 4.1.1.2 for the lymphohematopoietic cancer

20   data, the Science Advisory Board panel that reviewed the draft assessment recommended that

21   EPA not rely on the published grouped data but, rather, do additional analyses using the

22   individual data (SAB, 2007). Consequently, it was determined that using the individual data, a

23   better way to address the supralinearity (the categorical data appear fairly linear; however, based

24   on the continuous data, the exposure-response relationship does ultimately tend to plateau at the

25   higher exposures) of the data (while avoiding the extreme low-exposure curvature obtained with

26   the log cumulative exposure Cox regression model) might be to use a two-piece spline model,

27   and Dr. Steenland was commissioned to do the spline analyses. His findings are reported in

28   Appendix D (Section 1), and the results for the breast cancer incidence analyses are summarized

29   below. Note that, for the two-piece spline analyses, only the data from the subcohort with

30   interviews and for the invasive and in situ breast cancers combined were analyzed, because this

31   was the preferred dataset, as discussed above.

32   For the two-piece log-linear spline modeling approach, as described in Section 4.1.1.2

33   and discussed more fully in Appendix D, the Cox regression model was the underlying basis for

34   the splines which were fit to the breast cancer incidence exposure-response data (cumulative
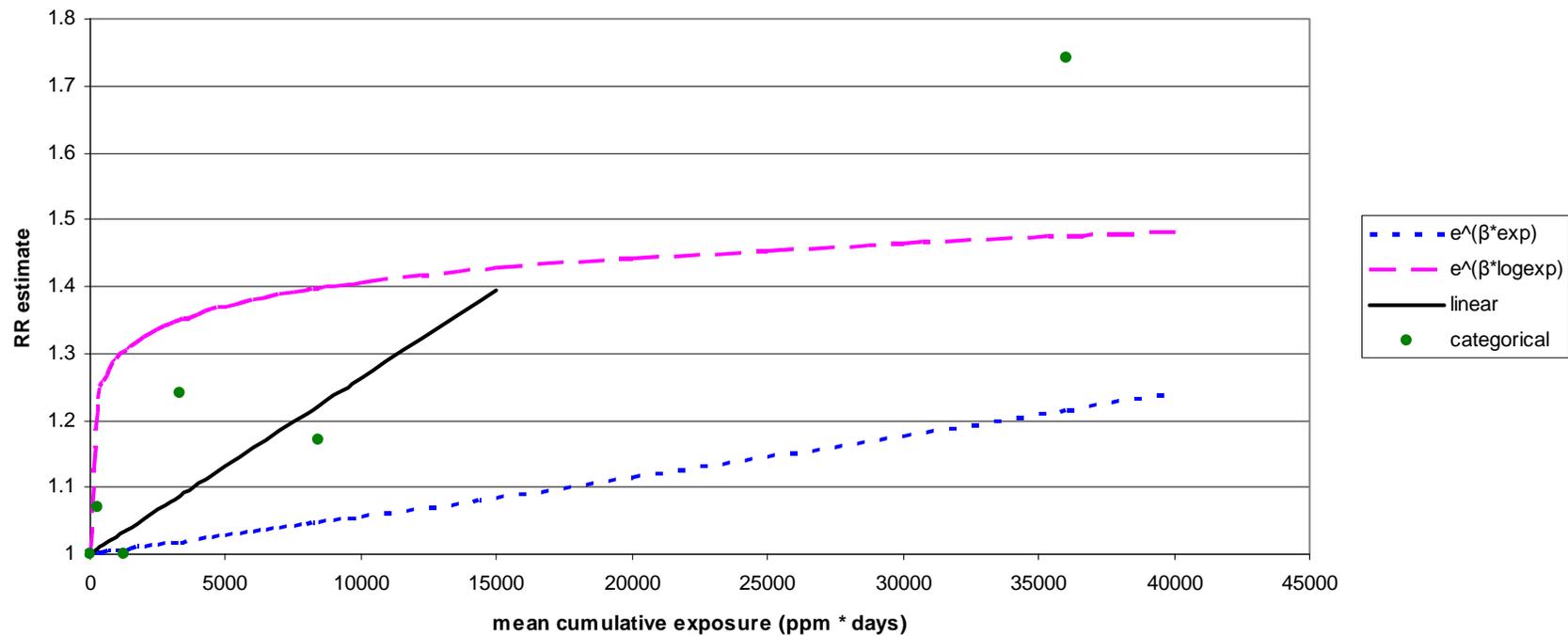
Figure 4-4. **RR estimate for breast cancer incidence in full cohort vs. mean exposure (with 15-year lag, unadjusted for continuous exposure).**

e^(β*exp): Cox regression results for $RR = e^{(\beta*exposure)}$; e^(β*logexp): Cox regression results for $RR = e^{(\beta*\ln(exposure))}$; categorical: Cox regression results for $RR = e^{(\beta*exposure)}$ with categorical exposures; linear: weighted linear regression of categorical results, excluding highest exposure group (see text).

Source: Steenland et al. (2003) (except for linear regression, which was done by EPA).
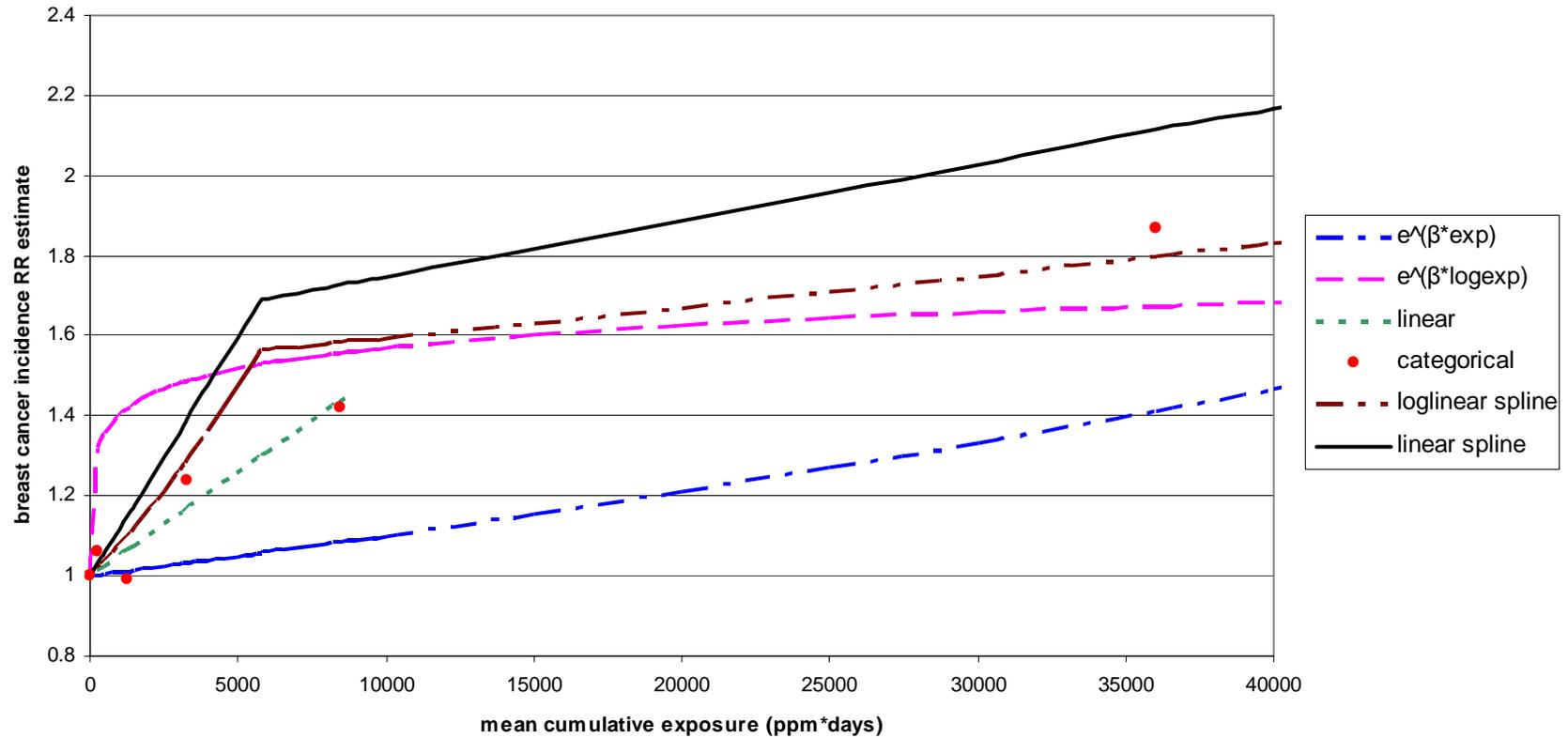
Figure 4-5. **RR estimate for breast cancer incidence in subcohort with interviews vs. mean exposure (with 15-year lag, unadjusted for continuous exposure).**

e^(β*exp): Cox regression results for RR = $e^{(\beta*exposure)}$; e^(β*logexp): Cox regression results for RR = $e^{(\beta*\ln(exposure))}$; categorical: Cox regression results for RR = $e^{(\beta*exposure)}$ with categorical exposures; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); log-linear and linear spline: 2-piece spline models, both with knots at 5800 ppm*days (see text)

Sources: Steenland et al. (2003) except for Steenland 2-piece spline models (see Appendix D) and linear regression, which was done by EPA.

1 exposure is used here, with a 15-year lag), and, thus, log RR is a function of two lines which join
2 at a single point of inflection, called a "knot". The shape of the two-piece spline model, in
3 particular the slope in the low-exposure region, depends on the location of the knot. For this
4 assessment, the knot was generally selected by trying different knots in increments of 1000 ppm
5 × days, starting at 1000 ppm × days, and choosing the one that resulted in the largest model
6 likelihood. In some cases, increments of 100 ppm × days were used between the increments of
7 1000 ppm × days to fine-tune the knot selection. The model likelihood did not actually change
8 much across the different trial knots (see Figure 1a of Appendix D), but it did change slightly,
9 and a knot of 5800 ppm × days for the breast cancer incidence data based on the largest
10 likelihood was chosen. The two-piece log-linear spline model with this knot provided a
11 statistically significant fit to the data ($p = 0.0003$; $p = 0.01$ for the addition of the exposure
12 terms), as well as a good visual fit (Figure 4-5). Using the resulting two-piece log-linear spline
13 model, a regression coefficient of 0.0000770 per ppm × day (SE = 0.0000317 per ppm × day)
14 was obtained for the low-exposure spline segment ($p = 0.02$).
15   A two-piece linear spline model was also fitted, using the just-published approach of
16 Langholz and Richardson (2010). This model is similar to the log-linear spline model discussed
17 above; however, for the linear spline model, the underlying basis for the splines is a linear model
18 (i.e., RR = 1 + β × z, where z represents the covariate data, including exposure, and β are the
19 parameters being estimated). The knot was selected as for the log-linear spline model, and the
20 same knot of 5800 ppm × days yielded the largest likelihood (Figure 1h of Appendix D) and was
21 also chosen for the two-piece linear spline model. The two-piece linear spline model with this
22 knot provided a statistically significant fit to the data ($p = 0.0001$; $p = 0.002$ for the addition of
23 the exposure terms), as well as a good visual fit (Figure 4-5). Using the resulting two-piece
24 linear spline model, a regression coefficient of 0.000119 per ppm × day (SE = 0.0000677 per
25 ppm × day)[15] was obtained for the low-exposure spline segment. Because this model provided a
26 better fit than the log-linear spline model, for both the full model and the addition of the
27 exposure terms, the two-piece linear spline model was selected as the preferred model for the
28 unit risk estimates for breast cancer incidence. For more discussion of the breast cancer
29 incidence exposure-response modeling and for a comparison of the results with those from a

---

[15] Confidence intervals were determined using the Wald approach. Confidence intervals for linear RR models, however, in contrast to those for the log-linear RR models, may not be symmetrical. EPA also evaluated application of a profile likelihood approach for the linear RR models (Langholz and Richardson, 2009), which allows for asymmetric CIs, for comparison with the Wald approach. Using the profile likelihood method, the 95% (one-sided) upper bound on the regression coefficient for the low-exposure spline segment is 0.000309 per ppm × day and the 95% (one-sided) lower bound is 0.000032 per ppm × day. This upper bound estimate of 0.000309 per ppm × day is 34% higher than the value of 0.000230 per ppm × day obtained using the Wald approach and employed in this assessment for the derivation of the unit risk estimates.

1   cubic spline Cox regression model and a square-root transformation Cox regression model[16], see

2   Section 1 of Appendix D.

3   Risk estimates based on the original linear regression analyses are also presented for

4   comparison. For the approach of using a weighted linear regression of the results from the Cox

5   regression model with categorical cumulative exposure (and a 15-year lag), excluding the highest

6   exposure group, the weights used for the ORs were the inverses of the variances, which were

7   calculated from the confidence intervals.[17] Mean and median exposures for the cumulative

8   exposure groups for the full cohort were kindly provided by Dr. Steenland (e-mail dated April

9   21, 2004, from Kyle Steenland, Emory University, to Jennifer Jinot, U.S. EPA).[18] The mean

10   values were used for the weighted regression analysis because the (arithmetic) mean exposures

11   best represent the model's linear relationship between exposure and cancer response.

12   Differences between means and medians were not large for the females, especially for the lower

13   four quintiles. If the median values had been used, a slightly larger regression coefficient would

14   have been obtained, resulting in slightly larger risk estimates. Although the exposure values are

15   for risk sets from the full cohort, they should be reasonably close to the values for the subcohort

16   with interviews. Using the weighted linear regression approach, a regression coefficient of

17   0.0000264 per ppm × day (SE = 0.0000269 per ppm × day) was obtained for the full cohort, and

18   a regression coefficient of 0.0000517 per ppm × day (SE = 0.0000369 per ppm × day) was

19   obtained for the subcohort of women with interviews. See Figures 4-4 and 4-5 for a depiction of

20   the resulting linear regression models.

21   The exposure level (EC$_x$) and the associated 95% lower confidence limit (LEC$_x$)

22   corresponding to an extra risk of 1% ($x = 0.01$) for breast cancer incidence in females (based on

23   invasive + in situ tumors in the subcohort with interviews) for the different models examined

24   above were estimated using the actuarial program (life-table analysis). As discussed in Section

25   4.1.1.2, a 1% extra risk level is a more reasonable response level for defining the POD for these

26   epidemiologic data than 10%. The results are presented in Table 4-7.

27   Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3),

28   which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,

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[16] The square-root transformation model was considered but rejected, because it was notably supralinear in the low-dose region (see Section 1.d of Appendix D). The cubic spline is too complicated a function for risk assessment (see Section 1.e of Appendix D).

[17] Equations for this weighted linear regression approach are presented in Rothman (1986) and summarized in Appendix F.

[18] Mean exposures for females with a 15-year lag for the exposure categories in Table 3 were 280; 1,241; 3,304; 8,423; and 36,022 ppm × days. Median values were 253; 1,193; 3,241; 7,741; and 26,597 ppm × days. These values are for the risk sets but should provide a good approximation to the full cohort values.

1 2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was

2 performed.

3

4   **Table 4-7. EC_{01}, LEC_{01}, and unit risk estimates for breast cancer incidence**
5   **in females—invasive and in situ[a]**

6

	With interviews			Full cohort		
Model	**EC_{01} (ppm)**	**LEC_{01} (ppm)**	**Unit risk (per ppm)**	**EC_{01} (ppm)**	**LEC_{01} (ppm)**	**Unit risk (per ppm)**
Cumulative exposure, 15-year lag[b]	0.135	0.0788	--[c]	0.237	0.115	--[c]
Log cumulative exposure, 15-year lag[b]	0.0000765	0.0000422	--[c]	0.000124	0.0000529	--[c]
Categorical; cumulative exposure, 15-year lag[b,d]	0.0257	0.0118	0.847	0.0503	0.0188	0.532
Low-exposure log-linear spline, cumulative exposure, 15-year lag[e]	0.0166	0.00991	1.01[f]	--[g]		
Low-exposure linear spline, cumulative exposure, 15-year lag[e]	0.0112	0.00576	**1.74**[f]	--[g]		

7   [a]All-cause mortality adjusted (to dying of something other than breast cancer or developing breast cancer). Unit risk
8   = 0.01/LEC_{01}. Note that the EC_{01} and LEC_{01} results presented here will not exactly match those presented in
9   Appendix D because, although the regression coefficients reported by Dr. Steenland in Appendix D were used, the
10 life-table analyses using 2004 all-cause mortality and 2002–2006 cause-specific mortality and incidence rates were
11 re-done to be more up-to-date; the results presented in Appendix D were based on life-table analyses using 2000
12 all-cause mortality rates and comparable cause-specific rates.
   [b]From Tables 4 and 5 of Steenland et al. (2003), Cox regression models.
   [c]Unit risk estimates are not presented for these models because these models were deemed unsuitable for the
    derivation of risks from (low) environmental exposure levels (see text).
   [d]Regression coefficient derived from linear regression of categorical results, as described in Section 4.1.2.3.
   [e]From low-exposure segment of two-piece spline analysis; see text and Table 2b of Appendix D for log-linear model
    or Table 2h for linear model; two-piece spline analyses not performed for the full cohort. The EC_{01} value is

appropriately below the value of 0.075 ppm roughly corresponding to the knot of 5800 ppm × days and, thus, in the range of the low-exposure segment..

[f]For unit risk estimates above 1, convert to risk per ppb. e.g., 1.74 per ppm = 1.74 × 10^{-3} per ppb.

[g]Not estimated.

1   The inhalation unit risk estimates for the different breast cancer incidence models considered
2   suitable for low-exposure extrapolation are presented in Table 4-7. As discussed above, the unit
3   risk estimate based on the two-piece linear spline model using cumulative exposure with a
4   15-year lag (i.e., 1.74 per ppm, or 1.74 × 10^{-3} per ppb) is the preferred estimate. The two-piece
5   log-linear spline model resulted in a unit risk estimate of 1.01
6   per ppm, while the linear regression approach yielded a unit risk estimate of 0.847 per ppm;
7   these alternate estimates are nearly 60% and 50%, respectively, of the estimate based on the
8   preferred two-piece linear spline model. EC_{01} and LEC_{01} estimates from the other models
9   examined are presented for comparison only, to illustrate the differences in model behavior at the
10   low end of the exposure-response range. Unit risk estimates are not presented for these other
11   models because, as discussed above, the log cumulative exposure Cox regression model was
12   considered overly supralinear and the cumulative exposure Cox regression model was considered
13   overly sublinear for the data in the lower exposure range (e.g., 1st 4 quintiles of exposure). As
14   one can see from the results for the subcohort with interviews, the standard Cox regression
15   cumulative exposure model, with its extreme sublinearity in the lower exposure region, yields a
16   notably higher EC_{01} estimate (0.135 ppm) than that from the two-piece linear spline model
17   (0.0112), while the log cumulative exposure model, with its extreme supralinearity in the lower
18   exposure region, yields a substantially lower EC_{01} estimate (0.0000765 ppm). Converting the
19   units, the preferred unit risk estimate of 1.74 per ppm corresponds to an estimate of 9.51 × 10^{-4}
20   per μg/m^{3} for breast cancer incidence.
21   As discussed above, the primary risk calculations for breast cancer incidence were based
22   on invasive and in situ tumors in the subcohort of women with interviews, and the primary
23   model was the two-piece linear spline model. For this assessment, the two-piece spline analyses
24   were not performed with the full cohort and the life-table analyses were not replicated for the
25   invasive cancers only. In the 2006 Draft Assessment (U.S. EPA, 2006b), however, comparison
26   analyses were done. Using the linear regression approach, the comparable unit risk estimate for
27   the full cohort was about 40% lower than the estimate based on the subcohort with interviews.
28   One would expect this value to be lower because of incomplete case ascertainment in the full
29   cohort. The corresponding unit risk estimate derived based on the subcohort results but using
30   invasive breast cancer only for the background incidence rates was about 17% lower than the
31   estimate based on invasive and in situ tumors, reflecting the difference between incidence rates
32   for invasive breast cancer only and for combined in situ and invasive breast cancer.

1   The unit risk estimate of **1.74 per ppm** (1.74×10^{-3} per ppb) is the preferred estimate for

2 female breast cancer risk because it is based on incidence data versus mortality data, it is based

3 on more cases ($n = 233$) than the mortality estimate ($n = 103$), and information on personal

4 breast cancer risk factors obtained from the interviews is taken into account. Furthermore, the

5 two-piece linear spline model, which uses the complete dataset with exposure as a continuous

6 variable, was statistically significant and provided a good visual fit to the data. Converting the

7 units, 1.74 per ppm corresponds to a unit risk of 9.51×10^{-4} per $\mu g/m^3$.

8

9 **4.1.3. Total Cancer Risk Estimates**

10   According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a),

11 cancer risk estimates are intended to reflect total cancer risk, not site-specific cancer risk;

12 therefore, an additional calculation was made to estimate the combined risk for (incident)

13 lymphoid and breast cancers, because females would be at risk for both cancer types. Assuming

14 that the tumor types are independent and that the risk estimates are approximately normally

15 distributed, one can estimate the 95% UCL (one-sided) on the total risk as the 95% UCL on the

16 sum of the MLEs of the risk estimates according to the formula

17

18   95% UCL = MLE + 1.645(SE),

19

20 where MLE is the MLE of total cancer risk (i.e., the sum of the individual MLEs) and the SE of

21 the sum of the MLEs is the square root of the sum of the individual variances (i.e., the variance

22 of the sum is the sum of the variances, and the SE is the square root of the variance). First, an

23 EC_{01} of 0.0078 ppm for the total cancer risk (i.e., lymphoid cancer incidence + breast cancer

24 incidence) was estimated, as summarized in Table 4-8.

25

26   **Table 4-8**. **Calculation of EC_{01} for total cancer risk**

27

Cancer type	EC_{01} (ppm)	$0.01/EC_{01}$ (per ppm)	EC_{01} for total risk (ppm)
Lymphoid	0.0254	0.394	--
Breast	0.0112	0.893	--
Total[a]	--	1.29	0.00775

28

29 [a]The total $0.01/EC_{01}$ value equals the sum of the individual $0.01/EC_{01}$ values; the EC_{01} for the total
30   cancer risk then equals $0.01/(0.01/EC_{01})$.

31

1
2   Then, a unit risk estimate of 2.3 per ppm for the total cancer risk (i.e., lymphoid cancer

3   incidence + breast cancer incidence) was derived, as shown in Table 4-9. An LEC_{01} estimate of

4   0.00441 ppm for the total cancer risk can be calculated as 0.01/(2.27 per ppm).

5   Thus, the total cancer unit risk estimate is 2.3 per ppm (or 2.3×10^{-3} per ppb; 1.2×10^{-3}

6   per $\mu g/m^3$) Recall that this is the unit risk estimate derived under the assumption that RR is

7   independent of age (Section 4.1.1.2). The preferred assumption of increased early-life

8   susceptibility, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), is

9

10   **Table 4-9**. **Calculation of total cancer unit risk estimate**

11

Cancer type	Unit risk estimate (per ppm)	$0.01/EC_{01}$ (per ppm)	SE^a (per ppm)	Variance	Total cancer unit risk estimate (per ppm)
Lymphoid	0.877	0.394	0.294	0.0864	--
Breast	1.74	0.893	0.515	0.265	--
Total	--	1.29	$(0.593)^b$	0.351	2.27^c

12
13   ^{a}SE = (unit risk – $0.01/EC_{01}$)/1.645.
14   ^{b}The SE of the total cancer risk is calculated as the square root of the sum of the variances (next column), not as the
15   sum of the SEs.
16   ^{c}Total cancer unit risk = $1.29 + 1.645 \times 0.593$.
17
18
19   considered in Section 4.4. While there are uncertainties regarding the assumption of a normal

20   distribution of risk estimates, the resulting unit risk estimate is appropriately bounded in the

21   roughly 2-fold range between estimates based on the sum of the individual MLEs (i.e., 1.29) and

22   the sum of the individual 95% UCLs (i.e., unit risk estimates, 2.6), or, more precisely in this

23   case, between the largest individual unit risk estimate (1.74) and the sum of the unit risk

24   estimates (2.6). Thus, any inaccuracy in the total cancer risk estimate resulting from the approach

25   used to combine risk estimates across cancer types is relatively minor.

26

27   **4.1.4. Sources of Uncertainty in the Cancer Risk Estimates**

28   The two major sources of uncertainty in quantitative cancer risk estimates are generally

29   interspecies extrapolation and high-dose to low-dose extrapolation. The risk estimates derived

30   from the Steenland et al. (2003, 2004) and additional Steenland (Appendix D) analyses are not

31   subject to interspecies uncertainty because they are based on human data. Furthermore, the

32   human-based estimates are less affected by high-dose to low-dose extrapolation than do rodent-

1   based estimates and, thus, uncertainty from that source is reduced somewhat. For example, the

2   average exposure in the NIOSH cohort was more than 10 times lower than the lowest exposure

3   level in a rodent bioassay after adjustment to continuous lifetime exposure. Nonetheless,

4   uncertainty remains in the extrapolation from occupational exposures to lower environmental

5   exposures. Although the actual exposure-response relationship at low exposure levels is

6   unknown, the clear evidence of EtO mutagenicity supports the linear low-exposure extrapolation

7   that was used (U.S. EPA, 2005a).

8   Other sources of uncertainty emanate from the epidemiologic studies and their analyses

9   (Steenland et al., 2003, 2004; Steenland analyses in Appendix D), including the retrospective

10   estimation of EtO exposures in the cohort, the modeling of the epidemiologic exposure-response

11   data, the proper dose metric for exposure-response analysis, and potential confounding or

12   modifying factors. Although these are common areas of uncertainty in epidemiologic studies,

13   they were generally well addressed in the NIOSH studies.

14   Regarding exposure estimation, the NIOSH investigators conducted a detailed

15   retrospective exposure assessment to estimate the individual worker exposures. They used

16   extensive data from 18 facilities, spanning a number of years, to develop a regression model

17   (Greife et al., 1988; Hornung et al., 1994). The model accounted for 85% of the variation in

18   average EtO exposure levels. Detailed work history data for the individual workers were

19   collected for the 1987 follow-up (Steenland et al., 1991). For the extended follow-up (Steenland

20   et al., 2003, 2004), additional information on the date last employed was obtained for those

21   workers still employed and exposed at the time of the original work history collection for the

22   plants still using EtO (25% of the cohort). It was then assumed that exposure for these workers

23   continued until the date of last employment and that their exposure level stayed the same as that

24   in their last job held at the time of the original data collection. Thus, there would be more

25   exposure misclassification in the extended follow-up. However, when the investigators

26   compared cumulative exposures estimated with and without the extended work histories, they

27   found little difference because exposure levels were very low by the mid-1980s and, therefore,

28   had little impact on cumulative exposure (Steenland et al., 2003, 2004). While the NIOSH

29   regression model performed well in estimating exposures in validation tests (Hornung et al.,

30   1994), there is, nonetheless, uncertainty associated with any retrospective exposure assessment,

31   and this can affect the ability to discriminate among exposure-response models.

32   With respect to the lymphohematopoietic cancer response, it is not clear exactly which

33   lymphohematopoietic cancer subtypes are related to EtO exposure, so analyses were done for

34   both lymphoid cancers and all lymphohematopoietic cancers (Steenland et al., 2004). The

35   associations observed for all lymphohematopoietic cancers was largely driven by the lymphoid

36   cancer responses, and, biologically, there is stronger support for an etiologic role for EtO in the

1 development of the more closely related lymphoid cancers than in the development of the more
2 diverse cancers in the aggregate all lymphohematopoietic cancer grouping; thus, the lymphoid
3 cancer analysis is the preferred analysis for the lymphohematopoietic cancers. Nonetheless, the
4 preferred unit risk estimate for all lymphohematopoietic cancers was similar (about 50% greater)
5 to that for the lymphoid cancers.
6 For the lymphoid cancer response (Steenland et al., 2004), all attempts at exposure-
7 response modeling are limited by the small number of cases ($n = 53$). The Cox proportional
8 hazards model used by Steenland et al. is commonly used for this type of analysis because
9 exposure can be modeled as a continuous variable, competing causes of mortality can be taken
10 into account, and potential confounding factors can be controlled for in the regression.
11 Normally, model dependence should be minimized by the practice, under EPA's 2005
12 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), of modeling only in the
13 observable range and then performing a linear extrapolation from the "POD" (in this case the
14 LEC_{01}). However, the log cumulative exposure Cox regression model with 15-year lag, which
15 provides the best fit to the overall data, is too steep in the low-exposure region and then plateaus
16 rapidly at higher exposures, making it difficult to derive stable risk estimates (i.e., estimates that
17 are not highly dependent on the POD). And the alternative cumulative exposure model, though
18 typically used for epidemiologic data, is too sublinear in the low-exposure region for these data,
19 which exhibit supralinearity. EPA attempted to fit two-piece log-linear and linear spline models
20 to the individual continuous data to address the supralinearity of the data while avoiding the
21 extreme low-exposure curvature of the log cumulative exposure model; however, these models
22 resulted in low-exposure slopes that appeared to be implausibly steep. The steep low-exposure
23 slopes are a manifestation of apparently high risks in workers with relatively low exposures;
24 however, this elevation is based on small numbers of cancer cases in that exposure range and we
25 have low confidence in the low-exposure slopes. The two-piece spline model with the knot at a
26 higher exposure level could have been used, but, without model likelihood as a basis for knot
27 selection, such selection becomes arbitrary, and with the knot at a higher exposure level which
28 had an apparent local maximum for the log-linear model (1600 ppm $\times$ days rather than 100 ppm
29 $\times$ days), the visual fit was problematic (Figure 4-1). Thus, EPA opted for a weighted linear
30 regression model based on the Cox regression categorical results, excluding the highest exposure
31 group, to reflect the exposure-response relationship in the exposure region below the "plateau".
32 The all lymphohematopoietic cancer dataset had more cases ($n = 74$) but was heavily dominated
33 by the lymphoid cancer response and conveyed the same problems for exposure-response
34 modeling; thus, a linear regression model, excluding the highest exposure group, was used for
35 this dataset as well.

1   The linear model is a parsimonious choice which assumes neither a sublinear nor a
2   supralinear exposure-response relationship and acknowledges the inherent imprecision in the
3   epidemiological data. The highest exposure group was excluded because it is less relevant to the
4   low-exposure risks of interest for low-exposure extrapolation and its inclusion would have overly
5   influenced the linear regression, resulting in a slope that would have underestimated the apparent
6   low-exposure risks. Excluding data can also become arbitrary, but EPA aimed to avoid an
7   arbitrary selection by using the *a priori* exposure groups presented by Steenland et al. (2004) and
8   excluding only the highest exposure group, with the exposures least relevant to low
9   environmental exposure levels. The linear regression has its own limitations, e.g., it is based on
10 categorical rather than continuous data and the slopes were not statistically significant ($p = 0.18$
11 for lymphoid cancers and $p = 0.075$ for all lymphohematopoietic cancers); nonetheless, it was
12 judged to be the most reasonable approach for deriving low-exposure risk estimates from the
13 available lymphohematopoietic cancer data.
14   Although the linear regression model seems to be a reasonable approach for best
15 reflecting the exposure-response results at the lower end of the exposure range, clearly there is
16 uncertainty regarding the exposure-response model, as suggested by the range of EC_{01} estimates
17 resulting from the different models (Table 4-3). The log cumulative exposure Cox regression
18 model, which was the best-fitting model overall, yields lower EC_{01} and LEC_{01} estimates, but the
19 estimates based on the linear regression model are preferred because the linear regression model
20 is more stable.
21   Another, more minor area of uncertainty related to the exposure-response modeling is the
22 lag period. The best-fitting models presented by Steenland et al. (2004) for
23 lymphohematopoietic cancer had a 15-year lag (lag periods of 0, 5, 10, 15, and 20 years were
24 considered). A 15-year lag period means that exposures in the 15 years prior to death or the end
25 of follow-up are not taken into account. In other words, in the best-fitting models, relevant
26 exposures for the development of the lymphohematopoietic cancers occurred over 15 years
27 before death. In addition, the analyses of the investigators indicate that the regression coefficient
28 for cumulative exposure might have decreased with follow-up, suggesting that the higher
29 exposure levels encountered by the workers in the more distant past are having less of an impact
30 on current risk. The regression coefficient for lymphoid cancers was 1.2×10^{-5} per ppm $\times$ day,
31 for both sexes with a 10-year lag, in the 1987 follow-up (Stayner et al., 1993) versus 4.7×10^{-6}
32 per ppm $\times$ day, for both sexes with a 15-year lag, in the 1998 follow-up (Steenland re-analyses in
33 Appendix D). A similar decrease was found in the regression coefficient for cumulative
34 exposure for all lymphohematopoietic cancers.
35   The life-table analysis used in this dose-response assessment accrues exposure over the
36 full lifetime for the cumulative exposure metric. If, in fact, exposures in the distant past cease to

1   have a meaningful impact on risk of lymphohematopoietic cancers, this approach would tend to

2   overestimate the unit risk. Thus, a comparison analysis was conducted to evaluate the impact of

3   ignoring exposures over 55 years in the past in the life-table analysis. The actual value of such a

4   cut-point, if warranted, is unknown. A value less than 55 years might not be appropriate because

5   exposures for some of the workers began in 1943, so any diminution of potency for past

6   exposures occurring since 1943 is already reflected in the regression coefficient with follow-up

7   through 1998, at least for those workers, although it is unknown what proportion of workers had

8   such early exposures and how long they survived. The comparison analysis for lymphoid cancer

9   yielded an LEC_{01} of 0.0156 ppm and a unit risk estimate of 0.64 per ppm, which is about 27%

10 less than the estimate obtained from the unrestricted life-table analysis. Because the appropriate

11 cut-point for excluding past exposures is unknown and the unit risk estimate from the linear

12 regression model is already substantially less than that obtained from the best-fitting log

13 cumulative exposure Cox regression model, the estimate from the full life-table analysis is

14 preferred. In any event, the preferred estimate is not appreciably different from the estimate

15 from the analysis which considered only the most recent 55 years of exposure in the life-table

16 analysis.

17   Several dose metrics (cumulative exposure, duration of exposure, maximum [8-hour

18 TWA] exposure, and average exposure) were analyzed by the Steenland et al. (2004), and

19 cumulative exposure was the best predictor of mortality from lymphohematopoietic cancers.

20 Cumulative exposure is considered a good measure of total exposure because it integrates

21 exposure (levels) over time.

22   Also, the important potential modifying/confounding factors of age, sex, race, and

23 calendar time were taken into account in the analysis, and the plants included in this cohort were

24 specifically selected for the absence of any known confounding exposures (Stayner et al., 1993).

25   With respect to the breast cancer mortality response (Steenland et al., 2004), the

26 exposure-response modeling was based on 103 deaths. As for the lymphohematopoietic cancer

27 responses, the exposure-response data for breast cancer mortality are fairly supralinear,

28 especially for the low-exposure groups. An attempt was again made to fit two-piece log-linear

29 and linear spline models to the individual continuous data to address the supralinearity of the

30 data while avoiding the extreme low-exposure curvature of the log cumulative exposure Cox

31 regression model; however, these models resulted in low-exposure slopes that appeared to be

32 implausibly steep and the model fits were not convincing (i.e., they were neither statistically

33 significant nor visually compelling; Figure 4-3). Thus, the same linear regression approach,

34 excluding the highest exposure group, was taken to obtain a regression coefficient for the life-

35 table analysis. As discussed above, the linear regression has its own limitations, e.g., it is based

36 on categorical rather than continuous data and the slope is not statistically significant ($p = 0.094$);

1   nonetheless, it was judged to be the most reasonable approach for deriving low-exposure risk

2   estimates from the available breast cancer mortality data.

3   For the lag period, the best-fitting model had a lag of 20 years, which was longest lag

4   period investigated. This is a commonly used lag period for solid tumors, which typically have

5   longer latency periods than lymphohematopoietic cancers. It is unknown whether a lag period

6   longer than 20 years would have provided a better model fit. The Steenland et al. (2004)

7   analysis took into account age, race, and calendar time. Other risk factors for breast cancer could

8   not be included in the mortality analysis, but many of these factors were considered in the breast

9   cancer incidence study (Steenland et al., 2003), as discussed below, and the preferred breast

10   cancer risk estimates are based on the breast cancer incidence data.

11   Steenland et al. (2003) conducted an incidence study for breast cancer; therefore, it was

12   not necessary to calculate unit risk estimates for breast cancer incidence indirectly from the

13   mortality data as was done for lymphohematopoietic cancer. Further advantages to using the

14   results from the incidence study are that more cases were available for the exposure-response

15   modeling (319 cases) and that the investigators were able to include data on potential

16   confounders in the modeling for the subcohort with interviews (233 cases). For the full cohort,

17   the continuous exposure Cox regression model providing the best fit to the data was again the log

18   cumulative exposure model. With breast cancer incidence, a 15-year lag provided the best model

19   fits. For the subcohort, the cumulative exposure and log cumulative exposure Cox regression

20   models fit nearly equally well. For both groups, the categorical Cox regression results suggest

21   that a linear model lying between the supralinear log cumulative exposure model and the

22   sublinear cumulative exposure model would better represent the low-exposure data than either of

23   the two presented continuous-variable models (Figures 4-4 and 4-5). Thus, for both groups, in

24   the original analyses based on the published summary data, a linear regression was fitted to the

25   categorical results, dropping the highest exposure group to provide a better fit to the lower-

26   exposure data. In addition, in subsequent analyses by Dr. Steenland (Appendix D) of the

27   individual data using exposure as a continuous variable, two-piece log-linear and linear spline

28   models were used to model the subcohort data; the two-piece linear spline model was the best-

29   fitting of these models and provided the preferred breast cancer incidence risk estimates.

30   Confidence intervals were determined using the Wald approach. Confidence intervals for

31   linear RR models, however, in contrast to those for the log-linear RR models, may not be

32   symmetrical. EPA also evaluated application of a profile likelihood approach for the linear RR

33   models (Langholz and Richardson, 2009), which allows for asymmetric CIs, for comparison with

34   the Wald approach. Using the profile likelihood method, the resulting unit risk estimate for

35   breast cancer incidence would have been 2.33 per ppm, slightly higher (34%) than the value of

36   1.74 per ppm obtained as the unit risk estimate for breast cancer incidence in this assessment.

1 These results suggest that if the profile likelihood method had been used for the linear RR
2 models in this assessment, the total cancer risk estimate, which incorporates the breast cancer
3 incidence estimate as a component, would be less than 34% higher than the total cancer risk
4 estimate presented here.

5   With respect to the two-piece spline models, the use of this model form is not intended to
6 imply that an abrupt change in biological response occurs at the knot but, rather, to allow
7 description of an exposure-response relationship in which the slope of the relationship differs
8 notably in the low-exposure versus high-exposure regions. The two-piece model is used here
9 primarily for its representation of the low-exposure data. The main uncertainty in the two-piece
10 spline models is in the selection of the knot, and the location of the knot is critical in defining the
11 low-exposure slope. The model likelihood was used to provide a statistical basis for knot
12 selection; although, as shown in Appendix D, the likelihood did not generally change
13 appreciably over a range of possible knots. Thus, because of the importance of knot selection, a
14 sensitivity analysis was done to examine the impacts of selecting different knots (Section 6 of
15 Appendix D). For the sensitivity analysis, the two-piece log-linear model was run with knots
16 roughly one increment (1000 ppm $\times$ days) below and one increment above the selected knot. For
17 breast cancer incidence, this sensitivity analysis yielded EC_{01} estimates of 0.0133 ppm and
18 0.0176 ppm, respectively, i.e., about 14% lower and 14% higher, respectively, than the EC_{01} of
19 0.0154 ppm obtained with the originally selected knot of 6000 ppm $\times$ days.[19]

20   As can be seen in Table 4-7, there is substantial variation in the EC_{01} estimates obtained
21 from the different models. The categorical data for breast cancer incidence do not display the
22 supralinearity in the lower exposure groups seen in the cases discussed above (some plateauing is
23 evident with the highest exposure group); thus, the difference between the EC_{01} estimates from
24 the standard cumulative exposure Cox regression model and the two-piece spline models or the
25 linear regression models are not as dramatic as seen in those cases (the EC_{01} estimates from the
26 latter three approaches are nearly within an order of magnitude of that of the cumulative
27 exposure model). For the subcohort with interviews, the two-piece spline models and the linear
28 regression approach gave similar results (the unit risk estimates spanned roughly a two-fold
29 range).

30   An area of uncertainty in the life-table analysis for breast cancer incidence pertains to the
31 rates used for the cause-specific background rate. The regression coefficients presented by
32 Steenland et al. (2003) represent invasive and in situ cases combined, where 6% of the cases are

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[19] about 12% lower and 17% higher, respectively, than the EC_{01} of 0.0151 ppm obtained with the more finely tuned knot of 5800 ppm $\times$ days (Appendix D). The EC_{01} value of 0.0166 presented in this assesssment (Table 4-7) is not directly comparable to the values in the sensitivity analysis because more recent background incidence and mortality rates were used in the lifetable analyses upon which the assessment estimates were based.

1   in situ, and the preferred unit risk estimates in this assessment are calculated similarly using
2   background rates for invasive and in situ cases combined. The regression coefficients for
3   invasive and in situ cases combined should be good approximations for regression coefficients
4   for invasive cases alone; however, it is uncertain how well they reflect the exposure-response
5   relationships for in situ cases alone. Diagnosed cases of in situ breast cancer would presumably
6   be remedied and not progress to invasive breast cancer, so double-counting is unlikely to be a
7   significant problem. Carcinoma in situ is a risk factor for invasive breast cancer; however, this
8   observation is most likely explained by the fact that these two types of breast cancer have other
9   breast cancer risk factors in common, some of which have been considered in the subcohort
10   analysis. One might hypothesize that EtO exposure could cause a more rapid progression to
11   invasive tumors; however, there is no specific evidence that this occurs. On the other hand, there
12   is some indication that in situ cases in the incidence study might have been diagnosed at
13   relatively low rates in comparison to the invasive cases. Steenland et al. (2003) reported that 6%
14   of the cases in their study are in situ; according to the National Cancer Institute, however, ductal
15   carcinoma in situ accounted for about 18% of newly diagnosed cases of breast cancer in 1998
16   (NCI, 2004b).
17   There are several possible explanations for this difference. One is that it reflects
18   differences in diagnosis with calendar time because the rate of diagnosis of carcinoma in situ has
19   increased over time with increased use of mammography. Another is that the difference is
20   partially a reflection of the age distribution in the cohort because the proportion of new cases
21   diagnosed as carcinoma in situ varies by age. A third possible explanation is that the low
22   proportion of in situ cases is at least partially a consequence of underascertainment of cases
23   because in situ cases will not be reported on death certificates, although, even if all 20 in situ
24   cases were in the subcohort with interviews, that would still be only 8.6% of the cases. In any
25   event, this is a relatively minor source of uncertainty, and a comparison of the unit risk estimates
26   using invasive + in situ breast cancer background rates and invasive-only background rates,
27   using EPA's original linear regression analyses in the 2006 Draft Assessment, found that the
28   estimate based on the invasive + in situ background rates was less than 20% higher than the
29   corresponding estimate using only invasive breast cancer background rates (U.S. EPA, 2006b).
30   The results for the subcohort with interviews are used for the primary breast cancer unit
31   risk calculations because, in addition to including the data on potential confounders, the
32   subcohort is considered to have full ascertainment of the breast cancer cases, whereas the full
33   cohort for the incidence study has incomplete case ascertainment, as illustrated by the fact that
34   death certificates were the only source of case ascertainment for 14% of the cases. Thus, risk
35   estimates based on the full cohort would be underestimated; nevertheless, these estimates were
36   calculated for comparison with the subcohort estimates using the original linear regression

1   analyses. The unit risk estimate based on the subcohort was about 60% higher than the

2   corresponding estimate from the full cohort (U.S. EPA, 2006b).

3   With respect to dose metrics for breast cancer incidence, models using duration provided

4   better model fits than those using cumulative exposure (Steenland et al., 2003); however,

5   duration is less useful for estimating unit risks and the cumulative exposure models also provided

6   statistically significant fits to the data, thus the cumulative exposure metric was used for the

7   quantitative risk estimates. Models using peak or average exposure did not fit as well.

8   Regarding potential confounders/modifying factors, analyses for the full cohort were

9   adjusted for age, race, and calendar time, and exposures to other chemicals in these plants were

10 reportedly minimal. For the subcohort with interviews, a number of specific breast cancer risk

11 factors were investigated, including body mass index, breast cancer in a first-degree relative,

12 parity, age at menopause, age at menarche, socioeconomic status, and diet; however, only parity

13 and breast cancer in a first-degree relative were determined to be important predictors of breast

14 cancer and were included in the final models.

15   Some additional sources of uncertainty are not so much inherent in the exposure-response

16 modeling or in the epidemiologic data themselves but, rather, arise in the process of obtaining

17 more general Agency risk estimates from the epidemiologic results. EPA cancer risk estimates

18 are typically derived to represent an upper bound on increased risk of cancer incidence for all

19 sites affected by an agent for the general population. From experimental animal studies, this is

20 accomplished by using tumor incidence data and summing across all the tumor sites that

21 demonstrate significantly increased incidences, customarily for the most sensitive sex and

22 species, to be protective of the general human population. However, in estimating comparable

23 risks from the NIOSH epidemiologic data, certain limitations are encountered. First, the study

24 reported by Steenland et al. (2004) is a retrospective mortality study, and cancer incidence data

25 are not available for lymphohematopoietic cancer (for breast cancer, a separate incidence study

26 [Steenland et al., 2003] was available). Second, these occupational epidemiology data represent

27 a healthy-worker cohort. Third, the epidemiologic study may not have sufficient statistical

28 power and follow-up time to observe associations for all the tumor sites that may be affected by

29 EtO.

30   The first limitation was addressed quantitatively in the life-table analysis for the

31 lymphohematopoietic cancer risk estimates. Although assumptions are made in using incidence

32 rates for the cause-specific background rates, as discussed in Section 4.1.1.3, the resulting

33 incidence-based estimates are believed to be better estimates of cancer incidence risk than are the

34 mortality-based estimates. Because of the relatively high survival rates for lymphoid cancers,

35 the incidence unit risk estimate is about 120% higher than (i.e., 2.2 times) the mortality-based

36 estimate.

1   The healthy-worker effect is often an issue in occupational epidemiology studies, but the
2   internal exposure-response analyses conducted by these investigators help address this concern,
3   at least partially. In terms of representing the general population, the NIOSH study cohort was
4   relatively diverse. It contained both female (55%) and male workers, and the workers were 79%
5   white, 16% black, and 5% "other." Furthermore, because of EtO's mutagenic mode of action,
6   increased early-life susceptibility is assumed and ADAFs are applied for exposure scenarios
7   involving early life (see Section 4.4).
8   With respect to other possible tumor sites of concern, the rodent data suggest that
9   lymphohematopoietic cancers are a major tumor type associated with EtO exposure in female
10   mice and in male and female rats. Thus, it is reasonable that this might be a tumor type of
11   concern in humans, too. Likewise, the mouse data suggest an increased risk of mammary gland
12   tumors from EtO exposure, and evidence of that can be seen in the Steenland et al. (2003, 2004)
13   study. However, the rodent data suggest associations between EtO exposure and other tumor
14   types as well, and, although site concordance across species is not generally assumed, it is
15   possible that the NIOSH study, despite its relatively large size and long follow-up (mean length
16   of follow-up was 26.8 years), had insufficient power to observe small increases in risk in certain
17   other sites. For example, the tumor site with the highest potency estimate in both male and
18   female mice was the lung. In the NIOSH study, one cannot rule out a small increase in the risk
19   of lung cancer, which has a high background rate.
20   To obtain the risk estimate for total cancer risk (2.3 per ppm, or 2.3×10^{-3} per ppb), the
21   preferred estimates for lymphoid cancer incidence and breast cancer incidence were combined.
22   While there are uncertainties in the approach used to combine the individual estimates, the
23   resulting unit risk estimate is appropriately bounded in the roughly 2-fold range between
24   estimates based on the sum of the individual MLEs of risk and the sum of the individual 95%
25   UCLs, and, thus, any inaccuracy in the total cancer unit risk estimate resulting from the approach
26   used is relatively minor. Because the breast cancer component of the total cancer risk estimate
27   applies only to females, the total cancer risk estimate is expected to overestimate the cancer risk
28   to males somewhat (the preferred unit risk estimate for lymphoid cancer alone was 0.877 per
29   ppm [or 8.77×10^{-4} per ppb], which is about 40% of the total cancer risk estimate).
30   Despite these uncertainties, the inhalation cancer unit risk estimate of 2.3 per ppm (or 2.3
31   $\times 10^{-3}$ per ppb) for the total cancer risk from lymphoid cancer incidence and female breast cancer
32   incidence has the advantages of being based on human data from a high-quality epidemiologic
33   study with individual exposure estimates for each worker. Furthermore, the breast cancer
34   component of the risk estimate, which contributes approximately 60% of the total cancer risk, is
35   based on a substantial number of incident cases (233 total, the vast majority of which were in the
36   exposure range below the knot of 5800 ppm×days [see Table 1 of Appendix D]).

1   A further area of uncertainty pertains to the assumption that RR is independent of age,
2   which is a common assumption in the dose-response modeling of epidemiological data and is an
3   underlying assumption in the Cox regression model. In the absence of data on early-life
4   susceptibility, EPA's *Supplemental Guidance* (U.S. EPA, 2005b) recommends that increased
5   early-life susceptibility be assumed for carcinogens with a mutagenic mode of action, and the
6   conclusion was made in Section 3.4 that the weight of evidence supports a mutagenic mode of
7   action for EtO. Thus, in accordance with the *Supplemental Guidance*, the alternate assumption
8   of increased early-life susceptibility is preferred as the basis for risk estimates in this assessment,
9   and risk estimates derived under this preferred assumption are presented in Section 4.4.
10

11   **4.1.5. Summary**

12   Under the common assumption that RR is independent of age, an inhalation unit risk
13   estimate for lymphoid cancer incidence of 0.877 per ppm (or 8.77×10^{-4} per ppb; 4.79×10^{-4} per
14   $\mu g/m^3$) was calculated using a life-table analysis and a weighted linear regression of the
15   categorical Cox regression results, excluding the highest exposure group, for excess lymphoid
16   cancer mortality from a high-quality occupational epidemiology study. Similarly an inhalation
17   unit risk estimate for female breast cancer incidence of 1.74 per ppm (or 1.74×10^{-3} per ppb;
18   9.51×10^{-4} per $\mu g/m^3$) was calculated using a life-table analysis and two-piece linear spline
19   modeling of the continuous data for excess breast cancer incidence from the same high-quality
20   occupational epidemiology study. The linear regression with the exclusion of the highest
21   exposure group for the lymphoid cancer results and the two-piece linear spline analysis for the
22   breast cancer incidence data were different modeling approaches used to address the
23   supralinearity of the exposure-response data in the two datasets. Low-dose linear extrapolation
24   was used, as warranted by the clear mutagenicity of EtO. An EC_{01} estimate of 0.0078 ppm, a
25   LEC_{01} estimate of 0.0044 ppm, and a unit risk estimate of 2.3 per ppm (or 2.3×10^{-3} per ppb; 1.2
26   $\times 10^{-3}$ per $\mu g/m^3$) were obtained for the total cancer risk combined across both cancer types.
27   Despite the uncertainties discussed above, this inhalation unit risk estimate has the advantages of
28   being based on human data from a high-quality epidemiologic study with individual exposure
29   estimates for each worker.
30   In the absence of data on early-life susceptibility, EPA's *Supplemental Guidance* (U.S.
31   EPA, 2005b) recommends that increased early-life susceptibility be assumed for carcinogens
32   with a mutagenic mode of action, and the conclusion was made in Section 3.4 that the weight of
33   evidence supports a mutagenic mode of action for EtO. Thus, in accordance with the
34   *Supplemental Guidance*, the alternate assumption of increased early-life susceptibility is
35   preferred as the basis for risk estimates in this assessment, and risk estimates derived under this
36   preferred assumption are presented in Section 4.4. Other than the use of the alternate assumption

1 about early-life susceptibility, the approach used to derive the estimates presented in Section 4.4

2 is identical to the approach used for the estimates derived here in Section 4.1, and the

3 comparisons made between various options and the issues and uncertainties discussed here in

4 Section 4.1 are applicable to the estimates derived in Section 4.4.

5

6 4.2.   INHALATION UNIT RISK DERIVED FROM EXPERIMENTAL ANIMAL
7   DATA

8 4.2.1. Overall Approach

9 Lifetime animal cancer bioassays of inhaled EtO have been carried out in three

10 laboratories, as described in Section 3.2. The data from these reports are presented in Tables 3-1

11 through 3-3. These studies have also been reviewed by the IARC (1994b) and Health Canada

12 (2001). Health Canada calculated the ED_{05} for each data set using the benchmark dose

13 methodology. The EOIC report (EOIC, 2001) tabulated only lymphatic tumors because they

14 constituted the predominant risk.

15 The overall approach in this derivation is to find a unit risk for each of the bioassays—

16 keeping data on males and females separate—from data on the incidence of all tumor types and

17 then to use the maximum of these values as the summary measure of the unit risk from animal

18 studies (i.e., the unit risk represents the most sensitive species and sex). The unit risk for the

19 animals in these bioassays is converted to a unit risk in humans by first determining the

20 continuous exposures in humans that are equivalent to the rodent bioassay exposures and then by

21 assuming that the lifetime incidence in humans is equivalent to lifetime incidence in rodents, as

22 is commonly accepted in interspecies risk extrapolations. For cross-species scaling of exposure

23 levels (see Section 4.2.2 below), an assumption of ppm equivalence is used; thus, no interspecies

24 conversion is needed for the exposure concentrations. Bioassay exposure levels are adjusted to

25 equivalent continuous exposures by multiplying by (hours of exposure/24 hours) and by (5/7) for

26 the number of days exposed per week. The unit risk in humans (risk per unit air concentration)

27 is then assumed to be numerically equal to that in rodents (after adjustment to continuous

28 exposures); the calculations from the rodent bioassay data are shown in Tables 3-1 through 3-3.

29

30 4.2.2. Cross-Species Scaling

31 In the absence of chemical-specific information, EPA's 1994 inhalation dosimetry

32 methods (U.S. EPA, 1994) provide standard methods and default scaling factors for cross-

33 species scaling. Under EPA's methodology, EtO would be considered a Category 2 gas because

34 it is reactive and water soluble and has clear systemic distribution and effects. Dosimetry

35 equations for Category 2 gases are undergoing EPA re-evaluation and are not being used at this

36 time. For cross-species scaling of extrarespiratory effects, current practice is to treat Category 2

1   gases as Category 3 gases. For Category 3 gases, ppm equivalence is assumed (i.e., responses
2   across species are equivalent on a ppm exposure basis), unless the air:blood partition coefficient
3   for the experimental species is less than the coefficient for humans (U.S. EPA, 1994, p. 4–61).
4   In the case of EtO, measured air:blood partition coefficients are 78 in the mouse (Fennell and
5   Brown, 2001), 64 in the rat (Krishnan et al., 1992), and 61 in the human (Csanády et al., 2000);
6   thus, ppm equivalence for cross-species scaling to humans can be assumed for extrarespiratory
7   effects observed in mice and rats. The assumption of ppm equivalence is further supported by
8   the PBPK modeling of Fennell and Brown (2001), who reported that simulated blood AUCs for
9   EtO after 6 hours of exposure to concentrations between 1 ppm and 100 ppm were similar for
10 mice, rats, and humans and were linearly related to the exposure concentration (see Section 3.3.1
11 and Figure 3-2). This modeling was validated against measured blood EtO concentrations for
12 rodents and humans.   For Category 2 gases with respiratory effects, there is no clear guidance on
13 an interim approach. One suggested approach is to do cross-species scaling using both Category
14 1 and Category 3 gas equations and then decide which is most appropriate. In this document, the
15 preferred approach was to assume ppm equivalence was also valid for the lung tumors in mice
16 because of the clear systemic distribution of EtO (e.g., see Section 3.1). Treating EtO as a
17 Category 1 gas for cross-species scaling of the lung tumors would presume that the lung tumors
18 are arising only from the immediate and direct action of EtO as it comes into first contact with
19 the lung. In fact, some of the EtO dose contributing to lung tumors is likely attributable to
20 recirculation of systemic EtO through the lung.
21   If one were to treat EtO as a Category 1 gas for the cross-species scaling of the lung
22 tumor response as a bounding exercise, EPA's 1994 inhalation dosimetry methods present
23 equations for estimating the $RGDR_{PU}$, i.e., the regional gas dose ratio for the pulmonary region,
24 which acts as an adjustment factor for estimating human equivalent exposure concentrations
25 from experimental animal exposure concentrations (adjusted for continuous exposure) (U.S.
26 EPA, 1994, pp. 4–49 to 4–51). These equations rely on parameters describing mass transport of
27 the gas (EtO) in the extrathoracic and tracheobronchial regions for both the experimental animal
28 species (mouse) and humans. Without experimental data for these parameters, it seems
29 reasonable to estimate $RGDR_{PU}$ using a simplified equation and the adjusted alveolar ventilation
30 rates of Fennell and Brown (2001). Fennell and Brown adjusted the alveolar ventilation rates to
31 reflect limited pulmonary uptake of EtO, a phenomenon commonly observed for highly water-
32 soluble gases (Johanson and Filser, 1992). The adjusted ventilation rates were then used by
33 Fennell and Brown in their PBPK modeling simulations, and good fits to blood concentration
34 data were reported for both the mouse and human models. In this document, the adjusted
35 alveolar ventilation rates were used to estimate the $RGDR_{PU}$ as follows:
36

1          $RGDR_{PU} = (RGD_{PU})_m/(RGD_{PU})_h = (Q_{alv}/SA_{PU})_m/(Q_{alv}/SA_{PU})_h,$ (4-3)

2 where:

3   RGD_{PU} = regional gas dose to the pulmonary region,

4   Q_{alv} = (adjusted) alveolar ventilation rate,

5   SA_{PU} = surface area of the pulmonary region, and

6   the subscripts "m" and "h" denote mouse and human values.

7

8 Then, using adjusted alveolar ventilation rates from Fennell and Brown (2001) and surface area

9 values from EPA (U.S. EPA, 1994, p. 4–26),

10

11          $RGDR_{PU} = ((0.78 \text{ L/h})/(0.05 \text{ m}^2))/((255 \text{ L/h})/(54.0 \text{ m}^2)) = 3.3.$ (4-4)

12

13 Using this value for the $RGDR_{PU}$ would increase the human equivalent concentration about

14 threefold, resulting in a decreased risk for lung tumors of about threefold, as a lower bound. The

15 true value of the $RGDR_{PU}$ is expected to be between 1 and 3, and any adjustment to the lung

16 tumor risks would still be expected to result in unit risk estimates roughly within the range of the

17 rodent unit risk estimates derived later in Section 4.2 under the assumption of ppm equivalence.

18

19 **4.2.3. Dose-Response Modeling Methods**

20   In this document the following steps were used:

21   1. *Extract the incidence data presented in the original studies.* In order to crudely adjust

22 for early mortality in the analysis of the NTP (1987) data, the incidence data have been corrected

23 for a specific tumor type by eliminating the animals that died prior to the occurrence of the first

24 tumor or prior to 52 weeks, whichever was earlier. It was not possible to make this adjustment

25 with the other studies where data on individual animals were not available. With these

26 exceptions, the tumor incidence data in Tables 3-1 through 3-3 match the original data.

27   2. *Fit the multistage model to the dose-response data using the Tox_Risk program.*

28 The likelihood-ratio test was used to determine the lowest value of the multistage polynomial

29 degree that provided the best fit to the data while requiring selection of the most parsimonious

30 model. In this procedure, if a good fit to the data in the neighborhood of the POD is not obtained

31 with the multistage model because of a nonmonotonic reduction in risk at the highest dose tested

32 (as sometimes occurs when there is early mortality from other causes), that data point is

33 eliminated and the model is fit again to the remaining data. Such a deletion was found necessary

34 in two cases (mammary tumors in the NTP study and mononuclear cell leukemia in the Lynch

35 study). The goodness-of-fit measures for the dose-response curves and the parameters derived

36 from them are shown in Appendix G.

1   In the NTP bioassay, where the individual animal data were available, a time-to-tumor
2   analysis was undertaken to account for early mortality. The general model used in this analysis
3   is the multistage Weibull model:

4

5    $$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)*(t - t_0)^z], \qquad (4\text{-}5)$$

6

7   where $P(d,t)$ represents the probability of a tumor by age t (in bioassay weeks) for dose d (i.e.,
8   human equivalent exposure), and the parameter ranges are restricted as follows: $z \geq 1$, $t_0 \geq 0$,
9   and $q_i \geq 0$ for $I = 0, 1, ..., k$. The parameter t_0 represents the time between when a potentially
10   fatal tumor becomes observable and when it causes death. The analyses were conducted using
11   the computer software Tox_Risk version 3.5, which is based on methods developed by Krewski
12   et al. (1983). Parameters are estimated in Tox_Risk using the method of maximum likelihood.
13   Tumor types can be categorized by tumor context as either fatal or incidental. Incidental
14   tumors are those tumors thought not to have caused the death of an animal, whereas fatal tumors
15   are thought to have resulted in animal death. Tumors at all sites were treated as incidental
16   (although it was recognized that this may not have been the case, the experimental data are not
17   detailed enough to conclude otherwise). The parameter t_0 was set equal to 0 because there were
18   insufficient data to reliably estimate it.
19   The likelihood-ratio test was used to determine the lowest value of the multistage
20   polynomial degree k that provided the best fit to the data while requiring selection of the most
21   parsimonious model. The one-stage Weibull (i.e., $k = 1$) was determined to be the most optimal
22   value for all the tumor types analyzed.
23   3. *Select the POD and calculate the unit risk for each tumor site.* The effective
24   concentration that causes a 10% extra risk for tumor incidence, EC_{10}, and the 95% lower bound
25   of that concentration, LEC_{10}, are derived from the dose-response model. The LEC_{10} is then used
26   as the POD for a linear low-dose extrapolation, and the unit risk is calculated as $0.1/LEC_{10}$. This
27   is the procedure specified in the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,
28   2005a) for agents such as EtO that have direct mutagenic activity. See Section 3.4 for a
29   discussion of the mode of action for EtO. Tables 3-1 through 3-3 present the unit risk estimates
30   for the individual tumor sites in each bioassay.
31   4. *Develop a unit risk estimate based on the incidence of all tumors combined.* This
32   method assumes that occurrences of tumors at multiple sites are independent and, further, that
33   the risk estimate for each tumor type is normally distributed. Then, at a given exposure level, the
34   maximum likelihood estimates (MLEs) of extra risk due to each tumor type are added to obtain
35   the MLE of total cancer risk. The variances corresponding to each tumor type are added to give

1 the variance associated with the sum of the MLEs. The one-sided 95% upper confidence limit

2 (UCL) of the MLE for the combined risk is then calculated as:

3

4 $$95\% \text{ UCL} = \text{MLE} + 1.645(\text{SE}),\tag{4-6}$$

5

6 where SE is the standard error and is the square root of the summed variance. (Note that as a

7 precursor to this step, when Tox _Risk is used to fit the incidence of a single tumor type, it

8 provides the MLE and 95% UCL of extra risk at a specific dose. The standard error in the MLE

9 is determined using the above formula). The calculation is repeated for a few exposure levels,

10 and the exposure yielding a value of 0.1 for the upper bound on extra risk is determined by

11 interpolation. The unit risk is then the slope of the linear extrapolation from this POD. The

12 results are given in Table 4-10.

13

14 **Table 4-10. Upper-bound unit risks (per μg/m^3) obtained by combining**
15 **tumor sites**

16

Combination method[a]	NTP (1987) female mouse	Lynch et al. (1982, 1984a) male rat	Snellings et al. (1984)[b]	
			Male rat	Female rat
U.c.b. on sum of risks[c]	2.71×10^{-5}	4.17×10^{-5}	2.19×10^{-5}	3.37×10^{-5}
Sum of unit risks[d]	4.12×10^{-5}	3.66×10^{-5}	2.88×10^{-5}	3.54×10^{-5}
Time-to-tumor analysis and u.c.b on sum of risks[c]	4.55×10^{-5}	–	–	–

17
18 [a]Unit risk in these methods is the slope of the straight line extrapolation from a point of departure at the dose
19 corresponding to a value of 0.1 for the 95% upper confidence bound on total extra risk.
20 [b]Includes data on brain tumors from the analysis by Garman et al. (1985). See Table 3-3.
21 [c]U.c.b. = 95% upper confidence bound. At a given dose, the MLE of the combined extra risk was determined by
22 summing the MLE of risk due to each tumor type. The variance associated with this value was determined by
23 summing over the variances due to each tumor type.
24 [d]Sum of values in last column of Tables 3-1 through 3-3.
25
26
27 **4.2.4. Description of Experimental Animal Studies**

28   NTP (1987) exposed male and female B6C3F$_1$ mice to concentrations of 0, 50, and 100

29 ppm for 6 hours per day, 5 days per week, for 102 weeks. An elevated incidence of lung

30 carcinomas was found in males, and elevated lung carcinomas, malignant lymphomas, uterine

31 adenocarcinomas, and mammary carcinomas were found in females. These data are shown in

32 Table 3-1.

1   Lynch et al. (1982, 1984a) exposed male F344 rats to 0, 50, and 100 ppm for 7 hours per

2   day, 5 days per week, for 2 years. They found excess incidence of tumors at three sites:

3   mononuclear cell leukemia in the spleen, testicular peritoneal mesothelioma, and brain glioma.

4   In this study the survival in the high-dose group (19%) was less than that of controls (49%),

5   which reduced the incidence of leukemias. In the animals in the high-dose group that survived to

6   the termination of the experiment, the incidence of leukemias was statistically significantly

7   higher than for controls ($p < 0.01$). The incidence data are shown in Table 3-2, uncorrected for

8   the high-dose-group mortality. If the individual animal data were available to perform the

9   correction, the incidence would be higher. Therefore, using these data results in an

10   underestimate of risk.

11   Snellings et al. (1984) exposed male and female F344 rats to 0, 10, 33, and 100 ppm for 6

12   hours per day, 5 days per week, for 2 years and described their results for all sites except the

13   brain. In two subsequent publications for the same study, Garman et al. (1985, 1986) described

14   the development of brain tumors in a different set of F344 rats. The Snellings et al. publication

15   reported an elevated incidence of splenic mononuclear cell leukemia and peritoneal

16   mesothelioma in males and an elevated incidence of splenic mononuclear cell leukemia in

17   females. The mortality was higher in the 100 ppm groups than the other three groups for both

18   males and females. The incidences in the animals killed after 24 months in Snellings et al.

19   (1984) are shown in Table 3-3. Table 3-3 also presents the brain tumor incidence data for male

20   and female rats from the Garman et al. (1985, 1986) publications. The brain tumor incidence

21   was lower than that of the other tumors, particularly the splenic mononuclear cell leukemias.

22

23   **4.2.5. Results of Data Analysis of Experimental Animal Studies**

24   The unit risks calculated from the individual site-sex-bioassay data sets are presented in

25   Tables 3-1 through 3-3. The highest unit risk of any individual site is 3.23×10^{-5} per $\mu g/m^3$, and

26   it is for mononuclear cell leukemia in the female rats of the Snellings et al. (1984) study.

27   Table 4-11 presents the results of the time-to-tumor method applied to the individual

28   animals in the NTP bioassay, compared with the results from the dose group incidence data in

29   Table 3-1. This comparison was done for each tumor type separately. The time-to-tumor

30   method of analyzing the individual animals results in generally higher unit risk estimates than

31   does the analysis of dose group data, as shown in Table 4-11. The ratio is not large (less than

32   2.2) across the tumor types. (In the case of mammary tumors this ratio is actually less than 1. It

33   must be noted that the incidence at the highest dose [where the incidence was substantially less

34   than at the intermediate dose] was deleted from the analysis of grouped data, whereas it was

35   retained in the time-to-tumor analysis. Therefore, the comparison for the mammary tumors is

36   not a strictly valid comparison of methods.) The results also show the extent to which a time-to-

1 tumor analysis of individual animal data increases the risk estimated from data on dose groups.

2 It is expected that if individual animal data were available for the Lynch et al. (1982, 1984a) and

3 the Snellings et al. (1984) bioassays, then the time-to-tumor analysis would also result in higher

4 estimates because both those studies also showed early mortality in the highest dose group.

5

6 **Table 4-11. Unit risk values from multistage Weibull[a] time-to-tumor**
7 **modeling of mouse tumor incidence in the NTP (1987) study**
8

Tumor type	Unit risk, $0.1/LEC_{10}$ (per $\mu g/m^3$) from time to tumor analysis	Unit risk, $0.1/LEC_{10}$ (per $\mu g/m^3$) (Table 3-1)[b]	Ratio of unit risks time-to-tumor/ grouped data
Males			
Lung: alveolar/bronchiolar adenoma and carcinoma	3.01×10^{-5}	2.22×10^{-5}	1.4
Females			
Lung: alveolar/bronchiolar adenoma and carcinoma	2.40×10^{-5}	1.10×10^{-5}	2.2
Malignant lymphoma	1.43×10^{-5}	7.18×10^{-6}	2.0
Uterine carcinoma	6.69×10^{-6}	4.33×10^{-6}	1.5
Mammary carcinoma	8.69×10^{-6}	1.87×10^{-5}	0.5

9
10 [a]$P(d,t)$. $1 - \exp[-(q_0 + q_1d + q_2d^2 + ... + q_kd^k)*(t - t_0)^z]$, where d is inhaled ethylene oxide concentration in ppm, t is
11 weeks until death with tumor. In all cases, $k = 1$ provided the optimal model.
12 [b]Incidence data modeled using multistage model without taking time to tumor into account.

13

14

15 The results of combining tumor types are summarized in Table 4-10. The sums of the

16 individual unit risks tabulated in Tables 3-1 to 3-3 are given in the second row of Table 4-10.

17 Note that as expected they are greater than the unit risks computed from the upper bound on the

18 sum of risks for all data sets except for the Lynch et al. (1982, 1984a) data. The reason for this

19 exception is not known, but the differences are small. It is likely that the problem arises from the

20 methodology used to combine the risks across tumor sites. In an attempt to be consistent with

21 the new two-step methodology (i.e., modeling in the observable range to a POD and then doing a

22 linear extrapolation to zero extra risk at zero exposure), the exposure concentration at which the

23 sum of the independent tumor site risks yielded a 95% upper bound on 10% extra risk was

24 estimated and used as the POD. Summing risks in this way results in a POD for the combined

25 tumor risk that is different (lower) than the points of departure for each individual tumor site

1   risk. Thus, the risk estimate for the sum is not strictly comparable to the individual risks that

2   constitute it. These tumor-site-specific risks were based on points of departure individually

3   calculated to correspond with a 10% extra risk. In any event, adding the upper bound risks of

4   individual tumor sites should overestimate the upper bound of the sum, and the latter is the

5   preferred measure of the total cancer risk since it avoids the overestimate. However, for the

6   exceptional Lynch et al. (1982, 1984a) data, the sum of upper bounds, 3.66×10^{-5} per µg/m^3, is

7   already an overestimate of the total risk, and this value is preferred over the anomalously high

8   value of 4.17×10^{-5} per µg/m^3 corresponding to the upper bound on the sum of risks. The latter

9   value is considered to be an excessive overestimate and is therefore not carried over into the

10   summary Table 4-12. For the Snellings et al. (1984) data sets, the upper confidence bound on

11   the sum of risks is used in the summary Table 4-12. The results of the sum-of-risks calculations

12   on the NTP bioassay time-to-tumor data are included in the third row of Table 4-10. The

13   estimate for the NTP female mice is 4.55×10^{-5} per µg/m^3, which is higher than the other two

14   measures of total tumor risk in that bioassay. This value is preferable to the other measures

15   because it utilizes the individual animal data available for that bioassay.

16

17   **Table 4-12**. **Summary of unit risk estimates (per µg/m^3) in animal bioassays**

18

Assay	Males	Females
NTP (1987), B6C3F$_1$ mice	3.01×10^{-5a}	4.55×10^{-5b}
Lynch et al. (1982, 1984a), F344 rats	3.66×10^{-5c}	–
Snellings et al. (1984), F344 rats	2.19×10^{-5d}	3.37×10^{-5d}

19
20 [a]From time-to-tumor analysis of lung adenomas and carcinomas, Table 4-11.
21 [b]Upper bound on sum of risks from the time-to-tumor analysis of the NTP data, Table 4-10.
22 [c]Sum of (upper bound) unit risks (see text for explanation), Table 4-10.
23 [d]Upper bound on sum of risks, Table 4-10.
24
25
26   <u>Summary of results</u>. The summary of unit risks from the five data sets is shown in

27   Table 4-12. The data set giving the highest risk (4.55×10^{-5} per µg/m^3) is the NTP (1987) data

28   on combined tumors in female mice. The other values are within about a factor of 2 of the

29   highest value.

30

31 **4.3.**   **SUMMARY OF INHALATION UNIT RISK ESTIMATES—NOT ACCOUNTING**
32   **FOR ASSUMED INCREASED EARLY-LIFE SUSCEPTIBILITY**

33   For both humans and laboratory animals, tumors occur at multiple sites. In humans, there

34   was a combination of tumors having lymphohematopoietic, in particular lymphoid, origins in

1   both sexes and breast cancer in females, and, in rodents, lymphohematopoietic tumors, mammary
2   carcinomas, and tumors of other sites were observed. From human data, an extra cancer unit risk
3   estimate of 4.79×10^{-4} per μg/m^3 (8.77×10^{-4} per ppb) was calculated for lymphoid cancer
4   incidence, and a unit risk estimate of 9.31×10^{-4} per μg/m^3 (1.74×10^{-3} per ppb) was calculated
5   for breast cancer incidence in females. The total extra cancer unit risk estimate was 1.2×10^{-3}
6   per μg/m^3 (2.3×10^{-3} per ppb) for both cancer types combined (EC_{01} = 0.0078 ppm; LEC_{01} =
7   0.0043 ppm). Unit risk estimates derived from the three chronic rodent bioassays for EtO ranged
8   from 2.2×10^{-5} per μg/m^3 to 4.6×10^{-5} per μg/m^3, over an order of magnitude lower than the
9   estimates based on human data.
10   Adequate human data, if available, are considered to provide a more appropriate basis
11   than rodent data for estimating human risks (U.S. EPA, 2005a), primarily because uncertainties
12   in extrapolating quantitative risks from rodents to humans are avoided. Although there is a
13   sizeable difference between the rodent-based and the human-based estimates, the human data are
14   from a large, high-quality study, with EtO exposure estimates for the individual workers and
15   little reported exposure to chemicals other than EtO. Therefore, the total extra cancer unit risk
16   estimate of 1.2×10^{-3} per μg/m^3 (2.3×10^{-3} per ppb) calculated for lymphoid cancers and breast
17   cancer combined is the preferred estimate of those estimates not taking assumed increased early-
18   life susceptibility into account (estimates accounting for assumed increased early-life
19   susceptibility are presented in Section 4.4). The unit risk estimate is intended to be an upper
20   bound on cancer risk for use with exposures below the POD (i.e., the LEC_{01}). The unit risk
21   estimate should not generally be used above the POD; however, in the case of this total extra
22   cancer unit risk, which is based on cancer type-specific unit risk estimates from two linear
23   models, the estimate should be valid for exposures up to about 0.060 ppm (110 μg/m^3), which is
24   the minimum of the limits for the lymphoid cancer unit risk estimate (0.060 ppm; see Section
25   4.1.1.2) and the breast cancer unit risk estimate (0.075 ppm; see Section 4.1.2.3).
26   Because a mutagenic mode of action for EtO carcinogenicity (see Section 3.3.2) is
27   "sufficiently supported in (laboratory) animals" and "relevant to humans", and as there are no
28   chemical-specific data to evaluate the differences between adults and children, increased early-
29   life susceptibility should be assumed and, if there is early-life exposure, the age-dependent
30   adjustment factors (ADAFs) should be applied, as appropriate, in accordance with EPA's
31   *Supplemental Guidance* (U.S. EPA, 2005b; see Section 4.4 below for more details on the
32   application of ADAFs).
33

4.4. ADJUSTMENTS FOR POTENTIAL INCREASED EARLY-LIFE SUSCEPTIBILITY

There are no chemical-specific data on age-specific susceptibility to EtO-induced carcinogenesis. However, there is sufficient weight of evidence to conclude that EtO operates through a mutagenic mode of action (Section 3.4.1). In such circumstances (i.e., the absence of chemical-specific data on age-specific susceptibility but sufficient evidence of a mutagenic mode of action), U.S. EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) recommends the assumption of increased early-life susceptibility and the application of default age-dependent adjustment factors (ADAFs) to adjust for this potential increased susceptibility from early-life exposure. See the *Supplemental Guidance* for detailed information on the general application of these adjustment factors. In brief, the *Supplemental Guidance* establishes ADAFs for three specific age groups. The current ADAFs and their age groupings are 10 for <2 years, 3 for 2 to <16 years, and 1 for 16 years and above (U.S. EPA, 2005b). For risk assessments based on specific exposure assessments, the 10-fold and 3-fold adjustments to the unit risk estimates are to be combined with age-specific exposure estimates when estimating cancer risks from early-life (<16 years age) exposure.

These ADAFs, however, were formulated based on comparisons of the ratios of cancer potency estimates from juvenile-only exposures to cancer potency estimates from adult-only exposures from rodent bioassay datasets with appropriate exposure scenarios, and they are designed to be applied to cancer potency estimates derived from adult-only exposures. Thus, alternate life-table analyses were conducted to derive comparable adult-exposure-only unit risk estimates to which ADAFs would be applied to account for early-life exposure. For these alternate life-table analyses, it was assumed that RR is independent of age for adults, which represent the life-stage for which the exposure-response data and the Cox regression modeling results from the NIOSH cohort study specifically pertain, but that there is increased early-life susceptibility, based on the weight-of-evidence-based conclusion that EtO carcinogenicity has a mutagenic MOA (Section 3.4), which supersedes the assumption that RR is independent of age for all ages including children.

In the alternate analyses, exposure in the life-table was taken to start at age 16 years, the age cut-point that was established in EPA's *Supplemental Guidance* (U.S. EPA, 2005b), to derive an adult-exposure-only unit risk estimate to which ADAFs would be applied to account for early-life exposure. Other than the age at which exposure was initiated, the life-table analyses are identical to those conducted for the results presented in Section 4.1. Adult-exposure-only unit risk estimates were derived for both cancer incidence and mortality for both lymphoid and breast cancers. Alternate estimates were not derived for all lymphohematopoietic cancers because lymphoid cancer was the preferred endpoint (see Section 4.1.1.2). Incidence

1 estimates are preferred over mortality estimates, but both are calculated here for comparison and
2 because mortality estimates are sometimes used in addition to incidence estimates in benefit-cost
3 analyses. For each cancer endpoint, the same exposure-response model was used as that which
4 was selected for the unit risk estimates in Section 4.1 (i.e., linear regression of the categorical
5 results, excluding the highest exposure category, for lymphoid cancer and breast cancer mortality
6 and two-piece linear spline model for breast cancer incidence). The results are presented in
7 Table 4-13 along with the unit risk estimates derived assuming that RR was independent of age
8 for all ages (Section 4.1) for comparison. As can be seen in Table 4-13, the unit risk estimates
9 for adult-only exposures range from about 66% to about 72% of the unit risk estimates derived
10 under the assumption of age independence across all ages.
11
12 **Table 4-13. EC_{01}, LEC_{01}, and unit risk estimates for adult-only exposures**
13

Cancer response	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate[a] (per ppm)	Lifetime-exposure unit risk estimate under assumption of age independence[b] (per ppm)
Lymphoid cancer mortality (both sexes)	0.0787	0.0352	0.284	0.397
Lymphoid cancer incidence (both sexes)	0.0364	0.0163	0.613	0.877
Breast cancer mortality (females)	0.0590	0.0297	0.337	0.513
Breast cancer incidence (females)	0.0167	0.00863	1.16[c]	1.74[c]

14 [a]Unit risk estimate = $0.01/LEC_{01}$.
15 [b]From Tables 4-2, 4-5, and 4-7 of Section 4.1.
16 [c]For unit risk estimates above 1, convert to risk per ppb. e.g., 1.16 per ppm = 1.16×10^{-3} per ppb.
17
18
19   According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a),
20 cancer risk estimates are intended to reflect total cancer risk, not site-specific cancer risk;
21 therefore, an additional calculation was made to estimate the combined risk for (incident)
22 lymphoid and breast cancers from adult-only exposures, because females would be at risk for
23 both cancer types. Assuming that the tumor types are independent and that the risk estimates are
24 approximately normally distributed, this calculation can be made as described in Section 4.1.3.

1 First, an EC_{01} of 0.0114 ppm for the total cancer risk (i.e., lymphoid cancer incidence + breast

2 cancer incidence) from adult-only exposure was estimated, as summarized in Table 4-14.

3

4

5 **Table 4-14. Calculation of EC_{01} for total cancer risk from adult-only**

6 **exposure**

7

Cancer type	EC_{01} (ppm)	$0.01/EC_{01}$ (per ppm)	EC_{01} for total risk (ppm)
Lymphoid	0.0364	0.275	--
Breast	0.0167	0.599	--
Total[a]	--	0.874	0.0114

8   [a]The total $0.01/EC_{01}$ value equals the sum of the individual $0.01/EC_{01}$ values; the EC_{01} for the
9   total cancer risk then equals $0.01/(0.01/EC_{01})$.

10

11

12   Then, a unit risk estimate of 1.5 per ppm for the total cancer risk (i.e., lymphoid cancer

13 incidence + breast cancer incidence) from adult-only exposure was derived, as shown in Table 4-

14 15. An LEC_{01} estimate of 0.00654 ppm for the total cancer risk can be calculated as $0.01/(1.53$

15 per ppm).

16

17 **Table 4-15. Calculation of total cancer unit risk estimate from adult-only**

18 **exposure**

19

Cancer type	Unit risk estimate (per ppm)	$0.01/EC_{01}$ (per ppm)	SE^a (per ppm)	Variance	Total cancer unit risk estimate (per ppm)
Lymphoid	0.613	0.275	0.205	0.0422	--
Breast	1.16	0.599	0.340	0.115	--
Total	--	0.874	(0.397)[b]	0.158	1.53[c]

20 [a]SE = (unit risk – $0.01/EC_{01}$)/1.645.
21 [b]The SE of the total cancer risk is calculated as the square root of the sum of the variances (next column), not as the
22   sum of the SEs.
23 [c]Total cancer unit risk = 0.874 + 1.645 × 0.397.

24

25   Thus, the total cancer unit risk estimate from adult-only exposure is 1.53 per ppm (or

26 1.53×10^{-3} per ppb; 8.36×10^{-4} per $\mu g/m^3$). While there are uncertainties regarding the

27 assumption of a normal distribution of risk estimates, the resulting unit risk estimate is

28 appropriately bounded in the roughly 2-fold range between estimates based on the sum of the

29 individual MLEs (i.e., 0.874) and the sum of the individual 95% UCLs (i.e., unit risk estimates,

1   1.77), or, more precisely in this case, between the largest individual unit risk estimate (1.16) and

2   the sum of the unit risk estimates (1.77), and, thus, any inaccuracy in the total cancer risk

3   estimate resulting from the approach used to combine risk estimates across cancer types is

4   relatively minor.

5   When EPA derives unit risk estimates from rodent bioassay data, there is a blurring of the

6   distinction between lifetime and adult-only exposures because the relative amount of time that a

7   rodent spends as a juvenile is negligible ($< 8\%$) compared to its lifespan. (According to the

8   *Supplemental Guidance*, puberty begins around 5-7 weeks of age in rats and around 4-6 weeks in

9   mice [U.S. EPA, 2005b].) Thus, when exposure in a rodent is initiated at 5-8 weeks, as in the

10 typical rodent bioassay, and the bioassay is terminated after 104 weeks of exposure, the unit risk

11 estimate derived from the resulting cancer incidence data is considered a unit risk estimate from

12 lifetime exposure, except when the ADAFs were formulated and are applied, in which case the

13 same estimate is considered to apply to adult-only exposure. Yet, when adult exposures are

14 considered in the application of ADAFs, the adult-only-exposure unit risk estimate is pro-rated

15 over the full default human lifespan of 70 years, presumably because that is how adult exposures

16 are treated when a unit risk estimate calculated in the same manner from the same bioassay

17 exposure paradigm is taken as a lifetime unit risk estimate.

18   However, in humans, a greater proportion of time is spent in childhood (e.g., 16 of 70

19 years $= 23\%$), and the distinction between lifetime exposure and adult-only exposure cannot be

20 ignored. Thus, adult-only-exposure unit risk estimates were calculated distinct from the lifetime

21 estimates that were derived in Section 4.1 under the assumption of age independence for all ages.

22 In addition, the adult-only-exposure unit risk estimates need to be re-scaled to a 70-year lifespan

23 in order to be used in the ADAF calculations and risk estimate calculations involving less-than-

24 lifetime exposure scenarios in the standard manner, which includes pro-rating even adult-based

25 unit risk estimates over 70 years. Thus, the adult-only-exposure unit risk estimates are

26 multiplied by 70/54 to re-scale the 54-year adult period of the 70-year default lifespan to 70

27 years. Then, for example, if a risk estimate were calculated for a less-than-lifetime exposure

28 scenario involving exposure only for the full adult period of 54 years, the re-scaled unit risk

29 estimate would be multiplied by 54/70 in the standard calculation and the adult-only-exposure

30 unit risk estimate would be appropriately reproduced. Without re-scaling the adult-only-

31 exposure unit risk estimates, the example calculation just described for exposure only for the full

32 adult period of 54 years would result in a risk estimate 77% (i.e., 54/70) of that obtained directly

33 from the adult-only-exposure unit risk estimates, which would be illogical. The re-scaled adult-

34 based unit risk estimates for use in ADAF calculations and risk estimate calculations involving

35 less-than-lifetime exposure scenarios are presented in Table 4-16. Re-scaled LEC_{01} and EC_{01}

1 estimates for adult-based total cancer risk are 5.0×10^{-3} ppm (9.2 µg/m^3) and 8.8×10^{-3} ppm (16

2 µg/m^3).

3

4 **Table 4-16**. **Adult-based unit risk estimates for use in ADAF calculations and**
5 **risk estimate calculations involving less-than-lifetime exposure scenarios** [*]

6

Cancer response	Adult-based unit risk estimate (per ppm)	Adult-based unit risk estimate (per µg/m^3)
Lymphoid cancer mortality	0.368	2.01×10^{-4}
Lymphoid cancer incidence	0.795	4.35×10^{-4}
Breast cancer mortality	0.436	2.39×10^{-4}
Breast cancer incidence	1.50[a]	8.21×10^{-4}
Total cancer incidence	1.98[a]	1.08×10^{-3}

7
8 [a]For unit risk estimates above 1, convert to risk per ppb. e.g., 1.16 per ppm = 1.16×10^{-3} per ppb.

9
10

11 An example calculation illustrating the application of the ADAFs to the human-data-
12 derived adult-based (re-scaled as discussed above) unit risk estimate for EtO for a lifetime
13 exposure scenario is presented below. For inhalation exposures, assuming ppm equivalence
14 across age groups, i.e., equivalent risk from equivalent exposure levels, independent of body
15 size, the ADAF calculation is fairly straightforward. Thus, the ADAF-adjusted lifetime total
16 cancer unit risk estimate is calculated as follows:

17

18 total cancer risk from exposure to constant EtO exposure level of 1 µg/m^3 from ages 0-70:

19

		unit risk	exposure	duration	partial
Age group	ADAF	(per µg/m^3)	conc (µg/m^3)	adjustment	risk
0 - < 2 years	10	1.08×10^{-3}	1	2 years/70 years	3.09×10^{-4}
2 - < 16 years	3	1.08×10^{-3}	1	14 years/70 years	6.48×10^{-4}
≥ 16 years	1	1.08×10^{-3}	1	54 years/70 years	$\underline{8.33 \times 10^{-4}}$
				total lifetime risk =	1.80×10^{-3}

26

27 The partial risk for each age group is the product of the values in columns 2-5 [e.g., $10 \times (8.36 \times$
28 $10^{-4}) \times 1 \times 2/70 = 2.39 \times 10^{-4}$], and the total risk is the sum of the partial risks.

1

2   This 70-year risk estimate for a constant exposure of 1 μg/m^3 is equivalent to a **lifetime**

3   **unit risk estimate of 1.8 × 10^{-3} per μg/m^3** (3.3 per ppm, or 3.3 × 10^{-3} per ppb), adjusted for

4   potential increased early-life susceptibility, assuming a 70-year lifetime and constant exposure

5   across age groups. Note that because of the use of the re-scaled adult-based unit risk estimate,

6   the partial risk for the ≥ 16 years age group is the same as would be obtained for a 1 μg/m^3

7   constant exposure directly from the total cancer adult-only-exposure unit risk estimate of 8.36 ×

8   10^{-4} per μg/m^3 that was presented above, as it should be (the small difference in the 2nd decimal

9   place is due to round-off error).

10   In addition to the uncertainties discussed above for the inhalation unit risk estimate, there

11   are uncertainties in the application of ADAFs to adjust for potential increased early-life

12   susceptibility. The ADAFs reflect an expectation of increased risk from early-life exposure to

13   carcinogens with a mutagenic mode of action (U.S EPA, 2005b), but they are general adjustment

14   factors and are not specific to EtO. With respect to the breast cancer estimates, for example,

15   evidence suggests that puberty/early adulthood is a particularly susceptible life-stage for breast

16   cancer induction (U.S. EPA, 2005b; Russo and Russo, 1999); however, EPA has not, at this time,

17   developed alternate ADAFs to reflect such a pattern of increased early-life susceptibility, and

18   there is currently no EPA guidance on an alternate approach for adjusting for early-life

19   susceptibility to potential breast carcinogens.

20

21   **4.5.   INHALATION UNIT RISK ESTIMATES—CONCLUSIONS**

22   For both humans and laboratory animals, tumors occur at multiple sites. In humans, there

23   was a combination of tumors having lymphohematopoietic, in particular lymphoid, origins in

24   both sexes and breast cancer in females, and, in rodents, lymphohematopoietic tumors, mammary

25   carcinomas, and tumors of other sites were observed. From human data, an extra cancer unit risk

26   estimate of 4.79 × 10^{-4} per μg/m^3 (8.77 × 10^{-4} per ppb) was calculated for lymphoid cancer

27   incidence, and a unit risk estimate of 9.49 × 10^{-4} per μg/m^3 (1.74 × 10^{-3} per ppb) was calculated

28   for breast cancer incidence in females, under the assumption that RR is independent of age for all

29   ages (Section 4.1). The total extra cancer unit risk estimate was 1.24 × 10^{-3} per μg/m^3 (2.27 ×

30   10^{-3} per ppb) for both cancer types combined (EC$_{01}$ = 0.00775 ppm; LEC$_{01}$ = 0.00441 ppm).

31   Unit risk estimates derived from the three chronic rodent bioassays for EtO ranged from 2.2 ×

32   10^{-5} per μg/m^3 to 4.6 × 10^{-5} per μg/m^3, over an order of magnitude lower than the estimates

33   based on human data.

34   Because a mutagenic mode of action for EtO carcinogenicity (see Section 3.3.2) is

35   "sufficiently supported in (laboratory) animals" and "relevant to humans", and as there are no

36   chemical-specific data to evaluate the differences between adults and children, increased early-

1 life susceptibility should be assumed, in accordance with EPA's *Supplemental Guidance* (U.S.

2 EPA, 2005b). This assumption of increased early-life susceptibility supersedes the assumption

3 of age independence under which the human-data-based estimates presented in the previous

4 paragraph were derived. Thus, as described in Section 4.4, adult-only-exposure unit risk

5 estimates were calculated from the human data under an alternate assumption that RR is

6 independent of age for adults, which represent the life-stage for which the data upon which the

7 exposure-response modeling was conducted pertain. These adult-only-exposure unit risk

8 estimates were then re-scaled to a 70-year basis for use in the standard ADAF calculations and

9 risk estimate calculations involving less-than-lifetime exposure scenarios. The resulting adult-

10 based unit risk estimates were 4.35×10^{-4} per μg/m^3 (7.95×10^{-4} per ppb) for lymphoid cancer

11 incidence and 8.21×10^{-4} per μg/m^3 (1.50×10^{-3} per ppb) for breast cancer incidence in females.

12 The adult-based total extra cancer unit risk estimate for use in ADAF calculations and risk

13 estimate calculations involving less-than-lifetime exposure scenarios was 1.08×10^{-3} per μg/m^3

14 (1.98×10^{-3} per ppb) for both cancer types combined.

15 For exposure scenarios involving early-life exposure, the age-dependent adjustment

16 factors (ADAFs) should be applied, in accordance with EPA's *Supplemental Guidance* (U.S.

17 EPA, 2005b). Applying the ADAFs to obtain a full lifetime unit risk estimate yields

18

$$19 \qquad 1.98/\text{ppm} \times ((10 \times 2 \text{ years}/70 \text{ years}) + (3 \times 14/70) + (1 \times 54/70)) \qquad (4\text{-}7)$$
$$20 \qquad = 3.29/\text{ppm} = 1.80 \times 10^{-3}/(\mu g/m^3).$$

21

22 Applying the ADAFs to the unit risk estimates derived from the three chronic rodent bioassays

23 for EtO yields estimates ranging from 3.7×10^{-5} per μg/m^3 to 7.6×10^{-5} per μg/m^3, still over an

24 order of magnitude lower than the estimate based on human data.

25 Adequate human data, if available, are considered to provide a more appropriate basis

26 than rodent data for estimating human risks (U.S. EPA, 2005a), primarily because uncertainties

27 in extrapolating quantitative risks from rodents to humans are avoided. Although there is a

28 sizeable difference between the rodent-based and the human-based estimates, the human data are

29 from a large, high-quality study, with EtO exposure estimates for the individual workers and

30 little reported exposure to chemicals other than EtO. Therefore, the **full lifetime total extra**

31 **cancer unit risk estimate of 1.8×10^{-3} per μg/m^3 (3.3×10^{-3} per ppb)** calculated for lymphoid

32 cancers and breast cancer combined and applying the ADAFs is the preferred lifetime unit risk

33 estimate. For less-than-lifetime exposure scenarios, the human-data-derived (re-scaled) adult-

34 based unit risk estimate of 1.1×10^{-3} per μg/m^3 (2.0×10^{-3} per ppb) should be used, in

35 conjunction with the ADAFs if early-life exposures occur.

1   The unit risk estimate is intended to be an upper bound on cancer risk for use with
2   exposures below the POD (i.e., the LEC_{01}). The unit risk estimate should not generally be used
3   above the POD; however, in the case of this total extra cancer unit risk, which is based on cancer
4   type-specific unit risk estimates from two linear models, the estimate should be valid for
5   exposures up to about 0.060 ppm (110 $\mu g/m^3$), which is the minimum of the limits for the
6   lymphoid cancer unit risk estimate (0.060 ppm: see Section 4.1.1.2) and the breast cancer unit
7   risk estimate (0.075 ppm; see Section 4.1.2.3).
8   Using the above full lifetime unit risk estimate of 3.3×10^{-3} per ppb (1.8×10^{-3} per
9    $\mu g/m^3$), the lifetime chronic exposure level of EtO corresponding to an increased cancer risk of
10   10^{-6} can be estimated as follows:

11

12    $$(10^{-6})/(3.3/ppm) = 3.0 \times 10^{-7} \text{ ppm} = 0.00030 \text{ ppb} = 0.0006 \text{ } \mu g/m^3. \qquad (4\text{-}8)$$

13

14   The inhalation unit risk estimate presented above, which is calculated based on a linear
15   extrapolation from the POD (LEC_{01}), is expected to provide an upper bound on the risk of cancer
16   incidence. However, estimates of "central tendency" for the risk below the POD are also
17   presented. Adult-based extra risk estimates per ppm for some of the cancer responses, based on
18   linear extrapolation from the adult-only-exposure EC_{01} (i.e., $0.01/EC_{01}$) and re-scaling to a 70-
19   year basis for use in ADAF calculations and risk estimate calculations involving less-than-
20   lifetime exposure scenarios (see Section 4.4), are reported in Table 4-17. The adult-only-
21   exposure EC_{01}s were from the linear regression models for lymphoid cancers and breast cancer
22   mortality and from the two-piece linear spline model (low-dose segment) for breast cancer
23   incidence. (Note that, for each of these models, the low-exposure extrapolated estimates are a
24   straight linear continuation of the linear models used above the PODs, and, thus, the statistical
25   properties of the models are preserved.) These estimates are dependent on the suitability of the
26   EC_{01} estimates as well as on the applicability of the linear low-dose extrapolation. The
27   assumption of low-dose linearity is supported by the mutagenicity of EtO (see Section 3.4). If
28   these estimates are to be used, ADAFs should be applied if early-life exposure occurs, in
29   accordance with EPA's *Supplemental Guidance*.
30
31   **Table 4-17**. **Adult-based extra risk estimates per ppm based on adult-only-**
32   **exposure $EC_{01}s^a$**
33

Cancer response	EC_{01} (ppm)	Adult-based $0.01/EC_{01}$ (per ppm)[b]
Lymphoid cancer mortality (both sexes)	0.0787	0.165

Lymphoid cancer incidence (both sexes)	0.0364	0.356
Breast cancer mortality (females)	0.0590	0.219
Breast cancer incidence (females)	0.0167	0.776

1

2 [a]ADAFs should be applied if early-life exposure occurs, in accordance with EPA's *Supplemental Guidance*.

3 [b]These estimates are calculated as $0.01/EC_{01}$ for the adult-only-exposure extra risk estimate per ppm re-scaled to a

4 70-year basis by multiplying by 70/54 (see Section 4.4).

5

6

7   As can be seen by comparing the adult-based re-scaled $0.01/EC_{01}$ estimates in Table 4-17

8   with the adult-based unit risk estimates in Table 4-16, the $0.01/EC_{01}$ estimates are about 45% of

9   the unit risk estimates for the lymphoid cancer responses and about 50% of the unit risk

10 estimates for the breast cancer responses.

11   Finally, it should be noted that some investigators have posited that the high and variable

12 background levels of endogenous EtO-induced DNA damage in the body (see Section 3.3.3.1)

13 may overwhelm any contribution from low levels of exogenous EtO exposure (SAB, 2007;

14 Marsden et al., 2009). It is true that the existence of these high and variable background levels

15 may make it hard to observe statistically significant increases in risk from low levels of

16 exogenous exposure. However, there is clear evidence of carcinogenic hazard from the rodent

17 bioassays and strong evidence from human studies (Section 3.5), and the

18 genotoxicity/mutagenicity of EtO (Section 3.4) supports low-dose linear extrapolation of risk

19 estimates from those studies (U.S. EPA, 2005a). In fact, as noted in Section 3.3.3.1, Marsden et

20 al. (2009), using sensitive detection techniques and an approach designed to separately quantify

21 both endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO treatment

22 in rats, reported increases in exogenous adducts in DNA of spleen and liver consistent with a

23 linear dose-response relationship ($p < 0.05$), down to the lowest dose administered (0.0001

24 mg/kg injected i.p. daily for 3 days, which is a very low dose compared to the LOAELs in the

25 carcinogenicity bioassays; see Appendix C). Furthermore, while the contributions to DNA

26 damage from low exogenous EtO exposures may be relatively small compared to those from

27 endogenous EtO exposure, low levels of exogenous EtO may nonetheless be responsible for

28 levels of risk (above background risk). This is not inconsistent with the much higher levels of

29 background cancer risk, to which endogenous EtO may contribute, for the two cancer types

30 observed in the human studies—lymphoid cancers have a background lifetime incidence risk on

1 the order of 3%, while the background lifetime incidence risk for breast cancer is on the order of

2 15%.[20]

3

4 **4.6.   COMPARISON WITH OTHER PUBLISHED RISK ESTIMATES**

5   The unit risk values derived in this document are compared with other recent risk

6 estimates presented in the published literature (Table 4-18).

7

8 **4.6.1. Unit Risk Estimates Based on Human Studies**

9   Kirman et al. (2004) used leukemia data only and pooled data from both the Stayner et al.

10 (1993) and the UCC studies (Teta et al., 1993, 1999). Based on the assumption that leukemias

11 are due to chromosome translocations, requiring two independent events (chromosome breaks),

12 the Kirman et al. (2004) proposed that two independent EtO-induced events are required for

13 EtO-induced leukemias and used a dose-squared model, yielding a unit risk value of 4.5×10^{-8}

14 $(\mu g/m^3)^{-1}$ as their preferred estimate.

15
16

---

[20] These background lifetime incidence values were obtained from the lifetable analysis, based on SEER rates, as discussed in Sections 4.1.1.3 and 4.1.2.3. For lymphoid cancer, for example, see the value of Ro at the bottom of the lifetable analysis in Appendix E.

Table 4-18. Comparison of unit risk estimates

Assessments	Data source	Inhalation unit risk estimate[a] (per µg/m^3)
Based on human data		
U.S. EPA (this document)	Lymphoid cancer incidence in sterilizer workers (NIOSH)[b]	7.2×10^{-4}
	Breast cancer incidence in female sterilizer workers (NIOSH)[c]	1.4×10^{-3}
	Total cancer risk based on the NIOSH data	1.8×10^{-3}
Kirman et al. (2004)	Leukemia mortality in combined NIOSH and UCC cohorts (earlier follow-ups)	4.5×10^{-8} Range of 1.4×10^{-8} to 1.4×10^{-7} [d]
Valdez-Flores et al. (2010)	multiple individual cancer endpoints, including all lymphohematopoietic, lymphoid, and breast cancers, in combined updated NIOSH and updated UCC cohorts	5.5×10^{-7} to 1.6×10^{-6} [e]
Based on rodent data		
U.S. EPA (this document)	Female mouse tumors	7.6×10^{-5}
Kirman et al. (2004)	Mononuclear cell leukemia in rats and lymphomas in mice	2.6×10^{-8} to 1.5×10^{-5} [f]

3

[a]Because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and in the absence of chemical-specific data, EPA assumes increased early-life susceptibility, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), and for the EPA lifetime unit risk estimates presented in this table, ADAFs have been applied, as described in Section 4.4. The corresponding adult-based unit risk estimates are 4.4×10^{-4} $(\mu g/m^3)^{-1}$ for human-based lymphoid cancer incidence, 8.2×10^{-4} $(\mu g/m^3)^{-1}$ for human-based breast cancer incidence, 1.1×10^{-3} $(\mu g/m^3)^{-1}$ for human-based total cancer incidence, and 4.6×10^{-5} $(\mu g/m^3)^{-1}$ for rodent-based total cancer incidence.   The non-EPA estimates in the table are shown as reported and do not account for potential increased early-life susceptibility for lifetime exposures that include childhood, with the exception of the Valdez-Flores et al. estimates, which are purported to include the ADAFs, but the ADAFs were in fact misapplied and have essentially no impact (see Appendix A.3.20).

[b]For lymphoid cancer mortality, the ADAF-adjusted lifetime unit risk estimate is 3.3×10^{-4} $(\mu g/m^3)^{-1}$ and the adult-based unit risk estimate is 2.0×10^{-4} $(\mu g/m^3)^{-1}$.

[c]For breast cancer mortality, the ADAF-adjusted lifetime unit risk estimate is 4.0×10^{-4} $(\mu g/m^3)^{-1}$ and the adult-based unit risk estimate is 2.4×10^{-4} $(\mu g/m^3)^{-1}$.

[d]Estimates based on linear extrapolation from EC0001 - EC000001 obtained from the quadratic model.

[e]Estimates based on range of EC(1/million)s of 0.001 – 0.003 ppm obtained from the model $RR = e^{\beta * exposure}$ for relevant cancer endpoints.

4
5
6   The Kirman et al. (2004) values are different from those in the current document because

7 of the different assumptions inherent in the Kirman et al. approach and because the study used

8 unpublished data from earlier follow-ups of the two cohorts. A key difference is that EPA uses a

9 linear model rather than a quadratic (dose-squared) model in the range of observation. Then,

10 EPA uses a higher extra risk level (1%) for establishing the POD, whereas Kirman et al. used a

11 risk level of 10^{-5} for their best estimate and a risk range of 10^{-4} to 10^{-6} for their range of values.

12 The extra risk level and the corresponding POD are not critical with the linear model, but with

13 the quadratic model used by Kirman et al., the lower the risk level and, hence, the POD, the

14 greater the impact of the quadratic model and the lower the resulting unit risk estimates.

15   In addition, EPA (1) uses data for lymphoid cancers (and female breast cancers) rather

16 than leukemias, (2) includes ages up to 85 years in the life-table analysis rather than stopping at

17 70 years, (3) calculates unit risk estimates for cancer incidence as well as mortality, (4) uses a

18 lower bound as the POD rather than the maximum likelihood estimate, (5) uses the results of

19 lagged analyses rather than unlagged analyses, and (6) uses adult-based unit risk estimates in

20 cojunction with ADAFs (see Section 4.4) to derive the lifetime unit risk estimates.

21   Another key difference is that Kirman et al. relied on earlier NIOSH results (Stayner et

22 al., 1993), whereas EPA uses the results of NIOSH's more recent follow-up of the cohort

23 (Steenland et al., 2004). Kirman et al. (2004) claim that a quadratic dose-response model

24 provided the best fit to the data in the observable range and that this provides support for their

25 assumed mode of action. However, the 2004 NIOSH data for lymphohematopoietic cancers

26 suggest a supralinear exposure-response relationship (see Section 4.1.1.2 and Figures 4-1 and

27 4-2), which is inconsistent with a dose-squared model. Furthermore, EPA's review of the mode

28 of action evidence does not support the mode of action assumed by Kirman et al. (see

29 Section 3.4).

30   The Valdez-Flores et al. (2010) unit risk estimates (Table 4-18) are similarly much lower

31 than those in the current document because of the different assumptions used. A key difference

32 is that EPA uses a linear model or a two-piece linear spline model in the range of observation

33 rather than an exponential model ($RR = e^{\beta*exposure}$), which was used by Valdez-Flores et al.

34 despite its lack of fit. Then, EPA uses a higher extra risk level (1%) for establishing the POD for

35 linear extrapolation, whereas Valdez-Flores et al. (2010) used a risk level of 10^{-6}. In addition,

36 EPA (1) includes ages up to 85 years in the life-table analysis rather than stopping at 70 years,

37 (2) calculates unit risk estimates for cancer incidence as well as mortality, (3) uses a lower bound

1 as the POD rather than the maximum likelihood estimate, and (4) uses the results of lagged

2 analyses rather than unlagged analyses. See Appendix A.3.20 for a more detailed discussion of

3 the differences between the EPA and Valdez-Flores et al. (2010) analyses.

4

5 **4.6.2. Unit Risk Estimates Based on Laboratory Animal Studies**

6   Kirman et al. (2004) also used linear and dose-squared extrapolation models to derive

7 unit risk estimates based on the rat mononuclear cell leukemia data and the mouse lymphoma

8 data. First, they used the multistage model to calculate the LEC_{10} (LEC_{01} for the male mouse

9 lymphoma data) for the POD from the observable range. Then, using these PODs for linear

10 extrapolation, Kirman et al. obtained a unit risk range of 3.9×10^{-6} $(\mu g/m^3)^{-1}$ to 1.5×10^{-5}

11 $(\mu g/m^3)^{-1}$. Alternatively, Kirman et al. used a quadratic extrapolation model below the

12 observable range to estimate secondary points of departure (LEC_{01}–LEC_{000001}; LEC_{001}–LEC_{000001}

13 for the male mouse) for final linear low-dose extrapolation, yielding unit risks ranging from 2.6

14 $\times 10^{-8}$ $(\mu g/m^3)^{-1}$ to 4.9×10^{-6} $(\mu g/m^3)^{-1}$. These values are all smaller than the unit risks derived

15 from the rodent data in this document.

16

17 **4.7.   RISK ESTIMATES FOR SOME OCCUPATIONAL EXPOSURE SCENARIOS**

18   The unit risk estimates derived in the preceding sections were developed for

19 environmental exposure levels, where maximum modeled levels are on the order of $1–2$ $\mu g/m^3$

20 (e-mail dated October 3, 2005, from Mark Morris, U.S. EPA, to Jennifer Jinot, U.S. EPA), and

21 are not applicable to higher exposures, including some occupational exposure scenarios. As

22 such, extra risk estimates were calculated for a number of occupational exposure scenarios of

23 possible concern. For these scenarios, exposure-response models from the NIOSH cohort were

24 used in conjunction with the life-table program, as previously discussed in Section 4.1. A

25 35-year exposure occurring between ages 20 and 55 years was assumed, and exposure levels

26 ranging from 0.1 to 1 ppm 8-hour TWA were examined (i.e., ranging from about 1,300 to

27 13,000 ppm × days). (Note that the current Occupational Safety and Health Administration

28 Permissible Exposure Limit is 1 ppm [8-hour TWA].)

29   For lymphoid cancer mortality in both sexes, the best-fitting (natural) log cumulative

30 exposure Cox regression model (Steenland re-analyses in Appendix D; see also Section 4.1.1.2),

31 lagged 15 years, was used. For lymphoid cancer incidence, the exposure-response relationship

32 was assumed to be the same as for mortality (see Section 4.1.1.3). The extra risk results for

33 lymphoid cancer mortality and incidence in both sexes are presented in Table 4-19. As can be

34 seen in Table 4-19, the extra risks for these occupational exposure levels are in the "plateau"

35 region of the exposure-response relationships and increase less than proportionately with

36 exposure. (For occupational exposures less than about 1,000 ppm × days, or about 0.08 ppm

1   8-hour TWA for 35 years, risk estimates are no longer in the plateau region [see Figure 4-1] but
2   rather in the steep low-exposure region, which is a region of greater uncertainty for the log
3   cumulative exposure model, and one might want to use the linear regression of the categorical
4   results that was used for lower exposures [see Section 4.1.1.2; Appendix D]). Furthermore, if
5   one is using the linear model in this range and also estimating risks for exposure levels in the
6   range between about 0.08 and 0.6 ppm (near where the linear and log cumulative exposure Cox
7   regression models meet) 8-hour TWA, one might want to use the linear model for the entire
8   range up to 0.6 ppm 8-hour TWA to avoid a discontinuity between the two models; thus, results
9   for the linear model for exposure levels up to 0.6 ppm 8-hour TWA are also presented in Table
10   4-19. While the best-fitting model would generally be preferred in the exposure range between
11   0.08 and 0.6 ppm 8-hour TWA, there is model uncertainty, so the use of either model could be
12   justified. For exposures higher than where the linear and log cumulative exposure Cox
13   regression models meet, the log cumulative exposure model exclusively is recommended.]

Table 4-19. Extra risk estimates for lymphoid cancer in both sexes for various occupational exposure levels[a]

8-hour TWA (ppm)	Lymphoid cancer mortality				Lymphoid cancer incidence[b]			
	Log cumulative exposure Cox regression model[c]		Linear regression model[d]		Log cumulative exposure Cox regression model[c]		Linear regression model[d]	
	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL
0.1	0.014	0.032	0.003	0.007	0.031	**0.071**	0.007	**0.016**
0.2	0.016	0.038	0.007	0.014	0.035	**0.084**	0.014	**0.031**
0.3	0.017	0.042	0.010	0.022	0.038	**0.093**	0.021	**0.047**
0.4	0.018	0.045	0.013	0.029	0.040	**0.099**	0.028	**0.062**
0.5	0.018	0.047	0.016	0.036	0.042	**0.10**	0.035	**0.076**
0.6	0.019	0.049	0.019	0.042	0.043	**0.11**	0.042	**0.090**
0.7	0.019	0.051	--	0.049	0.044	**0.11**	--	--
0.8	0.020	0.052	--	--	0.045	**0.12**	--	--
0.9	0.020	0.054	--	--	0.046	**0.12**	--	--
1.0	0.021	0.055	--	--	0.047	**0.12**	--	--

[a]Assuming a 35-year exposure between ages 20 and 55 years (see Section 4.7).
[b]Assumes same exposure-response relationship as for lymphoid cancer mortality.
[c]From the best-fitting log cumulative exposure Cox regression model for lymphoid cancer mortality in both sexes; 15-year lag (Appendix D; see also Section 4.1.1.2).
[d]Linear regression of categorical results for both sexes (Appendix D; 15-year lag), excluding the highest exposure group (See Section 4.1.1.2); extra risk estimates from the linear model are provided only up to the exposure level where the linear model meets the log cumulative Cox regression model.

1   For breast cancer, incidence data were available from the NIOSH incidence study and,
2   thus, only incidence estimates were calculated. In addition to being the preferred type of cancer
3   risk estimate, the breast cancer incidence risk estimates are based on more cases than were
4   available in the mortality study and the incidence data (for the subcohort with interviews) are
5   adjusted for a number of breast cancer risk factors (see Section 4.1.2.3). In terms of the
6   incidence data, the subcohort data are preferred to the full cohort data because the subcohort data
7   are adjusted for these potential confounders and also because the full cohort data have
8   incomplete ascertainment of breast cancer cases. For breast cancer incidence in the subcohort
9   with interviews, a number of Cox regression exposure-response models fit almost equally well
10   (Steenland et al., 2003; see also Section 4.1.2.3). These include a log cumulative exposure
11   model and a cumulative exposure model, both with a 15-year lag, and a log cumulative exposure
12   model with no lag. The latter model was omitted from the calculations because the inclusion of a
13   15-year lag for the development of breast cancer was considered more biologically realistic than
14   not including a lag. Steenland et al. (2003) also provide a duration-of-exposure Cox regression
15   model with a marginally better fit; however, models using duration of exposure are less useful
16   for estimating exposure-related risks, and duration of exposure and cumulative exposure are
17   correlated. Thus, only the lagged cumulative exposure models are considered here.
18   The extra risk results for breast cancer incidence in females from the lagged cumulative
19   exposure Cox regression models listed above are presented in Table 4-20. As can be seen in
20   Table 4-20, the extra risk estimates for the lagged log cumulative and cumulative exposure
21   models differ substantially. Furthermore, the categorical Cox regression results for breast cancer
22   incidence in the subcohort with interviews suggest that, for the lowest four exposure quintiles,
23   the log cumulative exposure model overestimates the RR, while the cumulative exposure model
24   generally underestimates the RR, with the categorical results largely falling between the RR
25   estimates of those two models (see Figure 4-5). (The lowest four exposure quintiles represent
26   individual worker exposures ranging from 0 to about 15,000 ppm $\times$ days, which covers the range
27   of cumulative exposures for the occupational exposure scenarios of interest in this assessment.)
28   Therefore, the two-piece linear spline model was also used to calculate the extra risk estimates
29   (see Section 4.1.2.3). In addition, this model provided a better fit to the data than that of the log
30   cumulative exposure model, as indicated by a lower AIC value (1950.9 for two-piece linear
31   spline model versus 1956.2 for the log cumulative exposure Cox regression model; Appendix D).
32   Extra risk estimates using the two-piece linear spline model are also presented in Table 4-20 and
33   are the preferred estimates because, in addition to providing a better overall fit to the data, the
34   two-piece linear spline model best represents the categorical RR results for exposures below
35   about 15,000 ppm $\times$ days (see Figure 4-5).

1   Extra risk estimates for a 45-year exposure to the same exposure levels were nearly

2   identical to those from the 35-year exposure for both lymphoid cancer in both sexes and breast

3   cancer in females (results not shown). With the 15-year lag, the assumption of an additional 10

4   years of exposure only negligibly affects the risks above age 70 and has little impact on lifetime

5   risk. For exposure scenarios of 35−45 years but with 8-hour TWAs falling between those

6   presented in the tables, one can estimate the extra risk by interpolation. For exposure scenarios

7   with durations of exposure less than 30-35 years, one could roughly estimate extra risk by

8   calculating the cumulative exposure and finding the extra risk for a similar cumulative exposure

9   in Table 4-19 (or 4-20). For a more precise estimation, or for exposure scenarios of much

10   shorter duration or for specific age groups, one should do the calculation using a life-table

11   analysis, as presented in Appendix E but modified for the specific exposure scenarios.

Table 4-20. Extra risk estimates for breast cancer incidence in females for various occupational exposure levels[a,b]

8-hour TWA (ppm)	Log cumulative exposure Cox regression model[c]		Cumulative exposure Cox regression model[c]		Two-piece linear spline model[d]	
	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL
0.1	0.055	0.11	0.0013	0.0023	0.016	**0.031**
0.2	0.061	0.12	0.0026	0.0046	0.032	**0.061**
0.3	0.065	0.13	0.0040	0.0069	0.048	**0.090**
0.4	0.068	0.14	0.0053	0.0092	0.063	**0.118**
0.5	0.070	0.14	0.0067	0.012	0.075	**0.139**
0.6	0.072	0.14	0.0081	0.014	0.081	**0.150**
0.7	0.073	0.15	0.0095	0.017	0.086	**0.157**
0.8	0.074	0.15	0.011	0.019	0.089	**0.162**
0.9	0.076	0.15	0.012	0.022	0.093	**0.167**
1.0	0.077	0.16	0.014	0.024	0.095	**0.171**

[a]Assuming a 35-year exposure between ages 20 and 55 years.
[b]From incidence data for subcohort with interviews; invasive and in situ tumors (Steenland et al., 2003).
[c]Cox regression models from Steenland et al. (2003; Table 5), with 15-year lag.
[d]Two-piece linear spline model results for occupational exposures use both spline segments (Appendix D), knot at 5800 ppm × days; with 15-year lag. For the 95% UCL, for exposures below the knot, RR = 1 + (β1+ 1.645 × SE1) × exposure; for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp-knot) + 1.645 × sqrt(exp^2 × var1 + (exp-knot)^2 × var2 + 2 × exp × (exp-knot) × covar)), where exp = cumulative exposure, var = variance, covar = covariance (see Appendix D for the parameter values).

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APPENDIX A
CRITICAL REVIEW OF EPIDEMIOLOGIC EVIDENCE

[EDITORIAL NOTE: Please note that in this assessment document the responses to external peer review and public comments can be found in Appendix H.]

A.1. BACKGROUND

On the basis of studies indicating that EtO was a strong mutagen and that exposure to EtO produced increased chromosomal aberrations in human lymphocytes (Rapoport, 1948; Ehrenberg and Gustafsson, 1959; Ehrenberg and Hallstrom, 1967), Hogstedt and colleagues studied three small, independent cohorts of workers from Sweden. Reports on two of these cohorts (Hogstedt et al., 1979a, b, 1984) were reviewed in the earlier health assessment document (U.S. EPA, 1985). These two small cohorts plus a third group of EtO-exposed workers from a third independent plant in Sweden were then combined and studied as one cohort (Hogstedt et al., 1986; Hogstedt, 1988). A review of this reconstituted cohort study and subsequent independent studies is presented in Section A3.

Shortly after the third Hogstedt study was completed, another independent study of EtO-exposed employees was completed (Gardner et al., 1989) on a cohort of workers from four companies and eight hospitals in Great Britain, and it was followed by a third independent study on a cohort of exposed workers in eight chemical plants from the Federal Republic of Germany (Kiesselbach et al., 1990). A follow-up study of the Gardner et al. (1989) cohort was recently conducted by Coggon et al. (2004).

Greenberg et al. (1990) was the first in a series of studies of workers exposed to EtO at two chemical manufacturing facilities in the Kanawha Valley (South Charleston, WV). The workers at these two facilities were studied later by Teta et al. (1993, 1999), Benson and Teta (1993), and Swaen et al. (2009) and became the basis for several important quantitative risk assessment analyses (Teta et al., 1999; EOIC, 2001; Valdez-Flores et al., 2010).

Another independent study of EtO-exposed workers in 14 sterilizing plants from across the United States was completed by the National Institute for Occupational Safety and Health (Steenland et al., 1991; Stayner et al., 1993). The Stayner et al. (1993) paper presents the exposure-response analysis performed by the National Institute for Occupational Safety and Health (NIOSH) investigators. These same workers were studied again from a different perspective by Wong and Trent (1993). The NIOSH investigators recently completed a follow-up of the mortality study (Steenland et al., 2004) and a breast cancer incidence study based in the same cohort (Steenland et al., 2003). The results of the Steenland et al. (2003, 2004) analyses

are the basis for the quantitative assessment in this document, for reasons explained in the review and summary sections of this appendix.

Several additional studies of lesser importance have been done on EtO-exposed cohorts of workers in Sweden (Hagmar et al., 1991, 1995), Italy (Bisanti et al., 1993), Belgium (Swaen et al., 1996), and western New York State (Norman et al., 1995), and other parts of the United States (Olsen et al., 1997). These studies are discussed in the following review, but they provide limited information to the overall discussion of whether EtO induces cancer in humans.

The more important studies, which are discussed in detail in the summary, are those at two facilities in the Kanawha Valley in West Virginia (Greenberg et al., 1990; Benson and Teta, 1993; Teta et al., 1993, 1999; Swaen et al., 2009; Valdez-Flores et al., 2010) and at 14 sterilizing plants around the country (Stayner et al., 1993; Steenland et al., 1991, 2003, 2004). These studies indicate that a great deal of effort and care was expended to ensure that they were done well. They have sufficient follow-up to analyze latent effects, attempts were made to develop dose-response relationships using reasonable assumptions about early exposures to EtO, and the cohorts appear to be large enough to test for small differences.

A.2. INDIVIDUAL STUDIES

A.2.1. HOGSTEDT ET AL. (1986), HOGSTEDT (1988)

Hogstedt et al. (1986) combined workers from several cohorts for a total of 733 workers, including 378 workers from two separate and independent occupational cohort mortality studies by Hogstedt et al. (1979a, b) and 355 employees from a third EtO production plant who had not been previously examined. The combined cohort was followed until the end of 1982. The first cohort comprised employees from a small technical factory in Sweden where hospital equipment was sterilized with EtO. The second was from a production facility where EtO was produced by the chlorohydrin method from 1940 to 1963. The third was from a production facility where EtO was made by the direct oxidation method from 1963 to 1982.

In the update of the 1986 occupational mortality report (Hogstedt, 1988), the cohort inexplicably was reduced to 709 employees (539 men; 170 women). Follow-up for mortality was extended to the end of 1985. The author reported that 33 deaths from cancer had occurred, whereas only 20 were expected in the combined cohort. The excesses that are significant are due mainly to an increased risk of stomach cancer at one plant and an excess of blood and lymphatic malignancies at all three. Seven deaths from leukemia occurred, whereas only 0.8 were expected (standard mortality ratio [SMR] = 9.2). Ten deaths due to stomach cancer occurred versus only 1.8 expected (SMR = 5.46). The results tend to agree with those from clastogenic and short-term tests on EtO (Ehrenberg and Gustafsson, 1959). The authors believe that the large number of positive cytogenetic studies demonstrating increased numbers of chromosomal aberrations and

1 sister chromatid exchanges at low-level exposure to EtO indicate that the lymphatic and

2 hematopoietic systems are particularly sensitive to the genotoxic effects of EtO. They concluded

3 that the induction of malignancies even at low-level and intermittent exposures to EtO should be

4 "seriously considered by industry and regulating authorities."

5   The average air EtO concentrations in the three plants were as follows: In Plant 1

6 (Hogstedt et al., 1979b) in 1977, levels ranged from 2 to 70 ppm in the storage hall. The average

7 8-hour time-weighted average (TWA) concentration in the breathing zone of the employees was

8 calculated as 20 ppm +/− 10 ppm. Measured concentrations were 150 ppm on the floor outside

9 of the sterilized boxes and 1,500 ppm inside.

10   In Plant 2 (Hogstedt et al., 1979a), EtO was produced through the chlorohydrin process.

11 Between 1941 and 1947, levels probably averaged about 14 ppm, with occasional exposures up

12 to 715 ppm. Between 1948 and 1963, levels were in the range of 6 ppm to 28 ppm. After 1963,

13 when production of EtO came to an end, levels ranged from less than 1 ppm to as much as 6

14 ppm.

15   In Plant 3 (Hogstedt et al., 1986), the 355 employees were divided into subgroups.

16 Subgroup A had almost pure exposure to EtO. Subgroup B had principal exposure to EtO but

17 also exposure to propylene oxide, amines, sodium nitrate, formaldehyde, and 1,2-butene oxide.

18 Workers in the remaining subgroup C were maintenance and technical service personnel, who

19 had multiple exposures, including EtO. Concentration levels in Plant 3 are shown in Table A-1.

20

1 **Table A-1. Estimated 8-hour time-weighted average ethylene oxide**
2 **exposure, Plant 3**
3

Group	1963–1976	1977–1982
A (*n* = 128)	5–8 ppm	1–2 ppm
B (*n* = 69)	3 ppm	1 ppm
C (*n* = 158)	1–3 ppm	0.4–1.6 ppm

4
5 Source: Hogstedt et al. (1986).
6
7
8   In the earlier studies (Hogstedt et al., 1979a, b) of two of the plants that contributed
9 workers to this cohort, the authors allude to the fact that there was exposure to benzene, ethylene
10 workforce, no gender differences in risk were analyzed separately by the investigators. Of 16
11 patients with tumors in the two exposed cohorts, there were three cases of leukemia (0.2
12 expected), six cases of alimentary tract cancer, and four cases of urogenital cancer. Of the 11
13 cancer cases in the full-time exposed cohort, 5.9 were expected ($p < 0.05$). This study was
14 criticized by Divine and Amanollahi (1986) for several reasons. First, they believed that the
15 study's strongest evidence in support of a carcinogenic claim for EtO was only a "single case of
16 leukemia" in subgroup C of Plant 3, where the workers had multiple chemical exposures;
17 however, there were no cases in subgroups A or B of Plant 3. Hogstedt et al. (1986) countered
18 that the expectation of leukemia in these two subgroups were 0.04 and 0.02, respectively, and
19 that the appearance of a case could only happen if EtO had "outstanding carcinogenic properties
20 at low levels." Divine and Amanollahi also pointed out that a study (Morgan et al., 1981) of a
21 cohort similar to that of Plant 3 found no leukemia cases or evidence of excessive mortality.
22 Hogstedt et al. replied that Morgan et al. stated in their paper that the statistical power of their
23 study to detect an increased risk of leukemia was not strong.
24   Divine and Amanollahi (1986) also stated that the exposures to EtO were higher in
25 plants 1 and 2 than in Plant 3; therefore, combinations would "normally preclude comparisons
26 between the plants for similar causes of adverse health." This potential problem could be
27 resolved by structuring exposure gradients to analyze risk. Furthermore, they noted, Plant 1 was
28 a nonproduction facility involved in sterilization of equipment. Plant 2 used the chlorohydrin
29 process for making EtO, and Plant 3 used the direct oxygenation process. Although these
30 conditions are obviously different, they "are grouped together as analogous." This criticism
31 would, in most instances, be valid only because the methods for producing EtO differ and there
32 were differing exposures to multiple chemicals.

1   However, these concerns are not supported by the evidence. In all three plants the
2   leukemia risk was elevated, even if only slightly in Plant 3. This suggests that there may have
3   been a common exposure, possibly to EtO, endemic to all three plants that was responsible for
4   the measured excesses. Noteworthy is the elevated risk of leukemia seen in Plant 1 (3 observed
5   vs. 0.14 expected), where the exposures were almost exclusively to EtO in the sterilization of
6   equipment. The argument that Plant 1 leukemias form a "chance cluster," as Shore et al. (1993)
7   claim, and as such should be excluded from any analysis does not preclude the possibility that
8   these cases are in reality the result of exposure to EtO. Hogstedt argues that earlier remarks by
9   Ehrenberg and Gustafsson (1959) that EtO "constituted a potential cancer hazard" on the basis of
10   a considerable amount of evidence other than epidemiologic should have served as a warning
11   that the increased risk seen in Plant 1 was not necessarily a "chance cluster," and because the
12   chlorohydrin process was not in use in Plant 1, it cannot be due to exposure to a chemical in the
13   chlorohydrin process.
14
15   **A.2.2. GARDNER ET AL. (1989)**
16   Gardner et al. (1989) completed a cohort study of 2,876 men and women who had
17   potential exposure to EtO. The cohort was identified from employment records at four
18   companies that had produced or used EtO since the 1950s and from eight hospitals that have had
19   EtO clinical sterilizing units since the 1960s, and it was followed to December 31, 1987. All but
20   1 of the 1,012 women and 394 of the men in the cohort worked at one of the hospitals. The
21   remaining woman and 1,470 men made up the portion of the cohort from the four companies.
22   By the end of the follow-up, 226 members (8% of the total cohort) had died versus 258.8
23   expected. Eighty-five cancer deaths were observed versus 76.64 expected.
24   No clear excess risk of leukemia (3 observed vs. 2.09 expected), stomach cancer (5
25   observed vs. 5.95 expected), or breast cancer (4 observed vs. 5.91 expected) was present as of
26   the cut-off date. "Slight excesses" of deaths due to esophageal cancer (5 observed vs. 2.2
27   expected), lung cancer (29 observed vs. 24.55 expected), bladder cancer (4 observed vs. 2.04
28   expected), and non-Hodgkin lymphoma (NHL) (4 observed vs. 1.63 expected) were noted,
29   although an adjustment made to reflect local "variations in mortality" reduced the overall cancer
30   excess from 8 to only 3. According to the authors' published tabulations, all three leukemias
31   identified in this study fell into the longest latent category (20 years or longer), where only 0.35
32   were expected. All three were in the chemical plants. This finding initially would seem to be
33   consistent with experimental animal evidence demonstrating excess risks of hematopoietic
34   cancer in animals exposed to EtO. But the authors note that since other known leukemogens
35   were present in the workplace, the excess could have been due to a confounding effect.

1   The hospitals began using EtO during or after 1962, whereas all of the chemical

2 companies had handled EtO from or before 1960. In the hospitals there was occasional exposure

3 to formaldehyde and carbon tetrachloride but few other confounding agents. On the other hand,

4 the chemical workers were exposed to a wide range of compounds including chlorohydrin,

5 propylene oxide, styrene, and benzene. The earliest industrial hygiene surveys in 1977 indicated

6 that the TWA average exposures were less than 5 ppm in almost all jobs and less than 1 ppm in

7 many. No industrial hygiene data were available for any of the facilities prior to 1977, although

8 it is stated that peaks of exposure up to several hundred ppm occurred as a result of operating

9 difficulties in the chemical plants and during loading and unloading of sterilizers in the hospitals.

10 An odor threshold of 700 ppm was reported by both manufacturers and hospitals, according to

11 the authors. The authors assumed that past exposures were somewhat higher without knowing

12 precisely what they were. An attempt was made to classify exposures into a finite number of

13 subjectively derived categories (definite, possible, continual, intermittent, and unknown). This

14 exercise produced no discernable trends in risk of exposure to EtO. However, the exposure

15 status classification scheme was so vague as to be useless for determining risk by gradient of

16 exposure to EtO.

17   It is of interest that all three of the leukemia deaths entailed exposure to EtO, with very

18 little or no exposure to benzene, according to the authors. The findings are not inconsistent with

19 those of Hogstedt et al. (1986) and Hogstedt (1988). The possibility of a confounding effect

20 other than benzene in these chemical workers cannot entirely be ruled out. Other cancers were

21 slightly in excess, but overall there was little increased mortality from cancer in this cohort. It is

22 possible that if very low levels of exposure to EtO had prevailed throughout the history of these

23 hospitals and plants, the periods of observation necessary to observe an effect may not have been

24 long enough.

25   A follow-up study of this cohort conducted by Coggon et al. (2004) is discussed below.

26

27 **A.2.3. KIESSELBACH ET AL. (1990)**

28   Kiesselbach et al. (1990) carried out an occupational cohort mortality study of 2,658 men

29 from eight chemical plants in the Federal Republic of Germany (FRG) that were involved in the

30 production of EtO. The method of production is not stated. At least some of the plants that were

31 part of an earlier study by Thiess et al. (1982) were included. Each subject had to have been

32 exposed to EtO for at least 1 year sometime between 1928 and 1981 before person-years at risk

33 could start to accumulate. Most exposures occurred after 1950. By December 31, 1982, the

34 closing date of the study, 268 men had died (about 10% of the total cohort), 68 from malignant

35 neoplasms. The overall SMR for all causes was 0.87, and for total cancer the SMR was 0.97,

based on FRG rates. The authors reported that this deficit in total mortality indicates a healthy-worker effect.

The only remarkable findings here are slightly increased risks of death from stomach cancer (14 observed vs. 10.15 expected, SMR = 1.4), cancer of the esophagus (3 observed vs. 1.5 expected, SMR = 2), and cancer of the lung (23 observed vs. 19.86 expected, SMR = 1.2). Although the authors claimed that they looked at latency, only stomach cancer and total mortality has a latency analysis included. This was accomplished by not counting the first 10 years of follow-up in the parameter "years since first exposure." This study is limited by the lack of further latency analyses at other cancer sites. The risk of stomach cancer shows only a slight nonsignificant trend upward with increasing latency. Only two leukemias were recorded versus 2.35 expected.

This is a largely unremarkable study, with few findings of any significance. No actual exposure estimates are available. The categories of exposure that the authors constructed are "weak," "medium," and "strong." It is not known whether any of these categories is based on actual measurements. No explanation of how they were derived is provided except that the authors claim that the information is available on 67.2% of the members of the cohort. If the information was based on job categories, it should be kept in mind that exposures in jobs that were classified the same from one plant to the next may have produced entirely different exposures to EtO. The tabular data regarding these exposure categories shows that only 2.4% of all members of the cohort were considered "strongly" exposed to EtO. Although 71.6% were classified as "weak," the remaining 26% were considered as having "medium" exposure to EtO.

This is largely a study in progress, and further follow-up will be needed before any definite trends or conclusions can be drawn. The authors reported that only a median 15.5 years of follow-up had passed by the end of the cutoff date, whereas the median length of exposure was 9.6 years. Before any conclusions can be made from this study several additional years of follow-up would be needed with better characterization of exposure.

A.2.4. GREENBERG ET AL. (1990)

Greenberg et al. (1990) retrospectively studied the mortality experience of 2,174 men who were assigned to operations that used or produced EtO in either of two Union Carbide Corporation (UCC) chemical plants in West Virginia. In 1970 and 1971, EtO production at the two plants was phased out, but EtO was still used in the plants for the production of other chemicals. SMRs were calculated in comparison with the general U.S. population and the regional population. Results based on regional population death rates were found to be similar to those based on the U.S. general population. Follow-up began either on January 1, 1940, if exposure to EtO began sooner, or on the date when exposure began, if it occurred after January

1   1, 1940. Follow-up ended on December 31, 1978. Note that this cohort is thus a mixture of a

2   prevalent cohort and an incident cohort, and the prevalent part of the cohort may be especially

3   vulnerable to bias from the healthy worker survivor effect. The healthy worker survivor effect

4   might have occurred if workers who were employed before1940 and who were of greater

5   susceptibility preferentially developed a disease of interest prior to 1940 and were no longer

6   employed when cohort enumeration began. It appears that the chemical facilities began

7   operating in 1925, so the maximum latency for the development of a disease of interest between

8   the time of first exposure and cohort enumeration was 15 years; however, these early (pre-1940)

9   hires would also have had the highest EtO exposures (Swaen et al., 2009) and may thus have had

10   short latency periods as well. The healthy worker survivor effect bias can also dampen

11   exposure-response relationships (Applebaum et al., 2007). According to Greenberg et al. (1990),

12   slightly over 10% of the cohort was comprised of prevalent hires (223 of 2174). This is not a

13   large proportion, but, as noted above, these early hires would also have had the highest exposures

14   (Swaen et al., 2009). It is unknown how many workers employed before 1940 were no longer

15   employed when cohort enumeration began. Two years of pre-1940 exposure were reportedly

16   taken into account when categorizing the cohort into groups with ≥ 2 years exposure in the

17   different potential exposure categories (see below); however, it is unclear how pre-1940 years of

18   exposure were treated in other analyses, e.g., the analyses based on duration of exposure

19   (although presumably they were taken into account for those analyses as well).

20   Total deaths equaled 297 versus 375.9 expected (SMR = 0.79, $p < 0.05$). Only 60 total

21   cancer deaths were observed versus 74.6 expected (SMR = 0.81). These deficits in mortality

22   suggest a manifestation of the healthy-worker effect. In spite of this, nonsignificant elevated

23   risks of cancer of the liver, unspecified and primary, (3 observed vs. 1.8 expected, SMR = 1.7),

24   pancreas (7 observed vs. 4.1 expected, SMR = 1.7), and leukemia and aleukemia (7 observed vs.

25   3.0 expected, SMR = 2.3) were noted.

26   The authors also reported that in 1976, 3 years prior to the end of follow-up, an industrial

27   hygiene survey found that 8-hour TWA EtO levels averaged less than 1 ppm, although levels as

28   high as 66 ppm 8-hour TWA had been observed. In maintenance workers, levels averaged

29   between 1 and 5 ppm 8-hour TWA. Because of the lack of information about exposures before

30   1976 (e.g., when EtO was in production) , the authors developed a qualitative exposure

31   categorization scheme with 3 categories of exposure (low, intermediate, and high) on the basis of

32   the potential for exposure in each department. The number of workers in each exposure category

33   was not reported; however, it appears from Teta et al. (1003) (see below) that only 425 workers

34   were assigned to EtO production departments, which were apparently the only departments with

35   high potential exposure. No significant findings of a dose-response relationship were

36   discernable.

1   Except for two cases of leukemia, all the victims of pancreatic cancer and leukemia
2 began their work—and hence exposure to EtO—many years prior to their deaths. The leukemia
3 and pancreatic cancer deaths were concentrated in the chlorohydrin production department. Four
4 of the seven leukemia victims had been assigned to the chlorohydrin department; only 0.8 deaths
5 (SMR = 5.0) would have been expected in this department of only 278 workers. Six pancreatic
6 cancer victims were assigned to the chlorohydrin department, whereas only 0.98 deaths would
7 have been expected to occur (SMR = 6.1). All seven leukemia victims, including the four in the
8 chlorohydrin department, were listed by the authors as having only low potential exposure to
9 EtO. In contrast, among workers ever assigned to a department in the high exposure category,
10 no leukemia deaths and only one pancreatic cancer death occurred.
11   The authors hypothesized that the excesses in leukemia and pancreatic cancers were
12 associated with production of ethylene chlorohydrin or propylene chlorohydrin or both in the
13 chlorohydrin department. Some later follow-up studies (described below) were done of the
14 cohort excluding the chlorohydrin production workers (Teta et al., 1993) and of the chlorohydrin
15 production workers alone (Benson and Teta, 1993) to further examine this hypothesis.
16
17 **A.2.5. STEENLAND ET AL. (1991)**
18   In an industry-wide analysis by the National Institute for Occupational Safety and Health,
19 Steenland et al. (1991) studied EtO exposure in 18,254 workers (55% female) identified from
20 personnel files of 14 plants that had used EtO for sterilization of medical equipment, treating
21 spices, or testing sterilizers. Each of the 14 plants (from 75 facilities surveyed) that were
22 considered eligible for inclusion in the study had at least 400 person-years at risk prior to 1978.
23 Within each eligible facility, at least 3 months of exposure to EtO qualified an employee for
24 inclusion in the cohort. Employees, including all salaried workers, who were "judged never to
25 have been exposed to EtO" on the basis of industrial hygiene surveys were excluded. Follow-up
26 ended December 31, 1987. The cohort averaged 16 years of latency. Approximately 86%
27 achieved the 9-year latent point, but only 8% reached the 20-year latency category. The average
28 year of first exposure was 1970, and the average length of exposure was 4.9 years. The workers'
29 average age at entry was not provided, nor was an age breakdown. Nearly 55% of the cohort
30 were women.
31   Some 1,137 workers (6.4%) were found to be deceased at the end of the study period,
32 upon which the underlying cause of death was determined for all but 450. If a member was
33 determined to be alive as of January 1, 1979, but not after and no death record was found in the
34 National Death Index through December 31, 1987, then that member was assumed to be alive for
35 the purposes of the life-table analysis and person-years were accumulated until the cut-off date.
36 Altogether, 4.5% of the cohort fell into this category. This procedure would tend to increase the

1   expected deaths and, as a consequence, potentially bias the risk ratio downward if a sizable

2   number of deaths to such persons during this period remained undiscovered to the researchers.

3   In the total cohort no significantly increased risks of death from any site-specific cancer

4   were noted. Analyses by job categories and by duration of exposure indicated no excess risks of

5   cancer when compared with the rate in the general population. However, there was an increased

6   trend in the risk of hematopoietic cancers, all sites, with increasing lengths of time since first

7   exposure. After 20 years latency, the SMR was 1.76, based on 13 cases. The test for trend was

8   significant at $p = 0.03$. For men (45%), without regard for latency, the SMR for hematopoietic

9   cancer was a significant 1.55 ($p < 0.05$), based on 27 cases. Among men with long latency

10   (greater than 20 years) and the longest duration of exposure (greater than 7 years) the SMR for

11   hematopoietic cancers was 2.63, based on 7 deaths ($p < 0.05$).

12   The authors pointed out that the SMR for leukemia among men was 3.45, based on 5

13   deaths ($p < 0.05$), for deaths in the latter period of 1985 to 1987. For kidney cancer, the SMR

14   was 3.27, based on 6 deaths ($p < 0.05$), after 20 years latency. The authors also reported on a

15   significant excess risk ($p < 0.05$) of lymphosarcoma-reticulosarcoma in men (SMR = 2.6), based

16   on 7 deaths. Women had a lower nonsignificant rate. The risk of breast cancer was also

17   nonsignificant (SMR = 0.85 based on 42 cases). The authors hypothesized that men were more

18   heavily exposed to EtO than were women because "men have historically predominated in jobs

19   with higher levels of exposure." However, the lack of an association between EtO exposure and

20   lymphohematopoietic cancer in females was also observed in the exposure-response analyses of

21   this cohort, including in the highest exposure category, performed by Stayner et al. (1993) and

22   discussed below.

23   Industrial hygiene surveys indicated that sterilizer operators were exposed to an average

24   personal 8-hour TWA EtO level of 4.3 ppm, whereas all other workers averaged only 2 ppm,

25   based on 8-hour samples during the period 1976 to 1985. These latter employees primarily

26   worked in production and maintenance, in the warehouse, and in the laboratory. This was during

27   a time when engineering controls were being installed to reduce worker's exposure to EtO;

28   earlier exposures may have been somewhat higher. The authors reported that no evidence of

29   confounding exposure to other occupational carcinogens was documented.

30   The authors concluded that there was a trend toward an increased risk of death from

31   hematopoietic cancer with increasing lengths of time since the first exposure to EtO. This trend

32   might have been enhanced if the authors had added additional potential deaths identified from

33   the 820 (4.5%) "untraceable" members of the cohort from 1979 to 1987. The authors felt that

34   their results were not conclusive for the relatively rare cancers of a priori interest, based on the

35   limited number of cases and the short follow-up. The cohort averaged 16 years of latency and

36   86% had at least 9 years but only 8% reached the 20-year latent category.

1   Exposure-response analyses were conducted by Stayner et al. (1993) and are discussed

2   below. More recently, a follow-up mortality study (Steenland et al., 2004) and a breast cancer

3   incidence study (Steenland et al., 2003) of this cohort were conducted; these are also discussed

4   below.

5

6   **A.2.6. TETA ET AL. (1993)**

7   In a follow-up analysis of the cohort of 2,174 male UCC workers studied by Greenberg et

8   al. (1990), Teta and her colleagues excluded the 278 workers in the chlorohydrin unit in which

9   Greenberg and colleagues found a high risk of leukemia and pancreatic cancer, thereby removing

10   the potential confounding of the chlorohydrin production process. The 1,896 men in the

11   remaining cohort were followed for an additional 10 years, through all of 1988. (Among the 278

12   men who were excluded because they had worked in the chlorohydrin unit, 49 had also been

13   assigned to EtO production departments, which were considered high potential ETO exposure

14   departments, according to Greenberg et al. [1990]. Data were reportedly examined with and

15   without the inclusion of these 49 workers with overlapping assignments; however, the results of

16   these analyses are not fully presented). According to Benson and Teta (1993), 112 of the 278

17   excluded workers were employed before 1940, reducing the prevalent part of the remaining

18   cohort to 111 of 1,896 workers, or just under 6%. (It is unclear how pre-1940 years of exposure

19   were treated in the analyses based on duration of exposure, although presumably they were taken

20   into account.) The update did not include additional work histories for the study subjects. Teta

21   et al. (1993) note that duration of assignment to an EtO production unit was not affected by the

22   update because EtO was no longer in production at the two plants; however, assignment to EtO-

23   using departments might have been affected, and, according to Greenberg et al. (1990), some of

24   these departments had medium EtO exposure potential.

25   Teta et al. (1993) reported that the average duration of exposure was more than 5 years

26   and the average follow-up was 27 years. Furthermore, at least 10 years had elapsed since first

27   exposure for all the workers. The reanalysis demonstrated no increased risk of overall cancer, or

28   of leukemia, NHL, or cancers of the brain, pancreas, or stomach. The SMR for total deaths,

29   based on comparison with mortality from the general population, was 0.79 ($p < 0.01$; observed =

30   431). The SMR for total cancer was 0.86 (observed = 110). No site-specific cancers were

31   significantly elevated. Although the authors concluded that this study did not indicate any

32   significant trends of increasing site-specific cancer risk with increasing duration of potential

33   exposure to EtO, there appeared to be a nonsignificant increasing trend for leukemia and

34   aleukemia ($p = 0.28$, based on 5 cases) as well as stomach cancer ($p = 0.13$; 8 cases).

35   According to Greenberg et al. (1990), 8-hour TWA EtO levels averaged less than 1 ppm,

36   based on the 1976 monitoring (after EtO production at the plants had ceased), although levels as

1 high as 66 ppm 8-hour TWA were reported. Teta et al. estimated that in the 1960s, exposure in
2 the units producing EtO by direct oxidation ranged from 3 to 20 ppm 8-hour TWA, with peaks of
3 several hundred ppm. These estimates were based on an industrial hygiene survey conducted at
4 another UCC facility in Texas that used the same direct oxidation process as the two plants in
5 West Virginia from which the UCC EtO cohort was taken. Ethylene oxide was also produced
6 via the chlorohydrin process in a closed building during the years 1925 to 1957. Levels of
7 exposure to EtO would have been higher than in the direct oxidation production process because
8 of start-up difficulties, fewer engineering controls, less complex equipment, and the enclosed
9 building. Employee nausea, dizziness, and vomiting were documented in the medical
10 department in 1949. These acute effects occur in humans at exposures of several hundred ppm,
11 according to the authors.
12   During the time periods under investigation, the estimated exposure ranges for
13 departments using or producing EtO were >14 ppm from 1925 to 1939; 14 ppm from 1940 to
14 1956; 5–10 ppm from 1957 to 1973; and <1 ppm from 1974 to 1988, with frequent peaks of
15 several hundred ppm in the earliest period and some peaks of similar intensity in the 1940s to
16 mid-1950s. In the absence of monitoring data prior to 1976, these estimates cannot be
17 confirmed. Furthermore, workers were eliminated from the analysis if they had worked in the
18 chlorohydrin unit because of the assumption that the increased risks of leukemia and pancreatic
19 cancer were possibly due to exposure to something in the chlorohydrin process, as conjectured
20 by Greenberg et al. (1990). However, even when the potential confounding influence of the
21 chlorohydrin process is removed, there remains the suggestion of a trend of an increasing risk of
22 leukemia and aleukemia with increasing duration of exposure to EtO in the remaining cohort
23 members ($p = 0.28$, based on 5 cases).
24   The authors indicated that their findings do not confirm the findings in experimental
25 animal studies and are not consistent with the earliest results reported among EtO workers. They
26 also noted that they did not observe any significant trend of increasing risks of stomach cancer
27 ($n = 8$), leukemia ($n = 5$) or cancers of the pancreas or brain and nervous system with increasing
28 duration of exposure. No lagged exposure or latency analyses were conducted in this study.
29   In a later analysis, Teta et al. (1999) fitted Poisson regression dose-response models to
30 the UCC data (Teta et al., 1993) and to the NIOSH data (Steenland et al., 1991). They reported
31 that latency and lagging of dose did not appreciably affect the fitted models. Because Teta et al.
32 (1999) did not present risk ratios for the categories used to model the dose-response
33 relationships, the only comparison that could be made between the UCC and NIOSH data is
34 based on the fitted models. These models are almost identical for leukemia, but, for the
35 lymphoid category, the risk according to the fitted model for the UCC data decreased as a
36 function of dose, whereas the risk for the modeled NIOSH data increased as a function of dose.

1 However, the models are based on small numbers of cases (16 [5 UCC, 11 NIOSH] for

2 leukemia; 22 [3 UCC, 19 NIOSH] for lymphoid cancers), and no statistics are provided to assess

3 model goodness of fit or to compare across models. This analysis is superseded by the more

4 recent analysis by the same authors (Valdez-Flores et al., 2010) of the results of more recent

5 follow-up studies of these two cohorts (see discussion of the Swaen et al. [2009] study below).

6

7 **A.2.7. BENSON AND TETA (1993)**

8 In a companion mortality study (Benson and Teta, 1993), the remaining 278 employees

9 who were identified by Greenberg et al. (1990) as having worked at some time in the

10 chlorohydrin unit and who were not included in the cohort of Teta et al. (1993) were followed to

11 the end of 1988. Note that the prevalent part (i.e., those workers first employed before the cohort

12 enumeration date of 1 January 1940) of this reduced cohort is 112 of the 278 workers, or 40%,

13 and, therefore, the potential for bias from a healthy worker survivor effect, as discussed for the

14 Greenberg et al. (1990) study above (Section A.3.4), may be more pronounced in this study of

15 the chlorohydrin unit workers. It is unknown how many chlorohydrin unit workers employed

16 before 1940 were no longer employed when cohort enumeration began.

17 Altogether, 40 cancer deaths occurred versus 30.8 expected (SMR = 1.3) in the subcohort

18 of chlorohydrin workers. In Greenberg et al., significant elevated risks of pancreatic cancer and

19 leukemia and aleukemia occurred in only those workers assigned to the chlorohydrin process.

20 Benson and Teta noted a significantly increased risk of pancreatic cancer (SMR = 4.9, 8

21 observed deaths, $p < 0.05$) in the same group and a significantly increased risk of cancer in the

22 enlarged category of lymphohematopoietic cancer (SMR = 2.9, 8 observed deaths, $p < 0.05$),

23 which included leukemia and aleukemia, after an additional 10 years of follow-up.

24 The authors concluded that these cancers were likely work-related and some exposure in

25 the chlorohydrin unit, possibly to the chemical ethylene dichloride, was probably the cause.

26 They pointed out that Greenberg et al. found that the chlorohydrin unit was likely to be a low-

27 EtO exposure area in the West Virginia plants. The other possibility was bis-chloroethyl ether,

28 which the authors pointed out is rated by the International Agency for Research on Cancer

29 (IARC) as a group 3 ("not classifiable as to its carcinogenicity to humans") chemical.

30 Circumstantial evidence seems to support the authors' contention that ethylene dichloride is the

31 cause: IARC designated ethylene dichloride as a group 2B chemical ("possibly carcinogenic to

32 humans"), exposure was likely heavier throughout the history of the facility, and plant medical

33 records documented many accidental overexposures occurring to the pancreatic cancer victims

34 prior to diagnosis. However, this conclusion is disputed by Olsen et al. (1997). Their analysis is

35 discussed later.

36

A.2.8. STAYNER ET AL. (1993)

Stayner et al. (1993) provide an exposure-response analysis for the cohort study of EtO workers described by Steenland et al. (1991). Nothing was modified concerning the follow-up, cohort size, vital status, or cut-off date of the study. The exposure assessment and verification procedures were presented in Greife et al. (1988) and Hornung et al. (1994). Briefly, a regression model allows the estimation of exposure levels for time periods, facilities, and operations for which industrial hygiene data were unavailable. The data consisted of 2,700 individual time-weighted exposure values for workers' personal breathing zones, acquired from 18 facilities between 1976 and 1985. Arithmetic mean exposure levels by facility, year, and exposure category were calculated on the basis of grouping all sampled jobs into eight categories with similar potential for EtO exposure. The data were divided into two sets, one for developing the regression model and the second for testing it. Arithmetic means were logarithmically transformed and weighted linear regression models were fitted. Seven out of 23 independent variables tested for inclusion in the model were found to be significant predictors of EtO exposure and were included in the final model. This model predicted 85% of the variation in average EtO exposure levels.

Early historical exposures in jobs in the plants were estimated using this industrial hygiene-based regression model. In the Stayner et al. (1993) study, cumulative exposure for each worker was estimated by calculating the product of the average exposure in each job the worker held by the time spent in that job and then summing these over all the jobs held by that worker. This value became the cumulative exposure index for that employee and reflected the working lifetime total exposure to EtO. SMRs were generated based on standard life-table analysis. The three categories of cumulative exposure were less than 1,200 ppm-days, 1,200 to 8,500 ppm-days, and greater than 8,500 ppm-days. Additionally, the Cox proportional hazards model (SAS, 1986) was used to model the exposure-response relationship between EtO and various cancer types, using cumulative exposure as a continuous variable.

Stayner and colleagues noted a marginally significant increase in the risk of hematopoietic cancers, with an increase in cumulative exposure by both the life-table analysis as well as the Cox model, although the magnitude of the increased risk was not substantial. At the highest level—greater than 8,500 ppm-days of exposure—the SMR was a nonsignificant 1.24, based on 13 cases. However, 12 of these cases were in males, whereas only 6.12 were expected. Thus, in this highest-exposure category, a statistically significant ($p < 0.05$) SMR of 1.96 in males was produced. This dichotomy produced a deficit in females (1 observed vs. 4.5 expected, $p < 0.05$).

The Cox analysis produced a significantly positive trend with respect to lymphoid cell tumors (combination of lymphocytic leukemia and NHL) when EtO exposures were lagged

1   5 years. The authors stated that these data provide some support for the hypothesis that exposure

2   to EtO increases the risk of mortality from lymphatic and hematopoietic neoplasms. They

3   pointed out, however, that their data do not provide evidence for a positive association between

4   exposure to EtO and cancer of the stomach, brain, pancreas, or kidney or leukemia as a group.

5   Breast cancer was not analyzed in this report.

6   This cohort was not updated with vital status information on the "untraceables" (4.5%),

7   and cause of death information was not provided on deaths with unknown causes; thus, it lacks a

8   complete follow-up and, therefore, the risk estimates may be understated. Another potential

9   limiting factor is the information regarding industrial hygiene measurements of EtO that were

10   completed in the plants. According to the authors, the median length of exposure to EtO of the

11   cohort was 2.2 years and the median exposure was 3.2 ppm. It may be unreasonable to expect

12   any findings of increased significant risks because follow-up was too short to allow the

13   accumulation of mortality experience (average follow-up = 16 years; only 8% of cohort had

14   $\geq$ 20 years follow-up).

15   The authors also remind us that there is a lack of evidence for an exposure-response

16   relationship among females or for a sex-specific carcinogenic effect of EtO in either laboratory

17   animals or humans. In fact, the mortality rate from hematopoietic cancers among the women in

18   this cohort was lower than that of the general U.S. population. Therefore the contrast seen here

19   is unusual.

20   The positive findings are somewhat affected by the presence in the cohort of one heavily

21   exposed case (although the authors saw no reason to exclude it from the analysis), and there is a

22   lack of definite evidence for an effect on leukemia as a group. Despite these limitations, the

23   authors believe that their data provide support for the hypothesis that exposure to EtO increases

24   the risk of mortality from hematopoietic neoplasms.

25

26

27

28   **A.2.9. WONG AND TRENT (1993)**

29   This study is a reanalysis of the same cohort that was studied by Steenland et al. (1990)

30   and Stayner et al. (1993), with some differences. The cohort was incremented without

31   explanation by 474 to a total of 18,728 employees and followed one more year, to the end of

32   December 1988. This change in the cohort resulted in the addition of 176 observed deaths and

33   392.2 expected deaths. The finding of more than twice as many expected deaths as observed

34   deaths is baffling. A reduced total mortality of this magnitude suggests that many deaths may

35   have been overlooked. This resulted in a further reduction of the overall SMR to a significant

1 deficit of 0.73. Sixty additional cancer deaths were added versus 65.9 expected, for an SMR =
2 0.9, based on 403 total cancer deaths observed versus 446.2 expected.

3 The authors reported no significant increase in mortality at the cancer sites found to be of
4 most interest in previous studies, that is, stomach, leukemia, pancreas, brain and breast. They
5 also reported the lack of a dose-response relationship and correlation with duration of
6 employment or latency. They did report a statistically significant increased risk of NHL among
7 men (SMR = 2.47; observed = 16, expected = 6.47; $p < 0.05$) that was not dose-related and a
8 nonsignificant deficit of NHL among women (SMR = 0.32; observed = 2, expected = 6.27). The
9 authors concluded that the increase in men was not related to exposure to EtO but could in fact
10 have been related to the presence of acquired immune deficiency syndrome (AIDS) in the male
11 population. When this explanation was offered in a letter to the editor (Wong, 1991) regarding
12 the excess of NHL reported in Steenland et al. (1991), it was dismissed by Steenland and Stayner
13 (1993) as pure speculation. Steenland and Stayner responded that most of the NHL deaths
14 occurred prior to the AIDS epidemic, which began in the early 1980s. They also indicated that
15 there was no reason to suspect that these working populations would be at a higher risk for AIDS
16 than was the general population, the comparison group.

17 Wong and Trent also reported a slightly increased risk of cancer in other lymphatic tissue
18 (14 observed vs. 11.39 expected). In men, the risk was nonsignificantly higher (11 observed vs.
19 5.78 expected). Forty-three lymphopoietic cancers were observed versus 42 expected. In men,
20 the risk was higher (32 observed vs. 22.22 expected). Fourteen leukemia deaths were noted
21 versus 16.2 expected. The authors did not derive individual exposure estimates for exposure-
22 response analysis, such as in Stayner et al. (1993). Rather, they used duration of employment as
23 a surrogate for exposure.

24 This study has many of the same limitations as the Stayner et al. (1993) study. The
25 authors assumed that those individuals with an unknown vital status as of the cut-off date were
26 alive for the purposes of the analysis, and they were unable to obtain cause of death information
27 on 5% of the known deaths.

28 The differences between this cohort study and that of Stayner et al. (1993) are in the
29 methods of analysis. Stayner et al. used the 9th revision of the International Classification of
30 Diseases (ICD) to develop their site-specific cancer categories for comparison with expected
31 cancer mortality, whereas Wong and Trent used the 8th revision. This could account for some of
32 the differences in the observed numbers of site-specific cancers, because minor differences in the
33 coding of underlying cause of death could lead to a shifting of some unique causes from one site-
34 specific category to another. Furthermore, Wong and Trent did not analyze separately the
35 category "lymphoid" neoplasms, which includes lymphocytic leukemia and NHL, whereas
36 Stayner et al. (1993) did. Stayner et al. (1993) further developed cumulative exposure

1   information using exposure estimates, whereas Wong and Trent used length of employment as
2   their surrogate for exposure but did not code detailed employment histories.
3   Because Wong and Trent made no effort to quantify the exposures, as was the case in
4   Stayner et al. (1993), this study is less useful in determining a exposure-response relationship.
5   Furthermore, the assumption that a member of the cohort should be considered alive if a death
6   indication could not be found will potentially tend to bias risk ratios downward if, in fact, a large
7   portion of this group is deceased. In this study all untraceable persons were considered alive at
8   the end of the follow-up; therefore, the impact of the additional person-years of risk cannot be
9   gauged.
10
11   **A.2.10. BISANTI ET AL. (1993)**
12   These authors reported on a cohort mortality study of 1,971 male chemical workers
13   licensed to handle EtO by the Italian government, whom they followed retrospectively from
14   1940 to 1984. Altogether, 76 deaths had occurred in this group by the end of the study period,
15   whereas 98.8 were expected. Of those, 43 were due to cancer versus 33 expected. The cause of
16   one death remained unknown, and 16 workers were lost to follow-up. A group of 637
17   individuals from this cohort was licensed to handle only EtO; the remaining 1,334 had licenses
18   valid for handling other toxic gases as well. Date of licensing for handling EtO became the
19   initiating point of exposure to EtO, although it is likely that some of these workers had been
20   exposed previously to EtO. The regional population of Lombardia was used as the reference
21   group from which comparison death rates were obtained.
22   Although there were excess risks from almost all cancers, one of the greatest SMRs was
23   in the category known as "all hematopoietic cancers," where 6 observed deaths occurred when
24   only 2.4 were expected (SMR = 2.5). In the subgroup "lymphosarcoma, reticulosarcoma" there
25   were 4 observed deaths whereas only 0.6 were expected (SMR = 6.7, $p < 0.05$); the remaining 2
26   were leukemias. The authors note that five hematopoietic cancers occurred in the subgroup of
27   workers who were licensed to handle only EtO but no other chemicals versus only
28   0.7 hematopoietic cancers expected (SMR = 7.1, $p < 0.05$). These deaths occurred within 10
29   years from date of licensing (latent period), which is consistent with the shorter latent period
30   anticipated for this kind of cancer. According to the authors, all workers began their
31   employment in this industry when the levels of EtO were high, although no actual measurements
32   were available. The fact that this subgroup of workers was licensed only for handling EtO
33   reduces the likelihood of a confounding chemical influence.
34   The authors concluded that the excess risk of cancer of the lymphatic and hematopoietic
35   tissues in these particular EtO cohort members support the suggested hypothesis of a higher risk
36   of cancer found in earlier studies, but they added that the lack of exposure information on the

1   other industrial chemicals in the group that had a license for handling other toxic chemicals made

2   their findings inconclusive.

3   This study was of a healthy young cohort, and most person-years were contributed in the

4   latter years of observation. Many years of follow-up may be necessary in order to fully verify

5   any trend of excess risks for the site-specific cancers of interest and to measure latent effects.

6   Furthermore, the unusual deficit of total deaths versus expected contrasted with an excess of

7   cancer deaths versus expected raises a question about the potential for selection bias when the

8   members of this cohort were chosen for inclusion. Also, one of the study's major limitations is

9   the lack of exposure data.

10

11 **A.2.11. HAGMAR ET AL. (1991, 1995)**

12   Cancer incidence was studied in a cohort of 2,170 EtO-exposed workers from two plants

13 in Sweden that produced disposable medical equipment. To fit the definition for inclusion, the

14 subjects, 1,309 women and 861 men, had to have been employed for a minimum of 12 months

15 and some part of that employment had to have been during the period 1970–1985 in the case of

16 one plant and 1965–1985 in the case of the other. The risk ratios were not dichotomized by

17 gender. No records of anyone who left employment or died before January 1, 1972 in one plant

18 and January 1, 1975 in the other were included. Expected incidence rates were generated from

19 the Southern Swedish Regional Tumor Registries.

20   Because of a short follow-up period and the relative young age of the cohort, little

21 morbidity had occurred by the end of the cutoff date of December 31, 1990. Altogether, 40

22 cancers occurred, compared with 46.3 expected. After 10 years latency, 22 cases of cancers

23 were diagnosed versus 22.6 expected. However, 6 lymphohematopoietic tumors were observed

24 versus 3.37 expected, and when latency is considered, this figure falls to 3 versus 1.51 expected.

25 The authors pointed out that for leukemia the standard incidence ratio (SIR) is a nonsignificant

26 7.14, based on 2 cases in 930 subjects having at least 0.14 ppm-years of cumulative exposure to

27 EtO and a minimum of 10 years latency. The authors believed that the results provided some

28 minor evidence to support an association between exposure to EtO and an increased risk of

29 leukemia. However, for breast cancer, no increase in the risk was apparent for the total cohort

30 (SIR = 0.46, OBS = 5). Even in the 10 years or more latency period, the risk was less than

31 expected (SIR = 0.36, OBS = 2).

32   The authors made a reasonably good attempt to determine exposure levels during the

33 periods of employment in both plants for six job categories. Sterilizers in the years 1970–1972

34 were exposed to an average 40 ppm in both plants. These levels gradually dropped to 0.75 ppm

35 by 1985–1986. Packers and developmental engineers were the next highest exposed employees,

36 with levels in 1970–1972 of 20 to 35 ppm and by 1985–1986 of less than 0.2 ppm. During the

1 period 1964–1966 in the older plant, EtO levels averaged 75 ppm in sterilizers and 50 ppm in

2 packers. Peak exposures were estimated to have ranged from 500 to 1,000 ppm during the

3 unloading of autoclaves up to 1973. The levels gradually dropped to less than 0.2 ppm in both

4 plants by 1985–1986 in all job categories (developmental engineers, laboratory technicians,

5 repair men, store workers, controllers, foremen, and others) except sterilizers.

6 These exposure estimates were verified by measurement of hydroxy ethyl adducts to

7 N-terminal valine in hemoglobin in a sample of subjects from both plants. The adduct levels

8 reflect the average exposure during the few months prior to the measurement of EtO. The results

9 of this comparison were close except for sterilizers, whose air monitoring measurements were 2

10 to 3 times higher.

11 The authors pointed out two limitations in their study: a minority of subjects had a high

12 exposure to EtO, and the median follow-up (11.8 years) was insufficient to assess a biologically

13 relevant induction latency period. Although this study has good exposure information and the

14 authors used this information to develop an exposure index per employee, they did not evaluate

15 dose-response relationships that might have been present, nor did they follow the cohort long

16 enough to evaluate morbidity. The strength of this study is the development of the cumulative

17 exposure index as well as the absence of any potential confounding produced by the

18 chlorohydrin process, which was a problem in workers who produced and manufactured EtO in

19 other similar studies.

20

21 **A.2.12. NORMAN ET AL. (1995)**

22 These authors conducted a mortality/incidence study in a cohort of 1,132 workers, mainly

23 women (82%), who were exposed to EtO at some time during the period July 1, 1974, through

24 September 30, 1980. Follow-up was until December 31, 1987. Ethylene oxide was used at the

25 study plant to sterilize medical equipment and supplies that were assembled and packaged there.

26 This plant was selected for the study because in an earlier small study at this plant (Stolley et al.,

27 1984) there was an indication that in a sample of workers the average number of sister chromatid

28 exchanges was elevated over that of a control group selected from the nearby community.

29 Cancer morbidity was measured by comparing cancers occurring in this cohort with those

30 predicted from the National Cancer Institute's Surveillance, Epidemiology, and End Results

31 (SEER) Program for the period 1981–1985 and with average annual cancer incidence rates for

32 western New York for 1979–1984. Observed cancers were compared to expected cancers using

33 this method.

34 Only 28 cancer diagnoses were reported in the cohort; 12 were for breast cancers. Breast

35 cancer was the only cancer site in this study where the risk was significantly elevated, based on

36 the SEER rates (SIR = 2.55, $p < 0.05$). No significant excesses were seen at other cancer sites of

1   interest: leukemia (1 observed, 0.54 expected), brain (0 observed, 0.49 expected), pancreas

2   (2 observed, 0.51 expected) and stomach (0 observed, 0.42 expected). The authors offered no

3   explanation except chance as to why the risk of breast cancer was elevated in these workers.

4   In 1980, three 2-hour samples from the plant provided 8-hour TWA exposures to

5   sterilizer operators that ranged from 50 to 200 ppm. Corrective action reduced the levels to 5 to

6   20 ppm.

7   This study has little power to detect any significant risk of cancer at other sites because

8   morbidity was small, chiefly as a consequence of the short follow-up period. The mean number

9   of years from the beginning of follow-up to the end of the study was 11.4 years. In fact, the

10 authors stated that breast cancer was the only cancer site for which there was adequate power to

11 detect an increased relative risk. Additional weaknesses in this study include no historic

12 exposure information and too short a period of employment in some cases (<1month) to result in

13 breast cancer. The authors maintained that their study was inconclusive.

14

15 **A.2.13. SWAEN ET AL. (1996)**

16   A significant cluster of 10 Hodgkin lymphoma cases in the active white male workforce

17 of an unidentified large chemical manufacturing plant in Belgium led to a nested case control

18 study by Swaen et al. (1996) to determine which, if any, chemical agents within the plant may

19 have led to the increase. By comparison with regional cancer incidence rates, the SIR for this

20 disease was 4.97 (95% CI = 2.38–9.15) over a 23-year period, from 1966 to 1992. This

21 suggested that an occupational exposure may have produced the significant excess risk of

22 Hodgkin lymphoma seen in these workers.

23   The investigators randomly selected 200 individuals from a computerized sampling frame

24 of all men ever employed at the facility. From this list of 200, workers who were actively

25 employed at the time of diagnosis of each case were chosen as controls. No age matching was

26 done because the authors stated that age-specific incidence rates for Hodgkin lymphoma in the

27 United States were relatively flat for men between ages 18 and 65. The investigators felt that a

28 control could serve for more than one case.

29   Verification of the 10 cases revealed that 1 case was, in reality, a large-cell anaplastic

30 lymphoma. Two others could not be confirmed as Hodgkin lymphoma due to the lack of tissue.

31 The remaining 7 were confirmed as Hodgkin lymphoma. In the ensuing case-control analysis,

32 significant odds ratios (ORs) for Hodgkin lymphoma were observed for five chemicals, ammonia

33 (6 cases, OR = 5.6), benzene (5 cases, OR = 11), EtO (3 cases, OR = 8.5), NaOH (5 cases, OR =

34 8) and oleum (3 cases, OR = 6.9), based on the number of cases and controls known to be

35 exposed to the chemicals in question. This does not mean they were exposed only to the

36 chemical in question.

1   The availability of exposure information made it possible to calculate cumulative
2   exposure to the cases and controls of two chemicals, benzene and EtO. The cumulative exposure
3   for benzene-exposed cases was 397.4 ppm-months versus an expected 99.7 ppm-months for the
4   matched controls. The authors stated that one heavily exposed case was chiefly responsible for
5   the high cumulative total for all the benzene-exposed cases; however, it was not statistically
6   significant. Only a few studies have suggested that exposure to benzene could possibly be
7   related to an increase in the risk of Hodgkin lymphoma. The cumulative total exposure to EtO
8   for the cases was 500.2 ppm-months versus 60.2 for the matched controls, which was statistically
9   significant, the significance being due to one extreme case.
10   This study is limited because the authors enumerated only cases among active employees
11   of the workforce; therefore, the distinct possibility exists that they could have missed potential
12   cases in the inactive workers. It is possible that latent Hodgkin lymphoma cases could have been
13   identified in the controls after the controls left active employment. However, given that there
14   were many different possible exposures to the chemicals produced in the workplaces of these
15   employees, it is not likely that EtO or benzene could be considered solely responsible for the
16   excess risk of Hodgkin lymphoma in this working group.
17
18   **A.2.14. OLSEN ET AL. (1997)**
19   Olsen et al. (1997) studied 1,361 male employees of four plants in Texas, Michigan, and
20   Louisiana who were employed a minimum of 1 month sometime during the period 1940 through
21   1992 in the ethylene chlorohydrin and propylene chlorohydrin process areas. These areas were
22   located within the EtO and propylene oxide production plants. Some 300 deaths had occurred by
23   December 31, 1992.
24   Plant A in Texas produced EtO beginning in 1941 and ceased production in 1967.
25   Bis-chloroethyl ether, a byproduct of EtO continued to be produced at this plant until 1973. The
26   plant was demolished in 1974. Plant B, which was nearby, manufactured EtO from 1951 to 1971
27   and then again from 1975 until 1980. This plant continues to produce propylene oxide. The
28   Louisiana plant produced EtO and propylene oxide through the propylene chlorohydrin process
29   from 1959 until 1970, when it was converted to propylene oxide production. The Michigan plant
30   produced ethylene chlorohydrin and subsequently EtO beginning in 1936 and continuing into the
31   1950s. This plant produced propylene chlorohydrin and propylene oxide up to 1974.
32   The authors suggested that exposure to EtO was possible at the plants studied in this
33   report but that exposure was unlikely in the 278 chlorohydrin unit workers who were excluded
34   from the cohort studied by Teta et al. (1993). Unfortunately, no actual airborne measurements
35   were reported by Olsen et al., and thus only length of employment could be used as a surrogate
36   for exposure.

1   The SMR for all causes was 0.89 (300 observed). For total cancer the SMR was 0.94

2   (75 observed, 79.7 expected). There were 10 lymphohematopoietic cancers versus 7.7 expected

3   (SMR = 1.3). No significantly increased risks of any examined site-specific cancer (pancreatic,

4   lymphopoietic, hematopoietic, and leukemia) were noted even after a 25-year induction latency

5   period, although the SMR increased to 1.44 for lymphopoietic and hematopoietic cancer. When

6   only the ethylene chlorohydrin process was examined after 25 years latency, the SMR increased

7   to 1.94, based on six observed deaths. The data to support the latter observation by the authors

8   were not presented in tabular form.

9   The authors concluded that there was a weak, nonsignificant, positive association with

10   duration of employment for lymphopoietic and hematopoietic cancer with Poisson regression

11   modeling. They stated that the results of their study provide some assurance that their cohort has

12   not experienced a significant increased risk for pancreatic cancer and lymphopoietic and

13   hematopoietic cancer in ethylene chlorohydrin and propylene chlorohydrin workers. They

14   believed that this study contradicted the conclusions of Benson and Teta (1993) that ethylene

15   dichloride, perhaps in combination with chlorinated hydrocarbons, appeared to be the causal

16   agent in the increased risk of pancreatic cancer and hematopoietic cancer seen in their study.

17   They pointed out that ethylene dichloride is readily metabolized and rapidly eliminated from the

18   body after gavage or inhalation administration; therefore, they questioned whether experimental

19   gavage studies (NCI, 1978) are appropriate for studying the effects of ethylene dichloride in

20   humans. One study (Maltoni et al., 1980) found no evidence of tumor production in rats and

21   mice chronically exposed to ethylene dichloride vapor concentrations up to 150 ppm for 7 hours

22   a day. Also, because this chemical is a precursor in the production of vinyl chloride monomer,

23   the authors wondered why an increase in these two site-specific cancers had not shown up in

24   studies of vinyl chloride workers. However, they believe that an additional 5 to 10 years of

25   follow-up of this cohort would be necessary to confirm the lack of risk for the two types of

26   cancer described above.

27   Another major weakness of this study is the lack of any actual airborne measurements of

28   EtO and the chlorohydrin chemicals.

29

30   **A.2.15. STEENLAND ET AL. (2004)**

31   In an update of the earlier mortality studies of the same cohort of workers exposed to EtO

32   described by Steenland et al. (1991) and Stayner et al. (1993), an additional 11 years of follow-

33   up were added. This increased the number of deceased to 2,852. Work history data were

34   originally gathered in the mid-1980s. Approximately 25% of the cohort continued working into

35   the 1990s. Work histories on these individuals were extended to the last date employed. It was

36   assumed that these employees continued in the job they last held in the 1980s. Little difference

1   was noted when cumulative exposure was calculated with and without the extended work

2   histories, chiefly because the exposure levels after the mid-1980s were very low. Again overall,

3   no excess risk of hematopoietic cancer was noted based on external rates. However, as in the

4   earlier paper, exposure-response analyses reported positive trends for hematopoietic cancers

5   limited to males ($p = 0.02$ for the log of cumulative exposure with a 15-year lag) using internal

6   comparisons and Cox regression analysis.[21] (See Table A-2 for the categorical exposure results.)

7   The excess of these tumors was chiefly lymphoid (NHL, myeloma, lymphocytic

8   leukemia) (see Table A-3), as in the earlier paper. A positive trend was also observed for

9   Hodgkin lymphoma in males, although this was based on small numbers.

10

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[21] Valdez-Flores et al. (2009) suggest that Steenland et al. (2004) incorrectly used one degree of freedom in their evaluation of statistical significance and that a second degree of freedom should have been included for estimating the lag. However, Steenland et al. (2004) did not estimate the lag using the likelihood; rather, lagged exposure was treated as an alternate exposure metric.

Table A-2. Cox regression results for hematopoietic cancer mortality (15-year lag) in males

Cumulative exposure (ppm-days)	Odds ratio (95% CI)
0	1
>0–1,199	1.23 (0.32–4.73)
1,200–3,679	2.52 (0.69–9.22)
3,680–13,499	3.13 (0.95–10.37)
13,500+	3.42 (1.09–10.73)

Source: Steenland et al. (2004)

Table A-3. Cox regression results for lymphoid cell line tumors (15-year lag) in males

Cumulative exposure (ppm-days)	Odds ratio (95% CI)
0	1
>0–1,199	0.9 (0.16–5.24)
1,200–3,679	2.89 (0.65–12.86)
3,680–13,499	2.74 (0.65–11.55)
13,500+	3.76 (1.03–13.64)

Source: Steenland et al. (2004)

The hematopoietic cancer trends were somewhat weaker in this analysis than were those reported in the earlier studies of the same cohort. This is not unexpected because most of the cohort was not exposed after the mid-1980s, and the workers who were exposed in more recent years were exposed to much lower levels because EtO levels decreased substantially in the early 1980s. No association was found in females, although average exposures were only twice as high in males (37.8 ppm-years) as in females (18.2 ppm-years), and there was enough variability in female exposure estimates to expect to be able to see a similar trend if it existed. In later analyses conducted by Dr. Steenland and presented in Appendix D, the difference between the

1   male and female results was found not to be statisitically significant, and the same pattern of

2   lymphohematopoietic cancer results observed for males by Steenland et al. (2004) was observed

3   for the males and females combined (i.e., statistically significant positive trends for both

4   hematopoietic and lymphoid cancers using log cumulative exposure and a 15-year lag).

5   This study also reports a significant excess risk of breast cancer in the highest

6   cumulative-exposure quartile, with a 20-year lag (SMR = 2.07, 95% CI 1.1–3.54, n = 13) in

7   female employees. The results using internal Cox regression analyses with a 20-year lag time

8   produced an OR = 3.13 (95% CI 1.42–6.92) in the highest cumulative-exposure quartile. The

9   log of cumulative exposure with a 20-year lag was found to be the best model (p = 0.01) for the

10   analyses of breast cancer. As for hematopoietic cancer in males, cumulative exposure

11   untransformed showed a weaker trend (p = 0.16). A breast cancer incidence study of this cohort

12   is discussed in Steenland et al. (2003).

13

14   **A.2.16. STEENLAND ET AL. (2003)**

15   In a companion study on breast cancer incidence in women employees of the same cohort

16   discussed in Steenland et al. (2004), the authors elaborated on the breast cancer findings in a

17   subgroup of 7,576 women from the cohort (76% of the original cohort). They had to be

18   employed at least 1 year and exposed while employed in commercial sterilization facilities. The

19   average length of exposure was 10.7 years. Breast cancer incidence analyses were based on

20   319 cases identified via interview, death certificates, and cancer registries in the full cohort,

21   including 20 in situ carcinomas. Interviews on 5,139 women (68% of the study cohort) were

22   obtained; 22% could not be located. Using external referent rates (SEER), the SIR was 0.87 for

23   the entire cohort based on a 15-year lag time. When in situ cases were excluded, the overall SIR

24   increased to 0.94. In the top quintile of cumulative exposure, with a 15-year lag time, the SIR

25   was 1.27 (95% CI 0.94–1.69, n = 48). A significant positive linear trend of increasing risk with

26   increasing cumulative exposure was noted (p = 0.002) with a 15-year lag time. Breast cancer

27   incidence was believed to be underascertained owing to incomplete response and a lack of

28   coverage by regional cancer registries (68% were contacted directly and 50% worked in areas

29   with cancer registries). An internal nested case-control analysis, which is less subject to

30   concerns about underascertainment, produced a significant positive exposure-response with the

31   log of cumulative exposure and a 15-year lag time (p = 0.05). The top quintile was significant

32   with an OR of 1.74 (CI 1.16–2.65) based on all 319 cases (the entire cohort).

33   The authors also conducted separate analyses using the subcohort with interviews, for

34   which there was complete case ascertainment and additional information on potential

35   confounders. In the subcohort with interview data, the odds ratio for the top quintile equaled

36   1.87 (CI 1.12–3.1), based on 233 cases in the 5,139 women and controlled for with respect to

1 parity and breast cancer in a first-degree relative. Information on other risk factors was also

2 collected—e.g., body mass index, SES, diet, age at menopause, age at menarche, breast cancer in

3 a first-degree relative, and parity—but only parity and breast cancer in a first-degree relative

4 were significant in the model. Continuous cumulative exposure, as well as the log cumulative

5 exposure, lagged 15 years, produced p values for the regression coefficient of 0.02 and 0.03,

6 respectively, for the Cox regression model, taking into account age, race, year of birth, parity,

7 and breast cancer in a first-degree relative.

8 The authors concluded that their data suggest that exposure to EtO is associated with

9 breast cancer, but because of inconsistencies in exposure-response trends and possible biases due

10 to nonresponse and incomplete cancer ascertainment, the case for breast cancer is not conclusive.

11 However, monotonically increasing trends in categorical exposure-response relationships are not

12 always the norm owing to lack of precision in the estimates of exposure. Furthermore, positive

13 trends were observed in both the full cohort and the subcohort with interviews, lessening

14 concerns about nonresponse bias and case underascertainment.

15

16 **A.2.17. KARDOS ET AL. (2003)**

17 These authors reported on a study completed earlier by Muller and Bertok (1995) of

18 cancer among 299 female workers who were employed from 1976 to 1993 in a pediatric ward at

19 the county hospital in Eger, Hungary, where gas sterilizers were used. Their observation period

20 for cancer was begun in 1987 on the assumption that cancer deaths before 1987 were not due to

21 EtO, based on a paper by Lucas and Teta (1996). Information about the Muller and Bertok

22 (1995) study is unavailable because the paper is in Hungarian and no translated copy is available.

23 Kardos and his colleagues evaluated mortality among these women and found a statistically

24 significant excess of total cancer deaths in the period from 1987 to 1999 when compared with

25 expected deaths generated from three different comparison populations (Hungary, Heves County,

26 and city of Eger). Altogether, 11 deaths were observed compared with, respectively, 4.38, 4.03,

27 and 4.28 expected deaths. The SMRs are all significant at the $p < 0.01$ level. Site-specific rates

28 were not calculated. Among the 11 deaths were 3 breast cancer deaths and 1 lymphoid leukemia

29 death. The authors claim that their results confirm "predictions of an increased cancer risk for

30 the Eger hospital staff." They suggest an etiological role for EtO in the excess risk.

31

32 **A.2.18. TOMPA ET AL. (1999)**

33 The authors reported a cluster of 8 breast cancer cases and 8 other malignant tumor cases

34 that developed over a period of 12 years in 98 nurses who worked in a hospital in the city of

35 Eger, Hungary, and were exposed to EtO. These nurses were exposed for 5 to 15 years in a unit

36 using gas sterilizer equipment. The authors report that EtO concentrations were in the

1   neighborhood of 5 to 150 mg/m^3. The authors state that the high breast cancer incidence in the

2   hospital in Eger indicates a combined effect of exposure to EtO and naturally occurring

3   radioactive tap water, possibly due to the presence of radon. This case report study is discussed

4   further in the genotoxicity section.

5

6   **A.2.19. COGGON ET AL. (2004)**

7   Descriptive information about this cohort is available from the earlier study (Gardner et

8   al., 1989). This current update of the 1,864 men and 1,012 women described in the Gardner et

9   al. study were followed to December 31, 2000. This added 13 more years of follow-up resulting

10 in 565 observed deaths versus 607.6 expected. For total cancer, the observed number of deaths

11 equaled 188 versus 184.2 expected. For NHL, 7 deaths were observed versus 4.8 expected. For

12 leukemia, 5 deaths were observed versus 4.6 expected. All 5 leukemia deaths fell into the subset

13 with definite or continual exposure to EtO, where only 2.6 were expected. In fact, the total

14 number of deaths classified to the lymphohematopoietic cancer category was 17 with 12.9

15 expected. This increased risk was not significant. When definite exposure was established, the

16 authors found that the risk of lymphatic and hematopoietic cancer was increased with 9 observed

17 deaths versus 4.9 expected. Deaths from leukemia were also increased in chemical workers with

18 4 leukemia deaths versus 1.7 expected. No increase was seen in the risk of hematopoietic cancer

19 in the hospital sterilizing unit workers, who are mostly female. Another finding of little

20 significance was that of cancer of the breast. Only 11 deaths were recorded in this cohort up to

21 the cutoff date versus 13.1 expected. Since there were no female workers in the chemical

22 industry, the results on breast cancer reflect only work in hospital sterilizing units. The

23 researchers concluded that the risk of cancer must be low at the levels sustained by workers in

24 Great Britain over the last 10 or 20 years.

25

26 **A.2.20. SWAEN ET AL. (2009)**

27   Swaen et al. (2009) redefined and updated the cohort of 1,896 male UCC workers studied

28 by Teta et al. (1993), which was itself a follow-up of the 2,174 UCC workers originally studied

29 by Greenberg et al. (1990), excluding the 278 chlorohydrin unit workers because of potential

30 confounding. (However, confounding by chlorohydrin production has not been established, and

31 49 of those excluded workers were also employed in EtO production and thus had high potential

32 EtO exposures.) Specifically, Swaen et al. extended the cohort enumeration period from the end

33 of 1978 to the end of 1988 (workers hired after 1988 were not added to the cohort because they

34 were considered to have no appreciable EtO exposure), identifying 167 additional workers, and

35 conducted mortality follow-up of the resulting cohort of 2063 male workers through 2003. Work

36 histories were also extended through 1988; exposures after 1988 were considered negligible

1 compared to earlier exposure levels. Swaen et al. (2009) used an exposure assessment reportedly

2 based on the qualitative categorizations of potential EtO exposure in the different departments

3 developed by Greenberg et al. (1990) and time-period exposure estimates from Teta et al. (1993).

4 The exposure assessment matrix for the exposure estimates of Swaen et al. (2009) is presented in

5 Table A-5 below. Cumulative exposures for the individual workers were estimated by

6 multiplying the time (in months) a worker was assigned to a department by the estimated

7 exposure level for the department and summing across the assignments.

8

9   **Table A-5. Exposure assessment matrix from Swaen et al. (2009) – 8-hour TWA**
10   **exposures in ppm**

11

Time period	Exposure potential category		
	Low (most EtO user departments)	**Medium** (some EtO user departments)	**High** (EtO production departments)
1925–1939	17	28	70
1940–1956	7	14	21
1957–1973	5	7.5	10
1974–1988	0.3	0.65	1

12

13 **Source:** Swaen et al. (2009).

14
15

16   The exposure assessment used in this study was relatively crude, based on just a small

17 number of department-specific and time-period-specific categories, and with exposure estimates

18 for only a few of the categories derived from actual measurements. For the 1974-1988 time

19 period, based on measurements from environmental monitoring conducted in the (West Virginia)

20 plants since 1976, exposure estimates of 1 ppm and 0.3 ppm were chosen for the high and low

21 potential exposure departments, respectively, and the average of 0.65 ppm was taken for the

22 medium exposure departments. For the 1957-1973 time period, exposure estimates were based

23 on measurements from an air-sampling survey of 3 EtO direct-oxidation production units in a

24 UCC plant in Texas in the early 1960s (during this 1957-1973 time period, direct oxidation was

25 the only method used for EtO production at the West Virginia plants as well). The majority of

26 the 8-hour TWA results in these units were between 3 and 20 ppm, with levels between 5 and 10

27 ppm for operators. Because the West Virginia plants and equipment were much older than for

28 the Texas facility, the high end of the range of values for operators (10 ppm) was selected as the

29 exposure estimate for the high potential exposure departments, and the low end of the range (5

1 ppm) was selected for the low exposure departments (even though these were not EtO production

2 departments). The average of 7.5 ppm was taken for the medium exposure departments.

3   For the 1940-1956 time period, exposure estimates were derived from "rough" estimates

4 of exposure reported by Hogstedt et al. (1986) for a chlorohydrin-based EtO production unit in

5 an enclosed building, as was the West Virginia chlorohydrin-based EtO production. Hogstedt et

6 al. reportedly suggested EtO exposures were probably below 14 ppm from 1941 to 1947,

7 although much higher levels occasionally occurred, and levels from the 1950s to 1963 averaged

8 5 to 25 ppm. Thus, based on these values, 14 ppm was selected as the exposure estimate for the

9 medium potential exposure departments and values 50% higher (21 ppm) and 50% lower (7

10 ppm) were assigned to the high and low exposure departments, respectively. For the 1925-1939

11 time period, it was assumed that exposures in this earlier, start-up period would have been higher

12 than those in the subsequent 1940-1956 time period, so the 14 ppm estimate from the medium

13 exposure departments in the 1940-1956 time period was used as the exposure estimate for the

14 low exposure potential departments for the 1925-1939 time period. Then, the same ratio of 1:2

15 between the low and medium exposure departments from the 1940-1956 time period was used to

16 obtain an estimate of 28 ppm for the medium exposure potential departments for the 1925-1939

17 time period. A factor of 5 (half an order of magnitude) was used between the low and high

18 exposure departments to obtain a highly uncertain exposure estimate of 70 ppm for the high

19 exposure departments. Swaen et al. (2009) suggest that despite the high exposure estimates for

20 the 1925-1939 time period, the contribution of this time period to cumulative exposure estimates

21 is limited because only 98 workers (4.8% of the cohort) had employment histories before 1940.

22 It appears, then, that pre-1940 employment histories may have been missing for 13 of the

23 workers, because excluding the 112 pre-1940 chlorohydrin production workers (Benson and

24 Teta, 1993) from the original 223 pre-1940 workers (Greenberg et al., 1990) leaves 111 pre-1940

25 workers in the cohort.

26   At the end of the 2003 follow-up, 1,048 of the 2,063 workers had died and 23 were lost to

27 follow-up. In comparison with general population U.S. mortality rates, the all-cause mortality

28 SMR was 0.85 (95% CI = 0.80, 0.90) and the cancer SMR was 0.95 (95% CI = 0.84, 1.06).

29 None of the SMRs for specific cancer types showed any statistically significant increases. In

30 analyses stratified by hire date (pre- [inclusive] or post-1956), the SMR for leukemia was

31 elevated but not statistically significant (1.51; 95% CI 0.69, 2.87) in the early-hire group, based

32 on 9 deaths. In analyses stratified by duration of employment, no trends were apparent for any

33 of the lymphohematopoietic cancers, although in the 9+ years of employment subgroup, the

34 SMR for NHL was nonsignificantly increased (1.49; 95% CI 0.48, 3.48), based on 5 deaths. In

35 SMR analyses stratified by cumulative exposure, no trends were apparent for any of the

36 lymphohematopoietic cancers and there were no notable elevations for the highest cumulative

1 exposure category. Note that only 27 lymphohematopoietic cancer deaths (including 12

2 leukemias and 11 NHLs) were observed in the cohort.

3   Internal Cox proportional hazards modeling was also done for some disease categories

4 (all-cause mortality, leukemia mortality, and lymphoid cancer [NHL, lymphocytic leukemia, and

5 myeloma] mortality [17 deaths]), using cumulative exposure as the exposure metric. Year of

6 birth and year of hire were included as covariates in the Cox regression model. Year of hire was

7 reportedly included to adjust for potential cohort effects; however, it is unclear whether or not

8 this covariate was a statistically significant factor in the regression. Furthermore, because age at

9 hire is often correlated with exposure, including it in the regression model could overadjust and

10 attenuate the observed exposure-related effects. These internal analyses showed no evidence of

11 an exposure-response relationship, although, again, these analyses rely on small numbers of

12 cases and a crude exposure assessment, where there is a high potential for exposure

13 misclassification.

14   Swaen et al. (2009) note that one of the strengths of their study is the long average

15 follow-up time of the workers. These authors further note that, because the UCC cohort is a

16 much older population (50% deceased) than the NIOSH cohort (Steenland et al., 2004), the

17 number of expected deaths is less than 3 times larger for the NIOSH cohort even though the

18 sample size is almost 9 times larger. However, the long follow-up and aged cohort might be a

19 limitation, as well. Because the follow-up is extended well beyond the time period of non-

20 negligible exposures (pre-1989) for workers still employed and, especially, beyond the highest

21 exposures (e.g., pre-1940 or pre-1956), the follow-up is likely observing workers at the high tail

22 end of the distribution of latency times for EtO-associated lymphohematopoietic cancers. In

23 other words, workers that were at risk of developing lymphohematopoietic cancer as a result of

24 their EtO exposures would likely have developed the disease earlier. Meanwhile, having an

25 older cohort means that the background rates of lymphohematopoietic cancers are higher and,

26 thus, relative risks may be attenuated. Such attenuation was observed even in the younger

27 NIOSH cohort between the 1987 follow-up (Steenland et al., 1991) and the 1998 follow-up

28 (Steenland et al., 2004), when the follow-up was extended well beyond the period of significant

29 EtO exposures (exposure levels were considered very low by the mid-1980s).

30   Swaen et al. (2009) also note that their estimate of the average cumulative exposure for

31 the UCC cohort was more than twice the average cumulative exposure estimate for the NIOSH

32 cohort. However, there are substantial uncertainties in the exposure assessment, especially for

33 the early years when the highest exposures occurred. And despite the reported strengths of the

34 Swaen et al. (2009) study in terms of follow-up, cohort age, and high exposures, a limitation of

35 the study is the small cohort size. Based on data presented by Greenberg et al. (1990) and

36 Benson and Teta (1993), it appears that fewer than 900 workers were hired before 1956 (1104 of

1   the original cohort were hired before 1960 and 233 of those were then excluded because they

2   worked in the chlorohydrin unit) and would have been potentially exposed to the higher pre-1956

3   exposures levels. In the full cohort of 2063 men, only 27 lymphohematopoietic (17 lymphoid)

4   cancers were observed.

5   In alternate analyses of the UCC data, Valdez-Flores et al. (2010) fitted Cox proportional

6   hazards models and conducted categorical exposure-response analyses using a larger set of

7   cancer endpoints. These investigators also performed the same analyses using the data from the

8   last follow-up of the NIOSH cohort (Steenland et al., 2004) and from the two cohorts combined,

9   analyzing the sexes both separately and together. Valdez-Flores et al. (2010) reported that they

10 found no evidence of exposure-response relationships for cumulative exposure with either the

11 Cox model or categorical analyses for all of the cohort/endpoint datasets examined (endpoints

12 included all lymphohematopoietic cancers, lymphoid cancers, and female breast cancer, the latter

13 in the NIOSH cohort only). These investigators suggest that a review of the data from the

14 NIOSH and UCC studies supports combining them, but it should be recognized that the exposure

15 assessment conducted for the UCC cohort is much cruder, especially for the highest exposures,

16 (see above) than the NIOSH exposure assessment (which was based on a validated regression

17 model; see A.3.8 above); thus, the results of exposure-response analyses of the combined cohort

18 data are considered to have greater uncertainty than those from analyses of the NIOSH cohort

19 alone, despite the additional cases contributed by the UCC cohort (e.g., the UCC cohort

20 contributes 17 cases of lymphoid cancer to the 53 from the NIOSH cohort; however, as discussed

21 above, it should also be noted that some of these UCC cases are occurring in older workers, with

22 longer post-exposure follow-up, and, thus, may reflect background disease more than exposure-

23 related disease).

24   Notable differences between the Steenland et al. (2004) and the Valdez-Flores et al.

25 (2010) analyses exist. A major difference is that Valdez-Flores et al. (2010) used only

26 cumulative exposure in the Cox regression model, so they considered only a sublinear exposure-

27 response relationship, whereas Steenland et al. (2004) also used log cumulative exposure, which

28 provides a supralinear exposure-response relationship model structure (see, e.g., Figure 4-1,

29 illustrating the difference between the cumulative exposure and log cumulative exposure Cox

30 regression models ($RR = e^{\beta \times exposure}$) for the lymphoid cancers from Steenland et al. [2004]).

31 Valdez-Flores et al. (2010) objected to the log cumulative exposure model for a number of

32 reasons, the primary one being that the use of log cumulative exposure forces the exposure-

33 response relationship to be supralinear regardless of the observed data. This is correct but no

34 different from the use of cumulative exposure imposing a *sublinear* exposure-response

35 relationship. And Steenland et al. (2004) used log cumulative exposure specifically when the

36 cumulative exposure Cox regression model didn't yield statistically significant results and the

1 categorical analyses suggested increases in risk that were more consistent with an underlying

2 supralinear exposure-response relationship. With log cumulative exposure, Steenland et al.

3 (2004) observed statistically significant fits to the exposure-response data for all

4 lymphohematopoietic cancers in males, lymphoid cancers in males, and breast cancer in females,

5 none of which yielded statistically significant fits with the cumulative exposure (sublinear

6 exposure-response) model, supporting the apparent supralinearity of the data.[22]

7 Another key difference between the Steenland et al. (2004) and the Valdez-Flores et al.

8 (2010) analyses is that Valdez-Flores et al. (2010) present results only for unlagged analyses.

9 Valdez-Flores et al. (2010) state that their Cox regression results with different lag times were

10 similar to the unlagged results. Because the Valdez-Flores et al. (2010) categorical results are

11 for unlagged analyses, however, their referent groups are different from those used by Steenland

12 et al. (2004). Valdez-Flores et al. (2010) used the lowest exposure quintile (providing there were

13 sufficient data) as the referent group, whereas Steenland et al. (2004) used the no-exposure

14 (lagged-out) group as the referent. Because the NIOSH cohort data have an underlying

15 supralinear exposure-response relationship, the increased risk in the lowest exposure group is

16 already notably elevated and using the lowest exposure quintile as a referent group would

17 attenuate the relative risk. Nonetheless, Valdez-Flores et al. (2010) observed statistically

18 significant increases in response rates in the highest exposure quintile relative to the lowest

19 exposure quintile for lymphohematopoietic and lymphoid cancers in males in the NIOSH cohort,

20 consistent with the categorical results of Steenland et al. (2004), as well as a statistically

21 significant increase in the highest exposure quintile for lymphoid cancers in males and females

22 combined in the NIOSH cohort, consistent with the results in Appendix D.[23]

23 Although Valdez-Flores et al. (2010) found no statistically significant exposure-response

24 relationships for any of the cohort/endpoint datasets that they analyzed using the cumulative

25 exposure Cox regression model, these investigators derived risk estimates from the positive

26 relationships for the purposes of comparing those estimates with EPA's 2006 draft risk estimates

27 (U.S. EPA, 2006b). Valdez-Flores et al. (2010) report that their estimate of the exposure level

28 associated with 10^{-6} risk of lymphohematopoietic cancer based on the male NIOSH cohort data is

29 1500 times larger than EPA's 2006 draft estimate (their exposure level estimate based on the

30 NIOSH and UCC male and female data combined was a further 3 times higher). Most of the

31 difference in magnitude between the Valdez-Flores et al. (2010) and the EPA 2006 draft

32 estimates is attributable to the difference in the models used. The Valdez-Flores et al. (2010)

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[22] This pattern of findings from the NIOSH cohort data for males (i.e., statistically significant fits with log cumulative exposure but not with cumulative exposure) was replicated for both the all lymphohematopoietic cancers and the lymphoid cancers when the NIOSH data on males and females were combined (see Appendix D).

[23] In Dr. Steenland's analyses of the NIOSH cohort data for both sexes combined, presented in Appendix D, the categorical results for all lymphohematopoietic cancers were also statistically significantly increased.

1   estimate is based on the sublinear Cox regression model, which EPA rejected as not providing a
2   good representation of the low-exposure data (EPA's 2006 draft risk estimate is based on a linear
3   model). In addition, Valdez-Flores et al. (2010) used maximum likelihood estimates, while EPA
4   uses upper bounds on risk (or lower bounds on exposure). Valdez-Flores et al. (2010) also
5   modeled down to 10^{-6} risk, whereas EPA modeled to 10^{-2} risk and used the LEC_{01} as a point of
6   departure (POD) for linear low-dose extrapolation. Valdez-Flores et al. (2010) suggest that
7   PODs should be within the range of observed exposures, and they chose a 10^{-6} risk level because
8   the corresponding exposure level was in the range of the observed occupational exposures
9   (converted to equivalent environmental exposures). The intention of EPA's 2005 *Guidelines for*
10 *Carcinogen Risk Assessment* (US EPA, 2005a), however, is for the POD to be at the low end of
11 the observable range *of responses*, i.e., a response level that might reasonably be observed to
12 have statistical significance with respect to background responses. The underlying assumption in
13 this approach is that one can have relative confidence in an exposure-response model in the
14 observable range, but there is less confidence in any empirical exposure-response model for
15 much lower exposures. The estimates also differ because Valdez-Flores et al. (2010) truncated
16 their life-table analysis at 70 years, while EPA uses a cut-off of 85 years.
17   A further reason for differences between the risk estimates of Valdez-Flores et al. (2010)
18 and EPA's 2006 draft result is that Valdez-Flores et al. (2010) estimated mortality risks, while
19 EPA estimates incidence risks. In a separate publication, Sielken and Valdez-Flores (2009a)
20 disagree with the assumption of similar exposure-response relationships for
21 lymphohematopoietic cancer incidence and mortality used by EPA in deriving incidence
22 estimates and assert that the methods used by EPA in calculating these estimates were
23 inappropriate. Sielken and Valdez-Flores (2009a) suggest that, except at high exposure levels,
24 the exposure-response data on all lymphohematopoietic cancers in males in the NIOSH cohort
25 are consistent with decreases in survival time as an explanation for the apparent increases in
26 mortality. For two of the four exposure groups, however, the best-fitting survival times were 0
27 years, which seems improbable. Moreover, Sielken and Valdez-Flores (2009a) have not
28 established that the excess mortality is due to decreased survival time; the data are also
29 consistent with increased mortality resulting from increased incidence. Furthermore, the rodent
30 bioassays show that EtO is a complete carcinogen (Section 3.2), and the mechanistic data
31 demonstrate that EtO is mutagenic (Section 3.3.3), with sufficient evidence for a mutagenic
32 mode of action (Section 3.4). Thus, EtO can be expected to act as an initiator in carcinogenesis,
33 and, consequently, be capable of inducing exposure-related increases in incidence. As for the
34 methods used by EPA in calculating the incidence estimates, EPA used adjustments to the life-
35 table analysis where warranted (U.S. EPA, 2006). EPA did not adjust the all-cause mortality
36 rates in the lymphohematopoietic cancer analyses, because "the lymphohematopoietic cancer

1   incidence rates are small when compared with the all-cause mortality rates" (U.S. EPA, 2006,
2   Section 4.1.1.3) and, thus, the impact of taking into account lymphohematopoietic cancer
3   incidence when calculating interval "survival" is negligible, as confirmed by Sielken and Valdez-
4   Flores' own calculations, presented in their Table 2 where the "multiplier" = 1 (Sielken and
5   Valdez-Flores, 2009a). On the other hand, for the breast cancer incidence analyses, where
6   incidence rates are higher, EPA adjusted the all-cause mortality rates to take into account breast
7   cancer incidence, effectively redefining interval "survival" (and thus the resulting population at
8   risk) as surviving the interval without developing an incident case of breast cancer (U.S. EPA,
9   2006, Section 4.1.2.3). Therefore, the concerns raised by Sielken and Valdez-Flores (2009a)
10   about using life-table analyses to derive incidence estimates do not apply to EPA's calculations.
11   Finally, the risk estimates of Valdez-Flores et al. (2010) and EPA's 2006 draft also differ
12   because Valdez-Flores et al. (2010), based on analyses in a separate publication by Sielken and
13   Valdez-Flores (2009b), misinterpreted the application of the age-dependent adjustment factors
14   (ADAFs) such that, even though they purported to apply the factors, this application had no
15   impact on the risk estimate. The ADAFs are default adjustment factors intended to be applied
16   directly to the unit risk estimates (i.e., risk per unit constant exposure, or "slope factors") in
17   conjunction with age-specific exposure level estimates (U.S. EPA, 2005b). For the purposes of
18   applying the ADAFs, the unit risk estimate is parsed, as a proportion of an assumed 70-year
19   lifespan, across age groups with different adjustment factors and/or exposure levels. The
20   ADAFs were not designed to be applied in life-table analyses, as was done by Sielken and
21   Valdez-Flores (2009b). In addition, the use of the 15-year lag in exposure in the life-table
22   analyses does not mean that there is no risk from exposures before age 15 years, as intimated by
23   Sielken and Valdez-Flores (2009b). Indeed, those exposures do not increase risk for cancer
24   occurring before 15 years of age; however, they do contribute to lifetime risk. The assumption
25   of increased early-life susceptibility that underlies the application of the ADAFs is that early-life
26   exposure increases the *lifetime* risk of cancer, not just the risk of cancer in early life, so it is
27   inappropriate to apply the ADAFs only to the age-specific hazard rates, as was done by Sielken
28   and Valdez-Flores (2009b). One might conceivably incorporate the ADAFs into the lifetable
29   analysis by weighting the age-specific exposures before they are aggregated into the cumulative
30   exposure, but such an integrated approach does not allow for the risks associated with less-than-
31   lifetime exposure scenarios to be calculated without redoing the lifetable analysis each time.
32

A.3. SUMMARY

The initial human studies by Hogstedt et al. (1979a, b, 1986) and Hogstedt (1988), in which positive findings of leukemia and blood-related cancers suggested a causal effect, have been followed by studies that either do not indicate any increased risks of cancer or else suggest a dose-related increased risk of cancer at certain sites. These are chiefly cancers of the lymphohematopoietic system and include leukemia, lymphosarcoma, reticulosarcoma and NHL. More recently, an association with breast cancer has also been suggested. However, the overall epidemiological evidence is not conclusive because of inadequacies and limitations in the epidemiological database. The main effects and limitations in the epidemiological studies of EtO are presented in Table A-4.

Exposure information, where available, indicates that levels of EtO probably were not high in these study cohorts. If a causal relationship exists between exposure to EtO and cancer, the reported EtO levels may have been too low to produce a significant finding. Exposures in the earlier years (prior to 1970) in most of the companies, hospitals, and other facilities where EtO was made or used are believed to have been in the range of 20 ppm, with excursions many times higher, although few actual measurements are available during this period. (One exception is the environmental study by Joyner (1964), who sampled airborne levels of EtO from 1960 to 1962 in a Texas City facility owned by Union Carbide.)

Almost all actual measurements of EtO were taken in the 1970s and 1980s at most plants and facilities in the United States and Europe, and levels have generally fallen to 5 ppm and below. Some plants may have never sustained high levels of airborne EtO. Assuming that there is a true risk of cancer associated with exposure to EtO, then the risk is not evident at the levels that existed in these plants except under certain conditions, possibly due to a lack of sensitivity in the available studies to detect associated cancers at low exposures.

Table A-4. Epidemiological studies of ethylene oxide and human cancer

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Sterilizers, production workers, Sweden Hogstedt et al., (1986); Hogstedt (1988)	709 (539 men, 170 women)	Plant 1: mean = 20 ppm in sterilizer room Plant 2: mean = 14 ppm in early years, less than 6 ppm later Plant 3: less than 8 ppm in early years, less than 2 ppm later	33 cancer deaths vs. 20 expected 7 leukemia deaths vs. 0.8 expected 10 stomach cancer deaths vs. 1.8 expected	Benzene, methyl formate, bis-(2-chloroethyl) ether, ethylene, ethylene chlorohydrin, ethylene dichloride, ethylene glycol, propylene oxide, amines, butylene oxide, formaldehyde, propylene, sodium	No personal exposure information from which to estimate dose No latency analysis Mixed exposure to other chemicals
Sterilizing workers in 8 hospitals and users in 4 companies, Great Britain Gardner et al. (1989)	2,876 (1,864 men, 1,012 women)	In early years, odor threshold of 700 ppm noted; in later years, 5 ppm or less was noted	3 leukemia deaths vs. 0.35 expected (after 20+ years latency) 5 esophageal cancer deaths vs. 2.2 expected 4 bladder cancer deaths vs. 2.04 expected 4 NHL deaths vs. 1.6 expected 29 lung cancer deaths vs. 24.6 expected	Aliphatic and aromatic alcohols, amines, anionic surfactants, asbestos, butadiene, benzene, cadmium oxide, dimethylmine, ethylene, ethylene chlorohydrin, ethylene glycol, formaldehyde, heavy fuel oils, methanol, methylene chloride, propylene, propylene oxide, styrene, tars, white spirit, carbon tetrachloride	Insufficient follow-up Exposure classification scheme vague, making it difficult to develop dose-response gradient No exposure measurements prior to 1977, so individual exposure estimates were not made Mixed exposure to several other chemicals

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Coggon et al. (2004) Update of Gardner et al. (1989)	Same cohort followed additional 13 years	Ibid.	Recent Findings 5 leukemia deaths vs. 2.6 expected (definite or continual exposure) 7 NHL vs. 4.8 expected 11 breast cancers vs. 13.1 expected 17 hematopoietic cancers vs. 12.9 expected 9 lymphatic and/or hematopoietic cancers vs. 4.9 expected (definite exposure)	Ibid.	Ibid. and, in addition, no latency evaluation

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Production workers (methods unspecified) from 8 chemical plants in West Germany Kiesselbach et al. (1990)	2,658 men	No exposure information available	14 stomach cancer deaths vs. 10.1 expected 3 esophageal cancer deaths vs. 1.5 expected 23 lung cancer deaths vs. 19.9 expected	Beta-naphthylamine, 4-amino-diphenyl, benzene, ethylene chlorohydrin, possibly alkylene oxide (ethylene oxide/propylene oxide), based on inclusion of plants that were part of a cohort study by Thiess et al. (1982)	Insufficient follow-up; few expected deaths in cancer sites of significance with which to analyze mortality Production methods not stated; information vague on what these plants do Latency analysis given only for total cancer and stomach cancer mortality Although categories of exposure are given, they are not based on actual measurements No actual measurement data are given; dose-response analysis is not possible

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Production workers and users at 2 chemical plants in West Virginia Greenberg et al. (1990)	2,174 men	Exposure prior to 1976 not known 1976 survey: average 8-hour TWA exposure levels less than 1 ppm; 1 – 5 ppm 8-hour TWA for maintenance workers	7 leukemia and aleukemia deaths vs. 3 expected; SMR = 2.3 3 liver cancer deaths vs. 1.8 expected; SMR = 1.7 7 pancreatic cancer deaths vs. 4.1 expected; SMR = 1.7 Suggestion of increasing risk of stomach cancer and leukemia/aleukemia with cumulative duration of potential exposure	Acetaldehyde, acetonitrile, acrolein, aldehydes, aliphatic and aromatic alcohols, alkanolamines, allyl chloride, amines, butadiene, benzene, bis-(chloroethyl) ether, ethylene dichloride, diethyl sulphate, dioxane, epichlorhydrin, ethylene, ethylene chlorohydrin, formaldehyde, glycol ethers, methylene chloride, propylene chlorohydrin, styrene, toluidine	Low exposure levels: average 8-hour TWA exposure levels to EtO less than 1 ppm (from a 1976 survey) No actual measurements of exposure to EtO for these plants exist prior to 1976 Exposure occurred to many other chemicals, some of which may be carcinogenic Lack of quantitative estimates of individual exposure levels
Same cohort as Greenberg et al. (1990) minus all chlorohydrin-exposed employees, followed an additional 10 years Teta et al. (1993)	1,896 men	Estimated exposure prior to 1956: 14+ ppm; after 1956: less than 10 ppm Prior to 1976, estimates were based on measurements taken at similar facilities	Trend of increasing risk of leukemia and aleukemia death with increasing duration of exposure	Same (except for chemicals specific to the chlorohydrin process)	Same

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Only the chlorohydrin-exposed employees from Greenberg et al. (1990) cohort, followed an additional 10 years Benson and Teta (1993)	278 men	Reported to be very low exposure to EtO in the chlorohydrin process	8 pancreatic cancer deaths vs. 1.63 expected ($p < 0.05$) 8 hematopoietic cancer deaths vs. 2.72 expected ($p < 0.05$) SMR = 2.9	Same	Same, and, in addition, very small cohort
Same cohort as for Teta et al. (1993) followed an additional 15 years plus cohort enumeration extended to end of 1988 (an additional 10 years), adding 167 workers Swaen et al. (2009)	2,063 men	Individual exposure estimates derived from an exposure matrix based on potential EtO exposure categorizations developed by Greenberg et al. (1990) and time-period exposure estimates developed by Teta et al. (1993), which relied on measurements taken at other facilities and guestimates for the time periods before 1974.	No statistically significant increases were observed for any cancer types No statistically significant trends were observed for the lymphohematopoietic cancer categories examined using Cox proportional hazards modeling 9 leukemia deaths in workers hired before 1956; SMR = 1.51 (95% CI 0.69, 2.87)	Same	Same Crude exposure assessment, especially for the early time periods Small cohort; thus, small numbers of specific cancers even though long follow-up time

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Sterilizers of medical equipment and spices; and manufacturers and testers of medical sterilization equipment, in 14 plants in the United States Steenland et al. (1991); Stayner et al. (1993)	18,254 (45% male, 55% female)	1938–1976 (estimated): 16 ppm for sterilizer operators, 5 ppm for remainder 1977–1985 (mean): 4.3 for sterilizers, 2 ppm for remainder Individual cumulative exposure estimates calculated for workers in 13 of the 14 facilities	36 (lympho)hematopoietic cancer deaths vs. 33.8 expected 8 lymphosarcoma and reticulosarcoma deaths vs. 5.3 expected After 20+ years latency, SMR = 1.76 for hematopoietic cancer, a significant trend with increasing latency ($p < 0.03$) Significantly increasing hematopoietic cancer and "lymphoid" cancer risks with cumulative exposure	No identified exposures to other chemicals	Potential bias due to lack of follow-up on "untraceable" members (4.5%) of the cohort Short duration of exposure and low median exposure levels Individual exposures were estimated prior to 1976 before first industrial hygiene survey was completed Short follow-up for most members of the cohort; only 8% had attained 20 years latency Little mortality (6.4%) had occurred in this large group of employees No exposure-response relationship among female workers

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Same cohort as Stayner et al. (1993) and Steenland et al. (1991), plus 474 additional members, followed 1 more year Wong and Trent (1993)	18,728 (45% male, 55% female)	Same as Steenland et al. (1991) and Stayner et al. (1993)	16 NHL deaths in men vs. 6.47 expected 43 lymphohematopoietic cancer deaths observed vs. 42 expected (in men 32 observed vs. 22.2 expected) 14 other lymphatic cancer deaths vs. 11.4 expected (in men 11 observed vs. 5.8 expected) 14 leukemia deaths vs. 16.2 expected	No identifiable exposures to other chemicals	All of the limitations of Steenland et al. (1991) apply here Although this group is the same as Steenland et al. (1991), an additional unexplained 474 employees were added It is questionable that one additional year of follow-up added 392.2 expected deaths but only 176 observed deaths No effort was made to develop exposure-response data such as in Stayner et al. (1993) on the basis of individual cumulative exposure data but only on duration of employment

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Steenland et al. (2004) Update of Steenland et al. (1991), Stayner et al. (1993)	18,254 (45% male, 55% female)	Same as Steenland et al. (1991), with extension of worker histories based on job held at end of initial exposure assessment for those still employed at end of 1991 study (25% of cohort)	With 15-year lag, in internal Cox regression analyses, OR = 3.42 ($p < 0.05$) in highest cumulative exposure group for (lympho)hematopoietic cancer in males; significant regression coefficient for continuous log cumulative exposure Similar results for "lymphoid" cancers in males For females, with 20-year lag, in internal Cox regression analyses, OR = 3.13 ($p < 0.05$) for breast cancer mortality in highest cumulative exposure group; significant regression coefficient for continuous log cumulative exposure	No identified exposures to other chemicals	Potential bias due to lack of follow-up on "untraceable" members (4.5% of the cohort) Individual exposures were estimated prior to 1976 before first industrial hygiene survey was completed No increase in hematopoietic cancer risk with increase in exposure in women

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Women employees from Steenland et al. (2004) employed in commercial sterilization facilities for at least 1 year Steenland et al. (2003)	7,576 women	Same as in Steenland et al. (2004) Minimum of 1 year	SIR = 0.87 319 cases of breast cancer SIR = 0.94 20 in situ cases excluded A positive trend in SIRs with 15-year lag time for cumulative exposure ($p = 0.002$) In internal nested case-control analysis, a positive exposure-response log of cumulative exposure with 15-year lag, top quintile had OR = 1.74, $p < 0.05$ Similar results in subcohort of 5,139 women with interviews (233 cases)	Same as in Steenland et al. (2004), Stayner et al. (1993)	Interviews were available for only 68% of the women; thus, there is underascertainment of cancer cases in full cohort. Also, there are potential nonresponse biases in the subcohort with interviews. Exposure-response trends not strictly monotonically increasing

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Chemical workers licensed to handle ethylene oxide and other toxic chemicals, Italy Bisanti et al. (1993)	1,971 men	Levels were said to be high at beginning of employment; no actual measurements were available 637 workers were licensed only to handle ethylene oxide and no other toxic chemicals	43 total cancer deaths vs. 33 expected 6 hematopoietic cancer deaths vs. 2.4 expected 4 lymphosarcoma and reticulosarcoma deaths vs. 0.6 expected 5 hematopoietic cancer deaths vs. 0.7 expected in group licensed to handle only ethylene oxide	Toxic gases, dimethyl sulphate, methylene chloride, carbon disulphide, phosgene, chlorine, alkalic cyanides, sulfur dioxide, anhydrous ammonia, hydrocyanic acid	Lack of exposure data Insufficient follow-up for this young cohort Potential selection bias Possible earlier exposure than date of licensing would indicate
Two plants that produced disposable medical equipment, Sweden Hagmar et al. (1991, 1995)	2,170 (861 men, 1,309 women)	1964–1966, 75 ppm in sterilizers, 50 ppm in packers 1970–1972, 40 ppm in sterilizers, 20–35 ppm in packers and engineers By 1985, levels had dropped to 0.2 ppm in all categories except sterilizers and to 0.75 ppm in sterilizers	6 lymphohematopoietic cancer cases vs. 3.37 expected Among subjects with at least 0.14 ppm-years of cumulative exposure and 10 years latency, the SIR for leukemia was 7.14, based on two cases	Fluorochlorocarbons, methyl formate (1:1 mixture with ethylene oxide)	Short followup period; authors recommend another 10 years of follow-up Youthful cohort—few cases and fewer deaths; unable to determine significance or relationships in categories Only a minority of subjects had high exposure to ethylene oxide

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Sterilizers of medical equipment and supplies that were assembled at this plant, New York Norman et al. (1995)	1,132 (204 men, 928 women)	In 1980, levels were 50–200 ppm (8-hr TWA); corrective action reduced levels to less than 20 ppm	Only 28 cancers were diagnosed 1 leukemia case vs. 0.54 expected 12 breast cancer cases vs. 4.7 expected ($p < 0.05$) 2 pancreatic cancer cases vs. 0.51 expected	No other chemical exposures cited	Little power to detect any significant risk chiefly because a short follow-up period produced few cancer cases Insufficient latency analysis
Nested case-control study; cases and controls from a large chemical production plant, Belgium Swaen et al. (1996)	10 cases of Hodgkin lymphoma (7 cases confirmed) and 200 controls; all male	Cumulative exposure to ethylene oxide in cases was 500.2 ppm-months vs. 60.2 ppm-months in controls	3 cases indicated exposure to EtO, producing an OR = 8.5 ($p < 0.05$)	Fertilizers, materials for synthetic fiber production, PVC, polystyrene, benzene, methane, acetone, ammonia, ammonium, sulfate, aniline, caprolactam, ethylene, Nah., oleum	This was a hypothesis-generating study; the authors were not looking for ethylene oxide exposure alone but for other chemical exposures as well to explain the excess risk Only one disease— Hodgkin lymphoma— was analyzed

Table A-4. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ Industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Four ethylene oxide production plants in 3 states utilizing the chlorohydrin process (both ethylene and propylene) Olsen et al. (1997)	1,361 men	No actual measurements were taken	10 lymphohematopoietic cancer deaths vs. 7.7 expected After 24 years, the SMR increased to 1.44, based on 6 observed deaths No increase in pancreatic cancer	Bis-chloroethyl ether, propylene oxide, ethylene chlorohydrin, propylene chlorohydrin, ethylene dichloride, chlorohydrin chemicals	No actual airborne measurements of ethylene oxide or other chemicals such as ethylene dichloride were reported; only length of employment was used as a surrogate Increase in risk of lymphocytic and hematopoietic cancers after a 25-year latency is not shown in tabular form An additional 5 to 10 years of follow-up is needed to confirm the presence or lack of risk of pancreatic cancer and lymphopoietic and hematopoietic cancers
Female worker at Markhot Fereng Provincial hospital and clinic of Eger in the Pediatric Department Kardos et al. (2003)	299 female employees	EtO sterilizing units with unknown elevated concentrations	11 cancer deaths observed compared with 4.38, 4.03, or 4.28 expected ($p < 0.01$), based on comparison populations of Hungary, Heves County, and city of Eger, respectively	No identifiable exposures to other chemicals	Underlying cause of death provided on all 11 cases but no expected deaths available by cause Possible exposure to natural radium, which permeates the region

1   The best evidence of an exposure-response relationship comes from the large, diverse

2   NIOSH study of sterilizer workers by Steenland et al. (2004, 1991) and Stayner et al. (1993).

3   This study estimated cumulative exposure (i.e., total lifetime occupational exposure to EtO) in

4   every member of the cohort. The investigators estimated exposures from the best available data

5   on airborne levels of EtO throughout the history of the plants and used a regression model to

6   estimate exposures for jobs/time periods where no measurements were available. This regression

7   model predicted 85% of the variation in average EtO exposure levels. An added advantage to

8   this study, besides its diversity, size, and comprehensive exposure assessment, is the absence of

9   other known confounding exposures in the plants, especially benzene.

10   In the recent follow-up of the NIOSH cohort, as in the earlier study, Steenland et al.

11   (2004) observed no overall excess of hematopoietic cancers (ICD-9 codes 200–208). In internal

12   analyses, however, they found a significant positive trend ($p = 0.02$) for hematopoietic cancers

13   for males only, using log cumulative exposure and a 15-year lag, based on 37 male cases. In the

14   Cox regression analysis using categorical cumulative exposure and a 15-year lag, a positive trend

15   was observed and the OR in the highest exposure quartile was statistically significant (OR =

16   3.42; 95% CI 1.09–10.73). Similar results were obtained for the "lymphoid" category

17   (lymphocytic leukemia, NHL, and myeloma). No evidence of a relationship between EtO

18   exposure and hematopoietic cancers in females in this cohort was observed. In later analyses

19   conducted by Dr. Steenland and presented in Appendix D, the difference between the male and

20   female results was found not to be statisitically significant, and the same pattern of

21   lymphohematopoietic cancer results observed for males by Steenland et al. (2004) was observed

22   for the males and females combined (i.e., statistically significant positive trends for both

23   hematopoietic [n = 74] and lymphoid [n = 53] cancers using log cumulative exposure and a 15-

24   year lag, as well as statistically signficant ORs in the highest exposure quartile for both

25   hematopoietic and lymphoid cancers).

26   In the analysis by Swaen et al. (2009) of male UCC workers, the authors discussed the

27   development of the exposure assessment matrix used in combination with worker histories to

28   estimate cumulative exposures for each worker in West Virginia UCC cohort. The exposure

29   matrix was based on the qualitative categorization of potential EtO exposure in the different

30   departments developed by Greenberg et al. (1990) and the time-period exposure estimates from

31   Teta et al. (1993). Eight-hour TWA concentrations (ppm) were estimated over four time periods

32   (1925–1939, 1940–1956, 1957–1973, and 1974–1978) at the two facilities for three exposure-

33   potential categories (high, medium, and low exposure departments). Average exposures in the

34   latter time period (1974–1978) were based on industrial hygiene monitoring conducted at the

35   locations where the study subjects worked. Estimates for the earlier time periods were inferred

1   from data on airborne exposure levels in "similar" manufacturing operations during the time

2   periods of interest. The estimates for the 1957-1973 time period were inferred from

3   measurements reported for the EtO production facility at Texas City studied by Joyner (1964),

4   and the estimates for the 1940-1956 time period were inferred from "rough" estimates of

5   exposure reported for the Swedish company described by Hogstedt et al. (1979b). Exposures for

6   the 1925-1939 time period were assumed to be greater than for the later time periods, but the

7   exposure estimates for this period are largely guesses.

8   This relatively crude exposure assessment formed the basis of the UCC exposure-

9   response analyses of the UCC study described in Swaen et al. (2009). Swaen et al. (2009)

10 conducted SMR analyses for the UCC workers stratified into those hired before and after

11 December 31, 1956; for three subgroups of employment duration; and for three subgroups of

12 cumulative exposure. These investigators also conducted Cox proportional hazards modeling for

13 leukemia mortality and lymphoid malignancy mortality. No statistically signficant excesses in

14 cancer risk or positive trends were reported. Despite the long follow-up of the UCC cohort, its

15 usefulness is limited by its small size (e.g., a total of 27 lymphohematopoietic cancer deaths were

16 observed).

17   Valdez-Flores et al. (2010) used the same exposure assessment to conduct further

18 exposure-response modeling of the UCC data. These authors used the Cox proportional hazards

19 model to model various cancer endpoints, using the UCC data, the NIOSH data (Steenland et al.,

20 2004), or the combined data from both cohorts. Using cumulative exposure as a continuous

21 variable, no statistically significant positive trends were observed from any of the analyses.

22 Unlike Steenland et al. (2004), Valdez-Flores et al. (2010) rejected the log cumulative exposure

23 model. Using cumulative exposure as a categorical variable, statistically significant increased

24 risks in the highest exposure quintile were reported for all lymphohemtopoietic cancers and for

25 lymphoid cancers in the NIOSH male workers, consistent with results reported by Steenland et

26 al. (2004). Statistically significant increased risks in the highest exposure quintile were also

27 reported for NHL in the NIOSH male workers and for lymphoid cancers and NHL in both sexes

28 combined in the NIOSH cohort.

29   The many different analyses of the UCC data are weakened by the reliance on the crude

30 exposure assessment. The NIOSH investigators, on the other hand, based their exposure

31 estimates on a comprehensive, validated regression model. Furthermore, the NIOSH cohort was

32 a much larger, more diversified group of workers who were exposed to fewer potential

33 confounders.

34   One other study that provides cumulative exposure estimates is the incidence study by

35 Hagmar et al. (1991, 1995). The short follow-up period and relative youthfulness of the cohort

1   produced little morbidity by the end of the study, although some support for an excess risk of

2   leukemia and lymphohematopoietic cancer had appeared.

3   In a separate analysis of the NIOSH cohort by Wong and Trent (1993), duration of

4   exposure to EtO was used as a surrogate for exposure. These authors did not find any positive

5   exposure-response relationships. They did observe an elevated significant risk of "NHL" in

6   males (SMR = 2.47, $p < 0.05$), based on 16 deaths, which was not dose- related or time-related.

7   However, a deficit in females remained.

8   Increases in the risk of hematopoietic cancers are also suggested in several other studies

9   (Gardner et al., 1989; Coggon et al., 2004; Norman et al., 1995; Bisanti et al., 1993; Swaen et al.,

10   1996; Olsen et al., 1997). However, in all these studies the deaths were few and the risk ratios

11   were mostly nonsignificant except at higher estimated exposures or after long observation

12   periods. They were not robust and there were potentially confounding influences, such as

13   exposure to benzene and/or chlorohydrin derivatives.

14   In those plants where there were no detectable risks (Kiesselbach et al., 1990; Norman et

15   al., 1995), the cohorts were generally relatively youthful or had not been followed for a sufficient

16   number of years to observe any effects from exposure to EtO. In the study by Olsen et al.

17   (1997), although a slight increase in the risk of cancer of the lymphopoietic and hematopoietic

18   system was evident, the authors stated that their study provided some assurance that working in

19   the chlorohydrin process had not produced significantly increased risks for pancreatic cancer or

20   lymphopoietic or hematopoietic cancer, thus contradicting the findings of Benson and Teta

21   (1993). This study lacks any measurement of airborne exposure to any of the chemicals

22   mentioned and the authors indicated that an additional 5 to 10 years of follow-up would be

23   needed to confirm the lack of a risk for the cancers described in their study.

24   Although the strongest evidence of a cancer risk is with cancer of the hematopoietic

25   system, there are indications that the risk of stomach cancer may have been elevated in some

26   studies (Hogstedt et al., 1979a, 1986; Kiesselbach et al., 1990; Teta et al., 1993); however, it

27   attained significance only in the study by Hogstedt et al. (1979a), with 9 observed versus 1.27

28   expected. It was reported by Shore et al. (1993) that this excess may have been due to the fact

29   that early workers at this plant "tasted" the chemical reaction product to assess the result of the

30   EtO synthesis. This reaction mix would have contained ethylene dichloride and bis-chloroethyl

31   ether. Ethylene dichloride is a suspected carcinogen, whereas bis-chloroethyl ether is not. This

32   increased risk of stomach cancer was not supported by analyses of intensity or duration of

33   exposure in the remaining studies, except that Benson and Teta (1993) suggested that exposure

34   to this chemical increased the risk of pancreatic cancer and perhaps hematopoietic cancer but not

35   stomach cancer.

1   A significant risk of pancreatic cancer first reported by Morgan et al. (1981) was also
2   reported by Greenberg et al. (1990) in his cohort of chemical workers, but only in those workers
3   assigned to the ethylene chlorohydrin production process, where the authors reported that
4   exposure to EtO was low. Benson and Teta (1993) attributed the increase in pancreatic cancer
5   seen in Greenberg et al. (1990) to exposure to ethylene dichloride in the chlorohydrin process.
6   However, Olson et al. (1997) refuted this finding in their study. The pancreatic cancers from the
7   study by Morgan et al. (1981) also occurred in workers in a chlorohydrin process of EtO
8   production. The possibility that exposure to a byproduct chemical such as ethylene dichloride
9   may have produced the elevated risks of pancreatic cancer seen in these workers cannot be ruled
10   out.
11   In addition to the cancer risks described above, some recent evidence indicates that
12   exposure to EtO may increase the risk of breast cancer. The study by Norman et al. (1995) of
13   women who sterilized medical equipment observed a significant twofold elevated risk of breast
14   cancer, based on 12 cases. A study by Tompa et al. (1999) reported on a cluster of breast cancers
15   occurring in Hungarian hospital workers exposed to EtO. In another Hungarian study of female
16   hospital workers by Kardos et al. (2003), 3 breast cancers were noted out of 11 deaths reported
17   by the authors. Although expected breast cancer deaths were not reported, the total expected
18   deaths calculated was just slightly more than 4, making this a significant finding for cancer in
19   this small cohort.
20   The most compelling evidence on breast cancer comes from the NIOSH cohort. In the
21   recent update of this cohort, no overall excess of breast cancer mortality was observed in the
22   female workers; however, a statistically significant SMR of 2.07 was observed in the highest
23   cumulative exposure quartile, with a 20-year lag. In internal Cox regression analyses, a positive
24   exposure-response ($p = 0.01$) was observed for log cumulative exposure with a 20-year lag,
25   based on 103 cases. Similar evidence of an excess risk of breast cancer was reported in a breast
26   cancer incidence study of a subgroup of 7,576 female workers from the NIOSH cohort who were
27   exposed for 1 year or longer (Steenland et al., 2003). A significant ($p = 0.002$) linear trend in
28   SIR was observed across cumulative exposure quintiles, with a 15-year lag. In internal Cox
29   regression analyses, there was a significant regression coefficient with log cumulative exposure
30   and a 15-year lag, based on 319 cases. Using categorical cumulative exposure, the OR of 1.74
31   was statistically significant in the highest exposure quintile. In a subcohort of 5,139 women with
32   interviews, similar results were obtained based on 233 cases, and the models for this subcohort
33   were also able to take information on other potential risk factors for breast cancer into account.
34   Additionally, the coefficient for continuous cumulative exposure was also significant ($p = 0.02$),
35   with a 15-year lag.

1   Several other studies with female employees in the defined cohorts reported no increased

2   risks of breast cancer due to exposure to EtO (Coggon et al., 2004; Hogstedt et al., 1986; Hagmar

3   et al., 1991, 1995). However, these studies have much lower statistical power than the NIOSH

4   studies, as evidenced by the much lower numbers of breast cancer cases that they report. The

5   largest number of cases in any of these other studies is 11 cases in the Coggon et al. (2004)

6   study. Furthermore, none of these other studies conducted internal (or external) exposure-

7   response analyses, which are the analyses that provided the strongest evidence in the NIOSH

8   studies.

9

10   **A.4. CONCLUSIONS**

11   Experimental evidence demonstrates that exposure to EtO in rodents produces

12   lymphohematopoietic cancers; therefore, an increase in the risk of lymphohematopoietic cancer

13   in humans should not be unexpected. An increase in mammary gland carcinomas was also

14   observed in mice. Although several human studies have indicated the possibility of a

15   carcinogenic effect from exposure to EtO, especially for lymphohematopoietic cancers, the total

16   weight of the epidemiologic evidence is not sufficient to support a causative determination. The

17   causality factors of temporality, coherence, and biological plausibility are satisfied. There is also

18   evidence of consistency and specificity in the elevated risk of lymphohematopoietic cancer as a

19   single entity in the human studies. The earlier significant risk of leukemia seen in the Hogstedt

20   studies was supported in some studies and not in others. In fact, not all human studies of EtO

21   have suggested an elevated risk of cancer and in those that do, the marginally elevated risks vary

22   from one site to another within the lymphohematopoietic system. When combined under the

23   rubric "lymphohematopoietic cancers," this loosely defined combination of blood malignancies

24   produces a slightly elevated risk of cancer in some studies but not in all. There is evidence of a

25   biological gradient in the significant dose-response relationship seen in the large, high-quality

26   Steenland et al. (2004) study.

27   The best evidence of a carcinogenic effect produced by exposure to EtO is found in the

28   NIOSH cohort of workers exposed to EtO in 14 sterilizer plants around the country (Steenland et

29   al., 1991, 2004; Stayner et al., 1993). A positive trend in the risk of lymphohematopoietic and

30   "lymphoid" neoplasms with increasing log cumulative exposure to EtO with a 15-year lag is

31   evident. But there are some limitations to concluding that this is a causal relationship at this

32   time. For example, there was a lack of dose-response relationship in females, although, as

33   presented in Appendix D, later calculations show that the difference in response between females

34   and males is not statistically significant and that significant increases are also observed with both

35   sexes combined.

1   An elevated risk of lymphohematopoietic cancers from exposure to EtO is also apparent

2   in several other studies. In some of these studies, confounding exposure to other chemicals

3   produced in the chlorohydrin process concurrent with EtO may have been partially responsible

4   for the excess risks. In other studies, where the chlorohydrin process was not present, there are

5   no known confounding influences that would produce a positive risk of lymphohematopoietic

6   cancer. Overall, the evidence on lymphohematopoietic cancers in humans is considered to be

7   strong but not sufficient to support a causal association.

8   There also exists the possibility that exposure to EtO may increase the risk of breast

9   cancer, based chiefly on the Steenland et al. (2003, 2004) studies discussed earlier, with some

10   corroborating evidence from the Norman et al. (1995) study of breast cancer in women exposed

11   to EtO. The risk of breast cancer was analyzed in a few other studies (Hagmar et al., 1991;

12   Hogstedt, 1988; Hogstedt et al., 1986; Coggon et al., 2004), and no increase in the risk of breast

13   cancer was found. However, these studies had far fewer cases to analyze, did not have

14   individual exposure estimates, and relied on external comparisons. The Steenland et al. (2003,

15   2004) studies, on the other hand, used the largest cohort of women potentially exposed to EtO

16   and clearly show significantly increased risks of breast cancer incidence and mortality, based on

17   internal exposure-response analyses. However, the authors suggest that the case is not

18   conclusive of a causal association "due to inconsistencies in exposure-response trends and

19   possible biases due to non-response and an incomplete cancer ascertainment." While these are

20   not decisive limitations—exposure-response relationships are often not strictly monotonically

21   increasing across finely dissected exposure categories, and the consistency of results between the

22   full cohort (less nonresponse bias) and the subcohort with interviews (full case ascertainment)

23   alleviates some of the concerns about those potential biases—the evidence for a causal

24   association between breast cancer and EtO exposure is less than conclusive at this time.

APPENDIX B
REFERENCES FOR FIGURE 3-3

The references in this list correspond to the additional data that was added to Figure 3-3 since the IARC (1994b) genetic toxicity profile was published. See the Figure 3-3 legend for details.

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APPENDIX C
GENOTOXICITY AND MUTAGENICITY OF ETHYLENE OXIDE

A summary of the available genotoxicity and mutagenicity data for ethylene oxide (EtO) is presented in Chapter 3 (Section 3.3.3). This appendix provides further details on the available genotoxicity and mutagenicity data and on some of the studies that are briefly mentioned in Chapter 3. The genotoxic potential of EtO is a key component of the assessment of its carcinogenicity. The relationship between genotoxicity/mutagenicity and carcinogenicity is based on the observations that genetic alterations are observed in almost all cancers and that many of these alterations have been shown to play an important role in carcinogenesis. Exposure to EtO has been found to result in a number of genotoxic effects in laboratory animal studies and in studies of humans exposed in occupational settings. In particular, EtO has been shown to alter or damage genetic material in such a manner that the genetic alterations are transmissible during cell division. Evidence of genotoxicity/mutagenicity provides strong mechanistic support for potential carcinogenicity in humans (Waters et al., 1999).

Since the first report of EtO's role in inducing sex-linked recessive lethals in *Drosophila* (Rapoport, 1948), numerous papers have been published on the mutagenicity of EtO in biological systems, spanning a whole range of assay systems, from bacteriophage to higher plants and animals (see Figure 3-3 in Chapter 3). EtO, being a mono-functional alkylating agent, is DNA–reactive, capable of forming DNA adducts and inducing mutations at both the chromosome and gene levels under appropriate conditions, as evidenced in numerous *in vitro* and *in vivo* studies (reviewed in Dellarco et al., 1990; Natarajan et al., 1995; Vogel and Natarajan, 1995; Thier and Bolt, 2000; Kolman et al., 1986, 2002; IARC, 2008). In prokaryotes (bacteria) and lower eukaryotes (yeasts and fungi), EtO induces DNA damage and gene mutations and conversions. In mammalian cells, EtO induces DNA adducts, unscheduled DNA synthesis, gene mutations, sister chromatid exchanges (SCEs), micronuclei, and chromosomal aberrations (Thier and Bolt, 2000; Natarajan et al., 1995; Preston et al., 1995; Dellarco et al., 1990; Walker et al., 1990; Ehrenberg and Hussain, 1981; IARC, 2008). The results of *in vivo* studies on the genotoxicity of EtO following ingestion, inhalation or injection have also been consistently positive (IARC, 1994b, 2008). Furthermore, *in vivo* exposure to EtO-induced gene mutations in the *Hprt* locus in mouse and rat splenic T-lymphocytes and SCEs in lymphocytes

1   from rabbits, rats, and monkeys, in bone marrow cells from mice and rats, and in rat spleen.

2   Increases in the frequency of gene mutation in the lung (*LacI* locus) (Sisk et al., 1997, Recio et

3   al., 2004) and in the *Hprt* locus in T-lymphocytes (Walker et al., 1997) in transgenic mice

4   exposed to EtO via inhalation have been observed at concentrations similar to those in

5   carcinogenesis bioassays (NTP, 1987). EtO has also induced heritable mutations or effects in

6   germ cells in rodents (Lewis et al., 1986; Generoso et al., 1990). In addition, significant

7   increases in the frequency of SCEs and chromosomal aberrations in peripheral blood

8   lymphocytes have been consistently reported in workers exposed to concentrations of EtO of

9   greater than 5ppm (TWA) (IARC [2008] and references therein). Thus, there is consistent

10 evidence that EtO interacts with the genome from both *in vitro* studies and *in vivo* studies of

11 laboratory animals and occupationally exposed humans. Based on these observations, exposure

12 to EtO is considered to cause cancer through a mutagenic mode of action (Chapter 3, Section

13 3.4).

14   The following sections provide further details on different genotoxicity test results

15 regarding the mutagenic potential of EtO.

16

17 **C.1.   DNA ADDUCTS**

18   Covalent binding of a chemical (direct-acting) or its electrophilic intermediates or

19 metabolites (indirect-acting chemicals following metabolic activation) with the nucleophilic sites

20 in DNA results in the formation of 'DNA adducts', which represent the biologically effective

21 dose of the chemical agent in question. Alkylating agents, such as EtO, are direct-acting

22 chemical agents which can transfer alkyl groups (e.g., ethyl groups) to nucleophilic sites in

23 DNA, alkylating the nucleotide bases. Alkylating agents are classified as S_N1-type or S_N2-type

24 depending on the substitution nucleophilicity (S_N). The S_N1-type chemicals follow first-order

25 kinetics (e.g., ethylnitrosourea [ENU] and methylnitrosourea or [MNU]), while the S_N2-type

26 agents exhibit an intermediate transition state (e.g., EtO and methyl methanesulfonate [MMS]).

27 EtO is a direct-acting S_N2 (substitution-nucleophilic-bimolecular)-type alkylating agent that

28 forms adducts with cellular macromolecules such as proteins (e.g., hemoglobin) and DNA. The

29 reactivity of an alkylating agent can be estimated by its Swain Scott substrate constant (*s*-value),

30 which ranges from 0 to 1 (Warwick, 1963). Alkylating agents such as EtO and MMS, which

31 have high '*s*' values (0.96 and >0.83, respectively), target the nucleophilic centers of ring

1   nitrogens (e.g., N7 of guanine and N3 of adenine) in DNA, while agents such as ENU with a low

2   's' values (0.26) target the less nucleophilic centers such as O^6 of guanine. EtO has a high

3   substrate constant favoring efficient alkylation at N7 of guanine (Warwick, 1963; Golberg, 1986;

4   Beranek, 1990). Due to the high nucleophilicity and steric availability of the N7 of guanine, EtO

5   predominantly forms the N7-hydroxyethylguanine (N7-HEG) adduct, although minor adducts

6   such as those forming at O^6 of guanine, N^1, N^3, and N^6 of adenine, and N^3 of cytosine, uracil and

7   thymine are found in some instances (Segerbäck, 1994).

8   Several methods have been developed since 1988 to detect EtO-induced DNA adducts *in*

9   *vitro* and *in vivo*. However, sensitivity and specificity of these methods have been the main

10   concern. These methods include immunochemical assays, fluorescence techniques, high

11   pressure liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS), ^{32}P-

12   postlabeling and electrochemical detection, with varying sensitivities for detection of EtO-DNA

13   adducts (Bolt et al., 1988, 1997; Uziel et al., 1992; van Delft et al., 1993, 1994; Kumar et al.,

14   1995; Saha et al., 1995; Leclercq et al., 1997; Marsden et al ., 2007, 2009; Huang et al., 2008;

15   Tompkins et al., 2008). In the following paragraphs, a brief summary of available methods is

16   provided to aid in the discussion of the DNA adduct data.

17   Van Delft et al. (1993) developed monoclonal antibodies against the imidazole ring of

18   N7-alkyldeoxyguanosine, with the limits of detection being 5-10, 1-2 and 20 adducts per 10^6

19   nucleotides, respectively, when used in the direct and competitive enzyme-linked

20   immunosorbant assay and in immunofluorescence microscopy. Later the same authors

21   developed an immunoslot-blot assay with increased sensitivity that detected 0.34 N7-HEG

22   adducts per 10^6 nucleotides (van Delft et al., 1994). Kumar et al. (1995) developed a ^{32}P-

23   postlabeling method using thin-layer chromatography (TLC) and HPLC, which detected 0.1 –

24   1.0 fmol 7-alkylguanine adducts in rats exposed to different alkenes. Despite occasional

25   inefficient labeling and poor recovery of adduct due to depurination, this method has potential

26   for use in measuring human exposure to alkenes or their corresponding epoxides as well as the

27   endogenously formed 7-alkylguanine adducts.

28   Bolt et al. (1997) developed a HPLC method involving derivatization with phenylglyoxal

29   and fluorescence detection, using 7-methylguanine as an internal standard, for measuring the

30   physiological background of the N7-HEG adduct in DNA isolated from human blood. Using

31   this method, the authors were able to detect N7-HEG levels in five individuals ranging between

1   2.1 and 5.8 pmol/mg DNA (mean 3.2). Furthermore, Leclercq et al. (1997) developed a method

2   based on DNA neutral thermal hydrolysis, adduct micro-concentration, and HPLC coupled to

3   single-ion monitoring electrospray mass spectrometry which has a detection limit of 1 fmol (10^-

4   ^{10} M), allowing the detection of 3 adducts/10^8 normal nucleotides. Using this method, Leclercq

5   et al. detected a dose-response relationship for N7-HEG after exposing calf thymus DNA and

6   blood samples to various doses of EtO. Marsden et al. (2007) used a highly sensitive LC-

7   MS/MS assay with selected reaction monitoring that offers a limit of detection of 0.1 fmol of

8   N7-HEG to establish background levels of N7-HEG (1.1-3.5 adducts/10^8 nucleotides) in tissues

9   of rats. Huang et al. (2008) developed an isotope-dilution on-line solid-phase extraction and

10 liquid chromatography coupled with tandem mass spectrometry method with reportedly excellent

11 accuracy, sensitivity and specificity to analyze N7-HEG in urine samples of nonsmokers. This

12 method also demonstrated high-throughput capacity for detecting EtO-DNA adducts and may be

13 particularly useful for future molecular epidemiology studies of individuals with low-dose EtO

14 exposure. Tompkins et al. (2008) used a high-performance liquid chromatography/electrospray

15 ionization tandem mass spectrometry and reported ~8 N7-HEG adducts/10^8 nucleotides in the

16 livers of control rats. This method was also capable of detecting the less prevalent but

17 potentially more biologically significant N1-hydroxyethyl-2'-deoxyadenosine (N1-HEA), O^6-

18 hydroxyethyl-2'-deoxyguanosine (O^6-HEG), N6-hydroxyethyl-2'-deoxyadenosine (N6-HEA)

19 and N3-hydroxyethyl-2'-deoxyuridine (N3-HEU) adducts. However, these minor adducts were

20 below the level of detection in control rat tissue DNA.

21   Overall, the sensitivity of EtO adduct detection depends on the method used for analysis.

22 Hence, use of appropriate methods is important when analyzing for these adducts and will be

23 highlighted in the following discussion.

24

25 **C.1.1 Detection of EtO Adducts in *In Vitro* and *In Vivo* Systems**

26   Numerous studies have been conducted to investigate the formation of DNA adducts

27 following EtO exposure, in a wide range of experimental models, including cell-free systems,

28 bacteria, fungi, *Drosophila* and experimental animals, as well as in exposed human subjects.

29 The following discussion is a review of the available studies of exposure to EtO and DNA adduct

30 formation in *in vitro* systems, laboratory animals, and humans (van Sittert and de Jong, 1985;

31 Bolt et al., 1988; Pauwels and Veulemans, 1998; Boysen et al., 2009).

1 **C.1.2. *In Vitro* DNA Binding Studies**

2   The capacity of EtO to bind to DNA and form DNA adducts has been documented in a

3 few *in vitro* studies. Segerbäck (1990) showed that ^{14}C-labeled EtO reacted *in vitro* with calf

4 thymus DNA to produce N7-HEG adduct as the predominant adduct, with relatively low

5 amounts of O^6-HEG and N3-(2-hydroxyethyl)adenine (N3-HEA) adducts. The levels of N3-

6 HEA and O^6-HEG are 4.4% and 0.5%, respectively, of the N7-HEG levels. Thus, the ratio of

7 N7-HEG, N3-HEA and O^6-HEG produced *in vitro* was 200:8.8:1, respectively. In the same

8 study, the *in vitro* reaction products of radiolabeled N-(2-hydroxyethyl)-N-nitrosourea

9 (HOEtNU) with calf thymus DNA exhibited a higher relative amount of O^6-HEG, which was

10 63% of the N7-HEG formed. The difference in reactivity towards the N7 and O^6 positions in

11 guanine by these two alkylating agents was explained by the difference in their '*s*' values. EtO,

12 with an *s*-values of 0.9, has a greater relative preference for reacting with N rather than O atoms

13 than does HOEtNU, with an *s*-values of 0.2.

14   In another study, Li et al. (1992) observed that EtO in aqueous solution incubated with

15 calf thymus DNA in vitro for 10 h produced several 2-hydroxyethyl (HE) DNA adducts whose

16 relative yields (nmol/mg DNA) were in the descending order: N7-HEG (330) > N3-HEA (39) >

17 N1-HEA (28), N6-HEA (6.2) > N3-HE-Cyt (3.1) > N3-HE-dThd (2.0) > N3-HEU (0.8). This *in*

18 *vitro* study did not detect the O^6-HEG adduct.

19

20 **C.1.3. *In Vivo* Studies – Animal Experiments**

21   Several studies evaluated N7-HEG levels following one or a range of doses with repeated

22 exposures of EtO given by inhalation or intraperitoneal injection in laboratory animals.

23 Segerbäck (1983) showed that in male CBA mice exposed by inhalation to ^{14}C-labeled EtO N7-

24 HEG adducts are formed in spleen, testes and liver with half lives of 24, 20, and 12 h,

25 respectively.

26   Walker et al. (1990) conducted a time-course study to investigate the formation and

27 persistence of N7-HEG adducts in various tissues such as brain, kidney, liver, spleen, lung and

28 kidney of male Fischer 344 rats exposed to one high dose of 300 ppm EtO by inhalation for 4

29 consecutive weeks (6 h/day, 5 days/wk) and sacrificed 1-10 days after the end of exposure. The

30 N7-HEG adduct was detectable in both target (brain, spleen and WBCs) and nontarget (kidney,

31 liver, lung and testis) tissues with maximum levels (1.5 times control levels) seen in brain

1   compared to other tissues 1 day after exposure. The similarities in N7-HEG levels in various

2   tissues are possibly due to efficient pulmonary uptake of EtO and rapid distribution by the

3   circulatory system. The N7-HEG adduct levels increased linearly for 3-5 days followed by a

4   slow removal from DNA with an apparent half-life of 7 days, suggesting that the adduct was

5   probably removed by spontaneous depurination. The calculated *in vivo* half-life for N7-HEG

6   formed by EtO confirms the persistence of this adduct and is consistent with another study in rats

7   exposed to another alkylating agent, N-nitrosomethyl-(2-hydroxyethyl)amine (Koepke et al.,

8   1988). Walker et al. (1990) suggested that the similarity in N7-HEG formation in the target as

9   well as non-target tissues could also be due to factors such as cell replication, location of the

10   adducts in the genome, and tissue susceptibility genes, which might be critical determinants

11   quantitatively affecting tissue-specific and/or dose-response relationships.

12   Using fluorescence-coupled HPLC, Walker et al. (1992a) measured N7-HEG levels in

13   DNA of target and nontarget tissues from male B6C3F1 mice and F344 rats exposed to 0, 3, 10,

14   33, 100, or 300 (rats only) ppm EtO by inhalation for 4 weeks (6 h/day, 5 days/week). Another

15   group of mice was exposed to 100 ppm EtO for 1, 3, 7, 14, or 28 days (5 days/week). The

16   authors reported linear dose-response relationships for N7-HEG in rat tissues following EtO

17   exposures between 10 and 100 ppm, with the slope increasing for exposures above 100 ppm. In

18   mice, only exposures to 100 ppm EtO resulted in significant increase in N7-HEG levels. Walker

19   et al. (1992a) observed N7-HEG adduct levels of 2-6 pmols/mg DNA in control mice and rats,

20   while in mice exposed to 100 ppm EtO, N7-HEG levels ranged from 17.5 ± 3.0 (testis) to 32.9 ±

21   1.9 (lung) pmol/mg DNA after 4 weeks of exposure. Rats and mice concurrently exposed to 100

22   ppm EtO for 4 weeks showed 2- to 3-fold lower N7-HEG levels in all tissues of mice compared

23   to rats, suggesting species differences in the susceptibility to EtO-induced genotoxicity. The

24   half-life of N7-HEG in mouse kidney DNA was 6.9 days, and in rat brain and lung it was 5.4-5.8

25   days. The half-lives of N7-HEG adducts in DNA from other tissues of mouse and rat were 1.0-

26   2.3 days and 2.9-4.8 days, respectively. The authors suggested that the slow linear removal of

27   N7-HEG adducts from the DNA was mainly due to chemical depurination, while the rapid

28   removal was due to loss by depurination and DNA repair. Rats exposed to 300 ppm EtO showed

29   O^6-HEG adducts at a steady-state concentration of ~1 pmol/mg DNA. Based on the results from

30   rats and mice, the authors suggested that DNA repair was saturated at the concentration of EtO

31   used in the time-course studies and that repeated exposures to lower concentrations of EtO

1   should lead to species- and tissue-specific differences in the levels of N7-HEG (Walker et al.,

2   1992a).

3   Wu et al (1999a) analyzed DNA from liver, brain, lung and spleen of B6C3F1 mice and

4   F-344 rats for N7-HEG adducts after exposure to EtO (0, 3, 10, 33 or 100 ppm) for 4 weeks (6

5   h/day, 5 days/week). The authors observed tissue- and species-specific dose-response

6   relationships of N7-HEG adducts in the EtO-exposed animals. Mice showed linear dose-

7   response relationships for N7-HEG adducts in liver, brain and spleen at exposures between 3 and

8   100 ppm, and sublinear responses in lung between 33 and 100 ppm EtO exposure. Rats showed

9   linear increases in adduct levels in liver and spleen DNA between 3 and 100 ppm EtO, and

10   sublinear responses in the brain and lung between 33 and 100 ppm EtO exposure. Overall, rats

11   and mice exposed to 3 ppm EtO showed 5.3- to 12.5- and 1.3- to 2.5-fold higher N7-HEG

12   adducts, respectively, compared to the corresponding unexposed control animals. Thus, results

13   from this study suggest species differences, with rats being more susceptible to adduct formation

14   than mice, at lower levels of EtO exposure. This study also showed a clear difference in N7-

15   HEG levels between unexposed and exposed mice at these lower exposure levels, unlike the

16   study of Walker et al. (1992a) discussed above, which is possibly due to the use of a highly

17   sensitive gas chromatography high-resolution mass spectrometry (GCHRS) assay in the Wu et al

18   (1999a) study.

19   Van Sittert et al (2000) exposed Lewis rats to 50, 100 and 200 ppm EtO by inhalation (4

20   weeks, 5 days/week, 6 h/day) and measured N7-HEG adducts 5, 21, 35 and 49 days after

21   cessation of exposure. The authors used mass spectrometry following neutral thermal hydrolysis

22   of DNA to release the N7-HEG adducts, which clearly show a difference between control and

23   EtO-exposed rats. The mean levels of liver N7-HEG immediately after cessation of exposure to

24   50, 100 and 200 ppm were estimated by extrapolation to be 310, 558 and 1202 adducts/10^8

25   nucleotides, respectively, while the mean level in control rats was 2.6 adducts/10^8 nucleotides.

26   By 49 days post-exposure, N7-HEG adducts had returned to near background levels. The N7-

27   HEG levels in liver DNA showed a linear response between 0 and 200 ppm EtO, suggesting that

28   detoxification and DNA repair processes were not saturated up to the highest exposure level

29   tested. The authors observed statistically significant linear relationships between mean N7-HEG

30   levels at 'day 0' post-exposure and (i) *Hprt* mutant frequencies at expression times of 21/22 and

31   49/50 days post-exposure, (ii) SCEs at 5 days post-exposure or (iii) high frequency cells

1   measured 5 days post-exposure. The authors also observed that SCEs and high frequency cells

2   continued to be present at 21-days post-exposure and significantly correlated with N7-HEG

3   adducts at that time. However, induction of micronuclei, chromosome breaks or translocations

4   did not show a dose-response relationship.

5   Nivard et al. (2003) showed that in male *Drosophila* flies EtO exposure (2-1000 ppm) by

6   inhalation for 24 h induced a linear dose-response relationship for N7-HEG adduct formation

7   (0.15 to 105.4 adducts/10^6 nucleotides) over the entire dose-range, as detected by ^{32}P-

8   postlabeling assay. The N7-HEG adducts were undetectable in controls, i.e., below the detection

9   limit of 1 adduct/10^8 nucleotides.

10   A study by Rusyn et al. (2005) tested the hypothesis that EtO exposure results in an

11 accumulation of apurinic/apyrimidinic (AP) sites in DNA and induces changes in expression of

12 genes involved in DNA base excision repair (BER). The authors exposed male Fisher 344 rats

13 by inhalation to 100 ppm EtO or ethylene (40 or 3000 ppm) for 1, 3 or 20 days (6h/day, 5

14 days/week) and sacrificed them 2, 6, 24 or 72 h after a single-day exposure. Brain and spleen

15 were considered as target sites for EtO-induced carcinogenesis, and liver as a non-target organ.

16 Rusyn et al. (2005) observed a time-dependent increase in N7-HEG in brain, spleen (target

17 organs) and liver (non-target organ) and in N-(2-hydroxyethyl)valine (HEVal) adducts in

18 hemoglobin. However, they could not detect any increase in AP sites in control or EtO-exposed

19 rats for any given duration or dose of exposure. Rats exposed to EtO for 1 day showed a 3-7-

20 fold decrease in expression of the DNA repair enzyme 3-methyladenine-DNA glycosylase in the

21 brain and spleen, while rats exposed to EtO for 20 days showed increased expression of hepatic

22 8-oxoguanine DNA glycosylase, 3-methyladenine-DNA glycosylase, AP endonuclease,

23 polymerase beta, and alkylguanine methyltransferase by 20-100%. Levels of brain AP

24 endonuclease and polymerase beta were increased by <20% only in rats exposed to 3000 ppm

25 ethylene for 20 days. Results from this study suggest that EtO-induced DNA damage is repaired

26 without accumulation of AP sites or involvement of the BER pathway in target organs. The

27 authors conclude that accumulation of AP sites is not likely a primary mechanism for

28 mutagenicity and carcinogenicity of EtO, and further suggest that minor DNA adducts such as

29 O^6-HEG or N1-HEA are likely to be involved in mutagenicity. In fact, in a previous study from

30 the same group (Walker et al., 1992a), steady-state concentrations of O^6-HEG were reported

31 after 4 weeks of exposure with 300 ppm EtO, a finding which warrants further investigation.

1   Marsden et al. (2007) have shown that intraperitoneal administration of a single or three

2   daily doses of EtO (0.01-1.0 mg/kg) induced dose-related increases in N7-HEG adduct levels in

3   male F344 rats, except at the lowest dose (0.01 mg/kg), where N7-HEG levels were similar to

4   endogenous levels detected in control animals. Further, they observed that N7-HEG adducts did

5   not accumulate in rats given three daily doses of EtO.

6   Recently, using a dual-isotope approach combining HPLC-accelerated mass spectrometry

7   with LC-MS/MS analysis, Marsden et al. (2009) observed linear dose-response relationships for

8   (^{14}C)N7-HEG adducts (0.002 to 4 adducts/10^8 nucleotides) in spleen, liver and stomach DNA of

9   F344 rats after exposure to low, occupationally relevant concentrations of (^{14}C)EtO (0, 0.0001,

10   0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 mg/kg daily for 3 consecutive days, with the rats killed

11   4 h after the last exposure). These results suggest that using of a highly sensitive assay it is

12   possible to measure the N7-HEG adducts resulting from low EtO exposures above the

13   background adduct levels.

14   Ottender and Lutz (1999) reviewed the quantitative relationship between DNA adduct

15   levels and tumor incidence in rodents that received repeated administration of EtO. The authors

16   observed a correlation with tumor incidence when the DNA adduct levels measured at a given

17   dose were normalized to the TD_{50} dose (the dose which results in 50% tumor incidence in a two-

18   year study). The calculated adduct level in mice associated with the hepatocellular TD_{50} was 812

19   N7-HEG adducts/10^8 normal nucleotides.

20

21   **C.1.4. *In Vivo* Studies - Human Subjects**

22   A few studies have examined the effect of EtO exposure on humans, particularly in

23   occupational settings, and these have been comprehensively reviewed by Kolman et al. (2002).

24   In that review, the authors examined the use of hemoglobin and DNA adducts as biomarkers of

25   EtO exposure and the roles of genetic polymorphisms and confounding factors. Kolman et al.

26   (2002) also described the genotoxic effects of EtO in mammalian cells and summarized the

27   genotoxic and carcinogenic effects of EtO in humans. Some of the relevant studies in humans

28   are briefly discussed below.

29   An immunoslot blot assay was used to analyze N7-HEG levels in white blood cell DNA

30   from individuals exposed to EtO (2-5 ppm) and from controls (van Delft et al., 1994). The

31   authors reported 0.1 and 0.065 N7-HEG adducts/10^6 nucleotides, respectively, in EtO-exposed

1　individuals (N=42) and controls (N=29) by this method. However, these differences were not

2　statistically significant.

3　　　In a study involving 58 sterilizer operators exposed to low and high levels of EtO ($\leq$ 32

4　and >32 ppm-hour, respectively) and 6 non-exposed controls from different hospitals, Yong et al.

5　(2007) examined N7-HEG adducts in granulocyte DNA. During the four-month study, the

6　cumulative exposure to EtO (ppm-hour) was estimated before the blood sample collection. After

7　adjusting for cigarette smoking and other potential confounders, the mean N7-HEG adduct levels

8　in the non-exposed, low, and high exposure groups were 3.8, 16.3, and 20.3 adducts/10^7

9　nucleotides, respectively, with considerable interindividual variation (range: 1.6-241.3

10　adducts/10^7 nucleotides). However, these differences in mean adduct level were not statistically

11　significant. The large variability across workers may reflect differences in their recent exposure

12　patterns because granulocytes have a lifespan of less than a day. Also, the study did not find a

13　significant correlation between the levels N7-HEG adducts and HEVal adducts.

14　　　Mayer et al. (1991) observed an apparent suppression of DNA repair capacity in EtO-

15　exposed individuals as measured by the DNA repair index, i.e., the ratio of unscheduled DNA

16　synthesis and N-acetoxy-2-acetylaminofluorene (NA-AAF)-DNA binding, ($p < 0.01$). In this

17　study, 34 sterilization unit workers of a large university hospital and 23 controls working in the

18　university library were used. Overall, this study demonstrates significant correlations between

19　EtO-induced hemoglobin adduct levels and SCEs and the number of high frequency cells, at low

20　levels of EtO exposure ($\leq$1 ppm), independent of smoking history.

21　　　In summary, EtO predominantly forms N7-HEG adducts. Minor adducts are O^6-HEG

22　adducts and reaction products with *N*1, *N*3 and *N*^{6} of adenine and with *N*3 of cytosine, uracil and

23　thymine *in vitro*. However, the minor adducts are not observed to the same extent *in vivo*, which

24　may reflect a limitation in the sensitivity of the adduct assays available to date. Repeated

25　inhalation exposure of EtO induces N7-HEG adducts in both target organs (brain, spleen and

26　white blood cells) and non-target organs (kidney, liver, and lung) in rodents, with an apparent

27　half-life of 3-6 days in rats and 1-3 days in mice (Walker et al., 1992a). The dose-response

28　relationship of N7-HEG and EtO exposure is influenced by the analytical method used, which

29　also affects the background (endogenous) levels of adducts observed in unexposed rodents.

30　Steady-state levels of O^6-HEG adducts (1 pmol/mg DNA) are detected in rats exposed by

31　inhalation to high doses of EtO (300 ppm) which are ~250-300 times lower than the N7-HEG

1   levels (Walker et al., 1992a). Although N7-HEG adducts are likely to be removed by

2   depurination forming apurinic/apyrimidinic (AP) sites, Rusyn et al. (2005) showed that DNA

3   damage induced by exposure to EtO is repaired without accumulation of AP sites and without

4   affecting base excision repair (BER) in target organs of Fischer rats. There are only two studies

5   available on EtO-induced DNA adducts in human populations. Although higher levels of N7-

6   HEG DNA adducts were observed in human white blood cells (van Delft et al., 1994) and

7   granulocytes (Yong et al., 2007) of exposed cases compared to controls, these differences were

8   not statistically significant, possibly due to high inter-individual variability.

9

10   **C.1.5. EtO-Hemoglobin Adducts**

11   Several studies have shown that EtO-induced hemoglobin adducts (e.g., HEVal) are good

12   biomarkers of exposure for this compound in human studies and that predicted hemoglobin

13   adduct levels resulting from exposure to ethylene or EtO are in agreement with measured values

14   (Britton et al., 1991; Walker et al., 1992b; Tates et al., 1999; Fennell et al., 2000; Yong et al.,

15   2001; Boogaard, 2002). Csanády et al. (2000) found a good agreement between the predicted

16   and measured hemoglobin adduct levels in humans. However, in rodents, hemoglobin adducts

17   were under-predicted by a factor of 2 to 3, while DNA adduct levels were comparable,

18   suggesting inconsistencies between the two biomarkers. Walker et al. (1993) also observed that

19   the relationships between HEVal and N7-HEG concentrations varied with length of exposure,

20   interval since exposure, species, and tissue, which may be due to differences in formation,

21   persistence, repair, and chemical depurination of the DNA adduct. Thus, Walker et al. (1993)

22   suggested that HEVal adducts do not provide accurate prediction of DNA adducts in specific

23   tissues of humans under actual exposure conditions. In summary, HEVal adducts do not appear

24   to be predictable markers for DNA adducts.

25

26   **C.2.   GENE MUTATIONS**

27   EtO has consistently yielded positive results, at both the gene and chromosome levels, in

28   a broad range of *in vitro* and *in vivo* mutational assays, including those performed in bacteria,

29   fungi, yeast, insects, plants, Drosophila and rodents, in both repair-deficient and proficient

30   organisms, and in mammalian cell cultures, including cells from humans (reviewed in Dellarco

31   et al., 1990; IARC, 1994b, 2008; Natarajan et al., 1995; Vogel and Natarajan, 1995; Thier and

1   Bolt, 2000; Kolman et al., 2002). The results of *in vivo* studies on the mutagenicity of EtO have

2   also been consistently positive following ingestion, inhalation, or injection (e.g., Tates et al.,

3   1999). Increases in the frequency of gene mutations in the lung (*LacI* locus) (Sisk et al., 1997),

4   in T-lymphocytes (*Hprt* locus) (Walker et al., 1997), and bone marrow and testes in B6C3F1

5   *LacI* transgenic mice (Recio et al., 2004) have been observed in mice exposed to EtO via

6   inhalation at concentrations similar to those used in the carcinogenesis bioassays (NTP, 1987),

7   clearly documenting that EtO is a DNA-reactive mutagenic agent. Furthermore, occupational

8   studies provide evidence for the genotoxic potential of EtO.

9

10   **C.2.1 Bacterial Systems**

11   Studies have been conducted to investigate the ability of EtO to induce gene mutations in

12   bacterial systems. Victorin and Stahlberg (1988) treated *Salmonella typhimurium* strain TA100

13   with EtO at concentrations of 1-200 ppm for 6 hours and demonstrated that EtO was mutagenic

14   in this system. In another study, Agurell et al. (1991) compared EtO and propylene oxide (two

15   alkylating agents) for genotoxic effectiveness in various test systems. The abilities of the two

16   compounds to induce point mutations in *S. typhimurium* strains TA 100 and TA1535 were

17   approximately equal. EtO induced a dose-dependent increase in the number of revertants in both

18   tester strains. No toxic effects were observed under the conditions tested.

19   In contrast, Agurell et al. (1991) found EtO to be 5-10 times more effective than

20   propylene oxide with respect to gene conversion and reverse mutation in the *S. cerevisiae* D7 and

21   *S. cerevisiae* RS112 strains. The greater effectiveness of EtO than propylene oxide in inducing

22   these types of mutations was probably due to the difference in these compounds' abilities to

23   cause strand breaks via alkylation of DNA-phosphate groups.

24   Mutagenicity studies of EtO have also been conducted using different *E. coli* strains.

25   Kolman (1985) investigated the influence of the *uvrB* and *umuC* genes on the induction of *LacI*-

26   mutants and nonsense mutants by EtO in the *LacI* gene of *E. coli* and found that *uvrB* gene

27   mutation was associated with higher mutation frequencies whereas *umuC* mutation did not

28   significantly affect the induction of *LacI* mutations. Thus, mutations induced by EtO were

29   enhanced by a lack of excision repair but not influenced by changes in error-prone repair. In

30   another study by the same group of authors (Kolman and Naslund, 1987), the mutagenicity of

31   EtO in *E. coli* B strains with different repair capacities was investigated. Deficiencies in

1 excision repair (*uvrA*, *polA*) led to considerable increases in mutation frequency compared to the

2 wild-type strain and strains deficient in error-prone repair (*recA*, *lexA*).

3   The induction of specific-locus mutations in the *adenine-3 (ad-3)* region of a two-

4 component heterokaryon (H-12) of *Neurospora crassa* by EtO was studied by de Serres and

5 Brockman (1995). The objective of this study was to compare EtO's mutational spectrum for

6 induced specific-locus mutations with those of other chemical mutagens. Conidial suspensions

7 were treated with five different concentrations of EtO (0.1-0.35%) for 3 h. The results from

8 these experiments showed (1) the dose-response curve for EtO-induced specific-locus mutations

9 in the *ad-3* region was linear, with an estimated slope of 1.49 ± 0.07, and (2) the maximum

10 forward-mutation frequency was between 10 and 100 *ad-3* mutations per 10^6 survivors. The

11 overall data demonstrate that EtO-induced *ad-3* mutations were a resultant of a high percentage

12 (96.9%) of gene/point mutations at the *ad-3A* and *ad-3B* loci.

13

14 **C.2.2. Mammalian Systems**

15   EtO has yielded positive results in virtually all *in vitro* mammalian cell culture systems

16 tested, including human cells (Dellarco et al., 1990; IARC, 1994b, 2008; Natarajan et al., 1995;

17 Vogel and Natarajan, 1995; Preston et al., 1999; Thier and Bolt, 2000; Kolman et al., 2002).

18 Only select *in vitro* studies of human cells will be reviewed here. For reviews of other *in vitro*

19 studies using mammalian cell cultures, see the aforementioned references.

20   Single base pair deletion and base substitution (both transitions and transversions)

21 mutations were observed in the *HPRT* gene in human diploid fibroblasts exposed to EtO

22 (Bastlova et al., 1993). Sequence analysis revealed that EtO induces many different kinds of

23 *HPRT* mutations — several mutants had large *HPRT* gene deletions, a few mutants showed

24 deletion of the entire *HPRT* gene, and other mutants had a truncated *HPRT* gene; overall, as

25 many as 50% were large deletions. In another study by the same group of authors (Lambert et

26 al., 1994), comparisons of the *HPRT* mutations in human diploid fibroblasts were made for three

27 urban air pollutants (acetaldehyde, benzo[a]pyrene and EtO). Large genomic deletions in the

28 *HPRT* gene were observed for acetaldehyde and EtO, whereas benzo(a)pyrene induced point

29 mutations. The authors concluded that the *HPRT* locus could be a useful target for the study of

30 chemical-specific mutational events (Lambert et al., 1994).

1   The effect of EtO as a pre-treatment or post-treatment to ionizing radiation was studied

2   by Kolman and Chovanec (2000). Human diploid VH-10 fibroblasts were either pre-exposed to

3   gamma-rays (0.66 Gy/min or 10 Gy/min) and then treated with EtO (2.5 mMh) or pre-treated

4   with EtO and then exposed to gamma-rays. Cell killing/cytotoxicity, DNA double-strand

5   breakage, and mutagenicity were studied in both types of exposures. The results of the study

6   indicate that pre-exposure of the cells to gamma-radiation (1 Gy) followed by treatment with EtO

7   (2.5 mMh) led to an additive interaction, irrespective of the dose rate. On the other hand, pre-

8   treatment with EtO followed by gamma-ray exposure resulted in an antagonistic effect, which

9   was most pronounced in the high dose group (10Gy/min). In this group, the mutant frequency

10   was half that of the sum of the mutant frequencies after the individual treatments. The authors

11   suggest that one possible explanation for the difference in the results is that DNA damage

12   induced by pre-exposure to gamma-radiation persisted into the EtO treatment phase, and EtO

13   might also prohibit DNA repair enzymes from operating, thus both treatments contributed to the

14   mutant frequency. However, when cells were exposed to gamma-radiation following EtO

15   treatment, the cells may have been able to repair, at least in part, the promutagenic lesions

16   induced by the gamma-rays.

17   The results of *in vivo* studies on the genotoxicity of EtO following ingestion, inhalation,

18   or injection have also been consistently positive (e.g., Tates et al., 1999). For example, increases

19   in the frequency of gene mutations in T-lymphocytes (*Hprt* locus) (Walker et al., 1997) and in

20   bone marrow and testes (*LacI* locus) (Recio et al., 2004) have been observed in transgenic mice

21   exposed to EtO via inhalation at concentrations similar to those in carcinogenesis bioassays with

22   this species (NTP, 1987). At somewhat higher concentrations than those used in the

23   carcinogenesis bioassays (200 ppm, but for only 4 weeks), increases in the frequency of gene

24   mutations have also been observed in the lung of transgenic mice (*LacI* locus) (Sisk et al., 1997)

25   and in T-lymphocytes of rats (*Hprt* locus) (Tates et al., 1999; van Sittert et al., 2000). These and

26   other key *in vivo* studies are discussed in more detail below.

27   An approach for determining mutational spectra in exon 3 of the *Hprt* gene in splenic T-

28   lymphocytes of B6C3F1 mice was developed by Walker and Skopek (1993). Mice (12 days old)

29   were given 2, 6, or 9 single i.p injections of 100 mg/kg EtO every other day or 30, 60, 90 or 120

30   mg/kg of EtO for 5 consecutive days to achieve different cumulative doses. In mice exposed

31   every other day, cumulative doses of 200, 600 and 900 mg/kg produced average mutant

1   frequencies of 15×10^{-6}, 45×10^{-6} and 73×10^{-6}, respectively, 8 weeks after dosing began.

2   However, in mice exposed daily, cumulative doses of 150, 300, 450, and 600 mg/kg yielded

3   average mutant frequencies were 4×10^{-6}, 8×10^{-6}, 11×10^{-6} and 16×10^{-6}, 20 weeks after

4   initiation of dosing. *Hprt* mutants obtained from mice exposed to 600 or 900 mg/kg EtO were

5   isolated and analyzed for mutations, specifically in exon 3. DNA sequencing showed base-pair

6   substitutions, transitions and transversions. The results suggested both modified guanine and

7   adenine bases being involved in EtO-induced mutagenesis.

8   The same group of authors (Walker et al., 1997) studied the *in vivo* mutagenicity of EtO

9   at the *Hprt* locus of T-lymphocytes following inhalation exposure of male B6C3F1 *LacI*

10   transgenic mice. Big Blue mice at 6-8 and 8-10 weeks of age were exposed to 0, 50, 100, or 200

11   ppm EtO for 4 weeks (6 h/day, 5 days/week). T-cells were isolated from the thymus and spleen

12   and cultured in the presence of concanavalin A, IL-2, and 6-thioguanine. Mice were sacrificed at

13   2 h, 2 weeks, and 8 weeks after exposure to 200 ppm EtO to determine a time course for the

14   expression of *Hprt*-negative lymphocytes in the thymus. The results of this study showed that

15   following two hours of exposure, the *Hprt* mutant frequency in the thymic lymphocytes of the

16   exposed mice was increased and reached an average maximum mutant frequency of 7.5 ± 0.9

17   $\times 10^{-6}$ at 2 weeks post-exposure when compared to $2.3 \pm 0.8 \times 10^{-6}$ in the thymic lymphocytes of

18   control mice. Dose-related increases in *Hprt* mutant frequency were found in thymic

19   lymphocytes from mice exposed to 100 and 200 ppm EtO. Furthermore, a greater mutagenic

20   efficiency (mutations per unit dose) was found at higher concentrations than at lower

21   concentrations of EtO in splenic T-cells. The average induced mutant frequencies in splenic T-

22   cells were 1.6, 4.6, and 11.9×10^{-6} following exposures to 50, 100, or 200 ppm EtO, respectively.

23   For the analysis of the *LacI* mutations, lymphocytes (both B- and T-cells) were isolated from the

24   spleen in the same animals. Two of three EtO-exposed mice at the 200 ppm exposure level

25   demonstrated an elevated *LacI* mutant frequency. The authors suggest that these elevations were

26   probably due to the *in vivo* replication of pre-existing mutants and not to the induction of new

27   mutations associated with EtO exposure. The results of this study indicate that repeated

28   inhalation exposures to high concentrations of EtO produce dose-related increases in mutations

29   at the *Hprt* locus of T-lymphocytes in male *LacI* transgenic mice.

30   *LacI* mutant frequencies as a result of exposure to EtO were further investigated by Sisk

31   et al. (1997). Male transgenic *LacI* B6C3F1 mice (n=15) were exposed to 0, 50, 100, or 200

1   ppm EtO for 4 weeks (6 h/day, 5 days/week) and were sacrificed at 0, 2, or 8 weeks after the last

2   EtO exposure. To determine the *LacI* mutant frequency, the *LacI* transgene was recovered from

3   several tissues, including lung, spleen, germ cells and bone marrow, selected because they were

4   the target sites for tumor formation (particularly lung tumors and lymphomas) in chronic

5   bioassays or germ cells. The results of this study indicate that the *LacI* mutant frequency in lung

6   was significantly increased at 8 weeks post-exposure to 200 ppm EtO. In contrast, no significant

7   increase in the *LacI* mutant frequencies was observed in the spleen, bone marrow or germ cells at

8   either 2 or 8 weeks following exposure. These results suggest that a 4-week inhalation exposure

9   to EtO is mutagenic in lung but not in other tissues examined under similar conditions. The

10   authors predict that the lack of mutagenic response in other tissues examined is probably because

11   of large deletions that were either not detected or recovered in the current lambda-based shuttle

12   vector systems. Based on the above study, the authors also suggest that the primary mechanism

13   of EtO-induced mutagenicity *in vivo* is likely through the induction of deletions.

14   Tates et al. (1999) exposed rats to EtO via three routes – a single intraperitoneal (i.p.)

15   injection (10-80 mg/kg), ingestion of drinking water (4 weeks at concentrations of 2, 5, and 10

16   mM), or inhalation (50, 100 or 200 ppm for 4 weeks, 5 days/week, 6 h/day). The goal of this

17   study was to measure the induction of *Hprt* mutations in splenic lymphocytes using a cloning

18   assay. Mutagenic effects of EtO following EtO administration via the three routes were

19   compared in the *Hprt* assay based on blood doses, which were determined from HEVal adduct

20   levels in hemoglobin. Exposure to EtO via both injection and ingestion of drinking water led to

21   a statistically significant dose-dependent induction of mutations (up to 2.3- and 2.5-fold

22   increases in mutant frequency compared to background, respectively). Exposure via inhalation

23   also caused a statistically significant increase in mutant frequency, although to a lesser extent (up

24   to 1.4-fold over background). Plotting of the mutagenicity data for the three exposure routes

25   against blood doses as a common denominator indicated that, at equal blood doses, the order of

26   increased mutant frequency was i.p. injection > ingestion (drinking water) > inhalation. In the

27   injection experiments, there was evidence for a saturation of detoxification processes at the

28   highest doses, although such effects were not seen following subchronic administration. Taken

29   together, the mutagenicity data from this study provide consistent results, showing that exposure

30   to EtO gives rise to a linear dose-dependent increase in mutant frequency.

1   In a study by Recio et al. (2004), male Big Blue (*LacI* transgenic) B6C3F1 mice were

2   exposed to 0, 25, 50, 100, or 200 ppm EtO (6 hours per day, 5 days per week) for 12, 24, and 48

3   weeks. An unambiguous mutagenic response in the bone marrow was observed only after 48

4   weeks, with dose-related *LacI* mutant frequencies of 7.3×10^{-5}, 11.3×10^{-5}, 9.3×10^{-5}, $14.1 \times$

5   10^{-5}, and 30.3×10^{-5}. The mutagenic response in bone marrow is consistent with a linear

6   exposure-response relationship, contrary to the assertion by Recio et al. (2004) which appears to

7   be based on a misleading plotting scale. Mutant frequencies from testes (seminiferous tubules)

8   were significantly greater than in controls at 25, 50, and 100 ppm (48-week exposure). No

9   difference between the control and treated groups was observed in the *LacI* mutant frequency

10   after 48 weeks of 200 ppm EtO exposure. The authors suggest that this was probably due to

11   testicular toxicity. Furthermore, a mutation spectrum analysis of induced mutations in bone

12   marrow indicated a decrease in mutations at G:C base pairs and an increase at A:T base pairs,

13   exclusively in A:T to T:A transversions; however, the mutation spectrum from testes was

14   similar to that of the untreated animals. The difference in mutation spectrum between the two

15   tissues was probably due to differences in the repair of the DNA adducts formed.

16   Mutations in oncogenes (*Kras, Hras*) and in the *p53* tumor suppressor gene have been

17   studied in tumor tissues of several types from B6C3F1 mice exposed to EtO. Hong et al. (2007)

18   obtained tumor tissues from lung, harderian gland and uterus from a 2-year study (NTP, 1987) in

19   which male and female mice were exposed to 0, 50, or 100 ppm EtO by inhalation 6h/day,

20   5days/week and from control mice from other NTP 2-year bioassays. The authors analyzed the

21   tissues for *Kras* mutations in codons 12, 13 and 61. A high frequency of *Kras* mutations (23/23

22   examined, 100%) was observed in EtO-induced lung neoplasms compared to spontaneous lung

23   neoplasms (27/108, 25%). EtO-induced lung neoplasms predominantly exhibited GGT-GTT

24   mutations in codon 12 (21/23), a transversion that was rare in spontaneous lung tumors (1/108).

25   A similar spectrum of *Kras* mutations was detected in EtO-induced lung neoplasms regardless of

26   histological subtype (adenomas or carcinomas) or dose group. In the case of Harderian gland

27   neoplasms, a high frequency (18/21, 86%) of *Kras* mutations was detected in EtO-induced

28   neoplasms compared to spontaneous tumors (2/27, 7%). The predominant mutations in EtO-

29   induced harderian gland neoplasms consisted of GGC to CGC transversions at codon 13 and

30   GGT to TGT transversions at codon 12, neither of which was observed in the spontaneous

31   tumors. When the six EtO-induced uterine neoplasms were examined (there were no uterine

1   tumors in the controls), the predominant mutation was a GGC to GGT transition in codon 13

2   (5/6, 83%). Based on the above results, the authors propose that the prominent targeting of

3   guanine bases in the lung and harderian gland neoplasms suggests that the formation of N7-HEG

4   adducts by EtO plays a role in the induction of these tumors. The authors further propose that

5   EtO can specifically target the *Kras* gene in multiple types of tissues and that this is a critical

6   component of EtO-induced tumorigenesis and is of potential relevance to humans.

7   In an earlier study by the same group of authors (Houle et al., 2006), mammary

8   carcinoma tissues from the same NTP study of mice exposed to EtO (0, 50 or 100 ppm)

9   mentioned above were examined for p53 protein expression and for *p53* (exons 5-8) and *Hras*

10   (codon 61) mutations. The authors supplemented the number of spontaneous mammary

11   carcinomas with tissues from female control mice in other NTP studies. P53 protein expression

12   was detected in 67% (8/12) of the EtO-induced mammary carcinomas and 42% (8/19) of the

13   spontaneous tumors; however, expression levels were about 6-times higher in the EtO-induced

14   than in the spontaneous tumors. *P53* mutations were observed in 67% (8/12) of the EtO-induced

15   mammary carcinomas and 42% (8/19) of the spontaneous tumors. *Hras* mutations were detected

16   in 33% (4/12) of the EtO-induced mammary carcinomas and 26% (5/19) of the spontaneous

17   tumors of the samples. While the mutation levels for these 2 genes weren't substantially elevated

18   in the EtO-induced mammary carcinomas compared to the spontaneous tumors, a shift in the

19   mutational spectrum was observed, with EtO-induced *Hras* mutations exhibiting a preference for

20   A-to-G and A-to-T transversions while spontaneous *Hras* mutations exhibited a preference for

21   C-to-A transversions and EtO-induced *p53* mutations exhibiting a base preference for guanine

22   while spontaneous *p53* mutations exhibited a preference for cytosine. In addition, concurrent

23   *Hras* and *p53* mutations were more common in the EtO-induced tumors than in the spontaneous

24   tumors. Based on the results of the above two studies, it is suggested that the purine bases serve

25   as primary targets for mutations induced by EtO, while mutations of these genes involving

26   cytosine appears to be a more common spontaneous event.

27   *In vivo* exposure to EtO also induced heritable mutations or effects in germ cells in

28   rodents (IARC, 1994b). EtO induces dominant lethal effects in mice and rats and heritable

29   translocations in mice (Lewis et al., 1986; Generoso et al., 1990). Generoso et al. (1986, 1988)

30   have reported that short bursts of EtO at high concentrations, such as those that my occur in the

1   workplace, may present a greater risk to germ cell damage than cumulative, long-term exposure

2   to lower levels.

3   Dominant-lethal mutations were investigated by Generoso et al. (1986) by conducting

4   two studies (dose-response and dose-rate) in mice exposed to different doses of EtO. Dominant-

5   lethal responses were assessed based on matings involving sperm exposed as late spermatids and

6   early spermatozoa, since these are the stages most sensitive to EtO exposure. In the dose-

7   response study, male mice were exposed by inhalation to 300 ppm, 400 ppm, or 500 ppm EtO, 6

8   hours per day, for 4 consecutive days. A dose-related increase in dominant-lethal mutations was

9   observed. In the dose-rate study, mice were given a total exposure of 1,800 ppm x hr per day,

10   also for 4 consecutive days, delivered either as 300 ppm in 6 hr, 600 ppm in 3 hr, or 1,200 ppm

11   in 1.5 hr. Dominant-lethal responses increased with increasing concentration level, indicating a

12   dose-rate effect for the production of dominant-lethal mutations.

13   In humans, workers occupationally exposed to EtO have been studied using different

14   physical and biological measures (Tates et al., 1991). Blood samples from 9 hospital workers

15   and 15 factory workers engaged in sterilization of medical equipment with EtO and from

16   matched controls were collected. Average exposure levels during 4 months (the lifespan of

17   erythrocytes) prior to blood sampling were estimated from levels of HEVal adducts in

18   hemoglobin. The adduct levels were significantly increased in hospital workers and factory

19   workers and corresponded to a 40-h time-weighted average of 0.025 ppm in hospital workers and

20   5 ppm in factory workers. Exposures were usually received in bursts, with EtO concentrations in

21   air ranging from 22 to 72 ppm in hospital workers and 14 to 400 ppm in factory workers. All

22   blood samples were analyzed for *HPRT* mutant frequencies, chromosomal aberrations,

23   micronuclei and SCEs. Mutant frequencies were significantly increased in factory workers but

24   not in hospital workers. The chromosomal aberration and SCE results are discussed in the

25   respective sections below.

26   The same authors (Tates et al., 1995) conducted another study of workers in an EtO

27   production facility. *HPRT* mutations were measured in three exposed groups and one unexposed

28   group (seven workers per group). Contrary to the earlier study, no significant differences in

29   mutant frequencies were observed between the groups; however, the authors stated that about 50

30   subjects per group would have been needed to detect a 50% increase.

1   Major et al. (2001) measured *HPRT* mutations in female nurses employed in hospitals in

2   Eger and Budapest, Hungary. This study was conducted to examine a possible causal

3   relationship between EtO exposure and a cluster of cancers (mostly breast) in nurses exposed to

4   EtO in the Eger hospital. Controls were female hospital workers in the respective cities. The

5   mean peak levels of EtO were 5 mg/m^3 (2.7 ppm) in Budapest and 10 mg/m^3 (5.4 ppm) in Eger.

6   *HPRT* variant frequencies in both controls and EtO-exposed workers in the Eger hospital were

7   higher than either group in the Budapest hospital, but there was no significant increase among

8   the EtO-exposed workers in either hospital when compared with the respective controls.

9   In summary, there is sufficient evidence for mutagenicity of EtO in various organisms

10   (prokaryotes, eukaryotes, *in vitro* and *in vivo* in rodents and *in vitro* in human cells) tested in a

11   variety of mutational assays. In addition, increases in mutations in specific oncogenes and tumor

12   suppressor genes in EtO-induced mouse tumors have been reported. Dominant-lethal mutations

13   have also been observed in several *in vivo* studies. Although data in humans are limited, there is

14   some evidence of increased frequencies of mutations from occupational studies.

15

16   **C.3.   CHROMOSOMAL ABERRATIONS**

17   The induction and persistence of EtO-induced chromosomal alterations have been studied

18   both in *in vitro* and *in vivo* systems in rodent and monkey models (Farooqi et al., 1993; Lorenti

19   Garcia et al., 2001; Kligerman et al., 1983; Lynch et al. 1984b). In addition, several studies

20   examined the association of chromosomal aberrations and EtO exposure in humans (Pero et al.,

21   1981; Stolley et al., 1984; Clare et al., 1985; Galloway et al., 1986; Sarto et al, 1984a; Theiss et

22   al., 1981; Lerda and Rizzi, 1992; WHO 2003). Chromosomal aberrations have been linked to an

23   increased risk of cancer in several large prospective studies (e.g., Liou et al., 1999; Hagmar et

24   al., 2004; Rossner et al., 2005; Boffetta et al., 2007). This section discusses key studies on EtO

25   and chromosomal aberrations.

26   Lorenti Garcia et al. (2001) studied the effect of EtO on the formation of chromosomal

27   aberrations in rat bone-marrow cells and splenocytes following *in vivo* exposure. Rats were

28   exposed to EtO either chronically by inhalation (50-200 ppm, 4 weeks, 5 days/week, 6 h/day) or

29   acutely by i.p. injection at dose levels of 50-100 ppm. Frequencies of both spontaneous and

30   EtO-induced chromosomal aberrations (and other endpoints, such as micronucleus formation and

31   SCEs, which are discussed in Sections 3.3.2.4 and 3.3.2.5) were determined in the splenocytes

1   and bone-marrow cells following *in vivo* mitogen stimulation. No significant increase in

2   chromosomal aberrations was observed from the chronic or acute exposures. In another study,

3   by Kligerman et al. (1983), no increase in chromosomal aberrations was observed in peripheral

4   blood lymphocytes from rats exposed to EtO by inhalation at concentrations of either 50, 150, or

5   450 ppm, for 6h per day, for 1 and 3 days.

6   A recent study by Donner et al. (2010) in mice, however, showed clear, statistically

7   significant increases in chromosomal aberrations with longer durations of exposure (≥ 12

8   weeks). Male B6C3F1 mice were exposed by inhalation to 0, 25, 50, 100, or 200 ppm EtO, 5

9   days/week, 6 hours/day, for 6, 12, 24, or 48 weeks. The frequency of total chromosomal

10   aberrations in peripheral blood lymphocytes was statistically significantly increased after 12

11   weeks exposure to 100 or 200 ppm EtO. By 48 weeks, statistically significant increases were

12   observed for all the exposure groups. In addition, reciprocal translocation frequencies were

13   statistically significantly increased in spermatocytes for all the exposure groups at 48 weeks.

14   Chromosomal aberrations in bone marrow cells were also reported in a study of acute EtO

15   exposure in mice (Farooqi et al., 1993). Female Swiss albino mice were administered single

16   doses of EtO in the range of 30 – 150 mg/kg by i.p. injection. A dose-related increase in

17   chromosomal aberrations in the bone marrow cells was observed.

18   Chromosomal aberrations induced by long-term exposures to inhaled EtO were also

19   investigated in the peripheral lymphocytes of cynomolgus monkeys (Lynch et al., 1984b).

20   Groups of 12 adult male monkeys were exposed at 0, 50, or 100 ppm EtO (7 hr/day, 5

21   days/week) for 2 years. Exposure to EtO at 100 ppm resulted in statistically significant increases

22   in chromosome-type aberrations in monkey lymphocytes, and exposure at both 50 and 100 ppm

23   resulted in statistically significant increases in chromatid-type aberrations and in chromosome-

24   and chromatid-type aberrations in combination. No differences in the number of gaps were

25   found.

26   Increases in chromosomal aberrations in peripheral blood lymphocytes have been

27   consistently reported in studies of workers exposed to high occupational concentrations of EtO

28   (> 5 ppm, TWA). Effects observed at lower concentrations have been mixed (WHO, 2003).

29   Chromosomal aberrations that have been detected in the peripheral blood lymphocytes of

30   workers include breaks, gaps, and exchanges and supernumerary chromosomes (Pero et al.,

1 1981; Stolley et al., 1984; Clare et al., 1985; Galloway et al., 1986; Sarto et al., 1984a; Thiess et

2 al., 1981; Lerda and Rizzi, 1992).

3   Clare et al. (1985) conducted chromosomal analyses of lymphocytes from 33 workers

4 employed in the manufacture of EtO. A slightly higher frequency of chromatid aberrations was

5 observed in workers exposed to EtO than in controls. Further, a positive correlation between

6 length of employment in the EtO-exposed group and the number of aberrations was observed. In

7 another study, Galloway et al. (1986) analyzed chromosomal aberration frequencies in 61

8 employees potentially exposed to EtO. Three work sites (I, II and III) with different historical

9 ambient levels of EtO were chosen for the study. Blood samples were drawn over a 24-month

10 period and aberrations were analyzed in 100 cells per sample after culture for 48–51 hours. At

11 work sites I and II, no consistent differences in aberration frequencies were found. However, at

12 work site III, aberration frequencies in potentially exposed individuals were significantly

13 increased when compared with controls. A previous study by the same group (Stolley et al.,

14 1984) showed an association between SCE frequency and EtO exposure. When the aberrations

15 were compared with the levels of SCEs, the authors found a weak overall association. In

16 addition, Lerda and Rizzi (1992) showed a significant increase in chromosomal aberration

17 frequencies in EtO-exposed individuals when compared with controls. Major et al. (1996)

18 studied hospital nurses exposed to low doses and high doses of EtO to identify changes in

19 structural and numerical chromosomal aberrations. Chromosomal aberrations were found to be

20 significantly elevated in both the low-dose and the high-dose exposure groups. Deletions and, to

21 a lesser extent, chromatid exchanges and dicentrics were detected in the low-dose exposure

22 group; however, in the high-dose group, in addition to the increased number of deletions, the

23 frequencies of dicentrics and rings showed a significant excess when compared with controls.

24 The authors suggest that a natural radioactivity from local tap water may have been a

25 confounding factor.

26   A study by Sarto et al. (1984a) showed significant increases in chromosomal aberrations

27 after exposure to EtO. Chromosomal aberrations were detected in the peripheral lymphocytes of

28 41 workers exposed to EtO in the sterilizing units of 8 hospitals in the Venice region compared

29 to 41 age- and smoking-matched controls. In another study of 28 EtO-exposed sterilizer workers

30 and 20 unexposed controls, Hogstedt et al. (1983) reported a statistically significant increase in

31 micronuclei, but not chromosomal breaks or gaps, in bone marrow cells (erythroblasts and

1 polychromatic erythrocytes) in the exposed workers, adjusted for age, smoking, drug intake, and

2 exposure to ionizing radiation. Tates et al. (1991) reported a significant increase in chromosomal

3 aberrations in hospital workers and in factory workers (details of this study are provided in the

4 section on gene mutations above). In a study involving small numbers (n = 4-12 per group) of

5 non-smoking males and females exposed to EtO through the sterilization of medical equipment,

6 Fuchs et al. (1994) reported 1.5-, 2.2- and 1.5-fold increases in DNA single-strand breaks in

7 peripheral blood mononuclear cells obtained from individuals exposed to EtO concentrations of

8 0.1-0.49 mg/m^3, 0.5 – 2.0 mg/m^3 and >2 mg/m^3, respectively.

9   In summary, the above data clearly indicate that EtO is genotoxic and can cause a variety

10 of chromosomal aberrations, including breaks, gaps and exchanges (reviewed in detail in Preston

11 et al. [1999]). Chromosomal aberrations have been observed in both *in vitro* and *in vivo* studies

12 in rodent models and mammalian cells. Increases in chromosomal aberrations in peripheral

13 blood lymphocytes have been consistently reported in studies of workers exposed to EtO.

14

15 **C.4.   MICRONUCLEUS FORMATION**

16   Micronucleus formation also demonstrates the genotoxic effects of a chemical. When

17 appropriate methods are used to identify the origin of the micronucleus (kinetochore-positive or

18 kinetochore-negative), this assay can provide information about a chemical's mechanism of

19 action, i.e., if a chemical causes direct DNA damage resulting from strand breaks (clastogen) or

20 indirect numerical changes (aneugen) resulting from spindle disruption. An association between

21 increased micronucleus frequency and cancer risk has been reported in at least one large

22 prospective study (Bonassi et al., 2007). Several *in vitro* and *in vivo* studies in both laboratory

23 animals (Applegren et al., 1978; Jenssen and Ramel, 1980; Lorenti Garcia et al. 2001) and

24 humans (Tates et al., 1991; Ribeiro et al., 1994; Sarto et al., 1990; Mayer et al., 1991) have been

25 conducted to explore the induction of micronuclei as a result of exposure to EtO.

26   Lorenti Garcia et al. (2001) studied the effect of EtO on the formation of micronuclei in

27 rat bone marrow cells and splenocytes following *in vivo* exposure. Rats were exposed to EtO

28 either subchronically by inhalation (50-200 ppm, 5 days/week, 6 h/day, for 4 weeks) or acutely

29 by i.p. injection at dose levels of 50 or 100 mg/kg. Spontaneous and induced frequencies of

30 micronuclei were determined in the bone marrow cells (only for acute EtO exposure) and

31 splenocytes following *in vitro* mitogen stimulation. Following chronic exposure, no significant

1 increase in micronuclei was observed in rat splenocytes. Following acute exposure, micronuclei

2 increased significantly in rat bone marrow cells as well as splenocytes.

3   The frequency of micronuclei in peripheral blood cells was increased in workers exposed

4 to relatively high (3.7 – 60.4 mg/m^3) levels of EtO (Tates et al., 1991; Ribeiro et al., 1994).

5 Schulte et al. (1992) did not observe increased micronuclei in the lymphocytes of hospital

6 workers with low levels of EtO exposure (up to 2.5 mg/m^3 8-hour TWAs). Sarto et al. (1990)

7 studied micronucleus formation in human exfoliated cells of buccal and nasal cavities to monitor

8 the genotoxic risk in a group of workers (n=9) chronically exposed to EtO (concentrations lower

9 than 0.38 ppm as time weighted average). The mean frequencies of micronucleated buccal cells

10 were similar to control values. The frequency of nasal micronucleated cells was higher than in

11 controls (0.77 vs 0.44); however, the difference was not statistically significant. In another

12 group of 3 subjects that were acutely exposed (concentration not provided) to EtO, buccal cavity

13 and nasal mucosa samples were taken 3, 9 or 16 days after acute exposure. The frequencies of

14 micronucleated buccal cells did not change, while the frequencies of micronucleated nasal cells

15 significantly increased.

16   Peripheral blood cells of 34 EtO-exposed workers at a sterilization plant and 23

17 unexposed controls were assessed for different biological markers such as EtO-hemoglobin

18 adducts, SCEs, micronuclei, chromosomal aberrations, DNA single-strand breaks and an index

19 of DNA repair (Mayer et al., 1991). Neither chromosomal aberrations nor micronuclei differed

20 significantly by exposure status, whether or not adjusted for smoking status.

21   In summary, increases in the frequency of micronuclei have been observed in *in vivo*

22 animal studies. The frequency of micronuclei in peripheral blood cells was also increased in

23 workers exposed to relatively high (3.7 – 60.4 mg/m^3) levels of EtO (Tates et al., 1991; Ribeiro

24 et al., 1994). However, in the majority of human studies involving exposures at lower levels, no

25 effects on the frequency of micronuclei were observed. Apparent inconsistencies in the data

26 could reflect the influence of peak exposures, differences in exposure measurement errors,

27 duration of exposure and/or smoking status.

28

29 **C.5.   SISTER CHROMATID EXCHANGES (SCEs)**

30   There is a significant body of evidence for the induction of SCEs as a result of exposure

31 to EtO. Studies have been conducted both in laboratory animals (Kligerman et al., 1983; Lynch

1   et al., 1984b; Kelsey et al., 1988; Lorenti Garcia et al., 2001; Yager and Benz, 1982; Ong et al.,

2   1993) and in humans (Garry et al., 1979; Galloway et al.,1986; Laurent et al., 1984; Sarto et al.,

3   1984a, 1984b; Stolley et al., 1984; Yager et al., 1983; Agurell et al., 1991). In particular,

4   several occupational exposure studies have yielded positive results when EtO-exposed workers

5   were studied. The following is a summary of both the animal and human studies.

6   Inhalation studies with rats have shown that exposures to EtO at 50 ppm or more for 3

7   days result in an increase in SCEs in peripheral blood lymphocytes (Kligerman et al., 1983).

8   Increased incidences of SCEs in the peripheral blood lymphocytes of monkeys exposed to EtO at

9   500 or 100 ppm were also reported by Lynch et al. (1984b). A follow-up study in these same

10   monkeys by Kelsey et al. (1988) indicated that the high SCE counts persisted for 6 years after

11   exposure.

12   Lorenti Garcia et al. (2001) studied the effect of EtO on the persistence of SCEs in rat

13   bone marrow cells and splenocytes following *in vivo* exposure. Rats were exposed to EtO either

14   chronically by inhalation (50-200 ppm, 5 days/week, 6 h/day, for 4 weeks) or acutely by i.p.

15   injection at dose levels of 50 or 100 mg/kg. Frequencies of SCEs were determined in the bone

16   marrow cells and splenocytes after *in vitro* mitogen stimulation. Following chronic exposure,

17   cytogenetic analyses were carried out at days 5 and 21 in the splenocytes. In these experiments,

18   EtO was effective in inducing SCEs, and marked increases in cells with high frequency SCEs

19   were observed which persisted until day 21 post-exposure. Following acute exposure, SCEs

20   were increased significantly in rat bone marrow cells as well as splenocytes.

21   New Zealand white male rabbits (n=4) were exposed in inhalation chambers to 0, 10, 50,

22   and 250 ppm EtO for 6 hours a day, 5 days a week, for 12 weeks (Yager and Benz, 1982).

23   Peripheral blood samples were drawn in three regimes (before the start of exposure, at intervals

24   during exposure, and up to 15 weeks after the end of exposure) to measure SCE rates. No

25   change in SCE rates was observed from exposure to 10 ppm; however, an increase was seen after

26   exposure to 50 and 250 ppm. Above-baseline levels were observed even after 15 weeks post-

27   exposure, although the levels were not as high as during exposure. These results indicate that

28   inhalation exposure to the EtO results in a dose-related increase in SCEs.

29   The ability of long-term exposures to inhaled EtO to induce SCEs in peripheral

30   lymphocytes of monkeys was investigated by Lynch et al. (1984b). Groups of 12 adult male

31   cynomolgus monkeys were exposed at 0, 50, or 100 ppm EtO (7 hr/day, 5 days/week) for 2

1   years. Statistically significant increases in SCE rates were observed in monkey lymphocytes in

2   both exposure groups. Both exposure groups had increased numbers of SCEs/metaphase as

3   compared to controls, and these numbers increased in a dose-dependent manner.

4   In an *in vitro* study of human cells, peripheral lymphocyte cultures were exposed to

5   methyl bromide, EtO, and propylene oxide, as well as diesel exhaust (Tucker et al., 1986). SCE

6   frequency was measured, and the frequency more than doubled in the cultures treated with EtO.

7   Agurell et al. (1991) also studied the effect of EtO on SCEs in human peripheral blood

8   lymphocytes *in vitro*. An increase in SCE frequency was observed as a result of exposure (0-20

9   mMh) to EtO. Similarly, Hallier et al. (1993) observed that the frequency of SCEs in human

10   peripheral blood lymphocytes exposed *in vitro* to EtO was higher in cells isolated from

11   individuals expressing low levels of glutathione S-transferase T1 than in cells from subjects

12   expressing higher levels of this enzyme.

13   Several studies of EtO-exposed workers have also reported an increased incidence of

14   SCEs in peripheral lymphocytes (e.g., Garry et al., 1979; Yager et al., 1983; Sarto et al., 1984a,

15   1984b; Galloway et al., 1986; Schulte et al., 1992).

16   Garry et al. (1979) analyzed SCEs in lymphocytes cultured from EtO-exposed individuals

17   as well as comparable controls. Significant increases in SCEs were observed at three weeks and

18   at eight weeks following exposure. Although this study does not describe the exact exposure

19   estimates, EtO was recognized as a mutagenic or genotoxic agent. Laurent et al. (1984) studied

20   SCE frequency in workers exposed to high levels of EtO in a hospital sterilization service.

21   Blood samples were obtained retrospectively from a group of 25 subjects exposed to high levels

22   of EtO for a period of two years. A significant increase in SCEs was observed in the exposed

23   group when compared with the control group. The authors concluded that the effect of exposure

24   to EtO was sufficient to produce a cumulative and, in some cases, a persistent genetic change.

25   Peripheral blood lymphocytes of nurses exposed to low and high concentrations of EtO

26   were studied by Major et al. (1996). SCEs were slightly elevated in the low-exposure group but

27   were significantly increased in the high-exposure group. Similarly, several studies by Sarto et al.

28   (1984a, 1984b 1987, 1990, 1991) showed significant increases in SCEs.

29   Tates et al. (1991) studied workers occupationally exposed to EtO using different

30   physical and biological measures. Blood samples from 9 hospital workers and 15 factory

31   workers engaged in sterilization of medical equipment with EtO and from matched controls were

1 collected. Exposures were usually received in bursts, with EtO concentrations in air ranging

2 from 22 to 72 ppm in hospital workers and 14 to 400 ppm in factory workers. The mean

3 frequency of SCEs was significantly elevated by 20% in hospital workers and by almost 100% in

4 factory workers. In contrast, no significant increase in SCEs was observed in lymphocytes of

5 workers who were accidentally exposed to high concentrations of EtO or of workers with low

6 exposure concentrations (Tates et al., 1995).

7   Schulte et al. (1992) observed a statistically significant increase in SCEs in 43 workers

8 exposed to EtO in U.S. hospitals compared to 8 unexposed hospital workers. The frequency of

9 SCEs was also significantly associated with cumulative EtO exposure in a regression analysis

10 that controlled for various potential confounding factors, including smoking. A similar

11 relationship was not observed in 22 Mexican hospital workers. Schulte et al. (1992)

12 hypothesized that the difference may have been due to longer shipping times of the Mexican

13 specimens for the cytogenetic assays.

14   In summary, significant increases in the frequency of SCEs were observed in rats and in

15 monkeys both by inhalation and intraperitoneal injection. In humans, multiple occupational

16 studies have reported positive responses, with significant increases in frequency of SCEs in

17 peripheral blood lymphocytes having been observed among individuals exposed to higher levels

18 of EtO. In some studies, increases in the frequency of SCEs have been observed to persist after

19 exposure has ceased. The results of studies of individual workers exposed to very low levels (<

20 0.9 mg/m^3) of EtO have been mixed.

21

22 **C.5.1. Other Endpoints (Genetic Polymorphism, Susceptibility)**

23   Dose-dependent effects of polymorphisms in the genes for epoxide hydrolase (*EPHX1*),

24 different subfamilies of glutathione-*S*-transferase (*GSTM1, GSTP1, GSTT1*) and various DNA

25 repair enzymes (*hOGG1, XRCC1, XRCC3*) on EtO-induced genotoxicity were evaluated by

26 Godderis et al. (2006). Peripheral blood mononuclear cells from 20 individuals were exposed to

27 3 doses of EtO (0.45, 0.67, 0.9 mM), and genotoxicity was evaluated by measuring comet tail

28 length and micronucleus frequencies in binucleated cells (MNBC). A dose-dependent increase

29 in tail length (indicating DNA strand breaks) was observed in exposed individuals compared to

30 controls. No change in MNBC was observed. None of the epoxide hydrolase or glutathione-*S*-

31 transferase polymorphisms had a significant influence on the tail length or MNBC results for any

EtO dose. Further analysis revealed a significant contribution of the *hOGG1* (involved in base excision repair) and *XRCC3* (involved in repair of cross-links and chromosomal double-strand breaks) genotypes to the inter-individual variability of EtO-induced increases in tail length. Homozygous *hOGG1[326]* wild type cells showed significantly lower effects of EtO on tail length compared to the heterozygous cells. Also, significantly higher tail lengths were found in EtO-exposed cells carrying at least one variant *XRCC3[241]* Met allele. For the latter effect, there was a significant interaction between the *XRCC3[241]* polymorphism and dose, signifying a greater impact of the polymorphism on DNA damage at higher doses.

In contrast to the findings of no significant effect of glutathione-*S*-transferase polymorphisms on DNA breaks and micronuclei production by Godderis et al. (2006), Hallier et al. (1993) observed that the frequency of SCEs in human peripheral blood lymphocytes exposed *in vitro* to EtO was higher in cells isolated from individuals expressing low levels of GSTT1 than in cells from subjects expressing higher levels of this enzyme. Similarly, Yong et al. (2001) measured approximately twofold greater EtO-hemoglobin adduct levels in occupationally exposed persons with a *GSTT1*-null genotype than in those with positive genotypes.

Primary and secondary cultures of lymphoblasts, breast epithelial cells, peripheral blood lymphocytes, keratinocytes and cervical epithelial cells were exposed to 0-100 mM EtO, and DNA damage was measured using the comet assay (Adam et al., 2005). A dose-dependent increase in DNA damage was observed in all cell types without notable cytotoxicity. Breast epithelial cells (26% increase in tail length) were more sensitive than keratinocytes (5% increase) and cervical epithelial cells (5% increase) but less sensitive than lymphoblasts (51% increase) and peripheral lymphocytes (71% increase) at the same dose of 20 mM.

C.6.   ENDOGENOUS PRODUCTION OF ETHYLENE AND EtO

Ethylene, a biological precursor of EtO, is ubiquitous in the environment as an air pollutant and is produced in plants, animals and humans (Abeles and Heggestad, 1973). Ethylene is generated *in vivo* endogenously during normal physiological processes such as (i) oxidation of methionine, (ii) oxidation of hemoglobin, (iii) lipid peroxidation and (iv) metabolism of intestinal bacteria (reviewed by IARC, 1994a; Thier and Bolt, 2000). Recently, Marsden et al. (2009) proposed that oxidative stress can induce the endogenous formation of ethylene, which can in turn be metabolized to EtO. Endogenous production of ethylene has been

1   documented in laboratory animals and in humans (Chandra and Spencer, 1963; Ehrenberg et al.,

2   1977; Shen et al., 1989; Filser et al., 1992).

3   Shen et al (1989) reported an endogenous production rate of 2.8 and 41 nmol/h ethylene

4   in Sprague-Dawley rats and humans, respectively, with similar thermodynamic partition

5   coefficients between the two species. Filser et al. (1992) reported a low degree of endogenous

6   production of ethylene (32 ±12 nmol/h) in healthy volunteers based on exhalation data. The

7   authors indicated that the endogenous levels of ethylene would account for ~66% of the

8   background level of EtO-hemoglobin adducts (HEVal), while the remaining one-third (15 ppb) is

9   contributed by exogenous environmental ethylene exposure. Although the percentage of

10 endogenous ethylene converted to EtO is not known, Tornqvist et al. (1989) have shown that in

11 fruit-store workers exposed to 0.3 ppm ethylene, only 3% is metabolized to EtO. Thus, the

12 amount of endogenous ethylene converted to EtO would be minimal. Furthermore, with

13 inadequate laboratory animal and human evidence available for ethylene as a carcinogen (IARC

14 1994a), exogenous ethylene exposure may not produce enough EtO to contribute significantly to

15 carcinogenicity under standard bioassay conditions (Walker et al., 2000).

16   Ethylene formed from endogenous sources is converted to EtO by cytochrome P450-

17 mediated metabolism (Tornqvist, 1996; IARC, 1994a). EtO formed from the endogenous

18 conversion of ethylene leads to 2-hydroxyethylation of DNA and forms N7-HEG adducts

19 contributing to the background levels of this adduct in unexposed humans and rodents.   As

20 shown in Table C-1, improvements in analytical methodology have led to the detection and

21 quantification of background N7-HEG adducts in DNA of unexposed experimental animals and

22 humans (Fost et al., 1989; Cushnir et al., 1991; Leutbecher et al., 1992; Walker et al., 1992a,

23 2000; Farmer et al., 1993; van Delft et al., 1993, 1994; Kumar et al., 1995; Bolt et al., 1997;

24 Zhao et al., 1997, 1999; Eide et al., 1999; Farmer and Shuker, 1999; Wu et al., 1999a, 1999b;

25 van Sittert et al., 2000; Swenberg et al., 2000, 2008; Marsden et al., 2007, 2009; Tompkins et al.,

26 2008). However, there is a wide variation in the levels of adducts detected in rodents and

27 humans which appears to depend on the type of the analytical method used. Even with the most

28 advanced techniques (Tompkins et al., 2008), minor DNA adducts such as O^6-HEG and N3-HEA

Table C-1. Levels of endogenous (background) N7-HEG adducts in unexposed human and experimental rodent tissues

Species	Tissue	Detection method	Adduct levels reported	*Adduccts/10^7 nucleotides	Reference
Human	Lymphocytes	GC/MS	8.5 pmol/mg DNA	28.05	Fost et al., 1989
Human	WBC	Immuno-slotblot	0.34 adducts/10^6 nucleotides	3.4	van Delft et al., 1994
Human	Blood	HPLC-fluorescence	3.2 pmol/mg DNA	10.56	Bolt et al., 1997
Human	Lymphocytes	GC/MS	2–19 adducts per 10^7 nucleotides	2.0–19	Wu et al., 1999b
Human	WBC	^{32}P/TLC/HPLC	0.6 adducts/10^7 nucleotides	0.6	Zhao et al., 1999
Human	WBC	^{32}P/TLC/HPLC	2.9 adducts/10^7 nucleotides	2.9	Zhao et al., 1999
Human	Lung	^{32}P/TLC/HPLC	4.0 adducts/10^7 nucleotides	4	Zhao et al., 1999
Rat	Lymphocytes	GC/MS	5.6 pmol/mg DNA	18.48	Fost et al., 1989
Mice/Rats	Control tissues	HPLC-fluorescence	2–6 pmol /mg DNA	8.58	Walker et al., 1992a
Rat	Liver, kidney, spleen	^{32}P/GC/MS	0.4 to 1.1 adducts/10^7 nucleotides	0.4–1.1	Eide et al., 1999
Mice/Rats	Spleen	GC/EC/NCI-HRMS	0.2 to 0.3 pmol/mmol guanine		Wu et al., 1999a
Rat	Control tissues	^{32}P/TLC/HPLC	0.6 to 0.9 adducts/10^7 nucleotides	0.6–0.9	Zhao et al., 1999
Rat	Liver	GC/MS	2.6 adducts/10^8 nucleotides	0.26	van Sittert et al., 2000
Rat	Control tissues	LC-MS/MS	1.1–3.5 adducts/10^8 nucleotides	0.11–0.35	Marsden et al., 2007
Rat	Liver	HPLC/ESI TMS	8 adducts/10^8 normal nucleotides	0.8	Tompkins et al., 2008
Rat	Spleen	HPLC/LC-MS/MS	0.08 adducts/10^{10} nucleotides	0.00008	Marsden et al., 2009

Table C-1. Levels of endogenous (background) N7-HEG adducts in unexposed human and experimental rodent tissues (continued)

Adduct levels are normalized using the formula: 1 pmol adducts/mg DNA = 3.3 adducts/10^7 normal nucleotides.
GC/MS, gas chromatography mass spectrometry; HPLC, high performance liquid chromatography; ^{32}P, ^{32}P-postlabeling assay; TLC, thin-layer chromatography; LC-MS, liquid chromatography mass spectrometry; ESI TMS, electrospray ionization tandem mass spectrometry; GC/EC/NCI-HRMS, gas chromatography/electron capture/negative chemical ionization high-resolution mass spectrometry.

1   were below the level of detection. Also, some researchers consistently demonstrated higher

2   background levels of DNA adducts (Walker et al., 1992a; Wu et al., 1999a). However, the

3   higher background levels in some of these studies are possibly due to the methodology used,

4   which may have caused an artifactual increase in the adduct levels.

5   Using sensitive detection techniques and an approach designed to separately quantify

6   both endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO

7   treatment in F344 rats, Marsden et al. (2009) recently reported increases in exogenous adducts

8   in DNA of spleen and liver consistent with a linear dose-response relationship ($p < 0.05$), down

9   to the lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days). Note that the

10   whole range of doses studied by Marsden et al. (2009) lies well below the dose corresponding

11   to the lowest LOAEL from an EtO cancer bioassay. For example, an approximate calculation

12   indicates that the low exposure level of 10 ppm for 6 hours/day used in the Snellings et al.

13   (1984) bioassay of F344 rats is equivalent to a daily dose of about 1.7 mg/kg, which is over 10

14   times higher than the largest daily dose of 0.1 mg/kg used by Marsden et al. (2009).[24]

15   In summary, endogenous ethylene and EtO production, which contribute to background

16   N7-HEG DNA adducts indicative of DNA damage, have been observed in unexposed rodents

17   and humans. Although a constant reduction in DNA damage *in vivo* is carried out by DNA

18   repair and DNA replicative synthesis, a certain steady-state background level of adducts is

19   measurable at all times. The quantitative relationships between the background DNA damage

20   and the spontaneous rates of mutation and cancer are not well established. Experimental

21   evidence is needed that can unequivocally measure artifact-free levels of background DNA

22   damage, including effects other than adducts, clearly establish mutagenic potency of such

23   background lesions, and demonstrate the organ- and cell type-specific requirements for the

24   primary DNA damage to be expressed as heritable genetic changes (Gupta and Lutz, 1999).

25   Some investigators have posited that the high and variable background levels of

26   endogenous EtO-induced DNA damage in the body may overwhelm any contribution from

27   exogenous EtO exposure (SAB, 2007; Marsden et al., 2009). It is true that the existence of

---

[24] This calculation uses the mean alveolar ventilation rate for rats of 52.9 mL/min/100 g reported by Brown et al. (1997). Changing the units, this rate is equivalent to approximately 0.032 m^3/hour/kg. For a 6-hour exposure, this results in an alveolar inhalation of 0.19 m^3/kg. 10 ppm EtO is equivalent to 18.3 mg/m^3, so a 6-hour exposure equates to about 3.48 mg/kg. IARC (2008) reports that measurements from Johanson and Filser (1992) indicate that only 50% of alveolar ventilation is available to be absorbed into the bloodstream, so the 6-hour exposure to 10 ppm EtO would approximate an absorbed daily dose of 1.7 mg/kg.

1 these high and variable background levels may make it hard to observe statistically significant

2 increases in risk from low levels of exogenous exposure. However, there is clear evidence of

3 carcinogenic hazard from the rodent bioassays and strong evidence from human studies

4 (Chapter 3, Section 3.5), and the genotoxicity/mutagenicity of EtO (Section 3.4) supports low-

5 dose linear extrapolation of risk estimates from those studies (U.S. EPA, 2005). In fact, as

6 discussed above, Marsden et al. (2009) reported increases in exogenous adducts in DNA of

7 spleen and liver consistent with a linear dose-response relationship ($p < 0.05$), down to the

8 lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days, which is a very low dose

9 compared to the LOAELs in the carcinogenicity bioassays). Furthermore, while the

10 contributions to cancer risk from low exogenous EtO exposures may be relatively small

11 compared to those from endogenous EtO exposure, low levels of exogenous EtO may

12 nonetheless be responsible for levels of risk (above background risk) that exceed *de minimis*

13 risk (e.g., $> 10^{-6}$). This is not inconsistent with the much higher levels of background cancer

14 risk, to which endogenous EtO may contribute, for the two cancer types observed in the human

15 studies—lymphoid cancers have a background lifetime incidence risk on the order of 3%,

16 whereas the background lifetime incidence risk for breast cancer is on the order of 15%.

17

18 **C.7.   CONCLUSIONS**

19   The overall available data from *in vitro* studies, laboratory animal studies, and human

20 studies indicate that EtO is both a mutagen and a genotoxicant. In addition, increases in

21 mutations in specific oncogenes and tumor suppressor genes in EtO-induced mouse tumors

22 have been reported. Stable translocations seen in human leukemias may arise from similar

23 DNA adducts that produce chromosome breaks, micronuclei, SCEs, and even gene mutations

24 observed in peripheral lymphocytes. Dominant lethal mutations, heritable translocations,

25 chromosomal aberrations, DNA damage, and adduct formation in rodent sperm cells have been

26 observed in a number of studies involving the exposure of rats and mice to EtO. Based upon

27 the likely role for DNA alkylation in the production of the genotoxic effects in germ cells in

28 laboratory animals exposed to EtO, as well as the lack of qualitative differences in the

29 metabolism of EtO between humans and laboratory animals, EtO can also be considered a

30 likely human germ cell mutagen (WHO, 2003). There is consistent evidence that EtO interacts

31 with the genome of cells within the circulatory system in occupationally exposed humans and

32 overwhelming evidence of carcinogenicity and genotoxicity in laboratory animals. Based on

1   these considerations, there is a strong weight of evidence suggesting that EtO would be

2   carcinogenic to humans (Chapter 3, Section 3.4).

APPENDIX D
RE-ANALYSES AND INTERPRETATION OF ETHYLENE OXIDE EXPOSURE-RESPONSE DATA

Kyle Steenland
May 27, 2010

(EDITORIAL NOTE: This Appendix contains the report submitted by Dr. Steenland summarizing the results of analyses that he conducted under contract to U.S EPA. The terminology originally used by Dr. Steenland to designate the different exposure-response model forms has been changed to be consistent with the terminology used in EPA's Ethylene Oxide Carcinogenicity Assessment. Models that are linear in log RR and which were previously referred to as "linear" models have been renamed "log-linear" models (except where it is stated that they are log RR models), and models of the form RR = 1 + β × exposure, which were previously referred to as "excess relative risk" (ERR) models have been renamed "linear" models.)

This report contains the results of re-analyses of the National Institute for Occupational Safety and Health cohort of workers exposed to ethylene oxide conducted for the U.S. Environmental Protection Agency. The report begins with an overview of the modeling strategy used, followed by the results of re-analyses of the breast cancer incidence, breast cancer mortality, lymphoid cancer mortality, and, finally, hematopoietic cancer mortality databases. Various models were used for these re-analyses, as discussed in this report. The report concludes with the results of some sensitivity analyses and discussions of the possible influences of the healthy worker survivor effect and exposure mis-measurement.

Introduction. Modeling strategy for ethylene oxide (ETO) risk assessment

The modeling strategy adopted here for ETO risk assessment relies principally on the usual epidemiologic models in which the log of the rate ratio (RR) is some function of exposure, in this case cumulative exposure with a lag to reflect a length of time which is likely necessary before an exposure can result in (observable or fatal) cancer. We have relied primarily on Cox regression as a flexible method of modeling the log RR; however we have also included some linear relative risk models. Cumulative exposure is typically the exposure metric of interest in predicting chronic disease.

1   For breast cancer incidence, we have relied principally on 2-piece linear models, in which log

2   RR (in the log-linear model) or RR (in the linear model) is a function of two lines which join

3   smoothly at a single point of inflection. Two-piece linear models may also be thought of as

4   linear splines with one knot, or point of inflection. They have been described as part of a

5   general description of exposure-response modeling by Steenland and Deddens (2004) and

6   have been used previously in risk assessment (e.g., see the risk assessment for dioxin by

7   Steenland et al. (2001)). The 2-piece log-linear model has the form log RR = β_0 +

8   β_1*cumexp + β_2*(max(0,cumexp-knot)), where cumexp is cumulative exposure, the last term

9   equals either 0 or cumexp-knot, whichever is greater, and the knot is the point of inflection or

10 point of change of slope for the 2 linear pieces. The slope of the last term is $\beta_1+\beta_2$ for

11 cumulative exposure values above the knot.

12

13 Log RR models are not linear when the log RR function is transformed via exponentiation

14 back to a non-logarithmic function, but they are nearly so in the low dose region of interest.

15 The splines are linear using the linear RR model.

16

17 "Plateau-like" exposure-response curves, in which the exposure-response curve begins

18 steeply but is attenuated at higher exposure, have been seen for many occupational

19 carcinogens. This may occur for a variety of reasons, including depletion of susceptible sub-

20 populations, mismeasurement at high exposure resulting in attenuation, and the healthy

21 worker survivor effect (Stayner et al., 2003). Attenuation of the exposure-response

22 relationship occurs for the breast cancer and (lympho) hematopoetic endpoints of interest for

23 ETO. For these endpoints, a simple linear model (often considered the default model), where

24 the log RR (for the log-linear model) or the RR increases linearly with cumulative exposure,

25 does not fit the data well, based on simple visual inspection of the categorical data.

26

27 Frequently, such plateau-like curves may be modeled by using the log of cumulative

28 exposure rather than cumulative exposure itself, but this has the disadvantage that the curve

29 is usually highly supra-linear at low doses. Two-piece linear spline models are particularly

30 useful in modeling exposure-response relationships in which the log RR or RR increases

31 initially with increasing exposure but then tends to increase less or plateau at high exposures.

32 The 2-piece linear models avoid this supra-linearity in the low-dose region (Steenland and

33 Deddens, 2004).

34

35 The shape of the 2-piece linear spline model, in particular the slope of the curve in the low-

36 dose region, depends on the choice of the point of inflection where the two linear pieces are

1   joined. Here we have chosen the point of inflection based on the best model likelihood,

2   trying a range of points of inflection (knots) across the range of exposure starting from 0 and

3   incrementing by 100 ppm-days (or 1000 ppm-days) intervals. The model likelihood often

4   does not change much across these different points of inflection, but it does change some and

5   we have chosen the point of inflection resulting in the best model likelihood. The model

6   likelihood used to find the best fit in all models used in this analysis is the usual partial

7   likelihood (Langholz and Richardson, 2010), as used with the Cox models, which maximizes

8   the probability, across all the cases, that a case fails (the numerator) relative to its case-

9   control risk set (which includes the case) (the denominator) and has the form

10

11    $L(\beta) = \varphi_{case}(Z;\beta)/ \Sigma_{j \text{ cases and controls}} \varphi_j(Zj;\beta),$

12

13   where $\varphi(Z;\beta)$ is some function of a vector of covariates Z and the parameters of interest β.

14   For example, for the linear RR model with only cumulative exposure in the model, $\varphi(Z;\beta) =$

15    $1 + z\beta$, where z is cumulative exposure and β is the exposure-response coefficient of interest.

16   For the log RR model, $\varphi(Z;\beta) = e^{(z\beta)}$.

17

18   While the 2-piece models work well for ETO breast cancer incidence, they do not for

19   hematopoetic cancer (and to a lesser extent for breast cancer mortality) because the best

20   knots are at very low doses and the resulting slopes for the first piece of the 2-piece model

21   are very steep, resulting in the same problem which occurs using log transform models (i.e.,

22   where the exposure metric is the log of cumulative exposure)). Risk for hematopoetic cancer

23   in fact increases quite steeply with very low exposure versus no exposure, and then plateaus

24   at higher exposures. This may be partly a result of the relatively small numbers of

25   hematopoetic cancers and the overall instability of the results. In this case, EPA's original

26   approach of a weighted regression through categorical RRs is a reasonable alternative to both

27   the log transform and 2-piece models.

28

29

30

1 **1. Breast cancer incidence based on the data with interviews**

2

3 **a. Distribution of exposure among ETO-exposed women in breast cancer incidence**
4 **cohort with interviews (n=5139)**

5

6 The estimated daily exposure to ETO across different jobs and time periods ranged from 0.05
7 ppm to 77 ppm. Exposure intensities from this broad range were multiplied by the length of
8 time in different jobs to get estimates of cumulative exposure. The duration of exposure had
9 a mean of 10.8 years (std dev 9.1), and a median of 7.4 years. The range was from 1.00 to
10 50.3 years. The 25th percentile was 2.8 years and the 75 percentile was 17.6 years.
11 Multiplying exposure intensity and exposure duration results in a wide range of cumulative
12 exposures.

13

14 Cumulative exposure at the end of follow-up, with no lag, had a mean of 13,524 ppm-days
15 (37.0 ppm-years), with a standard deviation of 13,254 ppm-days. These data are highly
16 skewed, with a range from 5 to 253,848 ppm-days. The 25th percentile is 926 ppm-days,
17 while the 75th is 10,206 ppm-days. Log transformation of these data results in an
18 approximately normal distribution of the data.

19

20 As a caveat, it should be remembered that cumulative exposure at the end of follow-up may
21 be misleading, as it is not relevant to standard analyses, all of which treat cumulative
22 exposure as a time-dependent variable which must be assessed at specific points in time. For
23 example, standard life table analyses calculate cumulative exposure at different times during
24 follow-up for each person. Subsequently, both person-time and disease events are put into
25 categories of cumulative exposure. A given person may pass through many such categories,
26 contributing person-time to each. Poisson regression, analogous to life table analyses (and
27 often based directly on output from life table programs), similarly relies on person-time (and
28 disease occurrence) categorized by cumulative exposure. Both these types of analyses are
29 inherently categorical.

30

31 In the analyses presented here, we have used Cox regression in which age is the time
32 variable. The basic approach is to compare each case to a set of 100 randomly chosen
33 controls, whose exposure is evaluated at the same age at which the case fails (gets disease or
34 dies of disease). Using 100 controls generally would be expected to give the same result as
35 the full risk set and shortens analysis time (Steenland and Deddens, 1997). Hence, again
36 cumulative exposure is time dependent. For the case who fails at an early age, the

1   cumulative exposure of the case and many of his or her controls at that same age may be low.

2   For the case who fails late in life, the cumulative exposure of the case and his or her controls

3   will be higher. When cumulative exposure is lagged so that no exposure is counted until

4   after a lag period (e.g., 15 years) is fulfilled, many cases and their respective controls will be

5   'lagged out', i.e., will have no cumulative exposure, if the case fails at an early age. Note

6   that Cox regression uses individual data, and there is no inherent categorization typical of life

7   table analyses and Poisson regression, although categorical analyses can still be done in Cox

8   regression and are often useful.

9

10 For these reasons, it is difficult to describe the cumulative exposure distribution of all

11 subjects in the Cox regression. Controls may appear more than once matched to different

12 cases, and their cumulative exposure will differ each time depending on the age of the case.

13 However, cases only appear once in the data and their exposure distribution can be easily

14 presented. In our situation, we have used Cox regression with a 15-year lag to analyze breast

15 cancer incidence. The exposure distribution of the cases, by deciles above the lagged out

16 category, is shown below. Creating deciles such that cases are equally distributed is a good a

17 priori way of creating categories in which rate ratios will have approximate equal variance, a

18 desirable feature. The lagged out cases are women who got incident breast cancer within 15

19 years of first exposure.

20

21

Table 1. Distribution of cases in Cox regression for breast cancer morbidity analysis after using a 15-year lag

Cumulative exposure, 15-year lag	Number of incident breast cancer cases
0 (Lagged out)	62
0–355 ppm-days	17
356–842 ppm-days	16
843–1361 ppm-days	17
1362–2187 ppm-days	17
2188–3772 ppm-days	17
3773–5522 ppm-days	18
5523–7891 ppm-days	16
7892–14483 ppm-days	17
14484–25112 ppm-days	17
>25112 ppm-days	18

b.1. Results of Cox regression analysis of breast cancer incidence using a variety of (log RR) models

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2003). Age was the time variable in proportional hazards (Cox) regression. For breast cancer incidence, family history of breast cancer, date of birth (quartiles), and parity were included in models along with exposure variables. For our exposure variable, we used cumulative exposure lagged 15 years, which was found in prior analyses to provide the best fit to the data (Steenland et al., 2003).

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a log transform model, a cubic spline model, and a square-root transform model. We also ran a number of analogous models using linear RR models.

The categorical analysis (log RR model) used deciles, as indicated in Table 2a. Deciles were used instead of the original quintiles from the publication (Steenland et al., 2003) because the

relatively large sample size enabled more extensive categorization. Results of the categorical

decile analysis are in Table 2a below.

Table 2a. Categorical analysis of breast cancer incidence by deciles (log RR model)

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CAT1	-0.09015	0.29318	0.0945	0.7585	0.914
CAT2	-0.08363	0.30341	0.0760	0.7828	0.920
CAT3	0.18536	0.29757	0.3880	0.5333	1.204
CAT4	0.12606	0.29995	0.1766	0.6743	1.134
CAT5	0.07900	0.29968	0.0695	0.7921	1.082
CAT6	0.37651	0.29675	1.6097	0.2045	1.457
CAT7	0.38177	0.31168	1.5003	0.2206	1.465
CAT8	0.25179	0.30640	0.6753	0.4112	1.286
CAT9	0.57845	0.31120	3.4551	0.0631	1.783
CAT10	0.80396	0.30766	6.8284	0.0090	2.234

    -2 LOG L   1936.910, df=15 (10 exposure terms, 5 covariates)

We then fit a cubic spline (restricted at the ends to be linear) which presents a description of

the data similar to the categorical analyses but using a smooth curve. The exposure metric

was cumulative exposure with a 15-year lag, which was found in earlier analyses to be the

optimal lag (Steenland et al., 2003). Five knots for the cubic spline were chosen using every

other midpoint from the categorical analysis (598, 1774, 4647, 11187, and 37668 ppm-days).

We then ran a 2-piece linear (log RR) model. The knot, or inflection point, was chosen to be

the one where the model likelihood was highest, which was at 5,800 ppm-days. To choose

this knot we looked at possible inflection points over the range 100 to 15,000 ppm-days by

100 ppm-day increments. Figure 1a shows the -2 log likelihood graphed against the knots.

In this figure the lower peak corresponds to the highest likelihood.

Figures 1b and 1c show the results of the 2-piece linear, the categorical, the linear, and the

cubic spline (log RR) models. In these figures the categorical points are the mid-points of

the categories in Table 1, with final category assigned the final cutpoint plus 50%.

1   It appears that the two-piece log-linear curve in Figure 1b approximates the shape of the
2   exposure-response seen in the decile and cubic spline (log RR) analyses, better than the log-
3   linear curve in Figure 1c.
4
5   The log-linear curve appears to have a low slope versus the other models, suggesting possible
6   influential observations in the upper tail of exposure. To further explore this, we excluded
7   from the analysis increasing amounts of the upper tail of the data using the log-linear model,
8   i.e., via excluding the upper 1%, 2.5%, 5%, 10%, 15%, 20%, and 27% of exposure, based
9   on the exposure distribution of the cases (the last amount, 27%, corresponds to excluding
10   subjects with cumulative exposure above 6000 ppm-days, which was close to the knot in the
11   2-piece log-linear model (5800 ppm-days). The ratios of the slope (coefficient) for the linear
12   term (log RR model) with these exclusions vs. the slope for the linear term (log RR model)
13   with no exclusions were 1.5, 2.3, 3.2, 3.2, 2.5, 3.1, 6.1, 9.2, respectively. As expected, the
14   slope increases markedly as the data are restricted to the lower range of exposure. For
15   example, a modified log-linear curve after excluding the upper 5% of the data is seen in
16   Figure 1d, along with the full log-linear curve from Figure 1c. Nonetheless, even the log-
17   linear curve from these truncated data has a markedly lower slope in the low-exposure region
18   than the 2-piece log-linear (or spline) curves. For example, inspection shows that the RR for
19   6000 ppm-days is about 1.2 for the log-linear curve from the truncated data and 1.6 from the
20   2-piece log-linear model. Use of the log-linear curve based on truncated data has the
21   disadvantage of having to choose rather arbitrarily where to truncate the data.   This
22   disadvantage is avoided by using the 2-piece log-linear model.
23
24   A 2-piece log-linear model, then, is preferred for estimating risk parsimoniously in the low-
25   exposure region. For comparison purposes, we also show the model using the logarithm of
26   exposure (Figure 1e), which we have not used for risk assessment because it is supra-linear in
27   the low-dose region.
28
29   We also fit a square-root transformation (square root of cumulative exposure, 15-year lag)
30   log RR model, which is shown in Figure 1f. This model also fit the breast cancer morbidity
31   well (it did not fit the other outcomes well and is not shown for them), and can be used for
32   risk assessment, but with the disadvantage that it is not linear or approximately linear in the
33   low-dose region. For this reason, we prefer the 2-piece log-linear curve, with is
34   approximately linear in the low-dose region (and strictly linear in the linear RR models
35   discussed below). Excess lifetime risk does not vary greatly between all these models (see

1   below), with the exception of the log RR model with a linear term for cumulative exposure,

2   which is below other excess risk estimates.

3

4

5

6

7
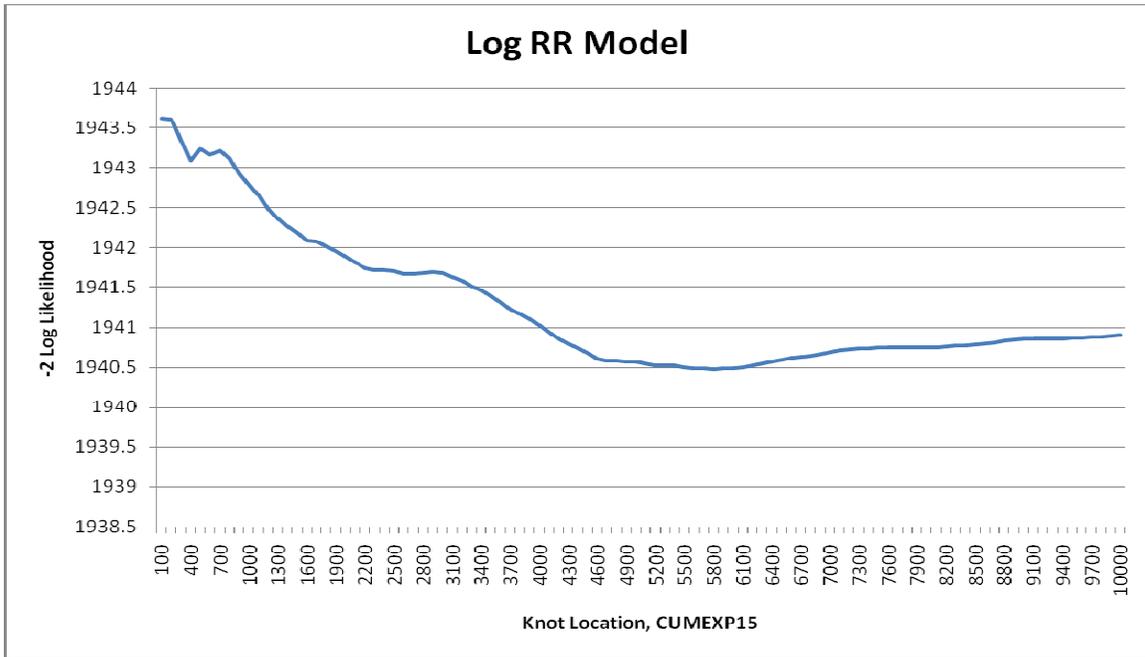
Figure 1a. Likelihoods vs knots, 2-piece linear log RR model for breast cancer morbidity.

10

11

12

1

RR
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9

0   10000   20000   30000   40000

CUMEXP15
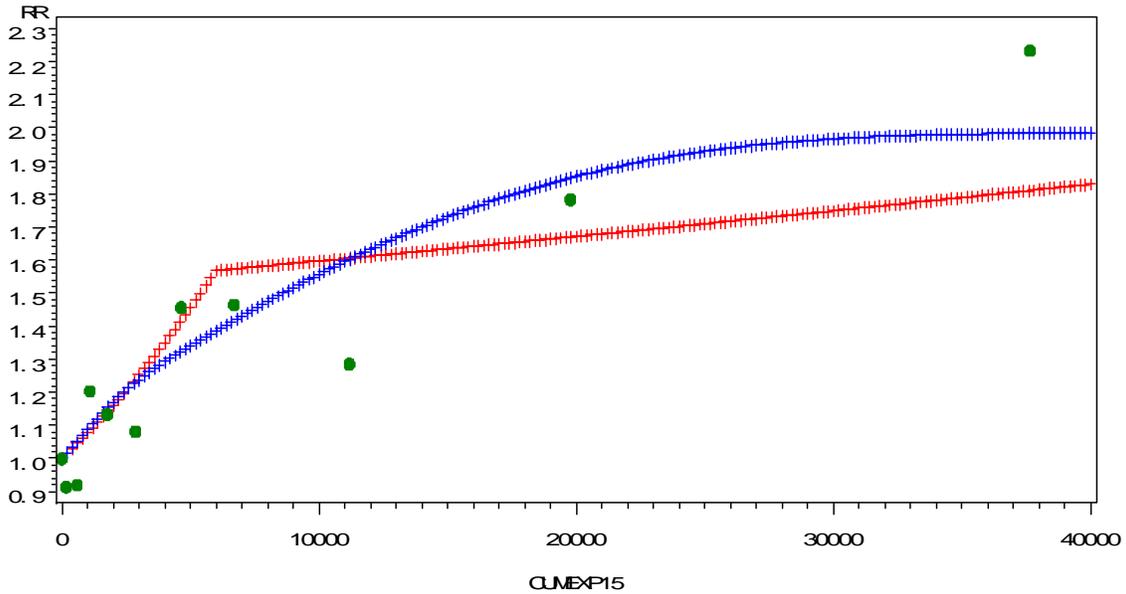
2   **Figure 1b. Breast cancer incidence.** Plot of the dose-response relationship for
3   continuous exposure generated using a **2-piece log-linear spline** overlayed with a
4   plot using restricted cubic (log RR) splines. Dots that represent the effect of
5   exposure grouped in deciles (log RR categorical model) are also presented in the
6   plot. Deciles formed by allocating cases approximately equally in ten groups,
7   above lagged-out cases, see Table 1 above. Y-axis is rate ratio, X axis is
8   cumulative exposure lagged 15 years, in ppm-days.
9
10
11

RR / CUMEXP15
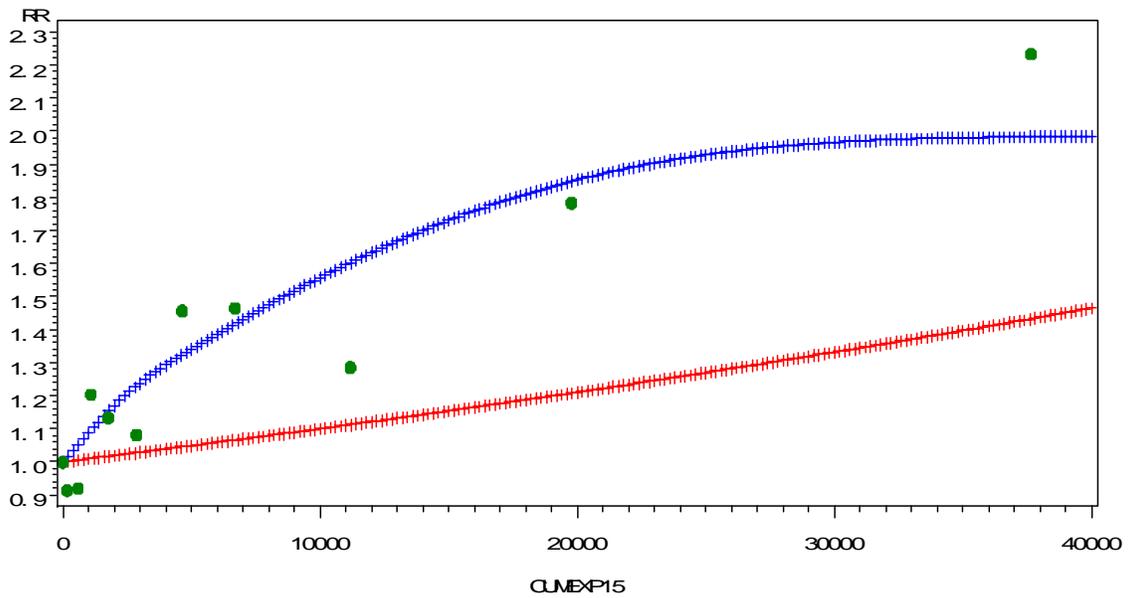
Figure 1c. Breast cancer incidence. Plot of a log-**linear** dose-response
relationship overlayed with a dose-response relationship generated using
restricted cubic log RR model with continuous exposure. Dots that represent the
effect of exposure grouped in deciles (log RR categorical model) are also
presented in the plot. Deciles formed by allocating cases approximately equally
in ten groups, above lagged-out cases.

Comparing log linear models, model with higher slope omits highest 5% of exposure
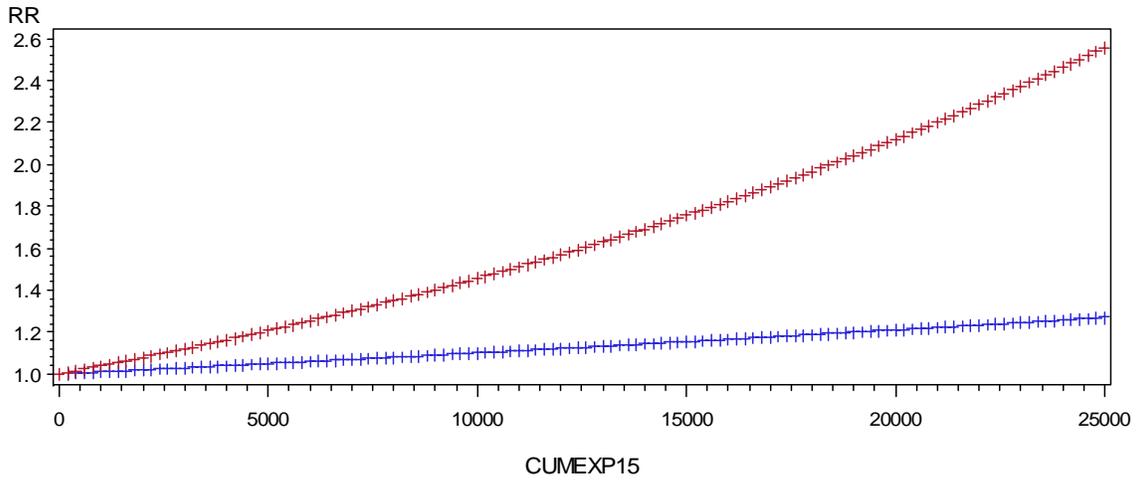
Figure 1d. **Breast cancer incidence.** Comparison of log-linear curve (log RR=β*cumexp) with all the data and the log-linear curve (higher slope) after excluding those in the top 5% of exposure (>27,500 ppm-days).

Breast cancer morbidity log transformed

-2 log likelihood is 1944.153
Categorical analyses overlayed
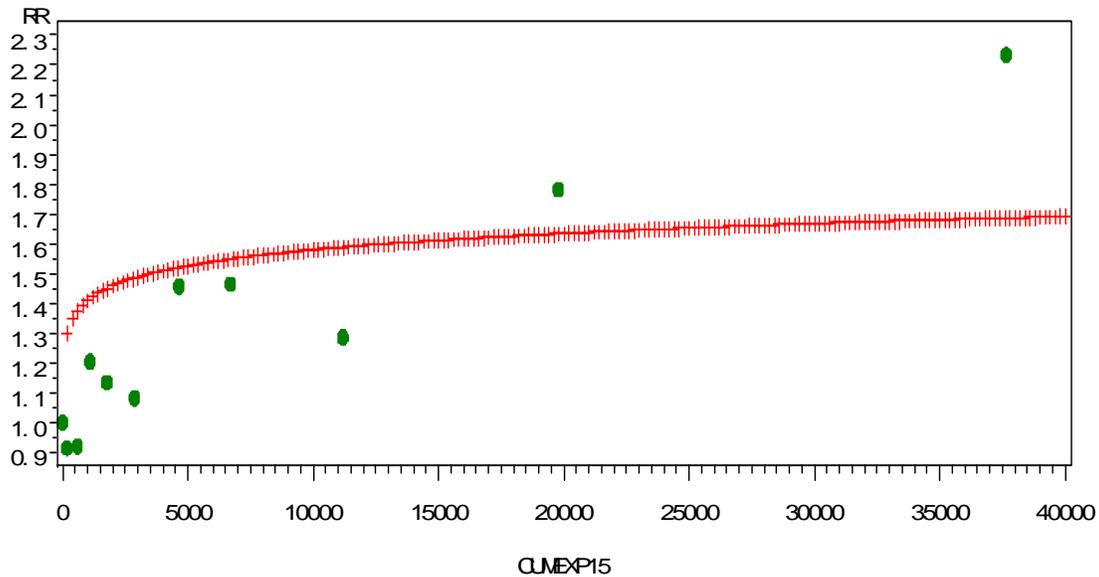
Figure 1e. Breast cancer incidence. Plot of a **logarithmic transformation** log
RR dose-response model (log RR = β*log(cumexp)) overlayed with a dose-
response relationship generated using categorical log RR analyses (deciles).
Deciles formed by allocating cases approximately equally in ten groups, above
lagged-out cases.

Breast cancer morbidity sqrt root transformed

-2 log likelihood is 1941.028
Categorical analyses overlayed
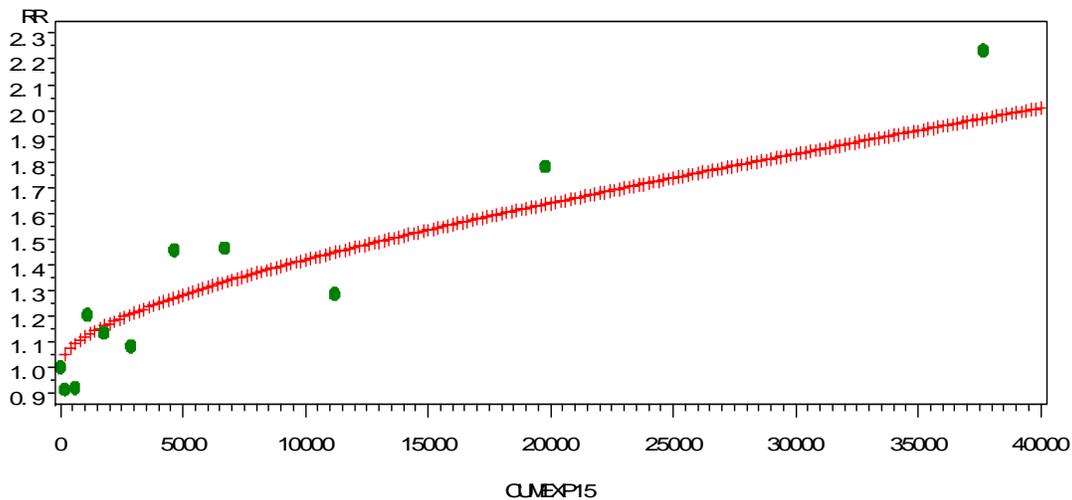
Figure 1f. Breast cancer incidence. Plot of a **square-root transformation** log RR dose–response model overlayed with a dose-response relationship generated using categorical log RR analyses (deciles). Deciles formed by allocating cases approximately equally in ten groups, above lagged-out cases.

Tables 2b, 2c, 2d, and 2e below present the model fit statistics for the 2-piece log-linear, the log-linear, the square root log RR model, and the log transform log RR model seen above. Table 2f summarizes the goodness-of-fit data with regard to the exposure term. Table 2f shows that the addition of exposure terms to the various models results in similar model fits. The exposure terms in the 2-piece log-linear improve model fit marginally better than those in the other models except the square root log RR model, with which the 2-piece log-linear model is tied. If one adds a degree of freedom to the chi-square test for the 2-piece log-linear model, on the assumption that the choice of the knot is equivalent to estimating another parameter, the p-value increases to 0.04, in the same range as the log-linear and log-transform log RR models. Our argument here, however, is not that the 2-piece log-linear model fits the data dramatically better than other models in purely statistical terms. Rather we believe that the fit conforms to the categorical and cubic spline models well in the low-exposure region of interest, and that the nearly linear exposure-response relationship in that region (strictly linear with the linear RR model) is a reason to prefer the 2-piece log-linear model to the other models. In particular, among the parametric models, the log transform and square root log RR models are supra-linear in the low-exposure region.

1

2 The effects of these departures from linearity in the low-exposure region can be seen in the

3 risk assessment results for the EC_{01} (estimate of effective concentration resulting in 1% extra

4 risk) in the next sections (c, d, and e). In these sections we use some of the results from the

5 exposure-response models to calculate EC_{01}s. We restrict these calculations to models which

6 appear most reasonable based on our results above, namely the 2-piece log-linear model, the

7 square root transform log RR model, and the cubic spline log RR model. While we do not

8 recommend the use of the cubic spline model for risk assessment due to its complexity, the

9 EC_{01} based on the cubic spline model provides a good comparison to other parametric

10 models.

11

12 **Table 2b. Fit of 2-piece log-linear model to breast cancer incidence data, Cox**
13 **regression[25]**

```
                            Without          With
            Criterion     Covariates      Covariates

            -2 LOG L       1967.813        1940.485
            AIC            1967.813        1954.485
            SBC            1967.813        1978.612


            Testing Global Null Hypothesis: BETA=0

      Test                   Chi-Square       DF      Pr > ChiSq

      Likelihood Ratio         27.3281         7         0.0003
      Score                    29.0949         7         0.0001
      Wald                     28.4426         7         0.0002


            Analysis of Maximum Likelihood Estimates

                    Parameter   Standard                            Hazard
    Variable         Estimate     Error   Chi-Square  Pr > ChiSq    Ratio

    LIN_0 (β1)      0.0000770  0.0000317     5.4642     0.0194       1.000
    LIN_1          -0.0000724  0.0000334     4.1818     0.0409       1.000
    DOB1             0.08770    0.21805      0.1618     0.6875       1.092
    DOB2             0.41958    0.24430      2.9496     0.0859       1.521
    DOB3             0.55168    0.29096      3.5950     0.0580       1.736
    PARITY1         -0.23398    0.18793      1.5502     0.2131       0.791
    FREL_BR_CAN1     0.47341    0.17934      6.9686     0.0083       1.605

Covariance lin0 and lin1 -1 * 10⁻⁹
```

47 Covariance lin0 and lin1 $-1 * 10^{-9}$

49

---

[25] For environmental exposures, only exposures below the knot are of interest. Below the knot, $RR = e^{(\beta1 * exposure)}$.

Table 2c. Fit of log-linear model to breast cancer incidence data, Cox regression ($RR = e^{(\beta \, * \, \text{exposure})}$)

```
                        Without          With
          Criterion    Covariates     Covariates

          -2 LOG L      1967.813       1944.675
          AIC           1967.813       1956.675
          SBC           1967.813       1977.356
```

```
              Testing Global Null Hypothesis: BETA=0

        Test                Chi-Square      DF      Pr > ChiSq

        Likelihood Ratio      23.1374        6        0.0008
        Score                 25.8389        6        0.0002
        Wald                  25.3594        6        0.0003
```

```
              Analysis of Maximum Likelihood Estimates

                 Parameter      Standard                                 Hazard
Variable          Estimate         Error    Chi-Square  Pr > ChiSq       Ratio

CUMEXP15 (β)    9.54826E-6    4.09902E-6       5.4261      0.0198         1.000
DOB1              0.13558       0.21676        0.3912      0.5316         1.145
DOB2              0.53147       0.23741        5.0116      0.0252         1.701
DOB3              0.74477       0.27425        7.3748      0.0066         2.106
PARITY1          -0.23394       0.18882        1.5351      0.2154         0.791
FREL_BR_CAN1      0.46449       0.17928        6.7126      0.0096         1.591
```

Table 2d. Fit of the square root transformation log RR model to breast cancer incidence data, Cox regression ($RR = e^{(\beta \, * \, \text{sqrt(exposure)})}$)

```
                     Model Fit Statistics

                        Without          With
          Criterion    Covariates     Covariates

          -2 LOG L      1967.813       1941.028
          AIC           1967.813       1953.028
          SBC           1967.813       1973.708
```

```
              Testing Global Null Hypothesis: BETA=0

        Test                Chi-Square      DF      Pr > ChiSq

        Likelihood Ratio      26.7851        6        0.0002
        Score                 28.9446        6        <.0001
        Wald                  28.5277        6        <.0001
```

```
              Analysis of Maximum Likelihood Estimates



                 Parameter      Standard
```

Variable	DF	Estimate	Error	Chi-Square	Pr > ChiSq
dob1	1	0.09778	0.21756	0.2020	0.6531
dob2	1	0.43872	0.24177	3.2929	0.0696
dob3	1	0.58623	0.28404	4.2596	0.0390
sqrtcumexp15 (β)	1	0.00349	0.00118	8.7489	0.0031
PARITY1	1	-0.22539	0.18787	1.4393	0.2302
FREL_BR_CAN1	1	0.46937	0.17922	6.8589	0.0088

Table 2e. Fit of the log transform model to breast cancer incidence data, Cox regression (RR = $e^{(\beta * \ln(\text{exposure}))}$)

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1944.176
AIC	1967.813	1956.176
SBC	1967.813	1976.856

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.6371	6	0.0006
Score	24.0044	6	0.0005
Wald	23.5651	6	0.0006

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.08605	0.21943	0.1538	0.6949	1.090
dob2	1	0.38780	0.25363	2.3378	0.1263	1.474
dob3	1	0.47303	0.31528	2.2509	0.1335	1.605
LCUMEXP15 (β)	1	0.04949	0.02288	4.6787	0.0305	1.051
PARITY1	1	-0.25908	0.18638	1.9322	0.1645	0.772
FREL_BR_CAN1	1	0.47620	0.17923	7.0595	0.0079	1.610

1

**Table 2f. Change in –2 log likelihood for log RR models for breast cancer
incidence, with addition of exposure term(s)[a]**

Log RR model	Change (chi square)	d.f.	*p*-value
Log transform	4.8	1	0.03
Linear	4.2	1	0.04
Categorical	12.0	10	0.29
Cubic spline	8.8	3	0.07
2-piece linear	8.4	2	0.01
Square root	7.7	1	0.01

[a]All models had 3 variables for date of birth, 1 for family history, and 1 for parity.

b.2. Linear relative risk models for breast cancer incidence

We also ran linear relative risk models for breast cancer incidence, using the techniques
described recently by Langholz and Richardson (2010) to use SAS to fit these models, using
the same data as used for the log RR models. The form of these linear RR models is
RR=1+βx, where x can be cumulative dose, the log of cumulative dose, a 2-piece linear
function of cumulative dose, etc.

Figure 1g below shows the different curves with the linear RR model, using cumulative
exposure lagged 15 years as the exposure metric. The categorical points in Figure 1g come
from the published categorical results for the log RR model (Steenland et al. 2003). The
midpoints for the 5 categories (above the lagged out referent, at 0 exposure) are the medians
of cumulative exposure (lagged 15 years), which were 253, 1193, 3241, 7741, and 26,597
ppm-days.

 Figure 1h shows the likelihood profile for different possible knots for the 2 piece linear
spline, with the search conducted by using increments of 100 ppm-days. For the 2 piece
linear spline model the best knot was 5800 ppm-days, as was the case for the 2-piece log-
linear model.

Table 2g shows the model fit statistics for the linear RR models. These models tend to fit
slightly better than their log RR counterparts, although generally the improvement in the chi

1 square does not attain significance at the 0.05 level. For the 2-piece linear model, the model

2 likelihood is 1936.9 vs a likelihood of 1940.5 for the 2-piece log-linear model. Among the

3 linear RR models, the 2-piece spline model fits better than the other models, although not

4 significantly so. Table 2h gives the exposure parameter values for the linear RR models.

5

6
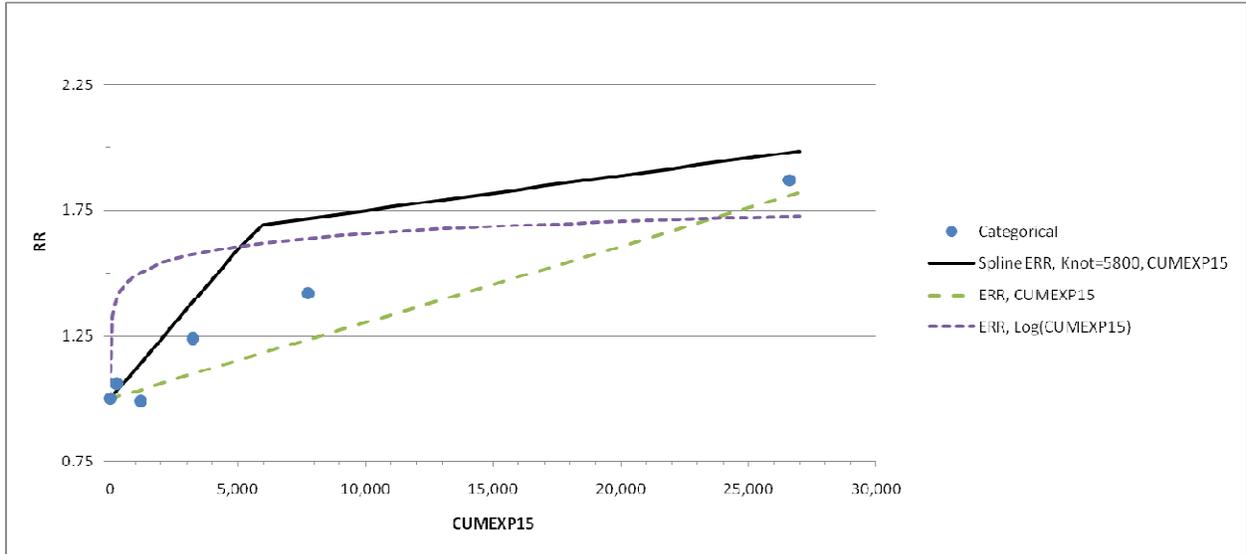
7 **Figure 1g. Breast cancer incidence exposure-response curves, linear RR**
8 **models (units are ppm-days, 15-year lag).**

9

10
11

1
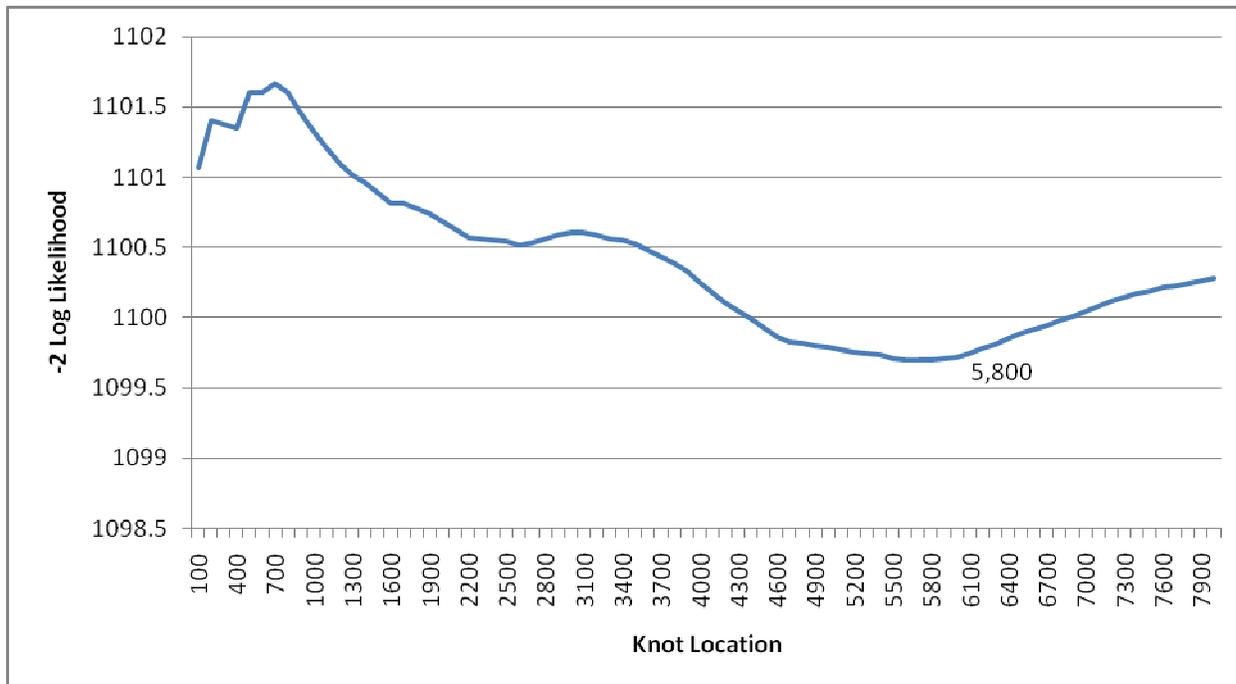
2   **Figure 1h. Knot location for Figure 1g above, 2-piece linear spline model**
3   **model, breast cancer incidence (units are ppm-days, 15-year lag).**
4

5

6   **Table 2g. Model fit statistics for linear RR models, breast cancer incidence[a]**
7

Linear RR Model	d.f. (full model)[b]	–2 Log likelihood (full model)	–2 LL (model without exposure)	–2 LL (model without any covariates)	*p*-value (full model)	*p*-value (for addition of exposure terms)[c]
CUMEXP15	6	1940.260	1949.06	1967.813	< 0.0001	0.0030
Log(CUMEXP15)	6	1942.267	1949.06	1967.813	0.0003	0.0096
Spline, knot = 5,800, CUMEXP15	7	1936.935	1949.06	1967.813	< 0.0001	0.0023

8
9   [a]For the linear RR models, all covariates were included linearly (i.e., additively). Including the non-exposure
10   covariates in the model multiplicatively instead did not improve model fit (e.g., for the 2-piece spline model,
11   inclusion of the non-exposure covariates multiplicatively instead of additively gave a -2 LL of 1940.4 (vs. 1936.9
12   for additive inclusion).
13 [b]Degrees of freedom for full model.
14 [c]Based on change in likelihood for breast cancer incidence linear RR models with addition of exposure term(s) to
15   model with date of birth, parity, and breast cancer in first degree relative. Degrees of freedom for addition of
16   exposure terms is (degrees of freedom for the full model – 5).

Table 2h. Model coefficients for linear RR models, breast cancer incidence

Linear RR Model	Parameter(s)	SE[c]
CUMEXP15	B = 0.000030402	SE = 0.000017549
Log(CUMEXP15)	B = 0.071322	SE = 0.039227
Spline, knot = 5,800, CUMEXP15[a, b]	B1 = 0.000119, B2 = –0.000105	SE1 = 0.000067727, SE2 = 0.000070478

[a] Covariance of 2 pieces of linear spline, $-4.64 * 10^{-9}$.

[b] For estimating risks from occupational exposures (Section 4.7 of the Carcinogenicity Assessment Document), both pieces of the 2-piece linear spline model were used. For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × (exp – knot)). For the 95% upper confidence limit, for exposures below the knot, RR = 1 + ((β1+ 1.645 × SE1) × exp); for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp-knot) + 1.645 × sqrt(exp^2 × var1 + (exp-knot)^2 × var2 + 2 × exp × (exp-knot) × covar)), where exp = cumulative exposure, var = variance, covar = covariance.

[c] Editorial note: As discussed in footnote 16 of Section 4.1.2.3 of this assessment, EPA became aware late in the preparation of this assessment that CIs for the linear RR models, in contrast to the log-linear models, may not be symmetrical and that the profile likelihood method rather than the Wald approach should have been used to calculate the CIs (Langholz and Richardson, 2010). For the linear spline model used in the assessment for the derivation of unit risk estimates, the 95% (one-sided) upper bound on the regression coefficient for the low-exposure spline segment using the profile likelihood method is 0.000309 per ppm × day and the 95% (one-sided) lower bound is 0.000032 per ppm × day. This upper bound estimate of 0.000309 per ppm × day is 34% higher than the value of 0.000230 per ppm × day obtained using the Wald approach and employed in this assessment for the derivation of the unit risk estimates.   Given the relatively small magnitude of the discrepancy and the advanced stage of the preparation of this assessment, it was determined not to revise the assessment to reflect the profile likelihood CIs.

c. Risk assessment for breast cancer incidence using the 2-piece log-linear spline

We used the 95% upper bound of the coefficient for the 1st piece of the linear term in the 2-piece log-linear model from Table 2b, which is 0.0000770 + 1.64*0.0000317 or 0.0001290, to calculate the LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their risk assessment. Here we used the same data on background breast cancer incidence and background all-cause mortality as used by EPA in their 2006 calculations. The rate ratio then, as a function of exposure, is RR = e$^{(0.0001290*cumexp15)}$. Note that the 2- piece log-linear model is linear for the log RR. Once this is exponentiated, it is no longer strictly linear, but is still approximately so, as can be seen in Fig 1a.

Use of the function RR = e$^{(0.0001290*cumexp15)}$ in the life-table analysis results in an excess risk of 0.01 when the daily exposure is 0.0090 ppm, which is the LEC_{01}. This is slightly lower

1 than the previous LEC_{01} of 0.0110 ppm in EPA's 2006 draft risk assessment (EPA, 2006,

2 Table 14).

3

4 Similar calculations were done for the EC_{01}, which resulted in a value of 0.0152 ppm.

5

6 **d. Risk assessment for breast cancer incidence using the square root transformation log**

7 **RR model**

8

9 Use of the 95% upper bound of the relative risk function, ie, $RR = e^{((0.000349 + .00118*1.64)*square}$

10 $^{root(cumexp15))}$, in the life-table analysis results in an excess risk of 0.01 when the daily exposure

11 is 0.00225 ppm, which is the LEC_{01}. This is about 5 times lower than the previous LEC_{01} of

12 0.0110 ppm in EPA' 2006 draft risk assessment (EPA, 2006, Table 14). The EC_{01} is 0.0060

13 ppm, which is about four times lower than the EPA's 2006 EC_{01}. The reason these estimates

14 are much lower than the EPA' is that the square root curve, as can be seen in Figure 1d, rises

15 very sharply (is supra-linear) in the low-dose region. In this sense, it shares the disadvantage

16 of the log transform model, and we recommend against using it as a basis for risk assessment

17 for that reason.

18

19 **e. Risk assessment for breast cancer incidence using the cubic spline curve log RR**

20 **model**

21

22 Risk assessment using the spline curve is more difficult due to the semi-parametric

23 complicated nature of the restricted cubic spline function. The cubic spline function for the

24 breast cancer incidence rate ratio is

25

```
26  RR=exp((ns_0*cumexp15) + ns_1*(((cumexp15-598)**3)*(cumexp15>= 598) -
27  ((37668-598) /(37668-11187)) *(((cumexp15-11187)**3) *(cumexp15>= 11187)) +
28  ((11187 -598)/(37668 - 11187)) *(((cumexp15-37668 )**3) *(cumexp15>= 37668))
29  ) +  ns_2*(((cumexp15-1774)**3)*(cumexp15>= 1774) - ((37668-1774) /(37668-
30  11187)) *(((cumexp15-11187)**3) *(cumexp15>= 11187)) + ((11187 -1774) /(37668
31  - 11187))*(((cumexp15-37668 )**3) *(cumexp15>= 37668)) ) + ns_3*(((cumexp15-
32  4647)**3)*(cumexp15>= 4647) - ((37668-4647) /(37668-11187)) *(((cumexp15-
33  11187)**3) *(cumexp15>= 11187)) + ((11187 -4647)/(37668 - 11187))
34  *(((cumexp15-37668 )**3) *(cumexp15>= 37668)) )).
```

35

36 The coefficients ns_0, ns_1, ns_2, and ns_3 used in this function are 0.00008294999811, -

37 0.00000000000310   0.00000000000425, and   -0.00000000000114, respectively. The

1   expression "cumexp15>=" is a logical statement whereby the term is 0 when "cumexp" is less

2   than the specified value.

3

4   Here we calculate only the EC_{01} (without the LEC_{01}) for comparison with the corresponding

5   EC_{01} from the 2-piece log-linear model. The point is to show that the cubic spline log RR model

6   and the 2-piece log-linear spline give similar answers, not to use the cubic spline for risk

7   assessment, given its relatively complicated formula above. Calculation of the LEC_{01} is also

8   particularly complicated because to do it correctly one must use not only the standard error for

9   four coefficients but also their covariances.

10

11 For breast cancer incidence, the EC_{01} using the cubic spline log RR model is 0.0138 ppm, similar

12 to the value of 0.0152 ppm using the 2-piece log-linear model.

13

14 **f. Risk assessment for breast cancer incidence using the 2-piece linear spline model**

15

16 Use of the function RR=1+ (0.000119+1.64*0.000067)*cumexp15 in the life-table analysis

17 results in an excess risk of 0.01 when the daily exposure is 0.0052 ppm, which is the LEC_{01},

18 which is about half of the value of 0.0110 ppm from the 2-piece log-linear spline model. The

19 corresponding EC_{01} is 0.0100 ppm.

20

21 **2. Breast cancer mortality**

22

23 **a. Exposure distribution among women and breast cancer deaths in the cohort**

24 **mortality study (n=9544)**

25

26 In the Cox regression analyses of Steenland et al. (2004), the data on breast cancer mortality

27 was found to be fit best using cumulative exposure with a 20-year lag.   Below is the

28 distribution of the 102 breast cancer deaths used in the analysis. The cutpoints are those used

29 in the published data (Steenland et al., 2004).

30

1

**Table 3. Distribution of cases in Cox regression analysis of breast cancer
mortality after using a 20-year lag**

Cumulative exposure, 20 year lag[a]	Number of breast cancer deaths
0 (Lagged out)	42
0–647 ppm-days	17
647–2779 ppm-days	16
2780–12321 ppm-days	15
12322+	12

[a]Mean exposures for females with a 20-year lag for the categorical exposure quartiles were
276; 1,453; 5,869; and 26,391 ppm × days. Median values were 250; 1,340; 5,300; and
26,676 ppm × days. These values are for the risk sets but should provide a good
approximation to the full cohort values.

Regarding the women in the cohort as a whole, cumulative exposure at the end of follow-up,

with no lag, had a mean of 8.2 ppm-years, with a standard deviation of 38.2. This

distribution was highly skewed; the median was 4.6 ppm-years.

**b. Results of Cox regression analysis of breast cancer mortality using a variety of log
RR models**

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al.

(2004). Age was the time variable in proportional hazards (Cox) regression. For breast

cancer mortality, only exposure variables were included in models. Cases and controls were

matched on sex (all female), date of birth, and race.

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a

log transform model, and a cubic spline model. We also ran a number of analogous models

using linear RR models (Section 2.c below).

The categorical log RR model for breast cancer mortality was run using the originally

published cutpoints to form four categories above the lagged-out group, as shown in Table 3.

To graph the categorical points, each category was assigned the mid-point of the category as

its exposure level, except for the last one which was assigned 50% more than the last

cutpoint 12,322 ppm-days.

1

2   For the 2-piece log-linear model , the single knot was chosen at 700 ppm-days based on a

3   comparison of likelihoods assessed every 100 ppm-days from 0 to 7000 (Figure 2a). We also

4   explored knots beyond 7000 ppm-days by looking at increments of 1000 ppm-days from 0 to

5   25,000 (Figure 2a' shows the results for knots up to 15,000 ppm-days). None of these

6   outperformed the knot at 700 ppm-days, although Figure 2a' suggests a local maximum

7   likelihood near 13,000 ppm-days.

-2 log likelihood for different knots for breast cancer mortality
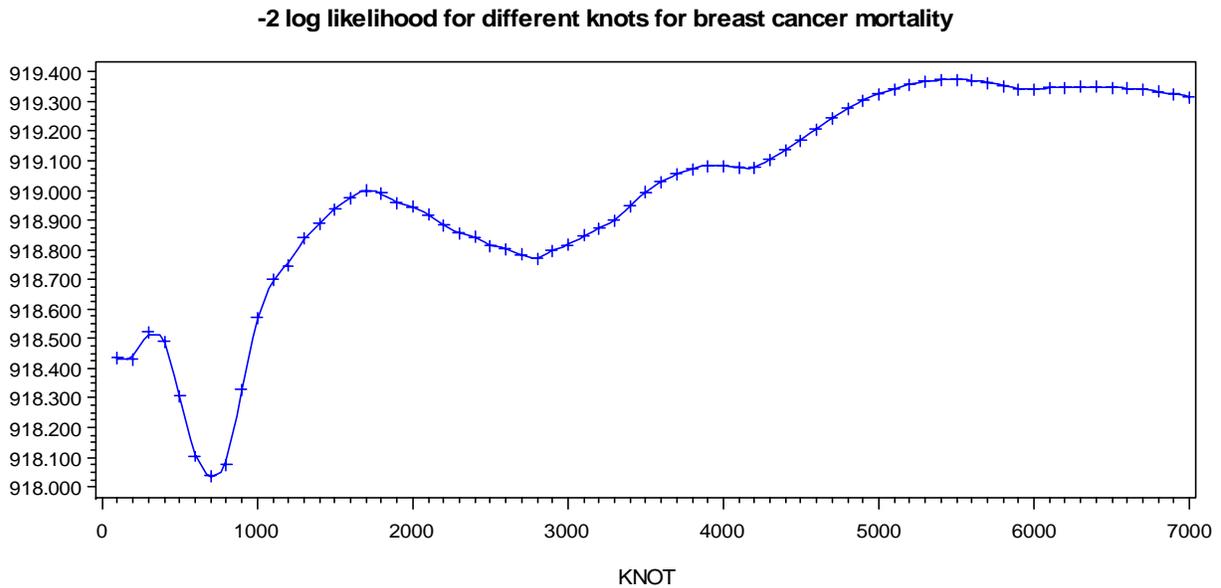
8   **Figure2a. Likelihoods vs knots for the 2-piece log-linear model, breast**
9   **cancer morality.**
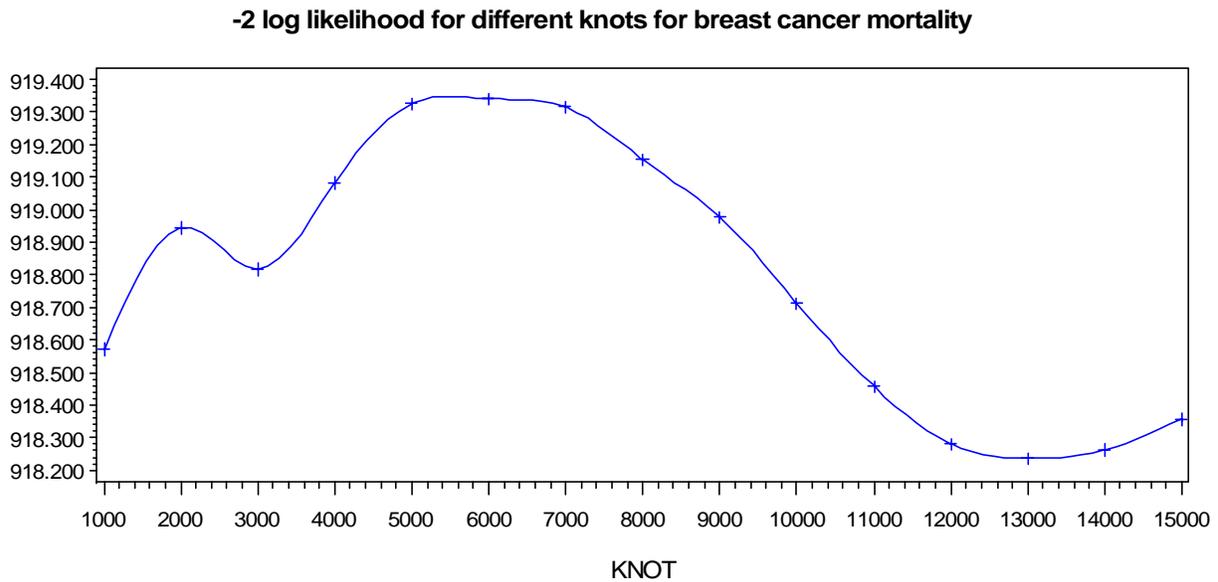
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11

12

-2 log likelihood for different knots for breast cancer mortality

1   **Figure2a'. Likelihoods vs knots for the 2-piece log-linear model, breast**
2   **cancer morality.**
3
4
5   In Figure 2b below, we show the categorical and 2-piece log-linear spline models, as well as
6   the log-linear model and the log-linear model after cutting out the top 5% of exposed
7   subjects.
8
9   The log-linear model was clearly highly sensitive to exclusion of the most highly exposed.
10   As a sensitivity analysis, we excluded 1%, 2.5%, 5%, and 10% of the upper tail of exposure.
11   The 5% cutoff was at 15,000 ppm-days, while the 10% cutoff was at 13,000 ppm-days. The
12   slope of the linear exposure-response relationship increased by 1.2, 1.6, 5.9 and 4.5 times,
13   respectively, with the exclusion of progressively more data. It would appear that the upper
14   5% of the exposure range most affects the linear slope, and it is responsible for the
15   attenuation seen in the exposure-response at high exposures.
16
17   The 2-piece log-linear spline model in Figure 2b fits reasonably well but appears to
18   underestimate the categorical RRs at higher exposures. This may be due to the influence of
19   the top 5% of the exposed, which appear to have a strong attenuating influence on the slope
20   (see below).
21

1   For comparison purposes, we also show the logarithmic transformation log RR model in

2   Figure 2c (which we have not used for risk assessment because it is supra-linear in the low

3   dose region).

4
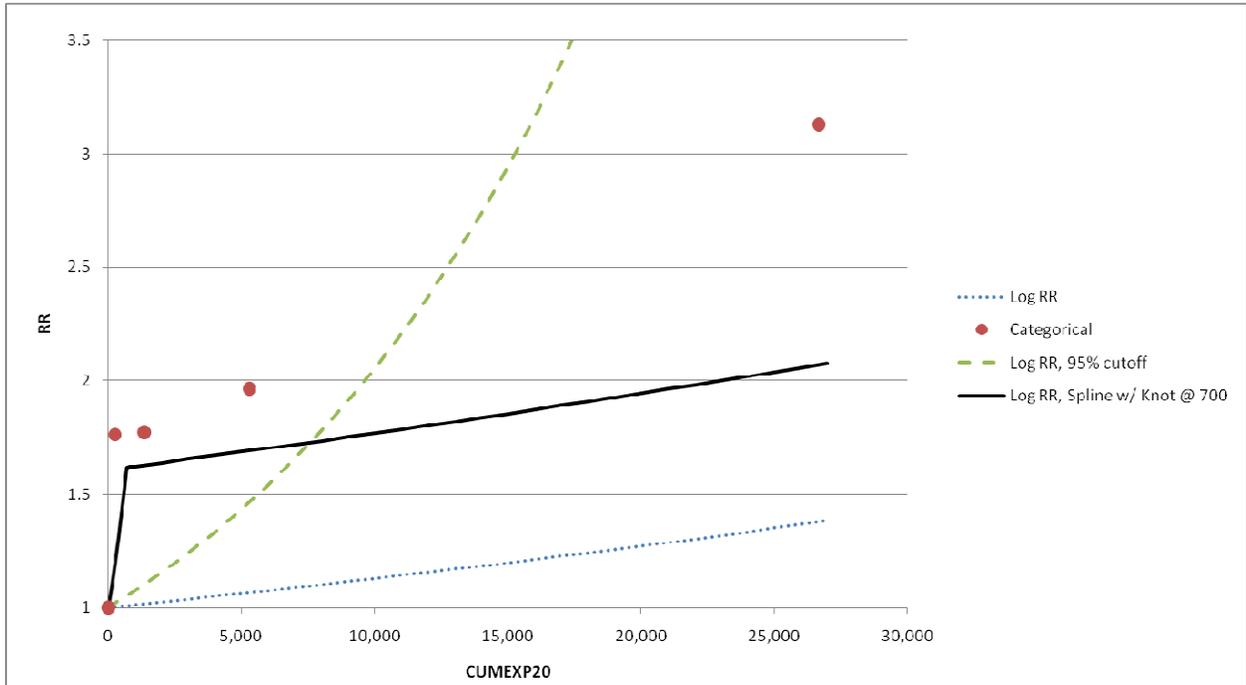
5

6   **Figure 2b. Plot of the dose-response relationship of continuous exposure**
7   **(lagged 20 years) for breast cancer mortality, with the two-piece linear**
8   **spline, the categorical, and the linear log RR models (labeled "log RR").** Also
9   shown is the log-linear curve (log RR=β*cumexp20) after cutting out the top 5%
10 of exposure subjects ('log RR 95% cutoff').

11

12

13

14

15

16

17

18

19

20

Breast cancer mortality log transformed
-2 log likelihood is 917.776
Categorical analyses overlayed
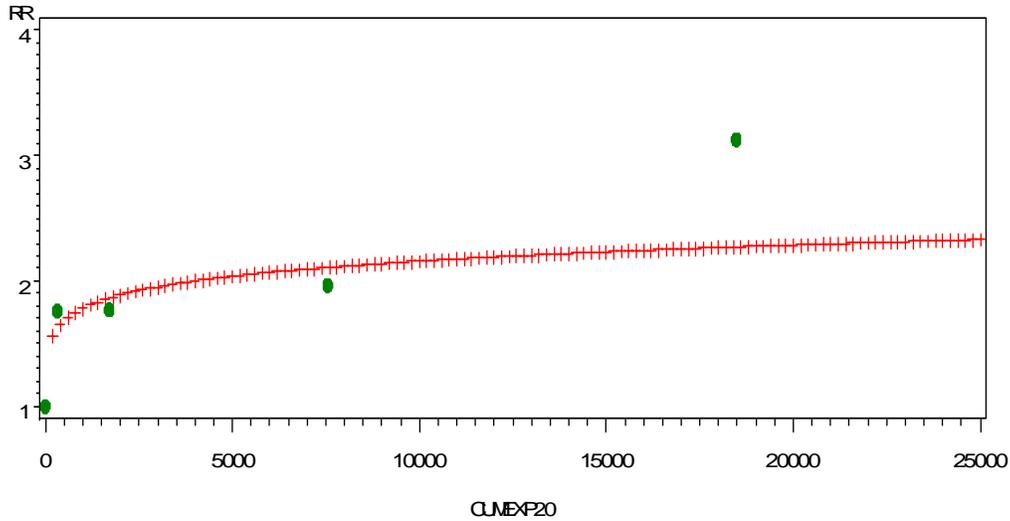
1
2   **Figure 2c. Plot of the dose-response relationship of continuous exposure**
3   **(lagged 20 years) for breast cancer mortality, generated using a logarithmic**
4   **transformation log RR model.** Dots that represent the effect of exposure
5   grouped in categories are also presented in the plot.
6
7
8   Outputs from the categorical, 2-piece linear spline, and linear log RR models are given
9   below.   The 2-piece log-linear model performed similarly to the log-linear model, but
10   appeared to fit the categorical log RR model points and the cubic spline log RR model much
11   better. The log-linear spline model is at the border of statistical significance (p=0.07). In
12   any case, models with relatively sparse data may not achieve conventional statistical
13   significance (at the 0.05 level) but still provide a good fit to the data, judged by conformity
14   with categorical and cubic spline analysis, and may still be useful for risk assessment.
15
16
17   **Table 4a. Categorical output breast cancer mortality,20-year lag (log RR**
18   **model)**
19

```
20                              Model Fit Statistics
21
22                                    Without              With
23                  Criterion        Covariates        Covariates
24
25                  -2 LOG L           923.433           915.509
```

```
                        AIC                    923.433        923.509
                        SBC                    923.433        934.009



                        Testing Global Null Hypothesis: BETA=0

                Test                  Chi-Square        DF        Pr > ChiSq

                Likelihood Ratio        7.9244          4          0.0944
                Score                   8.5160          4          0.0744
                Wald                    8.3993          4          0.0780



                        Analysis of Maximum Likelihood Estimates

                    Parameter    Standard                                Hazard
    Variable    DF   Estimate      Error    Chi-Square    Pr > ChiSq     Ratio

    CUM201       1    0.56653     0.33920      2.7894        0.0949       1.762
    CUM202       1    0.57236     0.35505      2.5987        0.1070       1.772
    CUM203       1    0.67537     0.37632      3.2207        0.0727       1.965
    CUM204       1    1.14110     0.40446      7.9598        0.0048       3.130
```

Table 4b. 2-piece log-linear spline, breast cancer mortality, 20-year lag, knot at 700 ppm-days

```
                        Model Fit Statistics

                              Without         With
                Criterion    Covariates     Covariates

                -2 LOG L       923.433        918.037
                AIC            923.433        922.037
                SBC            923.433        927.287



                Testing Global Null Hypothesis: BETA=0

            Test                  Chi-Square        DF      Pr > ChiSq

            Likelihood Ratio        5.3967          2        0.0673
            Score                   6.0153          2        0.0494
            Wald                    5.8857          2        0.0527



                Analysis of Maximum Likelihood Estimates

                    Parameter    Standard                                Hazard
        Parameter    Estimate      Error    Chi-Square    Pr > ChiSq     Ratio

        LIN_0       0.0006877    0.0004171     2.7178       0.0992        1.001
        LIN_1      -0.0006782    0.0004188     2.6229       0.1053        0.999
```

*covariance lin0 and lin1 -1.75 * 10^{-7}

Table 4c. Log-linear model, breast cancer mortality, 20-year lag

```
                              Model Fit Statistics

                          Without          With
            Criterion    Covariates     Covariates

            -2 LOG L       923.433        920.647
            AIC            923.433        922.647
            SBC            923.433        925.272


            Testing Global Null Hypothesis: BETA=0

       Test                  Chi-Square        DF      Pr > ChiSq

       Likelihood Ratio        2.7865          1         0.0951
       Score                   3.7383          1         0.0532
       Wald                    3.6046          1         0.0576


            Analysis of Maximum Likelihood Estimates

                   Parameter    Standard                             Hazard
       Variable    Estimate       Error   Chi-Square  Pr > ChiSq     Ratio

    CUMEXP20       0.0000122    6.40812E-6   3.6046      0.0576       1.000
```

Table 4d. Log transform log RR model, breast cancer mortality, 20-year lag

```
                              Model Fit Statistics

                          Without          With
            Criterion    Covariates     Covariates

            -2 LOG L       923.433        917.743
            AIC            923.433        919.743
            SBC            923.433        922.368


            Testing Global Null Hypothesis: BETA=0

       Test                  Chi-Square        DF      Pr > ChiSq

       Likelihood Ratio        5.6908          1         0.0171
       Score                   5.7676          1         0.0163
       Wald                    5.7688          1         0.0163
```

```
                    Analysis of Maximum Likelihood Estimates

                  Parameter    Standard                                Hazard
Parameter    DF    Estimate    Error       Chi-Square   Pr > ChiSq   Ratio

lcum20       1      0.08376     0.03487      5.7688       0.0163       1.087
```

Table 4e. 2-piece log-linear spline model, breast cancer mortality, 20-year lag, knot at 13,000 ppm-days

```
                        Model Fit Statistics

                              Without        With
                 Criterion   Covariates    Covariates

                 -2 LOG L      923.433       918.237
                 AIC           923.433       922.237
                 SBC           923.433       927.487


              Testing Global Null Hypothesis: BETA=0

      Test                 Chi-Square      DF      Pr > ChiSq

      Likelihood Ratio       5.1963        2        0.0744
      Score                  5.9044        2        0.0522
      Wald                   5.7813        2        0.0555


              Analysis of Maximum Likelihood Estimates

               Parameter    Standard                           Hazard
     Variable   Estimate    Error      Chi-Square  Pr > ChiSq   Ratio

     LIN_0      0.0000607   0.0000309    3.8539     0.0496       1.000
     LIN_1     -0.0000583   0.0000371    2.4761     0.1156       1.000
```

c. Linear relative risk models for breast cancer mortality

Finally, we also ran linear RR models for these data, as shown in Figure 2d below (denoted "ERR" models), which also includes the RRs from the log RR categorical model as shown in other graphs. Again, the linear curve, highly influenced by the upper 5% tail of exposure, underestimates the categorical points, while the log transform and 2-piece spline capture better the initial increase in risk followed by an attenuation. Parameter estimates for these models can be found in Table 4f.

1
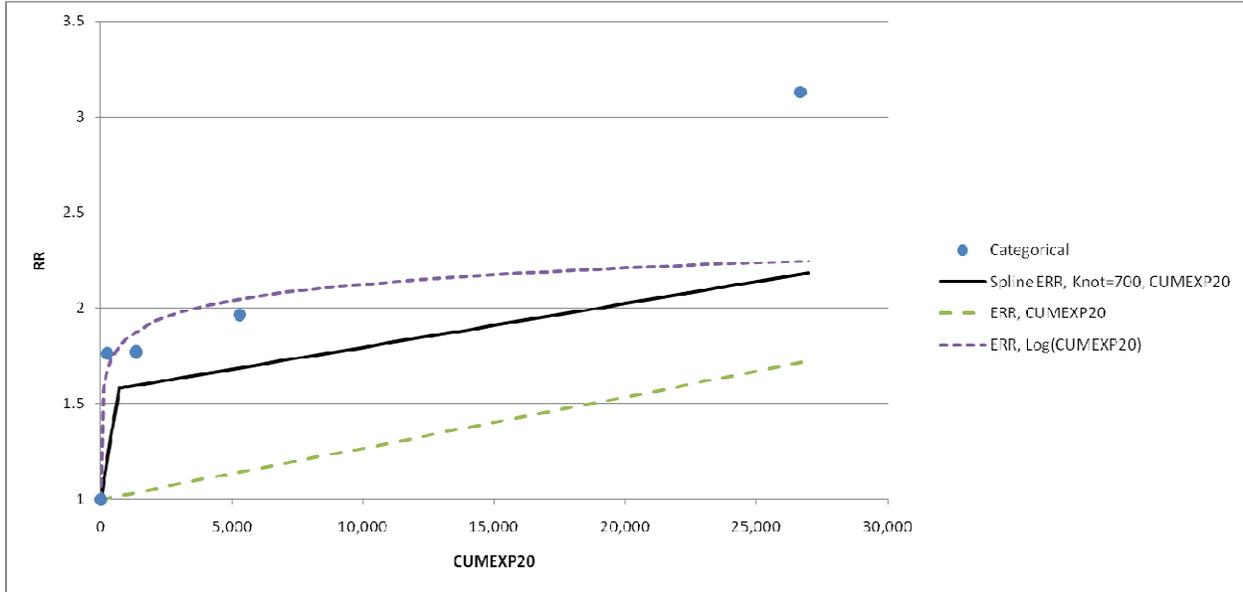
2

3   **Figure 2d. Linear RR models for breast cancer mortality.**

4

5

6   **Table 4f. Model results for breast cancer mortality, linear RR models[b]**

7

Linear RR Model	Parameter(s)	SE	–2 Log Likelihood
CUMEXP20	B = 0.000026779	0.000021537	920.122
Log(CUMEXP20)	B = 0.122090	SE = 0.061659	917.841
Spline, knot = 700, CUMEXP20[a]	B1 = 0.000830, B2 = –0.000807	SE1 = 0.000614, SE2 = 0.000619	918.058

8

9   [a]Covariance 2 pieces of spline, $-3.80*10^{-7}$.

10   [b]Editorial note: As discussed in footnote 16 of Section 4.1.2.3, EPA became aware late in the preparation of
11   this assessment that CIs for the linear RR models, in contrast to the log-linear models, may not be
12   symmetrical and that the profile likelihood method rather than the Wald approach should have been used to
13   calculate the CIs (Langholz and Richardson, 2010). The unit risk estimate for breast cancer mortality
14   presented in this assessment does not rely on any of the linear RR models, thus revised CIs calculated
15   using the profile likelihood method are not shown here.

16

17

18

19

20   **d. Risk assessment for breast cancer mortality using the 2-piece log-linear spline model**

21

1   We next used the 95% upper bound of the coefficient for the 1st piece of the linear term in the
2   2 piece log-linear model from Table 3b, which is 0.0006877 + 1.64*0.0004171, to calculate
3   the LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their 2006
4   draft risk assessment. Here we used the same data on background breast cancer mortality
5   and background all cause mortality as used by EPA in their 2006 calculations. The rate ratio,
6   then, as a function of exposure, is $RR = e^{(0.00137*cumexp20)}$. Note that the 2- piece log-linear
7   model is linear for the log of the rate ratio. Once this is exponentiated, it is no longer strictly
8   linear, but is still approximately so, as can be seen in Fig 2b.
9
10   Use of this function in the life-table analysis results in an excess risk of 0.01 when the daily
11   exposure is 0.0048 ppm, which is the LEC_{01}. This is substantially lower than the previous
12   LEC_{01} of 0.0195 ppm in EPA's 2006 draft risk assessment (EPA, 2006, Table 12).
13
14   Similar calculations were done to derive the EC_{01} which was 0.0095 ppm.
15
16   **e. Risk assessment for breast cancer mortality using the 2-piece linear spline model.**
17
18   The slope of the first segment of the 2-piece linear model was 21% higher than the slope of
19   the corresponding 2-piece log-linear spline (knot at 700 ppm-days). The slope coefficient
20   was 0.0008300, with a std. err. of 0.000614. The resulting EC_{01} and LEC_{01} were 0.0080 and
21   0.0037 ppm, respectively.
22
23   **3. Lymphoid cancer mortality (subset of all hematopoetic cancers combined)**
24   **(n=18,235).**
25
26   **a. Exposure distribution in cohort and among lymphoid cases in the cohort mortality**
27   **study**
28
29   In modeling lymphoid cancer, a subset of all (lympho)hematopoetic cancer, we used a 15-
30   year lag for cumulative exposure as in the prior publication (Steenland et al., 2004), and we
31   also used the same cutpoints as in the publication. Lymphoid cancer consists of
32   nonHodgkin's lymphoma, lymphocytic leukemia, and myeloma (ICD-9 200, 202, 203, 204).
33   The distribution of cases for lymphoid cancer mortality is seen below.
34

1

**Table 5. Exposure categories and case distribution for lymphoid cancer
   mortality**

4

Cumulative exposure, 15-year lag[a]	Male lymphoid cancer deaths	Female lymphoid cancer deaths	Total lymphoid cancer deaths
0 (Lagged out)	6	3	9
0–1200 ppm-days	2	8	10
1201–3680 ppm-days	4	7	11
3681–13,500 ppm-days	5	5	10
13,500+	10	3	13

5

6 [a]The means of the categories were 0, 446, 2,143, 7,335, and 39,927 ppm-days, respectively. The medians were
7   374, 1,985, 6,755, and 26,373 ppm-days, respectively. These values are for the full cohort.

8
9

**b. Results of Cox regression analysis of lymphoid cancer mortality using categorical,
cubic, 2-piece linear, log transform, and linear log RR models**

While the published results in Steenland et al. (2004) focused on males (Table 7 in Steenland
et al., 2004), in fact males and females do not differ greatly in categorical results using a 15-
year lag. A formal chunk test for four interaction terms between exposure and gender is not
close to significance (p = 0.58), although such tests are not very powerful in the face of
sparse data such as these. Table 7 below shows the categorical odds ratio results for men and
women separately and combined. In the analyses presented here, males and female are
combined.

1

Table 6. Lymphoid cancer mortality results by sex

3

Cumulative exposure, 15-year lag	Odds ratio (95% CI) males	Odds ratios (95% CI) females	Odds ratios (95% CI) combined
0 (Lagged out)	1.00	1.00	1.00
0–1200 ppm-days	0.91 (0.16–5.23)	2.25 (0.41-12.45)	1.75 (0.59-5.25)
1201-3680 ppm–days	2.89 (0.65–12.86)	3.26 (0.56-18.98)	3.15 (1.04-9.49)
3681-13,500 ppm–days	2.71 (0.65–11.55)	2.16 (0.34-13.59)	2.44 (0.80-7.50)
13,500+	3.76 (1.03–13.64)	1.83 (0.25–13,40)	3.00 (1.02–8.45)

4
5
6   Analyses used a case-control approach, with 100 controls per case, as in Steenland et al.
7   (2004). Age was the time variable in proportional hazards (Cox) regression. For lymphoid
8   cancer mortality, only exposure variables were included in the model. Cases and controls
9   were within risk sets matched on age, gender, and race.

10

11 Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a
12 log transform model, and a cubic spline model. We also ran a number of analogous models
13 using linear RR models (Section 3.c below).

14

15 The categorical log RR model for lymphoid cancer mortality was run using the originally
16 published cutpoints to form four categories above the lagged-out group, as shown in Table 6.
17 To graph the categorical points, each category was assigned the mid-point of the category as
18 its exposure level, except for the last one which was assigned 50% more than the last
19 cutpoint.

20

21 For the 2-piece log-linear model, the single knot was chosen at 100 ppm-days based on a
22 comparison of likelihoods assessed every 100 ppm-day from 100 to 15,000. The best
23 likelihood was at 100 ppm-days. Figure 3a below shows the likelihood vs the knots. Figure
24 3a also suggests a local maximum likelihood near 1600 ppm-days. Figure 3b shows the
25 categorical, cubic spline, and 2-piece linear log RR models.

26

1   Model results for the categorical and 2-piece linear log RR models are shown in Tables 7a

2   and 7b. Tables 7c and 7d give the results for the log transform model and linear log RR

3   models; the latter does not fit the data well.

4

5   Figure 3b shows the graphical results for the categorical, 2-piece linear, and log transform

6   log RR models. There is a very steep increase in risk at very low exposures. The knot for the

7   2-piece log-linear curve is a low 100 ppm-days. The steep slope at low exposures may be

8   unrealistic as a basis for risk assessment, dependent as it is on relatively sparse data in the

9   low-exposure region (e.g., only 19 cases in the non-exposed lagged-out referent group and

10   the lowest cumulative exposure group, up to 1200 ppm-days, combined).

11

12   We further explored the sensitivity of the log-linear model to high exposures, by excluding

13   progressively more of the upper tail of exposure. We excluded 5%, 10%, 20%, 30%, 40%,

14   and 55% of the upper tail of exposure. The 55% cutoff was at 2,000 ppm-days. The slope of

15   the log-linear exposure-response model increased by 0.4, 1.7, 7.9, 5.6, 26.7 and 113.7 times,

16   respectively, with the exclusion of progressively more data. It is clear that the curve changes

17   substantially once the top 20% of the exposure range is truncated.

-2 log likelihood for different knots for lymphoid cancer mortality

18
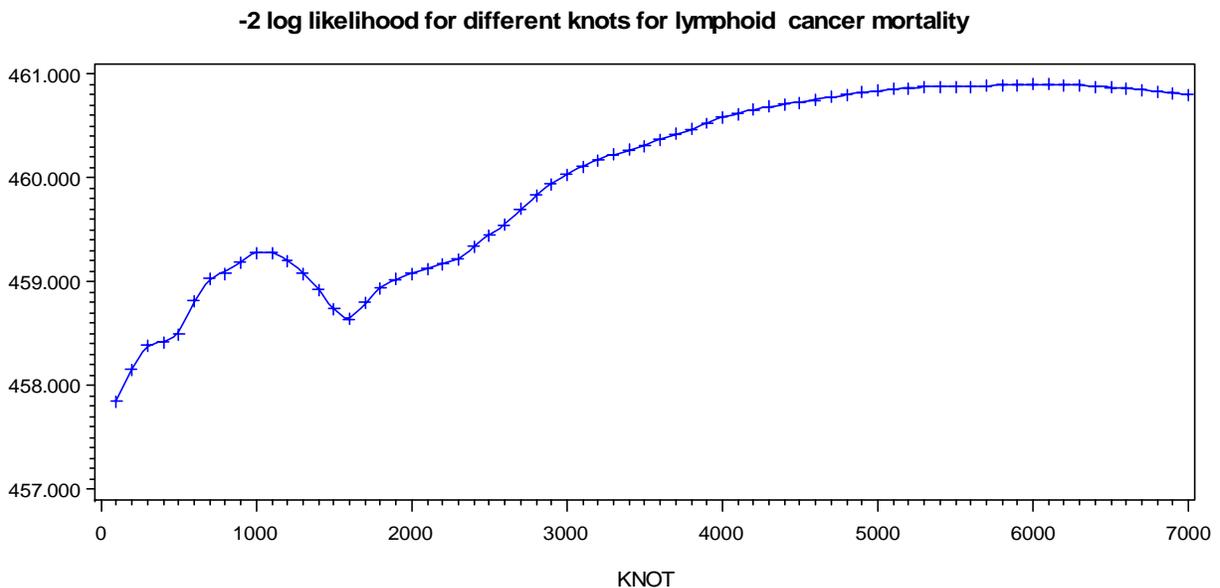
19   **Figure 3a. Likelihoods vs knots for 2-piece log-linear model, lymphoid**
20   **cancer mortality.**

21

22
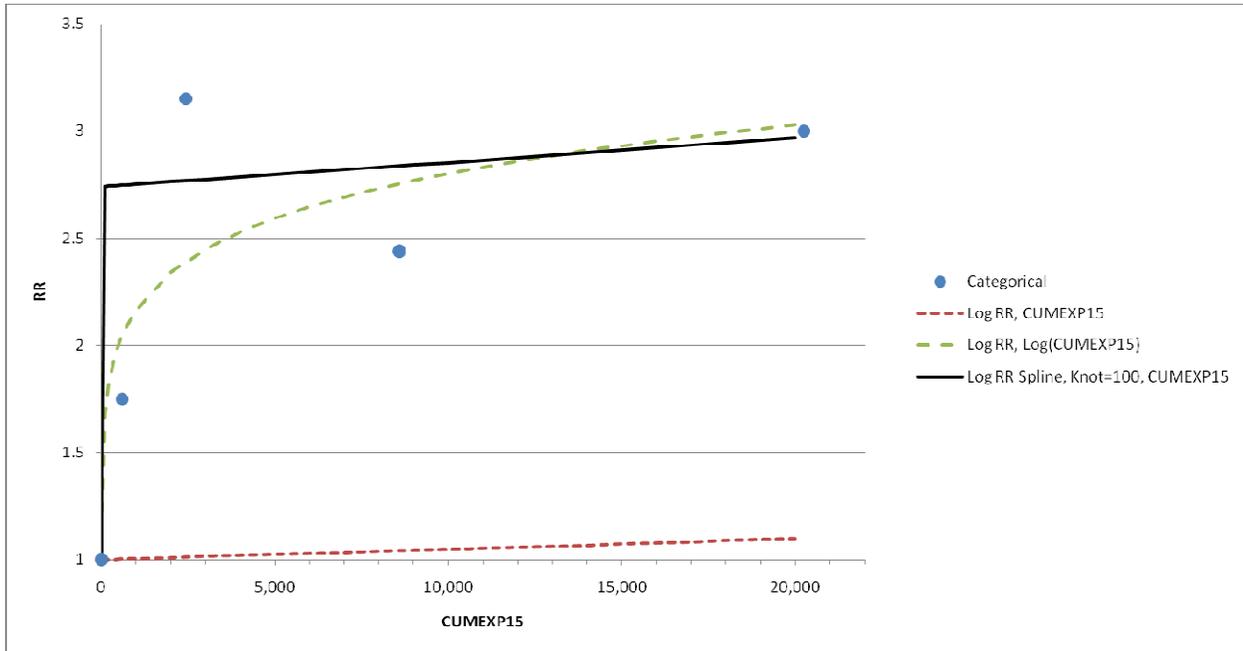
1
2 **Figure 3b. Plot of the exposure and lymphoid cancer mortality rate ratios**
3 **generated using a 2-piece log-linear spline model overlayed with log**
4 **transform log RR curve and categorical log RR model points.**
5
6
7
8 **Table 7a. Categorical results for lymphoid cancer mortality (log RR model),**
9 **men and women combined**
10
11
```
                           Model Fit Statistics

                           Without             With
          Criterion       Covariates        Covariates

          -2 LOG L          463.912           458.069
          AIC               463.912           466.069
          SBC               463.912           473.950



          Testing Global Null Hypothesis: BETA=0

      Test                Chi-Square        DF      Pr > ChiSq

      Likelihood Ratio      5.8435          4          0.2111
      Score                 5.7397          4          0.2195
      Wald                  5.6220          4          0.2292



          Analysis of Maximum Likelihood Estimates
```

```
                 Parameter    Standard                                    Hazard
   Variable   DF  Estimate      Error   Chi-Square  Pr > ChiSq   Ratio

   CUM151      1   0.56036     0.55981     1.0020      0.3168     1.75
   CUM152      1   1.14581     0.56351     4.1344      0.0420     3.15
   CUM153      1   0.89001     0.57391     2.4049      0.1210     2.44
   CUM154      1   1.09998     0.55112     3.9837      0.0459     3.00
```

Table 7b. Results of 2-piece log-linear spline model for lymphoid cancer mortality, men and women combined, knot at 100 ppm-days

```
                             Model Fit Statistics

                                   Without        With
                    Criterion     Covariates    Covariates

                    -2 LOG L       463.912        457.847
                    AIC            463.912        461.847
                    SBC            463.912        465.787




                    Testing Global Null Hypothesis: BETA=0

              Test                Chi-Square      DF      Pr > ChiSq

              Likelihood Ratio      6.0658         2        0.0482
              Score                 5.9648         2        0.0507
              Wald                  5.8246         2        0.0544


            Analysis of Maximum Likelihood Estimates

                 Parameter    Standard                                    Hazard
   Parameter     Estimate      Error    Chi-Square   Pr > ChiSq    Ratio

   LIN_0          0.01010     0.00493     4.1997       0.0404      1.010
   LIN_1         -0.01010     0.00493     4.1959       0.0405      0.990
```

Table 7c. Results of the log transform log RR model for lymphoid cancer mortality, both sexes combined

```
                            Model Fit Statistics

                                   Without          With
                       Criterion   Covariates      Covariates

                       -2 LOG L     463.912          458.426
                       AIC          463.912          460.426
                       SBC          463.912          462.396


                  Testing Global Null Hypothesis: BETA=0

             Test                 Chi-Square     DF      Pr > ChiSq

             Likelihood Ratio       5.4868        1        0.0192
             Score                  5.3479        1        0.0207
             Wald                   5.2936        1        0.0214


               Analysis of Maximum Likelihood Estimates

                      Parameter    Standard                                Hazard
Parameter    DF        Estimate      Error     Chi-Square    Pr > ChiSq     Ratio

lcum15       1         0.11184      0.04861       5.2936        0.0214       1.118
```

Table 7d. Results of the log-linear model for lymphoid cancer mortality, both sexes combined

```
                            Model Fit Statistics

                                   Without          With
                       Criterion   Covariates      Covariates

                       -2 LOG L     463.912          462.413
                       AIC          463.912          464.413
                       SBC          463.912          466.383


                  Testing Global Null Hypothesis: BETA=0

             Test                 Chi-Square     DF      Pr > ChiSq

             Likelihood Ratio       1.4998        1        0.2207
             Score                  2.0403        1        0.1532
             Wald                   1.9959        1        0.1577


               Analysis of Maximum Likelihood Estimates

                      Parameter    Standard                                Hazard
Parameter    DF        Estimate      Error     Chi-Square    Pr > ChiSq     Ratio

CUMEXP15     1      4.73679E-6    3.35285E-6      1.9959        0.1577       1.000
```

Table 7e. Results of 2-piece log-linear spline model for lymphoid cancer mortality, men and women combined, knot at 1600 ppm-days

```
                          Model Fit Statistics

                               Without          With
                Criterion     Covariates     Covariates

                -2 LOG L        463.912         458.640
                AIC             463.912         462.640
                SBC             463.912         466.581


                Testing Global Null Hypothesis: BETA=0

        Test                 Chi-Square      DF      Pr > ChiSq

        Likelihood Ratio       5.2722         2        0.0716
        Score                  5.2666         2        0.0718
        Wald                   5.1436         2        0.0764




                Analysis of Maximum Likelihood Estimates

                        Parameter      Standard                                Hazard
       Parameter   DF    Estimate        Error    Chi-Square    Pr > ChiSq     Ratio

       LIN_0        1    0.0004893     0.0002554     3.6713        0.0554       1.000
       LIN_1        1   -0.0004864     0.0002563     3.6014        0.0577       1.000
```

c. Results for linear relative risk models

Results for linear RR models are seen in Figure 3c (denoted as "ERR" models). They are quite similar to the log RR results in Figure 2b. Again there is a very steep rise in the exposure-response curve at very low exposures. The knot for the 2-piece linear curve is again at 100 ppm-days.

1
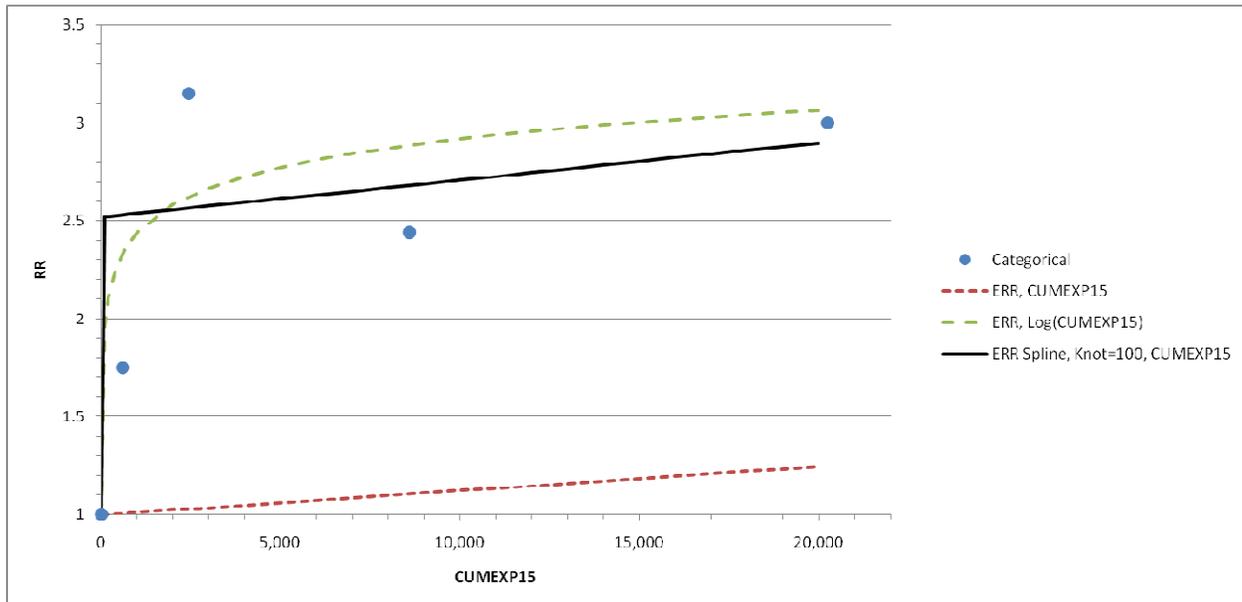
2

3   **Figure 3c. Linear RR models for lymphoid cancer.**

4

5

6   **d. Risk assessment for all lymphoid cancer mortality using the 2-piece log-linear spline**
7   **model**

8

9   We consider that none of the parametric models (either log RR or linear RR) generated for
10   the lymphoid cancer data (and the same is true for all hematopoetic cancer) are suitable for
11   EPA risk assessment because of the overly steep exposure-response relationship in the low-
12   dose range for the 2-piece models and log transform models (highly influenced by the sparse
13   number of deaths in the low-exposure region), and the overly shallow exposure-response
14   relationship for the linear and log-linear models, which are influenced highly by the upper
15   tail of exposures. A reasonable alternative approach is a weighted regression through the
16   categorical points (excluding the highest exposure group), an approach adopted originally by
17   EPA.

18

19   Nonetheless, we have used the 2-piece log-linear model to calculate the LEC_{01} and the EC_{01},
20   by way of illustrating the effect of the very steep exposure-response curve in the low-dose
21   region.

22

1   We used the 95% upper bound of the coefficient for the 1st piece of the linear term in the 2-

2   piece log-linear model from Table 6b, which is $0.01010 + 1.64*0.00493$, to calculate the

3   LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their 2006

4   draft risk assessment. Here we used the same data on lymphoid cancer mortality and

5   background all-cause mortality as used by EPA in their 2006 calculations. The predicted rate

6   ratio, then, as a function of exposure, is $RR = e^{((*0.01010 + 1.64*0.00493)*cumexp15))}$. Use of this RR

7   model in the life-table analysis results in an excess risk of 0.01 when the daily exposure (15-

8   year lag) is 0.0006 ppm, which is the LEC_{01}. This is much lower than the previous LEC_{01} of

9   0.0165 ppm for lymphoid cancer mortality in EPA's 2006 draft risk assessment (EPA, 2006,

10   Table 9).

11

12   A similar calculation was done for the EC_{01}, which resulted in a value of 0.0012 ppm.

13

14   **4. Hematopoetic cancer mortality (all hematopoetic cancers combined).**

15

16   **a. Exposure distribution in cohort and among all (lympho)hematopoetic cases in the**

17   **cohort mortality study**

18

19   In modeling hematopoetic cancer, we used a 15-year lag for cumulative exposure, as in the

20   prior publication (Steenland et al., 2004), and we also used the same cutpoints as in that

21   publication. The distribution of cases for hematopoetic cancer mortality is seen below.

22

23

Table 8. Exposure categories and case distribution for hematopoetic cancer mortality

Cumulative exposure, 15 year lag	Male hematopoetic cancer deaths	Female hematopoetic cancer deaths	Total hematopoetic cancer deaths
0 (Lagged out)	9	4	13
0–1200 ppm-days	4	13	17
1201–3680 ppm-days	5	10	15
3681–13,500 ppm-days	8	7	15
13,500+	11	3	14

[a]Mean exposures for both sexes combined with a 15-year lag for the categorical exposure quartiles were 446; 2,143; 7,335; and 39,927 ppm × days. Median values were 374; 1,985; 6,755; and 26,373 ppm × days. These values are for the full cohort.

b. Results of Cox regression analysis of hematopoetic cancer mortality using categorical, cubic, 2-piece linear, linear and log transform log RR models

While the published results of these data in Steenland et al. (2004) focused on males (Table 8 in Steenland et al. 2004)), in fact males and females do not differ greatly in categorical results using a 15 year lag. A formal chunk test for four interaction terms between exposure and gender is not close to significance (chi square 4.5, 4 df; p = 0.34), although such tests are not very powerful in the face of sparse data such as these. Table 10 below shows the categorical odds ratio results for men and women separately and combined. Males and females were combined in all analyses for hematopoetic cancer here.

Table 9. All hematopoetic cancer mortality categorical results by sex (log RR model)

Cumulative exposure, 15 year lag	Odds ratio (95% CI) males	Odds ratio (95% CI) females	Odds ratio (95% CI) combined
0 (Lagged out)	1.00	1.00	1.00
0–1200 ppm-days	1.23 (0.32–4.74)	3.76 (1.01–17.23)	2.33 (0.93–5.86)
1201–3680 ppm-days	2.53 (0.69–9.27)	4.93 (1.01–23.99)	3.46 (1.33–8.95)
3681–13,500 ppm-days	3.14 (0.95–10.37)	3.31,(0.64–17.16)	3.02 (1.16–7.89)
13,500+	3.42 (1.09–10.73)	2.11 (0.33–13.74)	2.96 (1.12–7.81)

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2004). Age was the time variable in proportional hazards (Cox) regression. For lymphoid cancer mortality, only exposure variables were included in the model. Cases and controls were matched within risk sets on age, gender, and race.

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a log transform model, and a cubic spline model. We also ran a number of analogous models using linear RR models (Section 4.c below).

The categorical log RR model for hematopoetic cancer mortality was run using the originally published cutpoints to form four categories above the lagged-out group, as shown in Table 9. To graph the categorical points, each category was assigned the mid-point of the category as its exposure level, except for the last one which was assigned 50% more than the last cutpoint.

For the 2-piece log-linear model, the single knot was chosen based on a comparison of likelihoods assessed every 100 ppm-days from 0 to 7,000 ppm-days. The best likelihood was at 500 ppm-days (Figure 4a). In Figure 4b below we show the categorical, 2-piece linear spline, and log transform log RR model results.

Model results for the categorical and 2-piece linear log RR models are shown in Tables 10a and 10b, and the results of the log transform and linear log RR models in Table 9c and Table

1   9d.. Again the linear model appears to substantially underestimate the exposure-response

2   relationship and does not provide a good model fit.

3

4   We further explored the sensitivity of the log-linear model to high exposures, by excluding

5   progressively more of the upper tail of exposure. We excluded 5%, 10%, 20%, 30%, 40%,

6   and 53% of the upper tail of exposure. The 53% cutoff was at 2,000 ppm-days. The slope of

7   the log-linear exposure-response model increased by 0.8, 1.0, 9.3, 28.6, 58.2, and 191.4

8   times, respectively, with the exclusion of progressively more data. It appears the curve is flat

9   in the top 20% of exposure.

10

-2 log likelihood for different knots for all hematopoetic cancer mortality
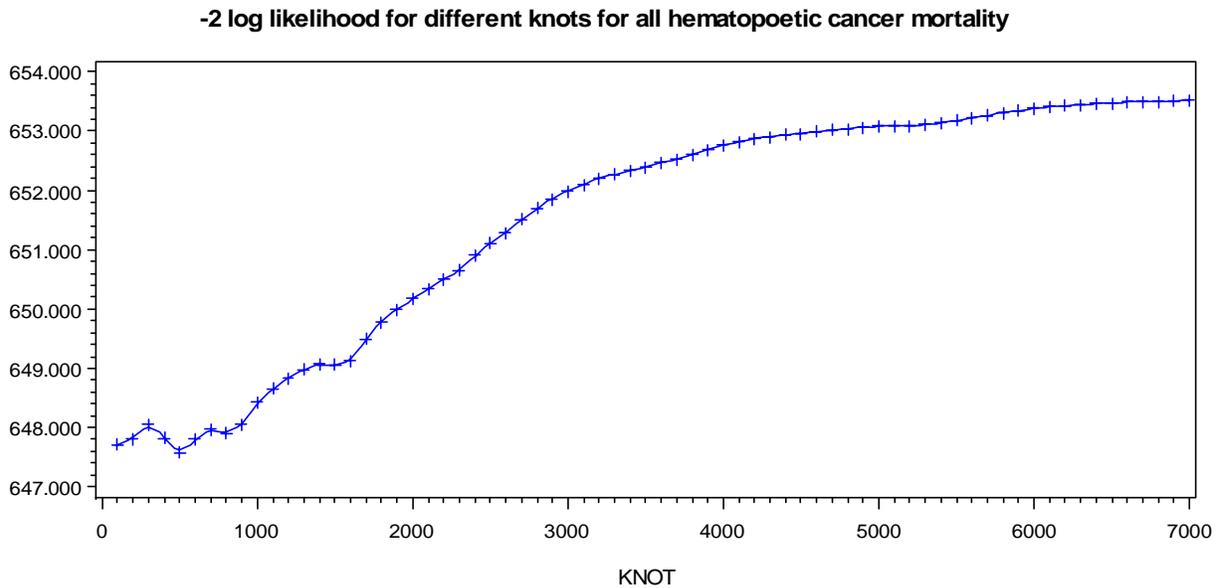
11

12   **Figure 4a. Likelihood vs knots for 2-piece log-linear model, all hematopoetic**
13   **cancer.**

14

15

16
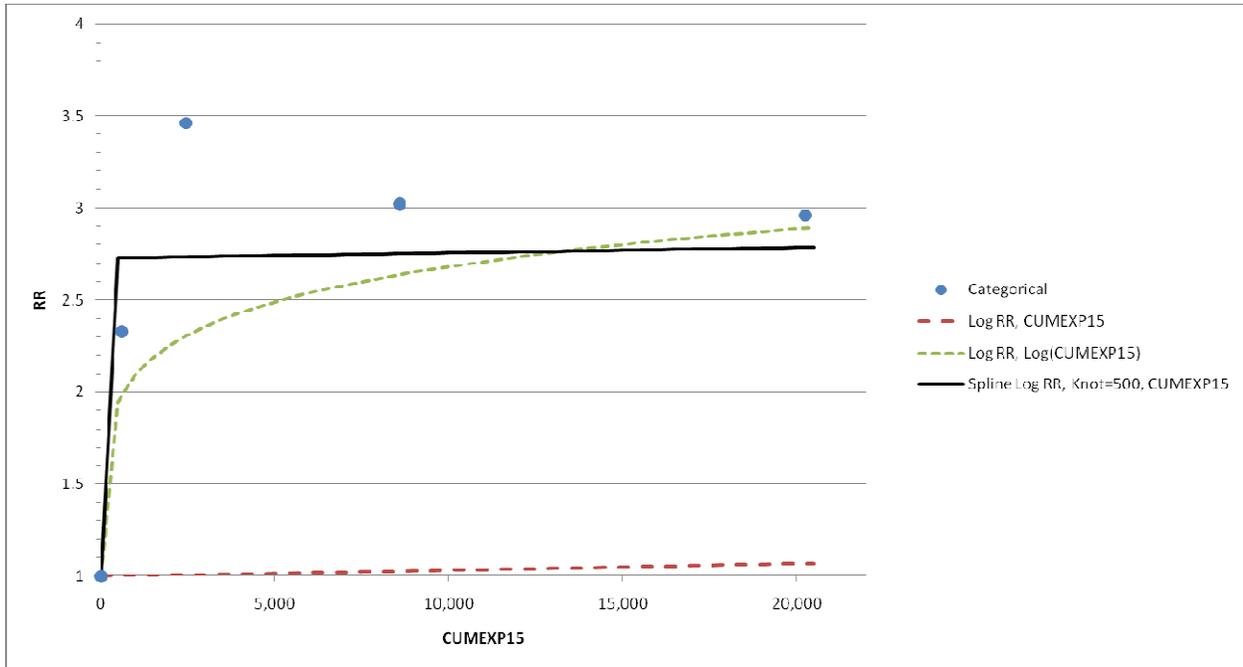
Figure 4b. Plot of exposure and rate ratios for all hematopoetic cancer generated using a 2-piece log-linear spline model and log transform, linear, and categorical log RR models.

Table 10a. Categorical results for all hematopoetic cancer mortality (log RR model), men and women combined, cumulative exposure with a 15-year lag

```
                         Model Fit Statistics

                            Without            With
            Criterion      Covariates       Covariates

            -2 LOG L         655.643          647.806
            AIC              655.643          655.806
            SBC              655.643          665.022



            Testing Global Null Hypothesis: BETA=0

       Test                Chi-Square       DF      Pr > ChiSq

       Likelihood Ratio       7.8371         4         0.0977
       Score                  7.3994         4         0.1162
       Wald                   7.2354         4         0.1240



            Analysis of Maximum Likelihood Estimates

          Parameter     Standard                         Hazard
```

```
Variable  DF     Estimate        Error   Chi-Square   Pr > ChiSq       Ratio

CUM151     1      0.84746      0.46956       3.2573       0.0711        2.33
CUM152     1      1.23989      0.48571       6.5166       0.0107        3.46
CUM153     1      1.10664      0.48943       5.1126       0.0238        3.02
CUM154     1      1.08360      0.49603       4.7723       0.0289        2.96
```

Table 10b. Results of 2-piece log-linear spline model for all hematopoetic cancer mortality, men and women combined, cumulative exposure with a 15-year lag

```
                         Model Fit Statistics

                                   Without          With
                    Criterion     Covariates      Covariates

                    -2 LOG L        655.643         647.581
                    AIC             655.643         651.581
                    SBC             655.643         656.189


                Testing Global Null Hypothesis: BETA=0

              Test                Chi-Square      DF      Pr > ChiSq

              Likelihood Ratio      8.0615        2         0.0178
              Score                 7.5092        2         0.0234
              Wald                  7.3467        2         0.0254


               Analysis of Maximum Likelihood Estimates

                    Parameter     Standard                                Hazard
    Parameter  DF    Estimate       Error    Chi-Square    Pr > ChiSq     Ratio

    spl1        1     0.00201     0.0007731     6.7457        0.0094       1.002
    spl2        1    -0.00201     0.0007738     6.7249        0.0095       0.998
```

Table 10c. Results of log-transform log RR model for all hematopoietic cancer mortality, men and women combined, cumulative exposure with a 15-year lag

```
                         Model Fit Statistics

                                   Without          With
                    Criterion     Covariates      Covariates


                    -2 LOG L        655.643         648.825
                    AIC             655.643         650.825
                    SBC             655.643         653.129
```

```
                         Testing Global Null Hypothesis: BETA=0

              Test                  Chi-Square        DF        Pr > ChiSq

              Likelihood Ratio        6.8177          1           0.0090
              Score                   6.6260          1           0.0100
              Wald                    6.5593          1           0.0104



                  Analysis of Maximum Likelihood Estimates

                    Parameter     Standard                                Hazard
    Parameter   DF   Estimate       Error     Chi-Square   Pr > ChiSq      Ratio

    lcum15       1    0.10706      0.04180       6.5593       0.0104        1.113
```

Table 10d. Results of log-linear model for all hematopoietic cancer morality, men and women combined, cumulative exposure with a 15-year lag

```
                              Model Fit Statistics

                                    Without         With
                    Criterion      Covariates     Covariates

                    -2 LOG L         655.643        654.922
                    AIC              655.643        656.922
                    SBC              655.643        659.226


                  Testing Global Null Hypothesis: BETA=0

              Test                  Chi-Square        DF        Pr > ChiSq

              Likelihood Ratio        0.7213          1           0.3957
              Score                   0.8783          1           0.3487
              Wald                    0.8739          1           0.3499


                  Analysis of Maximum Likelihood Estimates

                    Parameter     Standard                                Hazard
    Parameter   DF   Estimate       Error     Chi-Square   Pr > ChiSq      Ratio

    CUMEXP15     1  3.26052E-6   3.48788E-6     0.8739       0.3499        1.000
```

c. Results for linear relative risk models for hematopoetic cancer mortality

1

2   For completeness, we also present the results of the linear RR models below (Figure 4c;

3   linear RR models are denoted "ERR" models in the figure). They look much like their

4   counterparts for the log RR models. Again, the high slope of the exposure-response

5   relationship in the low-dose region for the 2-piece linear and log transform curves, and the

6   low overall slope of the linear curve, call into question the use of these models for risk

7   assessment.

8
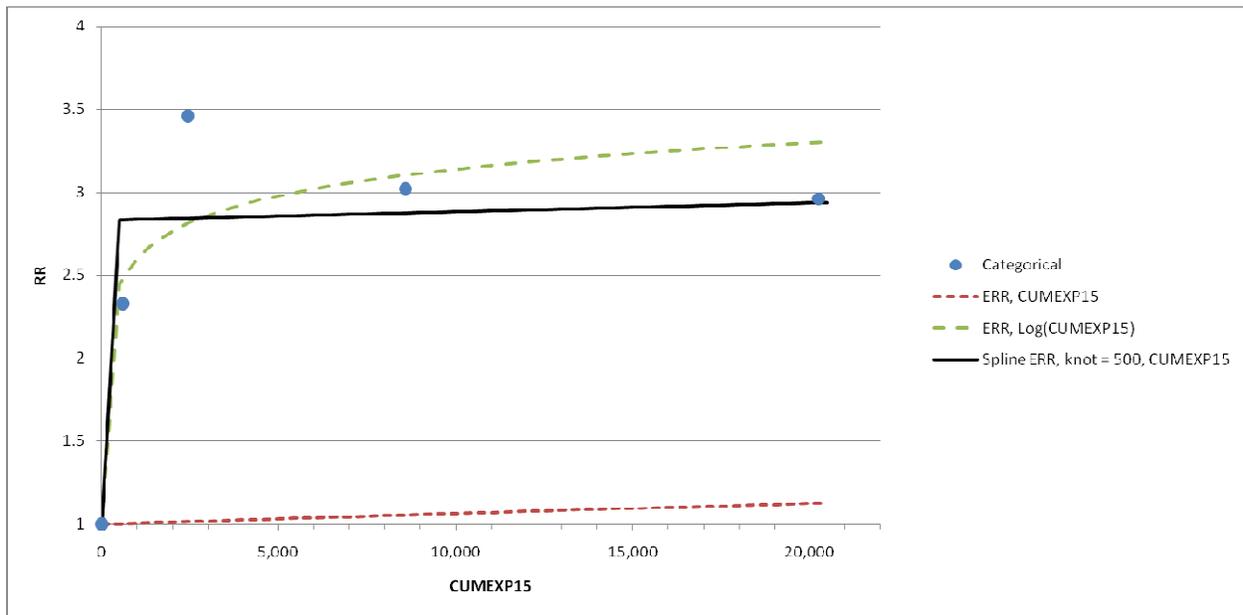
9

10   **Figure 4c. Linear RR models for hematopoetic cancer mortality.**

11

12

13   **d. Risk assessment for all hematopoetic cancer mortality using the 2-piece log-linear**

14   **spline model**

15

16   As was the case for lymphoid cancer (which is a subset of the hematopoetic cancers), we

17   consider that none of the parametric models (either log RR or ERR) generated for the

18   hematopoetic cancer data are suitable for EPA risk assessment because of the overly steep

19   exposure-response relationship in the low-dose range for the 2 piece models and the log

20   transform models (highly influenced by the sparse number of deaths in the low-exposure region),

21   and the overly shallow exposure-response relationship for the linear models, which are

22   influenced highly by the upper tail of exposures. A reasonable alternative approach is a

1   weighted regression through the categorical points (excluding the highest exposure group), an

2   approach adopted originally by EPA.

3

4   Nonetheless, we have used the 2-piece log-linear model to calculate the LEC_{01} and the EC_{01},

5   by way of illustrating the effect of the very steep exposure-response curve in the low-dose

6   region.

7

8   We used the 95% upper bound of the coefficient for the 1st piece of the linear term in the 2-

9   piece log-linear model from Table 9b, which is 0.00201 + 1.64*0.000773, or 0.003277, to

10   calculate the predicted LEC_{01} via the life-table analysis of excess risk used by EPA in

11   Appendix C of their 2006 draft risk assessment. Again, here we used the data on

12   hematopoeitic cancer mortality and background all-cause mortality as used in EPA's 2006

13   calculations. The predicted RR, then, as a function of exposure, is $RR = e^{(0.003277*cumexp15)}$ (up

14   to the knot of 500 ppm-days).

15

16   This results in an excess risk of 0.01 when the daily exposure (15-year lag) is 0.0032 ppm,

17   which is the LEC_{01}. This is notably lower than the previous LEC_{01} of 0.0109 ppm for

18   hematopoetic cancer mortality in EPA's 2006 draft risk assessment (EPA, 2006, Table 7).

19

20   Similar calculations were done for the EC_{01}, which resulted in a value of 0.0043 ppm.

21

22   **5. Summary table of EC_{01}s for different outcomes, using 2-piece linear models**

23

24   Table 11 below provides a summary of the current findings for EC_{01} and the prior EPA

25   findings for EC_{01}.

26

27   In general, findings are similar. As described above, the EC_{01} values based on the 2-piece

28   linear models were obtained by multiplying the background cancer rate by $e^{(beta*cumexp)}$ for log

29   RR models or by (1+beta*cumexp) for linear RR models, where the beta coefficient was for

30   the first piece of the 2-piece linear models, and cumexp was determined such that a daily

31   exposure would result in an excess risk of 1% above background, with risk calculated

32   through age 85 years (BIER methodology, spreadsheet obtained from EPA). In the case of

33   breast cancer incidence, following EPA's methods in the risk assessment, the life-table

34   values for all-cause mortality (within each 5-year age interval) were adjusted to account for

35   incident cases being withdrawn from the pool at risk entering the next age interval, by adding

36   the breast cancer incidence rate to the all-cause mortality rate and then subtracting breast

1 cancer mortality rate so that fatal breast cancer cases are not "counted" twice in this

2 adjustment.

3

4 As noted above, we believe the 2-piece spline models (either log RR or linear RR versions

5 are reasonable bases for risk assessment for the breast cancer incidence and mortality data.

6 They also result in EC_{01} values that are lower than but in the ballpark of the previous EPA

7 estimates using weighted regression for categorical points, excluding the highest exposure

8 quintile. However, this is not the case for the hematopoetic/lymphoid cancer data.

9

10 **Table 11. Summary of EC_{01} results (in ppm) in current analysis and previous**
11 **EPA risk assessment**

12

	EPA (2006) EC_{01}[a]	Steenland[a] LEC_{01} 2-piece spline	Steenland EC_{01} 2-piece spline
Breast cancer incidence[b] (log RR model, 15 year lag)	0.0238	0.009	0.0152
Breast cancer incidence (linear RR model, 15-year lag)[b]	--	0.0052	0.0100
Breast cancer mortality (log RR model, 20-year lag)	0.0387	0.0048	0.0096
Breast cancer mortality (linear RR model, 20 year lag)	--	0.0037	0.0080
Hematopoetic cancer mortality (log RR model, 15-yr lag)[c]	0.0238	0.0032	0.0043[d]
lymphoid cancer mortality (log RR model, 15-yr lag)[c]	0.0427	0.0006	0.0012[e]

13
14 [a]EPA (2006) EPA uses regression through categorical points, Steenland uses 2-piece spline models .
15 [b]Breast cancer incidence for the sub-group with interviews, see Steenland et al. (2004)
16 [c]For hematopoietic and lymphoid cancer, EPA EC_{01} calculated for males only, Steenland includes both men and
17   women.
18 [d]Using at knot at 500 ppm-days. 2-piece linear RR model results similar but not presented.
19 [e]Using knot at 100 ppm-days. 2-piece linear RR model results similar but not presented.
20
21
22 **6. Sensitivity of 2-piece linear curves to placement of knot**

23

24 By way of sensitivity analysis, we ran 2-piece log-linear models for all breast cancer incidence

25 with knots chosen at 5000, 5800 (optimal) and 7000 ppm-days, and for hematopoetic cancer

26 mortality for knots of 500 (optimal) and 1000. Results show the relatively large sensitivity to

27 the knot placement in the $EC_{.01}$.

1

Table 12. Exposure-response coefficients and EC_{01}s based on selection of different knots, using 2-piece log-linear models

	Coefficient first piece	−2 log-likelihood[b]	EC_{01}
Breast cancer incidence knot at 5000 ppm-days	0.0000860	1940.6	0.0133
Breast cancer incidence knot at 5800 ppm-days[a]	0.0000770	1940.5	0.0151
Breast cancer incidence knot at 7000 ppm-days	0.0000653	1940.7	0.0176
Hematopoetic cancer mortality knot at 500 ppm-days	0.00201	647.6	0.0043
Hematopoetic cancer mortality knot at 1000 ppm-days	0.00089	648.4	0.0098

[a]Knot used in analysis.
[b]Lower numbers equal better fit, linear RR model likelihoods not comparable to log RR likelihoods and are not
  shown here.

7. Possible influence of the Healthy Worker Survivor Effect

The healthy worker survivor effect is the effect of healthy workers remaining in the
workforce as sick workers leave, independently of any damaging effects of exposure. It is a
selection bias via which healthier workers remain in the workforce. It tends to create a
downward bias in exposure-response coefficients when the exposure metric is cumulative
exposure, which is by definition correlated with duration of exposure and almost always with
duration of employment (Steenland et al., 1996). Given a true effect of exposure on disease
incidence or mortality in the case of ethylene oxide, it is possible that the health worker
survivor effect has caused some negative bias in observed exposure-response coefficients.
However, there are no standard methods to correct for this bias, because leaving work is both
a confounder and an intermediate variable on a pathway between exposure and disease.
Therefore, standard analyses would need to adjust for employment status as a confounder,
but should not adjust for it because it is an intermediate variable. Robins (1992) has
proposed some solutions using G-estimation to address this problem, but to date these
solutions are not commonly used and can be difficult to implement.   The degree to which the
health worker survivor effect confounds measured exposure-response trends is not known,
but it is likely that lagging exposure, as has been done here, diminishes such confounding
(Arrighi and Hertz-Picciotto, 1994)

8. Possible influence of exposure mis-measurement

1

2   Exposure estimation in the ETO studies considered here is subject to errors in measurement.

3   The method for exposure estimation used here involved assigned estimated average

4   exposures in a given job, at a given time period in a given plant, to each worker in that job.

5   Estimated average exposures were taken from observed measurements in a given job, or

6   estimated likely average exposures in that job derived from a regression model based on

7   observed measurements (Hornung et al., 1994). Errors in measurement in this type of

8   situation are typically errors of the Berkson type, rather than classical errors (Armstrong,

9   1988, 1990). In Berkson errors, the model for errors is

10

11   $Exposure_{true} = exposure_{observed} + error,$

12

13   and the error is independent of the observed exposure. The classical error model is

14

15   $Exposure_{observed} = exposure_{true} + error,$

16

17   and the error is independent of the true exposure. Assuming the errors are unbiased, i.e.,

18   their expected value is 0, in the classical error model it is well known that measurement error

19   will bias exposure-response coefficients towards the null in regression analyses. However, in

20   the Berkson error model, exposure-response coefficients will be unbiased in linear regression

21   models, although their variance may be increased.   In log-linear regression models, such as

22   used here, Berkson error in some instances may result in biased exposure-response estimates

23   (Prentice, 1982; Deddens and Hornung, 1994). This may occur when the variance of the

24   errors increases with the true exposure level, which is often the case in occupational studies,

25   when the disease is relatively rare (also typical), and when the true exposure is distributed

26   log-normally (again typical of occupational exposures). In this situation, Steenland and

27   Deddens (2000) have shown that exposure-response coefficients using cumulative exposure

28   can be biased either upward or downward. The direction and degree of bias depends on the

29   degree of increase in the variance of exposure error as exposure level increases and on the

30   variance of duration of exposure.   When the standard deviation of duration of exposure is

31   less than or equal to its mean, as is the case in the ETO cohort studied here, simulations have

32   shown that the exposure-response coefficients are approximately unbiased (Steenland and

33   Deddens, 2000).   An added complication not considered in the simulations conducted by

34   Steenland and Deddens (2000) is the possible correlation between measurement error and

35   outcome. If this correlation is strong, which may occur when there is a strong exposure-

36   response relationship, it is important to take it into account. Estimating the effect of

1   exposure measurement in the presence of this correlation can be done using Bayesian models
2   and special software (WINBUGS), but the calculations are complex and require a good deal
3   of time.
4
5   Hornung et al. (1994) provide an estimate of the lognormal distribution of measured
6   exposure based on personal samples, as well as the likely distribution of error in assigning
7   the job-specific means to estimate individual exposures. Assignment of such job-specific
8   means was shown to involve some bias as well as random error. This provides a rich source
9   of information with which one could simulate the effect of measurement error on exposure-
10   response coefficients. Based on the exposure estimates used in the study, and some
11   assumptions about the error of such measurement in terms of bias and random error, as well
12   as the assumption of a Berkson error model, one could simulate what the true job-specific
13   exposure means were likely to have been, and then in turn simulate likely true personal
14   exposure distributions. Using the latter in exposure-response analysis, one could estimate the
15   true exposure-response coefficient. However, such analyses are rather involved and beyond
16   the scope of the current task.
17
18
19

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1 **APPENDIX E**
2 **LIFE-TABLE ANALYSIS**
3
4
5   A spreadsheet illustrating the extra risk calculation for the derivation of the LEC_{01} for
6 lymphoid cancer incidence is presented in Table E-1.

Table E-1. Extra risk calculation[a] for environmental exposure to 0.0114 ppm (the LEC_{01} for lymphoid cancer incidence)[b] using the weighted linear regression model based on the categorical cumulative exposure results of Steenland et al. (2004), re-analyzed by Steenland (2008; Appendix C), with a 15-year lag, as described in Section 4.1.1

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	Age interval	All cause mortality ($\times 10^5$/yr)	lymphoid cancer incidence ($\times 10^5$/yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	lymphoid cancer hazard rate (h)	Cond prob of lymphoid cancer incidence in interval (R0)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed lymphoid cancer hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of lymphoid cancer in interval (Rx)
1	<1	685.2	1.9	0.0069	0.9932	1.0000	0.0000	0.00002	0	0.00	0.00002	0.0069	0.9932	1.0000	0.00002
2	1–4	29.9	8.1	0.0012	0.9988	0.9932	0.0003	0.00032	0	0.00	0.00032	0.0012	0.9988	0.9932	0.00032
3	5–9	14.7	4.2	0.0007	0.9993	0.9920	0.0002	0.00021	0	0.00	0.00021	0.0007	0.9993	0.9920	0.00021
4	10–14	18.7	3.2	0.0009	0.9991	0.9913	0.0002	0.00016	0	0.00	0.00016	0.0009	0.9991	0.9913	0.00016
5	15–19	66.1	3.5	0.0033	0.9967	0.9903	0.0002	0.00017	2.5	31.64	0.00018	0.0033	0.9967	0.9903	0.00018
6	20–24	94	3.2	0.0047	0.9953	0.9871	0.0002	0.00016	7.5	94.92	0.00017	0.0047	0.9953	0.9871	0.00017
7	25–29	96	4.1	0.0048	0.9952	0.9824	0.0002	0.00020	12.5	158.20	0.00022	0.0048	0.9952	0.9824	0.00022
8	30–34	107.9	6.0	0.0054	0.9946	0.9777	0.0003	0.00029	17.5	221.49	0.00034	0.0054	0.9946	0.9777	0.00033
9	35–39	151.7	9.0	0.0076	0.9924	0.9725	0.0005	0.00044	22.5	284.77	0.00052	0.0077	0.9924	0.9724	0.00050
10	40–44	231.7	13.2	0.0116	0.9885	0.9651	0.0007	0.00063	27.5	348.05	0.00079	0.0117	0.9884	0.9650	0.00075
11	45–49	352.3	20.9	0.0176	0.9825	0.9540	0.0010	0.00099	32.5	411.33	0.00128	0.0179	0.9823	0.9538	0.00121
12	50–54	511.7	32.5	0.0256	0.9747	0.9373	0.0016	0.00150	37.5	474.61	0.00205	0.0260	0.9743	0.9369	0.00190
13	55–59	734.8	49.2	0.0367	0.9639	0.9137	0.0025	0.00221	42.5	537.90	0.00319	0.0375	0.9632	0.9128	0.00286
14	60–64	1140.1	70.1	0.0570	0.9446	0.8807	0.0035	0.00300	47.5	601.18	0.00467	0.0582	0.9435	0.8793	0.00399
15	65–69	1727.4	101.1	0.0864	0.9173	0.8319	0.0051	0.00403	52.5	664.46	0.00691	0.0882	0.9156	0.8296	0.00549
16	70–74	2676.4	128.7	0.1338	0.8747	0.7631	0.0064	0.00460	57.5	727.74	0.00902	0.1364	0.8725	0.7595	0.00640

Table E-1. Extra risk calculation[a] for environmental exposure to 0.0114 ppm (the LEC_{01} for lymphoid cancer incidence)[b] using the weighted linear regression model based on the categorical cumulative exposure results of Steenland et al. (2004), re-analyzed by Steenland (2008; Appendix C), with a 15-year lag, as described in Section 4.1.1 (continued)

Interval number (i)	Age interval	All cause mortality ($\times 10^5$/yr)	lymphoid cancer incidence ($\times 10^5$/yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	lymphoid cancer hazard rate (h)	Cond prob of lymphoid cancer incidence in interval (R0)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed lymphoid cancer hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of lymphoid cancer in interval (Rx)
17	75–59	4193.2	163.0	0.2097	0.8109	0.6675	0.0082	0.00491	62.5	791.02	0.01171	0.2132	0.8080	0.6627	0.00699
18	80–84	6717.2	179.8	0.3359	0.7147	0.5412	0.0090	0.00413	67.5	854.31	0.01323	0.3401	0.7117	0.5354	0.00601
							Ro =	0.02797						**Rx =**	0.03769

extra risk = (Rx−Ro)/(1−Ro) = 0.01001

Column A: interval index number (i).
Column B: 5-year age interval (except <1 and 1−4) up to age 85.
Column C: all-cause mortality rate for interval i ($\times 10^5$/year) (2004 data from NCHS).
Column D: lymphoid cancer incidence rate for interval i ($\times 10^5$/year) (2000-2004 SEER data).[c]
Column E: all-cause hazard rate for interval i (h^*_i) (= all-cause mortality rate × number of years in age interval).[d]
Column F: probability of surviving interval i without being diagnosed with lymphoid cancer (q_i) (= $\exp(-h^*_i)$).
Column G: probability of surviving up to interval i without having been diagnosed with lymphoid cancer (S_i) ($S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for i>1).
Column H: lymphoid cancer incidence hazard rate for interval i (h_i) (= lymphoid cancer incidence rate × number of years in interval).
Column I: conditional probability of being diagnosed with lymphoid cancer in interval i (= $(h_i/h^*_i) \times S_i \times (1-q_i)$), i.e., conditional upon surviving up to interval i without having been diagnosed with lymphoid cancer (Ro, the background lifetime probability of being diagnosed with lymphoid cancer = the sum of the conditional probabilities across the intervals).
Column J: exposure duration at mid-interval (taking into account 15-year lag) (xtime).
Column K: cumulative exposure mid-interval (xdose) (= exposure level (i.e., 0.0114 ppm) × 365/240 × 20/10 × xtime × 365) [365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures; xtime × 365 converts exposure duration in years to exposure duration in days).
Column L: lymphoid cancer incidence hazard rate in exposed people for interval i (hx_i) (= $h_i \times (1 + \beta \times xdose)$, where $\beta = 0.0002472 + (1.645 \times 0.0001854) = 0.0005522$) (0.0002472 per ppm × day is the regression coefficient obtained from the weighted linear regression model [see Section 4.1.1.2]). To

estimate the LEC_{01}, i.e., the 95% lower bound on the continuous exposure giving an extra risk of 1%, the 95% upper bound on the regression coefficient is used, i.e., MLE + 1.645 × SE].

Column M: all-cause hazard rate in exposed people for interval i (h^*x_i) (= h^*_i + (hx_i − h_i)).

Column N: probability of surviving interval i without being diagnosed with lymphoid cancer for exposed people (qx_i) (= $\exp(-h^*x_i)$).

Column O: probability of surviving up to interval i without having been diagnosed with lymphoid cancer for exposed people (Sx_i) (Sx_1 = 1; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for i>1).

Column P: conditional probability of being diagnosed with lymphoid cancer in interval i for exposed people (= (hx_i/h^*x_i) × Sx_i × (1−qx_i)) (Rx, the lifetime probability of being diagnosed with lymphoid cancer for exposed people = the sum of the conditional probabilities across the intervals).

[a]Using the methodology of BEIR IV (1988).

[b]The estimated 95% lower bound on the continuous exposure level that gives a 1% extra lifetime risk of lymphoid cancer incidence.

[c]Background cancer incidence rates are used to estimate extra risks for cancer incidence under the assumption that the exposure-response relationship for cancer incidence is the same as that for cancer mortality (see Section 4.1.1.3).

[d]For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval—the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. For the lymphoid cancer incidence calculations, this adjustment was ignored because the lymphoid cancer incidence rates are small when compared with the all-cause mortality rates. For the breast cancer incidence calculations, on the other hand, this adjustment was made in the all-cause hazard rate (see Section 4.1.2.3).

MLE = maximum likelihood estimate, SE = standard error.

APPENDIX F
EQUATIONS USED FOR WEIGHTED LINEAR REGRESSION
(source: Rothman [1986], p. 343-344)

6   linear model: RR = 1 + bX

7

8   where RR = rate ratio, X = exposure, and b = slope

9

10   b can be estimated from the following equation:

11

$$\hat{b} = \frac{\displaystyle\sum_{j=2}^{n} w_j x_j R\hat{R}_j - \sum_{j=2}^{n} w_j x_j}{\displaystyle\sum_{j=2}^{n} w_j x_j^{\,2}}$$

12

13

14   where j specifies the exposure category level and the reference category (j = 1) is ignored.

15

16   the standard error of the slope can be estimated as follows:

17

$$SE(\hat{b}) \approx \sqrt{\frac{1}{\displaystyle\sum_{j=2}^{n} w_j x_j^{\,2}}}$$

18

19

20   the weights, w_j, are estimated from the confidence intervals (as the inverse of the variance):

21

$$Var(R\hat{R}_j) \approx R\hat{R}_j^{\,2} Var[\ln(R\hat{R}_j)] \approx R\hat{R}_j^{\,2} \times \left[\frac{\ln(\overline{RR}_j) - \ln(\underline{RR}_j)}{2 \times 1.96}\right]^2$$

22

23

24   where $\overline{RR}_j$ is the 95% upper bound on the RR_j estimate (for the jth exposure category) and $\underline{RR}_j$

25   is the 95% lower bound on the RR_j estimate.

1   **APPENDIX G**
2 **MODEL PARAMETERS IN THE ANALYSIS OF ANIMAL TUMOR INCIDENCE**
3
4
5   **Table G-1. Analysis of grouped data, NTP mice study (NTP, 1987);[a]**
6   **multistage model parameters**
7

Tumor	Multistage[b] polynomial degree	q_0	q_1[c] $(mg/m^3)^{-1}$	q_2 $(mg/m^3)^{-2}$	q_3 $(mg/m^3)^{-2}$	*p* value (chi-square goodness of fit)
Males						
Lung adenomas plus carcinomas	1	2.52×10^{-1}	1.52×10^{-2}			0.92
Females						
Lung adenomas plus carcinomas	2	3.87×10^{-2}	0.0	4.80×10^{-4}		0.39
Malignant lymphoma	3	1.74×10^{-1}	0.0	0.0	1.13×10^{-5}	0.18
Uterine carcinoma	2	0.0	0.0	9.80×10^{-5}		0.90
Mammary carcinoma	1d	2.27×10^{-2}	1.09×10^{-2}			–

8
9 [a]The exposure concentrations were at 0, 50 ppm, and 100 ppm. These were adjusted to continuous exposure.
10 [b]$P(d) \ni 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is inhaled ethylene oxide exposure concentration.
11 [c]Even though q_1 is zero in some cases, the upper bound of q_1 is nonzero.
12 [d]The 100-ppm dose was deleted; the fit was perfect with only two points to fit.
13

1 **Table G-2. Analysis of grouped data, Lynch et al. (1982, 1984a) study of**
2 **male F344 rats;[a] multistage model parameters**
3

Tumor	Multistage[b] polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	p value (chi-square goodness of fit)
Splenic mononuclear cell leukemia	1[c]	3.12×10^{-1}	1.48×10^{-2}	–
Testicular peritoneal mesothelioma	1	3.54×10^{-2}	6.30×10^{-3}	0.34
Brain mixed-cell glioma	1	0	1.72×10^{-4}	0.96

4
5 [a]The exposure concentrations were at 0, 50 ppm, and 100 ppm. These were adjusted to continuous exposure.
6 [b]$P(d) \ni 1 - \exp[-(q_0 + q_1d + q_2d^2 + ... + q_kd^k)]$, where d is inhaled ethylene oxide exposure concentration.
7 [c]The 100-ppm dose was deleted; the fit was perfect with only two points to fit.
8

Table G-3. Analysis of grouped data, Snellings et al. (1984) and Garman et al. (1985) reports on F344 rats;[a] multistage model parameters

Tumor	Multistage[b] polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	*p* value (chi-square goodness of fit)
Males				
Splenic mononuclear cell leukemia	1	1.63×10^{-1}	8.56×10^{-3}	0.34
Testicular peritoneal mesothelioma	1	2.38×10^{-2}	4.74×10^{-3}	0.68
Primary brain tumors	1	5.88×10^{-3}	2.92×10^{-3}	0.46
Females				
Splenic mononuclear cell leukemia	1	1.08×10^{-1}	2.37×10^{-2}	0.75
Primary brain tumors	1	5.94×10^{-3}	1.65×10^{-3}	0.80

[a]The exposure concentrations were at 0, 10 ppm, 33 ppm, and 100 ppm. These were adjusted to continuous exposure.

[b]$P(d) \ni 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is inhaled ethylene oxide exposure concentration.

Table G-4. Time-to-tumor analysis of individual animal data, NTP mice study (NTP, 1987);[a] multistage-Weibull model[b] parameters

Tumor	Multistage polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	z
Males				
Lung adenomas plus carcinomas	1	3.44×10^{-1}	2.03×10^{-2}	5.39
Females				
Lung adenomas plus carcinomas	1	5.35×10^{-2}	1.76×10^{-2}	7.27
Malignant lymphoma	1	1.91×10^{-1}	8.80×10^{-3}	1.00
Uterine carcinoma	1	0.0	3.81×10^{-3}	3.93
Mammary carcinoma	1	3.78×10^{-2}	5.10×10^{-3}	1.00

[a]The exposure concentrations were at 0, 50 ppm, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d, t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)*(t - t_0)^z]$, where d is inhaled ethylene oxide exposure concentration.
The length of the study was 104 weeks. The times t and t_0 as expressed in the above formula are scaled so that the length of the study is 1.0. Then, q_0 is dimensionless, and the coefficients q_k are expressed in units of $(mg/m^3)^{-k}$.

APPENDIX H: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC

COMMENTS AND DISPOSITION

4   The assessment document entitled "Evaluation of the Carcinogenicity of Ethylene Oxide

5   (dated August 2006), has undergone a formal external peer review performed by scientists in

6   accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000b).   At the request of

7   ORD, the EPA Science Advisory Board (SAB) convened a panel of experts external to the

8   Agency to review the ethylene oxide (EtO) assessment document. An external peer review

9   meeting was held in January 2007, and a Final Peer Review Report was released in December

10   2007. The purpose of this assessment was to review the available data on the carcinogenicity of

11   EtO and evaluate the potential for lifetime cancer risk due to inhalation exposure.

12   The SAB panel was asked to comment on three main issues including carcinogenic

13   hazard, derivation of a cancer unit risk value for inhalation exposure to EtO and uncertainty

14   associated with the carcinogenicity assessment. The SAB panel was charged with answering a

15   number of questions that addressed key scientific issues. A summary of significant comments

16   made by the panel in response to the charge questions and EPA's response to these comments

17   arranged by charge question are provided below. A number of comments from the public were

18   also received. A summary of the public comments and EPA's responses are also included in a

19   separate section of this appendix.

20

21   **Science Advisory Board (SAB) Panel Comments:**

22   The statement of the issues as contained in the Agency's charge to the SAB panel are

23   listed below in italics followed by (1) the Panel's summary comments quoted directly from the

24   Executive Summary of the Panel's report and (2) the Agency's response to the comments.

25

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

27   *Do the available data and discussion in the draft document support the hazard conclusion*

28   *that EtO is carcinogenic to humans based on the weight-of-evidence descriptors in EPA's*

29   *2005 Guidelines for Carcinogen Risk Assessment? In your response, please include*

30   *consideration of the following:*

31

1   *1. a. EPA concluded that the epidemiological evidence on EtO carcinogenicity was strong, but*
2   *less than completely conclusive. Does the draft document provide sufficient description of the*
3   *studies, balanced treatment of positive and negative results, and a rigorous and transparent*
4   *analysis of the data used to assess the carcinogenic hazard of ethylene oxide (EtO) to*
5   *humans? Please comment on the EPA's characterization of the body of epidemiological data*
6   *reviewed. Considerations include: a) the consistency of the findings, including the*
7   *significance of differences in results using different exposure metrics, b) the utility of the*
8   *internal (based on exposure category) versus external (e.g., SMR and SIR) comparisons of*
9   *cancer rates, c) the magnitude of the risks, and d) the strength of the epidemiological evidence.*
10
11 SAB Panel Comment: A majority of the Panel agreed with the conclusion in the draft document
12 that the available evidence supports a descriptor of "Carcinogenic to Humans" although some
13 Panel members concluded that the descriptor "Likely to be Carcinogenic to Humans" was more
14 appropriate. There was consensus that the epidemiological data regarding ethylene oxide
15 carcinogenicity were not in and of themselves sufficient to provide convincing evidence of a
16 causal association between human exposure and cancer. Differing views as to the appropriate
17 descriptor for ethylene oxide were based on differences of opinion as to whether criteria
18 necessary for designation as "Carcinogenic to Humans" in the absence of conclusive evidence
19 from epidemiologic studies were met. The majority of Panel members thought that the combined
20 weight of the epidemiological, experimental animal, and mutagenicity evidence was sufficient to
21 conclude that EtO is carcinogenic to humans.
22   The Panel concluded that the assessment would be improved by: 1) a better introduction
23 to the hazard characterization section, including a brief description of the information that will be
24 presented; 2) a clear articulation of the criteria by which epidemiologic studies were judged as to
25 strengths and weaknesses; 3) addition of a more inclusive summary figure and/or table at the
26 beginning of section 3.0; and 4) inclusion of material now provided in Appendix A of the draft
27 assessment to within the main body of that assessment.
28   The Panel agreed with the EPA in their reliance on "internal" estimates of cancer rates
29 rather than "external" comparisons (SMR, SIR) due to well recognized limitations to the latter
30 method of analysis. The Draft Assessment characterizes the magnitude of the unit risk estimate
31 associated with EtO as "weak". This finding is substantiated by the epidemiologic evidence

1 where a relatively small number of excess cancers are found above background even among

2 highly exposed individuals. However, the magnitude of risk suggested by the unit risk estimate is

3 somewhat at odds with this concept. Subsequent recommendations in our report try to address

4 this apparent inconsistency.

5

6 <u>EPA Response:</u> EPA agrees with the recommendations of the majority of the Panel that the

7 combined weight of the epidemiological, experimental animal, and mutagenicity evidence

8 presented was sufficient to conclude that EtO is carcinogenic to humans. Some panel members

9 were of the opinion that the descriptor "Likely to be Carcinogenic to Humans" was more appropriate. In

10 response to the general comments related to improving the information in the assessment related

11 to the cancer descriptor, 1) the introduction to the hazard characterization section has been

12 revised and a brief description of the information presented has been added, 2) the criteria used

13 to evaluate epidemiological studies has been articulated, and 3) summary Table A-4 in Appendix

14 A has been cross-referenced at the beginning of Section 3. EPA considered the recommendation

15 to move the material in Appendix A of the draft assessment to the main body of the document,

16 but judged that the in-depth level of detail in Appendix A was not appropriate for the main body

17 of the document and that it was important to retain the format of presentation used in the draft

18 assessment. The Appendix A material is a detailed, critical review of the epidemiological

19 evidence for the toxicity of EtO. The Appendix is more than 50 pages long and describes details

20 of publications that document results of studies that address the effects on humans of exposure to

21 EtO. The main body of the document provides a summary of the findings of all the

22 epidemiological studies, referencing Appendix A for further details.

23 The basis for the assertion that the risk associated with EtO exposure is characterized in

24 the Draft Assessment as "weak" or the statement that "the magnitude of risk suggested by the

25 unit risk estimate is somewhat at odds with this concept" is unclear. The Draft Assessment did

26 not refer to or characterize the magnitude of the unit risk associated with EtO exposure as

27 "weak."

28

29 ***1.b. Are there additional key published studies or publicly available scientific reports that are***

30 ***missing from the draft document and that might be useful for the discussion of the***

31 ***carcinogenic hazard of EtO?***

1

2   <u>SAB Panel Comment:</u> The Panel agreed that the discussion of endogenous metabolic production

3   of ethylene oxide and the formation of background adducts should be expanded. The Panel

4   believed that the description of studies of DNA adduct formation resulting from EtO exposure

5   appears incomplete and superficial. This discussion should be expanded – both in

6   terms of the number of studies cited and the depth of the discussion. Since ethylene is

7   metabolized to EtO, some members recommended the inclusion of the ethylene

8   body of literature for consideration. Most members were hesitant about adding them to the

9   document, but if added, they cautioned that a discussion of the caveats associated with their

10   interpretation relative to ethylene oxide should be included.

11

12   <u>EPA Response:</u> The discussion of endogenous metabolic production of EtO and its significance

13   and contribution to the formation of background adducts in rodents and humans has been

14   expanded.The discussion of DNA adduct formation resulting from EtO exposure has also been

15   expanded to add depth and breadth. This section now includes a brief discussion of general DNA

16   adducts formation, sensitivity of the methods used to detect DNA adducts, and an in-depth

17   discussion of DNA adduct studies, both in vitro and in vivo, that have been conducted in

18   animals and humans. A discussion of the endogenous production of ethylene during normal

19   physiological processes and its metabolism to EtO under certain conditions has been added. EPA

20   agrees with the majority of the Panel that data on ethylene are not directly relevant and their

21   contribution to the assessment of the carcinogenicity of EtO may be minor. It should be noted

22   that the endogenous production of EtO due to the metabolism of endogenous ethylene will be

23   present in all test animals or subjects (including controls) and hence this factor is considered

24   inherently in the analysis of effects of EtO exposure.

25

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

27   *conclusions?*

28

29   <u>SAB Panel Comment:</u> The Panel agreed with the Draft Assessment conclusion of a mutagenic

30   mode of action. However, an expanded discussion of the formation of DNA adducts and

31   mutagenicity is warranted.

1

2 <u>EPA Response:</u> EPA agrees with the Panel and has expanded the discussion of DNA adduct

3 formation (see response to 1.b) and mutagenicity in the revised assessment document.

4

5 ***1.d. Does the hazard characterization discussion for EtO provide a scientifically-balanced and***

6 ***sound description that synthesizes the human, laboratory animal, and supporting (e.g., in***

7 ***vitro) evidence for human carcinogenic hazard?***

8

9 <u>SAB Panel Comment:</u> While some members of the Panel found the hazard characterization

10 section of the Draft Assessment to be satisfactory, a majority expressed concerns that this section

11 did not achieve the necessary level of rigor and balance. An issue in this characterization,

12 particularly in the face of epidemiological data that are not strongly conclusive, is whether the

13 presumed precursor events leading to cancer in animals, such as mutations and/or chromosomal

14 aberrations, are observed in humans. This issue needs to be addressed in greater detail.

15

16 <u>EPA Response:</u> The genotoxicity, mode of action, and hazard characterization sections have

17 been revised to provide a more complete and balanced discussion of EtO-induced precursor

18 events in animals and humans.

19

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

21 ***Do the available data and discussion in the draft document support the approaches taken by***

22 ***EPA in its derivation of cancer risk estimates for EtO? In your response, please include***

23 ***consideration of the following:***

24

25 ***2.a. EPA concluded that the epidemiological evidence alone was strong but less than***

26 ***completely conclusive (although EPA characterized the total evidence - from human,***

27 ***laboratory animal, and in vitro studies - as supporting a conclusion that EtO as "carcinogenic***

28 ***to humans"). Is the use of epidemiological data, in particular the Steenland et al. (2003,***

29 ***2004) data set, the most appropriate for estimating the magnitude of the carcinogenic risk to***

30 ***humans from environmental EtO exposures? Are the scientific justifications for using this***

31 ***data set transparently described? Is the basis for selecting the Steenland et al. data over other***

1   *available data (e.g., the Union Carbide data) for quantifying risk adequately described?*

2

3   SAB Panel Comment: The Panel concurred that the NIOSH cohort is the best single

4   epidemiological data set with which to study the relationship of cancer mortality to the full range

5   of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly consider

6   all of the epidemiological data in developing its final Assessment. In particular, the Panel

7   encourages the EPA to explore uses for the Greenberg et al. (1990) data including leukemia and

8   pancreatic cancer mortality and EtO exposures for 2174 Union Carbide workers from its two

9   Kanawha Valley, West Virginia facilities. (Also described in Teta et al. 1993; Teta et al., 1999).

10   The Panel encouraged the EPA to investigate potential instability that may result from

11   interaction between the chosen time metric for the dose response model and the treatment of time

12   in the estimated exposure (i.e., log cumulative exposure with 15 year lag) that is the independent

13   variable in that dose-response model.

14

15   EPA Response: EPA agrees with the judgment that the NIOSH cohort is the best single

16   epidemiological data set to use in the evaluation of the relationship between carcinogenicity and

17   exposure to EtO.

18   In regard to the possible use of other epidemiologic data, the assessment document

19   includes a detailed discussion of the studies of workers at the Union Carbide facilities in West

20   Virginia. In fact, the Greenberg et al. (1990) data are quite limited in the number of cancers.

21   Teta et al. (1993) extended the follow-up of the Union Carbide data for 10 years and split off the

22   278 chlorohydrin unit workers, where a three-fold significant excess of lymphohematopoetic

23   cancer was observed (8 vs. 2.7 expected, SMR 2.94, see Benson and Teta 1993), on the grounds

24   that the chlorohydrin unit workers were exposed to other potential carcinogens and likely had

25   low exposures to EtO. Teta et al. (1993) studied the remaining 1896 EtO production workers

26   who did not work in the chlorohydrin unit. This cohort is thus about a tenth of the size of the

27   NIOSH cohort. These data did not show an excess of lymphohematopoetic cancer (7 observed

28   vs. 11.8 expected) but continue to be limited by small numbers (e.g., fewer than 6 expected

29   deaths for non-Hodgkin lymphoma [NHL], although the exact number is not given).

30   Furthermore, these data are characterized by less extensive exposure assessment than the NIOSH

31   cohort. In part, this is inherent in a chemical production setting, where it is difficult to find

1   workers with relatively uniform work histories that involve relatively constant exposure to EtO.

2   As such, the exposure assessment used in the Union Carbide study was relatively crude, based on

3   just a small number of department-specific and time-period-specific categories, and with

4   exposure estimates for only a few of the categories derived from actual measurements (see

5   Section A.3.20 of Appendix A for the details). This is in contrast to sterilization plants, where

6   the NIOSH study was done, where workers can be grouped into relatively common jobs/work

7   zones, facilitating assignment of exposure. Furthermore, extensive sampling data (2350

8   measurements from 1975 to 1986, reduced to 205 annual job-specific means, representing 80%

9   of the data; another 20% were not included but used as a validation sample) were used in the

10   NIOSH effort to estimate exposure in different jobs and years. Such sampling data were not

11   used in estimating exposures in the Union Carbide cohort. Finally, the NIOSH regression model

12   for estimating EtO exposure included data not only on job/work zone, but also on variables such

13   as size of sterilizer, type of product, freshness of product, and exhaust systems for sterilizer.

14   This model explained 85% of the variance of the observed EtO sample. As a result, the exposure

15   estimates in the NIOSH data are likely to be more accurate. Because of the lack of comparability

16   in the exposure estimates across the two studies it is not possible to group together the NIOSH

17   cohort and the Union Carbide cohort for a rigorous combined quantitative exposure-response

18   analysis.

19   Teta et al. (1993) does not include any exposure-response analyses, but a later paper

20   (Teta et al. 1999) does. Teta et al. (1999) divide exposure into high, medium, and low intensity

21   of exposure and four time periods (1925-39, 1940-1956, 1957-1973, 1974-1988). The paper

22   does not give the exposure level assigned to each of the resulting twelve cells, nor any

23   justification for the chosen exposure levels. No published data describing how these estimates

24   were derived could be found.

25   Teta et al. (1999) also does not provide the number of observed leukemia deaths, but

26   models leukemia as a function of exposure using three categories of cumulative exposure and a

27   variety of models using continuous exposure. Assuming, as indicated, that the data are the same

28   as the 1988 follow-up reported by Teta et al. (1993), there are only 5 observed leukemia deaths

29   which suggests that the extensive modeling of the data that was done is highly uncertain.

30   The published (through 2006) Union Carbide data and analyses were not sufficient for

31   dose-response assessment of lymphohemaotpoetic cancer due to small numbers and the inherent

1   problem posed by the general assignment of exposure levels to subjects, adequate details of

2   which are not provided.

3   Since the peer review, follow-up of the Union Carbide cohort, without the chlorohydrin

4   production workers, has now been extended through 2003, and analyses of the data have been

5   published by Swaen et al. (2009) and Valdez-Flores et al. (2010). Swaen et al. (2009) used an

6   exposure assessment based on the qualitative categorizations of potential EtO exposure in the

7   different departments developed by Greenberg et al. (1990) and time-period exposure estimates

8   from Teta et al. (1993), which are the same generalized exposure estimates described above

9   based on a small number of department-specific and time-period-specific categories, and with

10 exposure estimates for only a few of the categories derived from actual measurements (additional

11 detailed discussion is provided in Appendix A of the final assessment document.) At the end of

12 the 2003 follow-up, only 27 lymphohematopoietic cancer deaths (including 12 leukemias and 11

13 NHLs) were observed in the cohort. Thus, even in the extended follow-up, the number of cases

14 is small compared to the NIOSH study, which had 74 lymphohematopoietic cancer deaths, 53

15 from lymphoid cancers. More importantly, as discussed above, the exposure assessment is

16 inherently problematic and much more rudimentary than that used for the NIOSH cohort. The

17 lack of comparability in the exposure estimates precludes a rigorous combined exposure-

18 response analysis of data from the two cohorts.

19   EPA requested that Professor Kyle Steenland, the principal investigator of the NIOSH

20 study, respond to the following excerpt from this comment from the SAB Panel:

21

22 "The Panel encouraged the EPA to investigate potential instability that may result from

23   interaction between the chosen time metric for the dose response model and the treatment of

24   time in the estimated exposure (e.g. log cumulative exposure with 15 year lag) that is the

25   independent variable in that dose-response model. "

26

27 Professor Steenland's response:

28

29 "This comment is difficult to understand, but appears to be a concern that the 15 year lag in the

30 exposure metric, which discounts the most recent exposure, may cause an over-reliance in the

31 exposure-response analysis on exposures which were estimated prior to 1979, which possibly are

1   less accurate. The reason they may be less accurate is because the NIOSH exposure model

2   assumed that the effect of calendar year was constant before 1979.   There are a couple of

3   comments to be made here. First, it is certain the much higher exposures took place before the

4   early 1980s when engineering controls were implemented, and that these exposures are likely to

5   compose the majority of the metric "cumulative exposure". Second such early exposures would

6   often, but not always, also be more biologically relevant than later exposures, given that there is

7   likely to be some latency period before a given exposure causes a cancer (the best fitting lag was

8   15 years in the analysis), and cancers occurred during the period 1980-2004, so that later lower

9   exposures were often discounted by the lag. But were such early exposures estimated

10   appreciably worse than later exposures by the NIOSH regression model?   The NIOSH

11   regression model was based on seven variables, one of which had 8 levels (job), one of which

12   had 5 levels (product types), and one of which was time or year.   All these variables were

13   statistically significant at the $p<.05$ level except one (aeration) which had a p value of 0.10.

14   Given that engineering controls were included in the model, the effect of calendar year was

15   thought to reflect improved work practices which got better year by year as employees and

16   managers became more conscious of the dangers of exposure. The effect of year only began in

17   1979, and was not apparent in the period 1975-1978 when there much less concern about the

18   dangers of ETO.   It would seem logical that prior to 1975 (when there were no sampling data to

19   include in the model), work practices also would have changed little year to year, given that

20   worker and management concern about the dangers of ETO was minimal or nonexistent.

21   Furthermore, data for the other variables in the model were available for years before 1979, and

22   hence were able to play a role in prediction of ETO prior to 1979, independent of the year effect,

23   which was constant prior to 1979. Hence, the model would be expected to perform reasonably

24   well in the period before sampling data were available, ie, prior to 1975, regardless of the

25   assumption that calendar year had no effect independent of the other variables in the model."

26

27   "In summary, there is obviously more uncertainty about the estimation of exposures prior to

28   1975 when there were no sampling data. This uncertainty is of some concern in the sense that

29   the majority of cumulative exposure metric for most workers is probably contributed by earlier,

30   higher exposures. The use of a 15 year lag does not, however, necessarily increase this

31   uncertainty, given that exposure in the lagged out period for most workers would be appreciably

1   lower than exposure before the lag came into effect. Furthermore, while the validity of the

2   NIOSH estimates before 1975 cannot be tested against sampling data, the NIOSH model would

3   be expected to permit reasonable estimation of exposure prior to 1975 based on other variables in

4   the model (job, type of product, size of sterilizer, exhaust of sterilizer, etc).”

5

6   “What if exposures prior to 1975 were estimated poorly? This raises the general question of

7   measurement error, which is more likely to have occurred in years before sampling data existed.

8   Measurement error is a complicated issue and its effects cannot be easily predicted. It does not

9   seem likely that the use of the 15 year lag, however, would appreciably increase whatever

10   measurement error occurred for early years of exposure before 1975. While it is possible that the

11   EPA should formally evaluate the likely effect of measurement error, this is a large task which

12   would take considerable amount of time and would necessarily depend on a large number of

13   assumptions about the error in the period before sampling data existed (as I have argued, it is

14   also largely independent of the use of a 15-year lag).”

15

16   *2.b. Assuming that Steenland et al. (2003, 2004) is the most appropriate data set, is the use of*

17   *a linear regression model fit to Steenland et al.'s categorical results for all*

18   *lymphohematopoietic cancer in males in only the lower exposure groups scientifically and*

19   *statistically appropriate for estimating potential human risk at the lower end of the observable*

20   *range? Is the use of the grouping of all lymphohematopoietic cancer for the purpose of*

21   *estimating risk appropriate? Are there other appropriate analytical approaches that should be*

22   *considered for estimating potential risk in the lower end of the observable range? Is EPA's*

23   *choice of a preferred model adequately supported and justified? In particular, has EPA*

24   *adequately explained its reasons for not using a quadratic model approach such as that of*

25   *Kirman et al. (2004) based? What recommendations would you make regarding low-dose*

26   *extrapolation below the observed range?*

27

28   SAB Panel Comment: The Panel identified several important shortcomings in the linear

29   regression modeling approach used to establish the point of departure for low dose extrapolation

30   of cancer risk due to EtO. The Panel was unanimous in its recommendation that the EPA develop

31   its risk models based on direct analysis of the individual exposure and cancer outcome data for

1   the NIOSH cohort rather than the approach based on published grouped data that is presently

2   used. The suggested analysis will require EPA to acquire or otherwise access individual data and

3   develop appropriate methods of analysis. The panel recommends that the Agency allocate the

4   appropriate resources to conduct this analysis.

5   The Panel was divided on whether low dose extrapolation of risk due to environmental

6   EtO exposure levels should be linear (following Cancer Guideline defaults for carcinogenic

7   agents operating via a mutagenic MOA) or whether plausible biological mechanisms argued for

8   a nonlinear form for the low dose response relationship. With appropriate discussion of the

9   statistical and biological uncertainties, several Panel members strongly advocated that both linear

10   and nonlinear calculations be considered in the final EtO Risk Assessment.

11   In conjunction with its recommendation to use the individual NIOSH cohort data to

12   model the relationship of cancer risk to exposures in the occupational range, the Panel

13   recommended that the Agency explore the use of the full NIOSH data set to estimate the cancer

14   slope coefficients that will in turn be used to extrapolate risk below the established point of

15   departure. The use of different data to estimate different dose response curves should be avoided

16   unless there is both strong biologic and statistical justification for doing so. The Panel believed

17   this justification was not made in the Agency's draft assessment.

18   Although the analysis based on total lymphohematopoietic (LH) cancers might have

19   value as part of a complete risk assessment, the rationale for this aggregate grouping needs to be

20   better justified. The Panel recommends that data be analyzed by subtype of LH cancers (e.g.

21   lymphoid, myeloid) and strong consideration be given to these more biologically justified

22   groupings as primary disease endpoints.

23   The Panel was divided in its views concerning the appropriateness of estimating the

24   population unit risk for LH cancer based only on the NIOSH data for males. Several Panel

25   members pointed out that a standard approach in cancer epidemiology and risk analysis begins

26   by conducting separate dose-response analyses on males and females and combining the data

27   only if the results are similar. Conducting separate analyses for males and females is also the

28   standard practice when analyzing data from animal carcinogenicity bioassays. A second

29   approach to dealing with the possibility of gender differences in response is to include gender as

30   a fixed effect in the statistical modeling of the data and determine whether gender or its

31   interaction with other predictors (e.g., gender x exposure) are significant explanatory variables. If

1   so, the combined model with the estimated gender effects could be used directly or separate,

2   gender-specific dose response analysis would be performed. If not, the gender effects could be

3   dropped and the model re-estimated for the combined male and female data. In addition, the

4   Agency should test whether the male/female differences are mitigated by use of alternate disease

5   endpoints discussed in the previous paragraph.

6

7   EPA Response: The categorical models which were published by Steenland et al. (2003, 2004)

8   and used by the Agency in its analysis are based on all the "individual exposure and cancer

9   outcome data." For the analysis of the categorical models, however, while all individual data

10   were used, the data are grouped into categories. Perhaps the argument is best cast as between

11   categorical data analysis which avoids parametric assumptions, and parametric models using

12   continuous exposure data which impose a specific parametric form to the exposure-response.

13   Additional detailed discussion of EPA's regression modeling approach is provided in the

14   response below.

15   The analysis of categorical data has its place in modeling, as it avoids parametric model

16   assumptions which can be restrictive. Categorical analysis, however, uses the average risk for

17   the category to represent the varying exposures within the category. Furthermore, risk

18   estimation in the end also requires fitting some kind of parametric curve (usually a line) to the

19   categorical points, so that estimates of increased risk per unit increase in exposure can be made.

20   In response to the SAB comments, EPA conducted extensive additional analysis and

21   critical review of alternative approaches to modeling this data set, including the development of

22   a range of alternative analyses using the individual-level exposure data. However, as explained

23   in detail in the text, the various alternative continuous models, including the spline models that

24   EPA initially believed would provide a sound approach to addressing SAB recommendations,

25   proved problematic in one or more ways. In particular, for lymphoid cancer, a number of models

26   predicted extremely steep slopes in the low dose region, suggesting that the spline modeling

27   approach was not able to place a realistic bound on low dose response levels. In consideration of

28   these results, EPA has retained the approach used in the Draft Assessment and has based the risk

29   estimates for lymphoid cancer on a linear regression using the categorical data.

30   EPA's approach of using a weighted regression of a line through the categorical points

31   follows well established procedures (Rothman, K.J. (1986), Van Wijingaarden, E; Hertz-

1   Picciotto, I. (2004)). In particular, this choice was reasonable because the best parametric fit in

2   the published articles was provided by a model using the log of cumulative exposure, which is

3   supra-linear in the low dose region. While it is true that cancer risk in this cohort rises relatively

4   quickly at the beginning and then plateaus at high exposures (a common feature of occupational

5   carcinogens, see Stayner et al. 2003, Scan J WkEnv Hlth), the log transform model is so supra-

6   linear in the low dose region that it was judged to be inappropriate as the basis for risk estimation

7   in that region. EPA chose to fit a weighted regression through all categorical points except the

8   last one, thereby avoiding the distortion of the slope estimate which would have necessarily

9   occurred if the last point – in the plateau region – had been included. The approach used by EPA

10   reflects the recognition that the exposure-response relationship changes over the range of

11   exposure levels and does not represent an arbitrary exclusion of data from the estimation process.

12   There are parametric models which may fit the data well and which may take into

13   account the steeper slope at lower exposures without imposing the extreme supra-linearity of the

14   log transform model. As recommended by the Panel, EPA collaborated with Professor Steenland

15   on the investigation of the use of a class of such models: the two-piece log-linear model, in

16   which the two pieces are constrained to join at a point, referred to as a 'knot,' where the slope

17   changes. Use of such a model is based on analysis of individual data rather than categorical data

18   and results in a linear slope (on the log relative risk [RR] scale) in the low dose region. A linear

19   slope on the log RR scale in the low dose region translates to a very nearly linear slope on the

20   RR scale in the low dose region. The coefficient estimates for the two-piece linear model are

21   based on all individual observations throughout the range of the data. Thus, the effects of the

22   high exposure level observations are entrained in the estimated overall model coefficients which

23   are used as the basis for estimates of risk at low exposure levels.

24   For the breast cancer incidence data, EPA determined it was able to implement the two-

25   piece linear approach which is consistent with the recommendation of the SAB to develop a

26   modeling approach using the individual-level exposure data across the entire range of the data.

27   This is the two-piece linear model discussed in Chapter 4 of the revised assessment document

28   which now forms the basis for EPA's unit risk estimate for breast cancer incidence.

29   In regard to end points other than breast cancer incidence, after considering the

30   comments, EPA made a reasonable choice in fitting the data to a weighted regression of the

31   published categorical points, omitting the category of highest exposure. In consultation with

1   Professor Steenland, who had access to the original data, EPA investigated alternative parametric

2   models which might provide a good fit to the data and avoid the supra-linearity of the log

3   transform model. The details of these analyses are described in the revised assessment document.

4   With regard to modeling without the high dose category, the data presented in the

5   original Steenland paper show plateauing of response so that an overall linear relationship is not

6   an appropriate fit to the entire data set. Analysis using the two piece linear approach clearly

7   demonstrated the plateauing behavior, but failed to provide an appropriately bounded response

8   slope for the low dose data. The mutagenic MOA of EtO supported the use of a model form that

9   is linear in the low dose range. Given this, the categorical regression developed over the range

10 of the data that is consistent with a linear low dose response provided an appropriate and sound

11 approach to modeling the data. EPA's draft Benchmark Dose Technical Guidance (2000)

12 recognizes analyses omitting the high dose data points, when not compatible with development

13 of appropriate descriptive statistical analyses, as an appropriate analytical approach.

14   EPA appreciates the care taken in the SAB review of EtO in presenting a range of

15 scientific perspectives on the issue of low dose extrapolation and recognizes the viewpoint

16 expressed by "several panel members" who "advocated the consideration of both linear and

17 nonlinear functional forms" in the EtO assessment. EPA has given consideration to such an

18 approach. EPA's judgement is that the addition of a non-linear dose response assessment to the

19 EtO assessment is not warranted. EPA observes that the quadratic or linear quadratic models

20 suggested for consideration by some SAB members would not provide a suitable description of

21 the EtO cancer dose response data that are analyzed in this assessment. The empirical data show

22 a supralinear dose response pattern (concave down shape) as opposed to an upward curving

23 relationship that would be implied by the quadratic and linear quadratic models indicating that

24 these models would not be appropriate for use in this assessment. EPA also notes that the

25 alternative viewpoint presented in the SAB report in support of a nonlinear approach for EtO

26 drew primarily on conjectures about mechanistic processes and did not present scientific data

27 specific to EtO to provide cogent biological support for a nonlinear dose response for EtO. EPA

28 believes that its scientific inference that a linear dose response relationship should be applied for

29 DNA-reactive, mutagenic compounds is consistent with available data for EtO.

1   As recommend by the Panel, the primary risk estimates are now based on the lymphoid

2   cancers. Analysis based on total lymphohematopoietic (LH) cancers is also included for

3   completeness and comparison.

4   Analyses by Dr. Steenland determined that there was not a statistically significant

5   difference between the LH results for males and females. Thus, in the revised assessment, unit

6   risk estimates based on male only LH cancer are not used. Unit risk estimates are now based on

7   lymphoid cancers for males and females combined and breast cancer in females.

8

9   **The following additional comments on page 31 of the SAB Panel report under "2.b.**

10   **Methods of Analysis", "7. Statistical issues", are quoted below followed by EPA's**

11   **responses**:

12   SAB Panel Comment:

13   7. Statistical issues

14

15   Pages 29-49 of the draft Evaluation outline the EPA's proposed approach to estimation of the

16   Inhalation Unit Risk for EtO. In addition to the general issues of estimation and model-based

17   extrapolation described above, there are a number of statistical assumptions and methods used in

18   this approach that deserve mention. Conditional on the cancer slope factor results from the

19   weighted least squares regression analysis, the life table (BEIR IV) approach to the

20   determination of the $LEC_{.01}$ is programmed correctly. The life table methodology that is the basis

21   for the BEIR IV algorithm is designed to estimate excess mortality and is not readily adapted to

22   modeling excess risk for events (incidence) that do not censor observation on the individual in

23   population under study. The methodology for substituting the mortality slope to an excess risk

24   computation for HL cancer incidence requires the assumption of a proportional rate of

25   incidence/mortality across the cancer types that are included in the grouped analysis. This is

26   generally not a viable assumption. The Panel therefore discourages the use of the BEIR IV

27   algorithm for extrapolation of the cancer mortality algorithm to estimation of excess cancer

28   incidence.

29   Several Panel members commented on the use of the upper confidence limit for the

30   estimated slope coefficient as the basis for estimating an $LEC_{.01}$. The Panel encourages the EPA

31   to present unit risk estimates based on the range of $EC_{.01}$ values corresponding to the lower 95%

1   confidence limit, the point estimate, and the upper 95% confidence limit for the estimated cancer

2   slope coefficients from the final dose-response models.

3

4   **EPA Response on using BEIR approach to estimate incidence risks:** In this assessment EPA

5   is developing estimates of the risk of cancer incidence, not mortality, as the cancers associated

6   with EtO exposure (lymphohematopoietic and breast cancers) have substantial survival rates.

7   The SAB provided the relevant comment that mathematically the BEIR formula would apply to

8   the case where there is a proportional rate of incidence/mortality across the cancer types that are

9   included in the grouped analysis. EPA considered this in its application of the BEIR formula.

10   EPA decided that the Panel's suggestion to not use the BEIR approach for development of

11   cancer incidence estimates for lymphohematopoietic cancer would not allow EPA to develop the

12   desired cancer incidence risk estimates. One possible alternative approach involving a crude

13   survival adjustment to the mortality-based estimates would yield results with greater uncertainty

14   than use of the BEIR approach. No alternative approaches were identified by the SAB. In the

15   absence of an appropriate alternative approach to estimate risks of cancer incidence, EPA has

16   retained the application of the BEIR approach, which it judges to provide a reasonable,

17   approximate, estimate of incidence risks. EPA recognizes the uncertainties and assumptions

18   outlined by the Panel and discusses these in the carcinogenicity assessment. However, EPA

19   notes that deriving mortality estimates as the sole cancer risk estimates for lymphohematopoietic

20   cancer would substantially underestimate cancer risk. In addition, EPA presents the mortality-

21   based estimates as well, for comparison, and reports that for lymphoid cancers the incidence unit

22   risk estimate is about 120% higher than (i.e., 2.2 times) the mortality-based estimate. This is

23   considered reasonable, given the high survival rates for lymphoid cancers.

24

25   **EPA Response on the use of upper and lower confidence limits:** EPA considered the SAB

26   comment encouraging the Agency to present a confidence interval range as well as a central

27   estimate for cancer slopes. The EtO cancer assessment presents an upper confidence value for

28   the slope, following EPA's Cancer Guidelines and consistent practice, as the basis for the

29   inhalation unit risk estimate for EtO. The assessment also provides a central estimate (maximum

30   likelihood estimate of the EC_{01}) for comparison and to provide information on the extent to

31   which the estimate is affected by statistical uncertainty. Lower bound confidence estimates on

1   potency have not been developed for EPA IRIS assessments, and EPA decided not to seek to

2   initiate development of such an approach in this assessment.

3

4   *2.c. Is the incorporation of age-dependent adjustment factors in the lifetime cancer unit risk*

5   *estimate, in accordance with EPA's Supplemental Guidance (U.S. 2005b), appropriate and*

6   *transparently described?*

7

8   <u>SAB Panel Comment:</u> In accordance with EPA guidance, the Draft Assessment applied an Age

9   Dependent Adjustment Factor (ADAF) to adjust the unit risk for early life exposure. While the

10 majority of the Panel felt that the application of a default value by the Agency was appropriate

11 due to lack of data, the description in the Draft Assessment was not adequate, particularly for

12 those not familiar with the EPA's Supplemental Guidance.

13

14 <u>EPA Response:</u> EPA agrees with the Panel and a new subsection detailing the application of the

15 ADAFs has been added to the assessment.

16

17 *2.d. Is the use of different models for estimation of potential carcinogenic risk to humans from*

18 *the higher exposure levels more typical of occupational exposures (versus the lower exposure*

19 *levels typical of environmental exposures) appropriate and transparently described in Section*

20 *4.5?*

21

22 <u>SAB Panel Comment:</u> While the method was transparently described, most of the Panel did not

23 agree with the estimation based on two different models for two different parts of the dose

24 response curve (see response to 2b). The use of different data to estimate different dose response

25 models curves should be avoided unless there is both strong biological and statistical justification

26 for doing so. The Panel believed this justification was not made in the Agency's draft report.

27

28 <u>EPA Response:</u> For the breast cancer incidence risk estimates, a single model, the 2-piece linear

29 model is now recommended for the occupational exposure scenarios. The 2-piece linear model

30 is a unitary model comprised of two linear pieces or segments with different slopes that are

31 joined at a point referred to as a 'knot.' The 2-piece linear model has the flexibility to represent

1   situations, such as with EtO, where the relationship between exposure level and response

2   changes over the range of exposure. For lymphoid cancer risk estimates, two models are

3   presented for the lower-exposure exposure scenarios, but just one of the models is recommended

4   for the higher-exposure exposure scenarios; users have the option of using a single model across

5   the range of exposure scenarios or of transitioning across models, depending on the exposure

6   scenarios of interest, and some guidance on choice of approach is provided in Section 4.7 of the

7   revised assessment. As discussed in the assessment, the log-cumulative exposure model, which

8   provides a good fit to the data in the plateau and is suitable for exposure scenarios with

9   cumulative exposures in that region, is not appropriate for the low-exposure region because such

10   a steep increase in slope is considered to be biologically implausible and the good statistical

11   global fit of the model shouldn't be over-interpreted to infer that the model provides a

12   meaningful fit to the low-exposure region. Likewise, the linear regression used to model the

13   lower-dose exposure groups is not intended to reflect the exposure-response relationship in the

14   higher-exposure region. Hence, for lymphoid cancer, the use of both models may be required to

15   cover a range of occupational exposure scenarios. Table 4-19 of the assessment shows how

16   results from the two models compare over a range of exposure scenarios for which either model

17   might be used.

18

19   *2.e. Are the methodologies used to estimate the carcinogenic risk based on rodent data*

20   *appropriate and transparently described? Is the use of "ppm equivalence" adequate for*

21   *interspecies scaling of EtO exposures from the rodent data to humans?*

22

23   <u>SAB Panel Comment:</u> The ppm equivalence method is a reasonable approach for interspecies

24   scaling of EtO exposures from rodent data to humans. If the use of animal data becomes more

25   important (i.e., the principal basis for the ethylene oxide unit risk value), more sophisticated

26   approaches such as PBPK modeling should be considered.

27

28   <u>EPA Response:</u> EPA appreciates the Panel's support for the use of the ppm equivalence method.

29   As the unit risk value is based on human data, the use of more sophisticated models is not

30   necessary.

31

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

2   *EPA's Risk Characterization Handbook requires that assessments address in a transparent*

3   *manner a number of important factors. Please comment on how well this assessment clearly*

4   *describes, characterizes and communicates the following:*

5   *a. The assessment approach employed;*

6   *b. The use of assumptions and their impact on the assessment;*

7   *c. The use of extrapolations and their impact on the assessment;*

8   *d. Plausible alternatives and the choices made among those alternatives;*

9   *e. The impact of one choice versus another on the assessment;*

10   *f. Significant data gaps and their implications for the assessment;*

11   *g. The scientific conclusions identified separately from default assumptions and policy calls;*

12   *h. The major risk conclusions and the assessor's confidence and uncertainties in them, and;*

13   *i. The relative strength of each risk assessment component and its impact on the overall*

14   *assessment.*

15

16   SAB Panel Comment: The Panel's report contained specific responses to charge questions 1 and

17   2. The report did not contain specific responses to question 3 and instead contained the following

18   statements regarding question 3:

19

20   "The Panel has responded to Charge Questions 1 and 2 and has tried to incorporate their

21   comments regarding Charge Question 3 within those responses. A separate response for

22   Charge Question 3 was not deemed necessary since issues of uncertainty were addressed

23   in the responses to charge questions 1 and 2."

24

25   *The following are detailed comments on the regression modeling used in the draft ethylene*

26   *oxide assessment quoted from the SAB Ethylene Oxide Panel report and the EPA response:*

27

28   SAB Panel Comment:

29   2. Linear regression model for categorical data

30

1   The Panel identified several important shortcomings in the linear regression modeling

2   approach used to establish the point of departure for low dose extrapolation of cancer risk due to

3   EtO. Based on its review of the methods and results presented at the January 17,18, 2007

4   meeting, the Panel was unanimous in its recommendation that the EPA develop its risk models

5   based on direct analysis of the individual exposure and cancer outcome data for the NIOSH

6   cohort. The Panel understands that these data are available to EPA analysts upon request to the

7   CDC/NIOSH. The Panel recognizes the burden that a reanalysis of the individual data places on

8   the EPA ORD staff but given the important implications of the risk assessment, this burden is

9   well justified to achieve the best scientific and statistical treatment of all the available

10   epidemiological data.

11   The following paragraphs present the statistical basis for the Panel's assessment of the

12   linear regression model approach and the use of categorized exposure and outcome data.

13   The approach described in the Draft Assessment uses a model based on categories

14   defined by cumulative exposure ranges for male subjects in the NIOSH cohort. Steenland et al.

15   identified several models that provide a significant ($p<0.05$) fit to the exposure data; however,

16   the EPA has elected to use model-based relative rate parameter estimates for categories of 15

17   year lagged, cumulative exposure. In Steenland, et al. (2004) this model was not one that

18   provided a significant fit to the NIOSH data (p=0.15 for the likelihood ratio test of $\underline{\beta}= \{\beta_1, \beta_2, \beta_3,$

19   $\beta_4\}=0$). The use of the weighted least squares regression fit of a linear regression line through the

20   three data points defined by the estimated rate ratios and mean cumulative exposures for the first

21   three exposure categories of the Steenland, et al. 15 year lag, cumulative exposure category

22   model is not a robust application of this technique. The Panel identified four weaknesses in the

23   approach.

24   a) Model-based dependent variable: The dependent variables are model-based estimates

25   of rate ratios for exposure categories. The rate ratio values used in the weighted least squares

26   regression are derived from a cumulative exposure model (15 year lag) in which the estimated

27   regression parameters in the proportional hazards regression model are not significantly different

28   from 0 at $\alpha=0.05$ (p=0.15). In Steenland et al. (2004), the only individually based (proportional

29   hazards) model that fits the data for males in the NIOSH cohort is a model for log of individual

30   exposure through t-15 years.

1   b) Grouped data regression: The weighted least squares fit applies estimates of variance

2   for the individual rate ratios under that assumption that these inverse weighting corrections

3   correctly adjust for heteroscedasticity of residuals in the underlying regression model.

4   Historically, models for grouped proportions applied adjustments of this type but it is by no

5   means a preferred technique when the underlying individual data are available. The "ecological

6   regression" model per Rothman (1998, Second edition) is subject to bias due to within group

7   heterogeneity of predictors and unmeasured confounders. The heterogeneity in the grouped

8   model involves the range of exposures within the collapsed categories. The unmeasured

9   confounders include variables (other than gender) that affect the potency of exposure or may

10   have produced gross misclassification based on the original exposure model estimation for the

11   individual (Hornung, et al., 1994).

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

13   1998 (Second edition) of Rothman (Rothman and Greenland, 1998) describes the

14   technique for estimating this risk from grouped data in Chapter 23. In that updated version of the

15   original monograph the model that is fitted is:

16

17        $$Expected(Rate/Exposure) = \hat{B}_0 + \hat{B}_1 * Mean(Exposure)$$

18

19   The objective is to estimate the rate ratio (for exposure 0=no, 1=yes, or equivalently for a one

20   unit increase in the exposure metric). That estimator is then:

21

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

23

24   The model estimated by the EPA method is:

25

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

27

28   In the former, the variance in the estimation of the rate ratio is a function of the variance of the

29   estimated slope and the variance in the estimated baseline hazard, represented by the estimated

30   intercept. This variance is present in the estimation of the baseline hazard in the Steenland, et al.

31   (2004) estimation of the rate ratios but is not present in the EPA adaptation to the linear rate ratio

1 model. The EPA approach permits no intercept (>0) for the background exposure or any
2 allowance for an effect of true non-zero exposures in the internal control group (exposures less
3 than 15 years).
4
5 In general, the use of categorical exposure ranges is not the optimal strategy for using
6 epidemiologic data. When continuous data are categorized and then used in dose response
7 modeling, it amounts to starting with a full range of exposures, collapsing that range into
8 somewhat arbitrary boundaries and then deriving a continuous dose response model for an even
9 larger range of exposures.
10
11 Categorizing continuous variables results in a host of issues:
12 • Assumption that the risk within the category boundaries is constant
13 • It is not known whether a given categorization is representative of the data since there are many
14 ways of categorizing.
15 • Loss of power and precision by spending degrees of freedom on each category
16 • Misclassification at category boundaries (this can be minimized by choosing cutpoints
17 where relatively few observations are present)
18 • Categorizations can be manipulated to show the desired results
19
20 The Panel acknowledged that techniques such as the linear regression method described
21 by Rothman (1998) or Poisson regression may be the most appropriate techniques when only
22 grouped or categorized data are available for estimating the dose/response model. However, the
23 original NIOSH cohort data are available at the individual level and this permits the use of
24 models such as the Cox regression models employed by Steenland et al. (2004) that utilize the
25 full information in the individual observations. If categories of exposure (as opposed to
26 individual exposure estimates) must be used, the crude rates should be computed for a large
27 number of equally spaced exposure ranges and the Rothman and Greenland (1998) model fitted
28 to these multiple points.
29
30

1   EPA Response: EPA agrees that it is may be preferable to develop risk models on the basis of

2   direct analysis of individual exposure and cancer outcome data. In fact, the Draft Assessment

3   document included the presentation of models based on fitting Cox regression models to

4   individual exposure-outcome data for EtO. These models provided reasonable fits to the data, as

5   described by Steenland et al. (2004) and in the Draft Assessment document. However, it was the

6   judgment of EPA that these models represented exposure-response relationships that were

7   excessively sensitive to changes in exposure level in the low dose region and thus were not

8   biologically realistic. That is, in the low dose region, these models would yield extremely large

9   changes in response for small changes in dose level. Accordingly, the judgment was that these

10   models would not be suitable as the basis for low-dose unit risk values. This is what led EPA to

11   use the regression methodology with the published grouped data.   The grouped data regression

12   methodology is considered to be a valid procedure for analysis of such data; therefore, EPA has

13   retained its use for some endpoints in the final assessment and implemented it as described by

14   Rothman (1986) (also described in Rothman and Greenland [1998], Rothman et al. [2008] and

15   Van Wijingaarden, E; Hertz-Picciotto, I. [2004]).

16   EPA also followed the Panel's recommendation and performed additional analyses of the

17   individual data in collaboration with Professor Steenland. The work performed by Professor

18   Steenland is described in Appendix D of the final assessment. Working with Professor

19   Steenland,alternative models based on direct analysis of all individual data using (1) linear

20   relative risk models (Langholz, B., and Richardson, D.B., Am J Epidemiol 2010) and (2) two-

21   piece linear and log-linear spline models (e.g., Rothman et al. Modern Epidemiology, 3rd

22   Edition, 2008) were developed and evaluated. In the final assessment, linear low dose risk

23   estimates based on the two-piece linear spline model (using the Langholz-Richardson linear

24   relative risk approach) were used for breast cancer incidence risk estimates. Additional responses

25   to specific comments follow:

26   a) Model-based dependent variable: EPA used dependent variables that are model-based

27   estimates of rate ratios for exposure categories which follows the Rothman (1986, page 343)

28   methodology. The rate ratio estimates were derived from the same data that produced significant

29   fits using the proportional hazard (or Cox) model with individual data (exposure as a continuous

30   variable). The continuous models were not used for risk estimation because of excessive

31   sensitivity in the low exposure range. The rate ratios for the exposure categories were not

1   statistically significant, likely due to loss of power as noted in the comment, but were used

2   because we were confident that they represented a real effect in the data (based on the significant

3   fit of the continuous models) due to exposure to EtO.

4

5   b) Grouped data regression: These comments correctly identify assumptions inherent in

6   the method. The assumptions do not, however, preclude the use of the Rothman model in the

7   context of the EtO cancer risk estimation. While there is the potential for some bias due to

8   within group heterogeneity in the EtO data, use of individual within group values results in

9   unbiased estimates of within group mean levels. EPA disagrees with the suggestion that

10   unmeasured confounders may have produced gross misclassification and somehow impaired the

11   exposure model estimation for individuals. The estimation performed by NIOSH to estimate

12   individual worker exposure (Hornung et al., 1994) was extensive and detailed. The resulting

13   model used to estimate worker exposure accounted for 85% of the variation in average EtO

14   exposure (see <u>Evaluation of the Carcinogenicity of Ethylene Oxide [2010]</u>, page 4-29). EPA

15   agrees with the Panel that the exposure analysis of Hornung et al. (1994) is an example of an

16   "exemplary quantitative analysis of likely errors in exposure estimates." In response to the

17   Panel's suggestion that the Hornung analysis represents an "invaluable opportunity" for further

18   analysis of the impact of possible errors in exposure estimation, EPA investigated the possible

19   use of the "errors in variables" approach (page 27 of the Panel report). Professor Steenland

20   visited the NIOSH offices in Cincinnati in order to review the data and assess whether it would

21   support an "errors in variables" analysis. Unfortunately, the electronic data files used in the

22   exposure analysis were no longer available, so that analysis based on the "errors in variables"

23   approach was not possible.

24   c) EPA reviewed the statistical procedure for modeling categorical data using the

25   methodology in Rothman (1986). This review confirmed that the Rothman procedure was

26   followed closely. The equations used, which are the same as those in Rothman (1986), pp.341-

27   344, are described in Appendix F of the <u>Evaluation of the Carcinogenicity of Ethylene Oxide</u>

28   <u>(2010)</u>. The equations are also provided in Van Wijingaarden, E; Hertz-Picciotto, I. (2004). The

29   linear model in Appendix F is identical to equation 16-6 in Rothman (1986) and the estimator of

30   the slope in Appendix F is identical to equation 16-7 in Rothman (1986). The Rothman

31   procedure, which is appropriate for case-control data such as the NIOSH data, is based on

1   estimating the effect at each response level relative to the reference or baseline level. This is the

2   lowest exposure category, for which the rate ratio is defined as 1.0, so in effect there is no

3   intercept term in the model. As described by Rothman (1986, page 345), variability in the

4   reference category is necessarily entrained in estimates of the slope. As Rothman points out, this

5   can result in loss of estimation efficiency but nevertheless yields in a valid estimate of trend.

6   Thus, while it is true, as the comment states, that this procedure may not be optimal in a

7   theoretical sense, it can provide a useful mechanism for estimating linear trend. The Panel

8   acknowledges that this approach may be the most appropriate when only grouped data are

9   available. EPA agrees but would add that when the objective is low dose risk estimation, the

10   approach may yield the most useful results from a pragmatic perspective. The availability of

11   individual data does not preclude the use of the Rothman grouped data regression methodology.

12   In the case of the EtO data, it was possible to derive theoretically correct models via

13   direct analysis of the individual data. In the case of the breast cancer incidence data, this

14   approach yielded a model that provided a suitable basis for risk estimation. For the other end

15   points (breast cancer mortality, lymphoid cancer incidence and mortality), however, the models

16   derived using all individual data were not suitable for risk estimation because of excessive

17   sensitivity in the low dose range. The large sensitivity of the models to small changes in low

18   dose values results in unstable low dose risk estimates lacking in biological plausibility and thus

19   the Rothman procedure was used.

20

21   ***Responses to SAB Panel 'bullet' comments:***

22   • Assumption that the risk within the category boundaries is constant.

23

24   *Response: EPA is not assuming that within category risk is constant. Instead, the assumption is*

25   *that observed risk within a category may be averaged over a category even though there may be*

26   *a trend within the category. This is a conventional approach in epidemiological analyses in*

27   *which categorical analysis is used.*

28

29   • It is not known whether a given categorization is representative of the data since there are many

30   ways of categorizing.

31

1   *Response: The data groupings and category rate estimates used in the EPA analyses were*

2   *obtained from the Steenland et al. publications and are thought to be objective representations of*

3   *the data. The categories were generally quartiles based on the distribution of cumulative*

4   *exposures for the cases of the cancer of interest, resulting in essentially the same number of*

5   *cancer cases per quartile, a typical approach in epidemiological studies.*

6

7   • Loss of power and precision by spending degrees of freedom on each category.

8

9   *Response: There is some loss of power and precision in categorization. This can result in a*

10   *failure to find a statistically significant effect when in fact there is a meaningful effect in the*

11   *data, as noted above.*

12

13   • Misclassification at category boundaries (this can be minimized by choosing cut points where

14   relatively few observations are present)

15

16   *Response: Misclassification can occur because of overall uncertainty in classification including*

17   *uncertainty that may arise at category boundaries. We believe that the extensive work done by*

18   *Steenland and co-workers who worked on the NIOSH data to define data categories and*

19   *category rate estimates has minimized problems of misclassification at the boundaries, which*

20   *are, in any event, expected to be a small part of overall misclassification.*

21

22   • Categorizations can be manipulated to show the desired results.

23

24   *Response: This may be possible but no manipulation of the EtO data was performed to show*

25   *"desired results." The data categories and category rate estimates used in the EPA analyses*

26   *were obtained from the Steenland et al. publications. The Panel's recommendation to use "a*

27   *large number of equally spaced exposure ranges" to determine categories was not feasible*

28   *because of the relatively small numbers of cases.*

29

30   *References:*

31   *Rothman, K.J. (1986) Modern epidemiology. Worcester, MA: Little, Brown and Co. p. 341–344.*

1   *Rothman, K.J. and Greenland, S. (1998)* Modern epidemiology*, Second Edition. Philadelphia,*

2   *PA: Lippincott Williams & Wilkens*

3   *Rothman, KJ. , Greenland, S. and Lash, T.L. (2008)* Modern epidemiology*, Third Edition.*

4   *Philadelphia, PA: Lippincott Williams & Wilkens*

5   *Van Wijingaarden, E; Hertz-Picciotto, I. (2004) A simple approach to performing quantitative*

6   *cancer risk assessment using published results from occupational epidemiology studies. Sci*

7   *Total Environ 332: 81-87.*

8   **Public Comments:**

9

10   A number of public comments were received that addressed a range of technical issues

11   related to the inhalation carcinogenicity of EtO. A number of comments were also received that

12   are generally directed at what are referred to as 'Risk Management' issues and, as such, are not

13   addressed here. In the following, summaries of comments on technical risk assessment issues

14   submitted by the public and responses are provided.

15

16   <u>**Comment 1.0:**</u> **The Draft Cancer Assessment Fails to Meet the Rigorous Standard of**

17   **Quality Required Under the Information Quality Act and Cancer Guidelines.** The Draft

18   Cancer Assessment is "influential information" as set forth under the Information Quality Act

19   (IQA) and therefore is subject to a rigorous standard of quality. EPA guidance and the

20   Guidelines for Carcinogen Risk Assessment (Cancer Guidelines) require a rigorous standard of

21   quality, which necessitates ensuring that the Draft Cancer Assessment uses scientifically

22   defensible analytical and statistical methods and has a higher degree of transparency than

23   information considered noninfluential, particularly regarding the application of uncertainty

24   factors in EPA's dose-response assessment and risk characterization. The Draft Cancer

25   Assessment demonstrably fails to meet either the standard set forth under the IQA or the Cancer

26   Guidelines. EPA must, therefore, substantially revise the assessment before the final EO

27   Integrated Risk Information System (IRIS) Risk Assessment (IRIS Assessment) is publicly

28   disseminated or relied upon for any regulatory purposes.

29

1   EPA RESPONSE: Comments received from the EPA Science Advisory Board and from the

2   public have been addressed and the EtO carcinogenicity assessment has been revised. It is

3   EPA's position that as a result of the extensive development, review, re-analysis and revision,

4   the final assessment follows the EPA Cancer Guidelines, uses scientifically defensible analytical

5   and statistical methods and meets a high standard of transparency. As such, the final assessment

6   is consistent with Information Quality Guidelines.

7

8   **Comment 2.0:** EPA failed to use all available epidemiologic data, including the Union Carbide

9   Corporation (UCC) data and all the National Institute of Occupational Safety and Health

10   (NIOSH) data that were available at the time EPA conducted its assessment.

11

12   EPA RESPONSE: The assessment describes and considers all relevant epidemiological data

13   available at the time the assessment was conducted, including all the NIOSH data and the UCC

14   data. The Union Carbide data and the publications that the ACC Panel referred to were evaluated

15   and included in the assessment. EPA also reviewed articles describing additional follow-up and

16   analysis of the Union Carbide data that have been published after the Panel's report was

17   finalized. Ultimately, EPA came to the conclusion that the shortcomings inherent in the Union

18   Carbide data are fundamental and as a consequence the data are not suitable for credible

19   quantitative analysis of the carcinogenic risk due to exposure to EtO. In particular, the crude

20   assignment of exposure levels to subjects in the UCC data necessitated by the lack of

21   quantitative exposure data. This method of exposure assignment is likely to have resulted in a

22   high degree of misclassification. In the NIOSH data, exposure estimates were based on a very

23   large number of exposure measurements and a sophisticated modeling approach (Hornung et al.

24   1994) which took into account job category and other factors such as product type, exhaust

25   controls, age of product, cubic feet of sterilizer, and degree of aeration. Hence prediction and

26   assignment of exposure levels for different workers in the NIOSH study would be expected to be

27   much better than the crude assignment methods used in the Union Carbide study. Although the

28   recent follow-up of the UCC data has now been reported, there still remain a rather small number

29   of cancers (27 hematopoetic cancers, vs. 79 in the NIOSH cohort, 12 vs. 31 Non Hodgkin's

30   lymphomas). Small numbers is a problem in general for rare hematopoetic cancers, but it is

31   more severe in the Union Carbide study. For example, there was a 50% excess of NHL in the 9+

1 duration category in the Union Carbide study but it was based on only 5 cases so that it was far

2 from statistically significant. Also, the UCC cohort is restricted to men, making impossible an

3 analysis of breast cancer, which was seen to have a significant increase among those with high

4 exposures in the NIOSH cohort. In sum, the Union Carbide and NIOSH cohorts are not

5 comparable on a number of levels, and the NIOSH cohort remains superior as a basis for risk

6 assessment analyses. In the NIOSH cohort, exposure-response analyses are likely to involve

7 much less misclassification of exposure and are based on greater numbers, and thus would be

8 expected to be more reliable.   Analyses of the important breast cancer endpoint are only possible

9 in the NIOSH cohort. There is also some concern about possible bias due to the healthy worker

10 survivor affect among a portion of the Union Carbide cohort.

11

12 **Comment 3.0:** EPA inappropriately based its evaluation on summaries of statistics available in

13 various publications, rather than the primary source data, review of which and reliance upon are

14 essential to conduct valid dose-response modeling. EPA should have based its calculations on

15 readily available NIOSH data for individual subjects from the cohort mortality study.

16

17 EPA RESPONSE: The statistics used in draft proposal were obtained from published journal

18 articles describing the analysis of the NIOSH data. They are summary and categorical statistics

19 that are commonly used in epidemiological research. The methodology for using such

20 categorical data to perform dose-response analysis is well established in the epidemiological

21 literature and is described in Rothman, KJ. (1986) Modern Epidemiology. Worcester, MA:

22 Little, Brown and Co. p. 343–344, and Van Wijingaarden, E; Hertz-Picciotto, I. (2004) "A

23 simple approach to performing quantitative cancer risk assessment using published results from

24 occupational epidemiology studies." Sci Total Environ 332: 81-87. The categorical and

25 summary statistics used by EPA are constructed from all the individual data in the NIOSH data.

26 It is possible to perform analyses and construct models via direct analysis of the individual data

27 and in some cases this is a preferable approach. In fact, the draft EPA assessment presented the

28 results of such analyses in the form of the Cox regression models that were based on direct

29 analysis of the individual data with exposure as a continuous variable. These models provided

30 reasonable fits to the data. However, it was the judgment of EPA that these models generated

31 estimates of risk in the low dose region that were excessively sensitive to changes in exposure

1 level and therefore would not be suitable as the basis for low-dose unit risk values. This is what

2 led EPA to use the regression methodology with the published grouped data. EPA, in

3 consultation with Professor Steenland, did perform analyses to fit additional models to the

4 continuous NIOSH data.   The work performed by Professor Steenland is described in Appendix

5 D of the final assessment. Working with Professor Steenland, EPA developed and evaluated sets

6 of models using all individual data using (1) linear relative risk models (Langholz, B., and

7 Richardson, D.B., Am J Epidemiol 2010) and (2) two-piece linear and log-linear spline models

8 (e.g., Rothman et al. Modern Epidemiology, 3rd Edition, 2008). In the final assessment, linear

9 low dose estimates based on the two-piece spline model and using the Langholz-Richardson

10 linear approach were used for breast cancer incidence risk estimates.

11

12 **Comment 4.0: EPA Statistical Analysis of the Data Is Flawed and Other Incorrect**

13 **Procedures Grossly Overestimate Risk.** Key flaws include:

14

15 **Comment 4.1:** EPA's risk assessments are invalid, based on linear regressions on odds ratios

16 (ORs), rather than on individual subject data;

17

18 EPA RESPONSE: The odds ratios referred to are summary statistics. Regression on categorical

19 or summary statistics such as odds ratios is a valid statistical approach. See the response to

20 comment 1.2 and response to the SAB Panel comment on this issue.

21

22 **Comment 4.2:** EPA fails to include all available epidemiologic data;

23

24 EPA RESPONSE: This refers to the use of the Union Carbide data. See response to Comment

25 2.0 and response to the SAB Panel comment on this issue.

26

27 **Comment 4.3:** EPA's rationale and methodology for exclusion of the highest exposure group is

28 inappropriate;

29

30 EPA RESPONSE: EPA did not use the data from the highest exposure group in estimating the

31 unit risk because it was evident that the relationship between exposure and response changed

1 over the range of exposure. The general pattern in the data indicated a steep increase in response

2 in the low exposure range with a leveling or plateau in the high exposure range. Inclusion of the

3 data from the highest exposure levels in either a Cox regression model or a linear regression

4 yielded overall estimated relationships that were not suitable for risk assessment. Although the

5 Cox regression models with log cumulative exposure provided adequate fits to the data,

6 estimates of risk in the low dose region were overly sensitive to changes in dose level and thus

7 not biologically realistic.   In order to obtain a suitable result for risk estimation at low

8 exposures, in the draft assessment, EPA used a linear regression estimated using data that

9 exclude the highest exposure group. For the final assessment, EPA investigated the use of two

10 piece linear models that modeled the data as a combination of two linear relationships or

11 segments, one that increased steeply in the lower dose region joined with a second that increased

12 at a smaller rate in the higher dose region. This approach has the advantage of including all the

13 data and incorporating into the overall model the change in the relationship over the observed

14 range of exposure*.*

15

16 **Comment 4.4:** EPA's use of the heterogeneous broad category of distinct diseases of

17 lymphohematopoietic (LH) cancers as the response increases sample size at the expense of

18 validity and, thereby, reduces the ability to identify a valid positive dose-response relationship.

19

20 EPA RESPONSE: EPA uses the narrower category of lymphoid cancer data for the primary risk

21 estimates in the final assessment.

22

23 **Comment 5.0: Certain Policy Decisions EPA Implements in the Draft Cancer Assessment**

24 **Are Scientifically Unsupported, Overly Conservative, Inappropriate and Have Not Been**

25 **Reviewed by a Science Advisory Board.** EPA made several policy decisions that compounded

26 greatly the inherent conservatism in the risk estimates. These include, among others: (1) EPA's

27 reliance on the lower bound of the point of departure, rather than the best estimate when using

28 human data; (2) use of background incidence rates with mortality-based relative rates, thereby

29 relying on unsupported assumptions that bias results; (3) EPA's assumption of an 85-year

30 lifetime of continuous exposure and cumulative risk, rather than the more traditional 70-year

31 lifetime; and (4) the application of adjustment factors for early-life exposures.

1

2 <u>EPA RESPONSE:</u> The EtO assessment has been reviewed by the EPA Science Advisory Board

3 and EPA has responded to their comments and revised the assessment. With regard to (1), use of

4 the lower bound on the point of departure is consistent with the EPA 2005 Cancer Guidelines;

5 (2), background incidence rates were used with mortality-based relative rates because EPA's

6 objective is to estimate incidence risk not mortality risk (3) EPA did not assume an 85-year

7 lifetime, rather exposures were considered up to age 85 (i.e., actual age-specific mortality and

8 disease rates <u>to age 85</u> were used in a life table analysis; because most individuals die before age

9 85 years, the overall average lifespan from the analysis is about 75 years); (4) EPA's application

10 of adjustment factors for early life exposures in the EtO assessment was in accordance with the

11 recommendations in EPA's Supplemental Cancer Guidelines and the scientific data supporting

12 the Guidelines. The application of these adjustment factors was endorsed by the Science

13 Advisory Board.

14

15 <u>**Comment 6.0:**</u> **EPA Improperly Relies Entirely on Males in Its Assessment of**

16 **Lymphohematopoietic (LH) Cancer Mortality.** To be scientifically defensible, EPA's LH

17 cancer risk characterization must include both males and females, consistent with a "weight-of-

18 evidence" approach that relies on *all* relevant information. In the NIOSH retrospective study,

19 increased risks of LH cancer were observed in males but not females, even though the NIOSH

20 cohort was large and diverse, and consisted of more women than men. EPA's exclusive reliance

21 on male data is scientifically unsound without a mechanistic justification for treating males and

22 females differently with respect to LH, which the analysis lacks.

23

24 <u>EPA RESPONSE:</u> In the final assessment, the lymphohematopoietic cancer unit risk estimates

25 are based on data for both sexes.

26

27 <u>**Comment 7.0:**</u> **EPA's Draft Risk Estimates for Occupational Exposure Levels Rely on**

28 **Invalid and/or Inappropriate Models.** The models used to estimate risks from occupational

29 exposure are flawed because they generate supralinear results, regardless of the observed data.

30 These estimates also suffer from the same invalid methodology used in the environmental risk

1   estimates. EPA must employ a dose-response model that would generate results consistent with

2   the observed data.

3

4   EPA RESPONSE: It is the underlying data that indicate a supralinear exposure-response

5   relationship, as suggested by the categorical results as well as by the poorer fits of the Cox

6   regression models with untransformed exposure data.

7

8   **Comment 8.0: EtO is Considered by Many to be a Weak Mutagen and EPA Should**

9   **Consider This in Proposing a Unit Risk Factor.** A chemical's mutagenic potency is

10 necessarily related to its carcinogenic potency. If genotoxicity is considered the means by which

11 a chemical induces cancer, it follows that it will not induce cancer under conditions where it does

12 not induce mutations, at either the chromosome or gene level, thus providing a mechanistic basis

13 for estimating carcinogenicity. EtO has been shown only to be a weak mutagen; therefore, it

14 should not be automatically considered a human carcinogen and certainly not a potent

15 carcinogen. In addition, no treatment-related tumors were observed in rats exposed to EtO, even

16 at the 100 ppm concentration level, at the 18 month sacrifice, and the most sensitive tumor type

17 (*i.e.,* splenic mononuclear cell leukemia) did not significantly increase in the exposed rats until

18 23 months, almost the end of their lifetime of exposures (Snellings *et al.*, 1984)). EPA's analysis

19 should have reconciled these findings with its estimation of EtO's carcinogenic potency, but the

20 analysis does not do so.

21

22 EPA RESPONSE: Mutagenic potency is certainly a factor in the evaluation of carcinogenic

23 potency. EPA has, however, emphasized the use of human epidemiological data in performing

24 the assessment of the carcinogenicity of EtO.

25

26

27 **Comment 9.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.**

28

29 **Comment 9.1:** The results of the Draft Cancer Assessment (resulting in negligible risk only at

30 levels less than a part per trillion), are not reasonable when compared with the results generated

1 for other substances that are considered potent mutagens and/or potent carcinogens, and do not

2 comport with the results of other assessments EPA has undertaken.

3

4 EPA RESPONSE: The procedures used in this assessment comport with those used in other

5 assessments EPA has undertaken. Differences in relative potency across chemicals based on

6 exposure levels may reflect differences in absorption, distribution, metabolism, excretion, or

7 pharmacodynamics of the chemicals.

8

9 **Comment 9.2**: The Draft Cancer Assessment grossly over predicts the observed number of

10 cancer mortalities in the study upon which it is based by more than 60-fold. Further,

11

12 EPA RESPONSE: The assessment is not intended, nor is it appropriate, for prediction of the

13 observed number of LH cancer mortalities in the NIOSH study. The potency estimates derived

14 in the assessment are constructed for use with low dose levels consistent with environmental

15 exposure and are not appropriate for use with exposures in occupational settings, as stated

16 explicitly in the document. Occupational exposure scenarios are addressed in Section 4.7 of the

17 assessment document. Extra risks associated with occupational exposures are in the 'plateau'

18 region of the exposure-response relationships and thus increase proportionately less than risks in

19 the low dose region.

20

21 **Comment 9.3:** EPA's *de minimis* value from the Draft Cancer Assessment is 2 to 3 orders of

22 magnitude below the endogenous level of EtO that is produced naturally in humans.

23

24 EPA RESPONSE: EPA's risk estimates are for risk above background. The issue of endogenous

25 levels is addressed in the final assessment.

26

27 **Comment 9.4**: EPA's draft unit risk values for EtO are unreasonably large, given the evidence

28 of carcinogenicity in a large body of epidemiology studies that is not conclusive, the weak

29 mutagenicity data, and the lack of cancer response in rodents until very late in life. EPA must

30 make the best use of all of the epidemiology, toxicology and genotoxicity data for EtO that

1   provide valid information on the relationship between exposure and cancer response to improve

2   the reasonableness of the unit risk values for EtO.

3

4   EPA RESPONSE: EPA believes that it has made the best use of the available information in

5   revising the assessment.

6

7   **Comment 10.0: The Draft Cancer Assessment Does Not Use the Best Available Science as**

8   **Required under the Information Quality Act and Cancer Guidelines.**

9

10   **Comment 10.1:** EPA based its evaluation on summaries of statistics available in various

11   publications. These data, however, are not sufficient to conduct valid dose-response modeling.

12   EPA should have based its calculations on readily available National Institute of Occupational

13   Safety and Health (NIOSH) data for individual subjects from the cohort mortality study.

14

15   EPA RESPONSE: The statistics used in draft proposal were obtained from published journal

16   articles describing the analysis of the NIOSH data. They are summary and categorical statistics

17   that are commonly used in epidemiological research and are suitable for dose-response analysis

18   and modeling. The methodology for using categorical data to perform dose-response analysis is

19   well established in the epidemiological literature and is described in Rothman, KJ. (1986)

20   Modern Epidemiology. Worcester, MA: Little, Brown and Co. p. 343–344, and Van

21   Wijingaarden, E; Hertz-Picciotto, I. (2004) "A simple approach to performing quantitative

22   cancer risk assessment using published results from occupational epidemiology studies." Sci

23   Total Environ 332: 81-87. The categorical and summary statistics used by EPA are constructed

24   from all the individual data in the NIOSH data. It is possible to perform analyses and construct

25   models via direct analysis of the individual data and in some cases this is a preferable approach.

26   In fact, the draft EPA assessment presented the results of such analyses in the form of the Cox

27   regression models that were based on direct analysis of the individual data with exposure as a

28   continuous variable. These models provided reasonable fits to the data. However, it was the

29   judgment of EPA that these models generated estimates of risk in the low dose region that were

30   excessively sensitive to changes in exposure level and therefore would not be suitable as the

31   basis for low-dose unit risk values. This is what led EPA to use the regression methodology with

1   the published grouped data. EPA, in consultation with Professor Steenland, did perform analyses

2   to fit additional models to the continuous NIOSH data.   The work performed by Professor

3   Steenland is described in Appendix D of the final assessment. Working with Professor

4   Steenland, EPA developed and evaluated sets of models using all individual data using (1) linear

5   relative risk models (Langholz, B., and Richardson, D.B., Am J Epidemiol 2010) and (2) two-

6   piece linear and log-linear spline models (e.g., Rothman et al. Modern Epidemiology, 3rd

7   Edition, 2008). In the final assessment, linear low dose estimates based on the two-piece spline

8   model and using the Langholz-Richardson linear approach were used for breast cancer incidence

9   risk estimates.

10

11 **Comment 10.2A:** EPA did not use all available epidemiologic data, including the Union Carbide

12 Corporation (UCC) data and all NIOSH data that were available at the time EPA conducted its

13 assessment. In particular, the Greenberg, *et al.* (1990) UCC study reported the consistency of the

14 death certificate diagnosis with a pathology review of medical records for leukemia cases, a

15 validation not conducted for cases in the NIOSH study.

16

17 EPA RESPONSE: EPA considered all the available epidemiological data, including NIOSH

18 data and the Union Carbide data and the publications that the ACC Panel referred to in its

19 comments. EPA also reviewed articles describing additional follow-up and analysis of the Union

20 Carbide data that have been published after the Panel's report was finalized. Ultimately, EPA

21 came to the conclusion that the shortcomings inherent in the Union Carbide data are fundamental

22 and as a consequence the data are not suitable for credible quantitative analysis of the

23 carcinogenic risk due to exposure to EtO. In particular, the rudimentary assignment of exposure

24 levels to subjects in the UCC data necessitated by the lack of quantitative exposure data is a

25 critical deficiency. This method of exposure assignment is likely to have resulted in a high

26 degree of misclassification. In the NIOSH data, exposure estimates were based on a very large

27 number of exposure measurements and a sophisticated modeling approach (Hornung et al. 1994)

28 which took into account job category and other factors such as product type, exhaust controls,

29 age of product, cubic feet of sterilizer, and degree of aeration. Hence prediction and assignment

30 of exposure levels for different workers in the NIOSH study would be expected to be much

31 better than the crude assignment methods used in the Union Carbide study. Although the recent

1 follow-up of the UCC data has now been reported, there still remain a rather small number of

2 cancers (27 hematopoetic cancers, vs. 79 in the NIOSH cohort, 12 vs. 31 non-Hodgkin

3 lymphomas). Small numbers is a problem in general for rare hematopoetic cancers, but it is

4 more severe in the Union Carbide study. For example, there was a 50% excess of NHL in the 9+

5 duration category in the Union Carbide study but it was based on only 5 cases so that it was far

6 from statistically significant. Also, the UCC cohort is restricted to men, making impossible an

7 analysis of breast cancer, which was seen to have a significant increase among those with high

8 exposures in the NIOSH cohort. In sum, the Union Carbide and NIOSH cohorts are not

9 comparable on a number of levels, and the NIOSH cohort remains superior as a basis for risk

10 assessment analyses. In the NIOSH cohort exposure-response analyses are likely to involve

11 much less misclassification of exposure and are based on greater numbers, and thus would be

12 expected to be more reliable.   Analyses of the important breast cancer endpoint are only possible

13 in the NIOSH cohort. There is also some concern about possible bias due to the healthy worker

14 survivor affect among a portion of the Union Carbide cohort.

15

16 **Comment 10.3:** EPA Should Not Have Relied Entirely on Males in Its Assessment of

17 Lymphohematopoietic (LH) Cancer Mortality. To be scientifically defensible, EPA's LH cancer

18 risk characterization must include both males and females, consistent with a "weight-of-

19 evidence" approach that relies on *all* relevant information. In the NIOSH

20 retrospective study, increased risks of LH cancer were observed in males but not females, even

21 though the NIOSH cohort was large and diverse, and consisted of more women than men. EPA's

22 exclusive reliance on male data is scientifically unsound because it lacks a mechanistic

23 justification for treating males and females differently with respect to LH.

24

25 EPA RESPONSE: In the final assessment, unit risk estimates for lymphohematopoietic cancers

26 are based on both sexes.

27

28 **Comment 11.0: EPA Should Recognize That EtO Is Both a Weak Mutagen and Weak**

29 **Animal Carcinogen.** If genotoxicity is considered the means by which a chemical induces

30 cancer, it follows that it will not induce a cancer under conditions where it does not induce

31 mutations, at either the chromosome or gene level, thus providing a mechanistic basis for

1   estimating carcinogenicity. A chemical's carcinogenic potency is necessarily related to its

2   mutagenic potency. EtO is a DNA-reactive genotoxic agent, as demonstrated by numerous *in*

3   *vitro* and *in vivo* studies. It is only weakly mutagenic. It is therefore not surprising that no

4   exposure-related tumors were observed in rats exposed to EtO, even at the 100 parts per million

5   concentration level, at the 18 month sacrifice, and the most sensitive tumor type (*i.e.,* splenic

6   mononuclear cell leukemia) did not significantly increase in the exposed rats until 23 months-

7   almost the end of their lifetime of exposures (Snellings *et al.*, 1984). EPA's analysis should have

8   reconciled these findings with its estimation of EtO's carcinogenic potency, but the analyses do

9   not do so.

10

11   EPA RESPONSE: It is not surprising that that there was no statistically significant increase in

12   tumors at 18 months in the Snellings et al. study. Because of the latency for cancer development,

13   tumors generally occur later in life. Furthermore, only 20 animals per sex per dose group were

14   killed at 18 months (and tissues from the animals in thetwo low- and mid-doses group only got

15   microscopically examined in the presence of a gross lesion) , so there is low power to detect an

16   effect.

17

18   **Comment 11.1:**   Among 26 alkylating agents studies by Vogel, *et al.* (1998), EtO showed the

19   second lowest carcinogenic potency.

20

21   EPA RESPONSE: The Vogel et al. (1998) study is not relevant to EPA's assessment of the

22   carcinogenicity of EtO. Most of the substances considered by Vogel et al. (1998) are

23   chemotherapeutic chemicals that are, by design, intended to be strong alkylating agents.

24   **Comment 11.2:**   Previous assessments of EtO inhalation time to tumor in rats showed that the

25   increased risks observed at higher experimental doses did not extend to the lowest experimental

26   dose. To comply with the Cancer Guidelines, EPA should include these and other relevant

27   animal data in a weight-of-evidence characterization of EtO.

28

29   EPA RESPONSE: The basis for the EtO unit risk estimation is human epidemiology data which

30   is the Agency's preferred approach when such data are available. The weight of evidence

31   characterization in EPA's assessment presents appropriate consideration of relevant animal data.

1

2 **Comment 12.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.**

3

4 **Comment 12.1:** The results of the Draft Cancer Assessment (resulting in negligible risk only at

5 levels less than a part per trillion (ppt)), are not scientifically defensible when compared with the

6 results generated for other substances that are considered potent mutagens and/or potent

7 carcinogens, and do not comport with the results of assessments EPA has undertaken.

8

9 EPA RESPONSE: The procedures used in this assessment comport with those used in other

10 assessments EPA has undertaken. Differences in relative potency across chemicals based on

11 exposure levels may reflect differences in absorption, distribution, metabolism, excretion, or

12 pharmacodynamics of the chemicals.

13

14 **Comment 12.2:** The results of the Draft Cancer Assessment are at odds with EPA's conclusion

15 that EtO is a potent (*de minimis* level < 1 ppt) human carcinogen and EtO's potency seen in

16 animal studies.

17

18 EPA RESPONSE: The risk estimates based on the rodent data are over an order of magnitude

19 lower than (~1/20) the estimate based on the human data, but human data are generally preferred

20 over rodent data for quantitative risk estimates because the uncertainties due to interspecies

21 extrapolation are avoided.

22

23

24 **Comment 12.3:** EPA's draft unit risk values for EtO are not applicable to the general public.

25 The Draft Cancer Assessment grossly over predicts the observed number of LH cancer

26 mortalities in the study upon which it is based by more than 60-fold. Further, EPA's *de minimis*

27 value is about 50 times lower than the lowest ambient concentration found at remote coastal

28 locations. Based upon PBPK simulations, endogenous concentrations of EtO in humans are

29 approximately 400-1700 times greater than EPA's proposed *de minimis* value of 0.00036 parts

30 per billion.

31

1   EPA RESPONSE: The assessment is not intended, nor is it appropriate, for prediction of the

2   observed number of LH cancer mortalities in the NIOSH study. The potency estimates derived

3   in the assessment are constructed for use with low dose levels consistent with environmental

4   exposure and are not appropriate for use with exposures in occupational settings, as stated

5   explicitly in the document. Occupational scenarios are addressed in Section 4.7 of the

6   assessment document. Extra risks associated with occupational exposures are in the 'plateau'

7   region of the exposure-response relationships and thus increase proportionately less than risks in

8   the low dose region. Endogenous and ambient concentrations of EtO could be contributing to

9   background rates of LH cancer and breast cancer incidences, which are appreciable. The EPA

10   values are not implausible upper bound estimates.

11

12   **Comment 12.4:**   EPA's draft unit risk values for EtO are unreasonably large, given the non-

13   conclusive evidence of carcinogenicity in a large body of epidemiology studies, the weak

14   mutagenicity data, and the lack of cancer response in rodents until very late in their exposure

15   lifetime. EPA must make the best use of all of the epidemiology, toxicology, and genotoxicity

16   data for EtO that provide valid information on the relationship between exposure and cancer

17   response to improve the reasonableness of the unit risk values for EtO.

18

19   EPA RESPONSE: The final unit risk values are based on appropriate human epidemiological

20   data, which is the Agency's preferred approach when, as is the case for EtO, such data are

21   available. The assertion that "a large body of epidemiology studies" provides "non-conclusive

22   evidence of carcinogenicity" of EtO is not supported by the NIOSH study which is, by far, the

23   largest and most comprehensive epidemiological study of the effects of exposure to EtO.

24

25   **Comment 13.0: Certain Policy Decisions EPA Implements in the Draft Cancer**

26   **Assessment Are Scientifically Unsupported, Unprecedented, Overly Conservative, and**

27   **Inappropriate.** EPA made several policy decisions that compounded greatly the inherent

28   conservatism in the risk estimates. These include, among others: (1) EPA's reliance on the lower

29   bound of the point of departure, rather than the best estimate when using human data, resulting in

30   a 2- to 3-fold overestimate of risk; (2) use of background incidence rates with mortality-based

31   relative rates, which rely on an unsupported assumption and which yields bias results; (3) EPA's

1 assumption of an 85-year lifetime of continuous exposure and cumulative risk, rather than the

2 more traditional 70-year lifetime, resulting in an increase in the lifetime excess risk estimate of

3 approximately 3-fold; and (4) the application of adjustment factors for early-life exposures.

4   Consequently, EPA's proposed unit risk value cannot be used reliably to estimate the

5 potential risk to the general public from low levels of EtO inhalation exposure with any

6 reasonable degree of confidence. As discussed in more detail below EPA should substantially

7 revise the Draft Cancer Assessment to address these numerous scientific deficiencies and flaws.

8

9 <u>EPA RESPONSE:</u> The Draft Assessment has been revised based on consideration of comments

10 received on the draft assessment from the Science Advisory Board Panel and the public and new

11 analyses undertaken since the draft assessment was released. Specific responses to the numbered

12 comments above:

13 (1) Use of the lower bound on the point of departure is consistent with current practice and the

14 2005 EPA Cancer Guidelines.

15 (2) Background incidence rates were used with mortality-based relative rates because EPA's

16 objective is to estimate incidence risk not mortality risk

17 (3) EPA did not assume an 85-year lifetime. EPA used death rates only to age 85 which, in

18 effect, assumed a *maximum* age of 85 years (i.e., actual age-specific mortality and disease rates

19 up <u>to age 85</u> were used in a life table analysis; because most individuals die before age 85 years,

20 the overall average lifespan from the analysis is about 75 years). Since survival beyond age 85 is

21 not uncommon, this is a conservative assumption with regard to estimating excess lifetime risk.

22 (4) The use of adjustment factors to account for early-life exposures is in accordance with the

23 recommendations of EPA's 2005 Supplemental Guidance and the scientific data supporting the

24 Guidance. The application of these factors was endorsed by the Science Advisory Board.

APPENDIX I: LIST OF REFERENCES ADDED AFTER THE EXTERNAL REVIEW DRAFT

Note: These references were added to the Carcinogenicity Assessment in response to the peer reviewers' and public comments, and for completeness. The added references have not changed the overall qualitative or quantitative conclusions. These references are also included in the reference list at the end of the main body of the assessment.

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