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Evaluation of the Inhalation Carcinogenicity of Ethylene Oxide

(CASRN 75-21-8)

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

October 2016

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PREFACE

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).

In their 2011 review of EPA's draft IRIS assessment of formaldehyde, the National Research Council (NRC) provided a number of recommendations related to the IRIS Program. In their report, the NRC encouraged EPA to continue with assessments in progress while also developing improvements to the IRIS Program. Consistent with this advice, EPA indicated that it would not go backwards in the assessment development process, but would focus on moving forward, while also phasing in improvements.

The EtO assessment was one of a group of chemical assessments that had already completed an external peer review at the time that the 2011 NRC recommendations were released. For this group of assessments, EPA focused on applying some of the short-term NRC recommendations. Thus, the EtO assessment does not incorporate recent revisions to the IRIS assessment format, such as the inclusion of a standard Preamble and the revised chapter structure, and it does not fully implement the longer-term NRC recommendations.

The assessment implements a number of other NRC recommendations. For example, the assessment is streamlined and uses tables, figures, and appendices to increase transparency and clarity. The assessment is also structured to have separate hazard identification and dose-response sections, and, in addition, an updated literature search was conducted using systematic approaches. Furthermore, consistent with the goal that assessments should provide a scientifically sound and transparent evaluation of the relevant scientific literature and presentation of the analyses performed, the assessment contains an expanded discussion on the rationales for study evaluation and selection, as well as for other key assessment decisions.

In 2013, the IRIS Program implemented a suite of enhancements to the IRIS process, one of which included separating the public comment period from the external peer review period. This enhancement allowed the IRIS Program to consider public comments and make revisions as needed prior to releasing a document for peer review. As a result, current and future assessments will include the *response to public comments* as an appendix in the *external review draft* sent for peer review, and then include a response to *peer review comments* in the *final assessment*. Given the complex history of the ethylene oxide assessment, which had multiple public comment periods and peer reviews, EPA is including all of the responses to comments (both public and peer review) in the appendices of this final assessment. More details on the development of the assessment can be found in the assessment introduction in Chapter 2.

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LIST OF ABBREVIATIONS

ADAF	age-dependent adjustment factor
AIC	Akaike information criterion
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
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 estimate
MOA	mode of action
NCEA	National Center for Environmental Assessment
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
O^6-HEG	O^6-hydroxyethylguanine
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

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Earlier drafts of this document were provided for review to EPA scientists, interagency reviewers from other federal agencies and the Executive Office of the President, the public, and independent scientists external to EPA. Summaries and EPA's dispositions of the comments received from the independent external peer reviewers and from the public are included in Appendices H (for the 2006 external review draft), K (for the 2013 public comment draft), and I (for the 2014 SAB review draft).

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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, there is strong evidence of an increased risk of cancer of the lymphohematopoietic system and of breast cancer in females. Increases in the risk of lymphohematopoietic cancer have been seen in most (but not all) of the epidemiological studies of EtO-exposed workers, 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 were reported for lymphohematopoietic cancer mortality, primarily in males and in particular for lymphoid cancer (i.e., non-Hodgkin lymphoma, myeloma, and lymphocytic leukemia), and for breast cancer mortality in females (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). There is supporting evidence for an association between EtO and breast cancer from other studies, but the database is more limited than that for lymphohematopoietic cancers, in part because there are not as many studies that include sufficient numbers of females.

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). The lines of evidence supporting this characterization include: (1) strong, but less than conclusive on its own, epidemiological evidence of lymphohematopoietic cancers and 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. Overall, confidence in the hazard characterization of EtO as "carcinogenic to humans" is high.

This document describes the derivation of inhalation unit risk estimates for cancer mortality and incidence based on the human data from the NIOSH study (Steenland et al., 2004; Steenland et al., 2003). This study was selected for the derivation of risk estimates because it is a high-quality study,[1] it is the largest of the available studies, and it has exposure estimates for the individual workers from a high-quality exposure assessment. Multiple modeling approaches were evaluated for the exposure-response data, including modeling the cancer response as a function of either categorical exposures or continuous individual exposure levels. Model selection for each cancer data set was primarily based on a preference for models of the individual-level continuous exposure data, prioritization of models that are more tuned to local behavior in the low-exposure data, and a weighing of statistical and biological considerations.

Unit risk estimates based on the human data were first derived under the common assumption that relative risk is independent of age. This assumption is later superseded by an assumption of increased early-life susceptibility, and it is the unit risk estimates derived under this latter assumption that are the ultimate estimates proposed in this assessment (presented further below).

Under the assumption that relative risk is independent of age, an LEC_{01} (lower 95% confidence limit on the EC_{01}, the estimated effective concentration associated with 1% extra risk) for excess lymphoid cancer mortality (Steenland et al., 2004) was calculated using a life-table analysis and the lower spline segment from a two-piece linear spline model. Linear low-dose extrapolation below the range of observations is supported by the conclusion that a mutagenic mode of action is operative in EtO carcinogenicity. Linear low-dose extrapolation from the LEC_{01} for lymphoid cancer mortality yielded a lifetime extra cancer unit risk estimate of 1.1×10^{-3} per $\mu g/m^3$ (2.0×10^{-3} per ppb)[2] of continuous EtO exposure. Applying the same lower-spline regression coefficient and life-table analysis to background lymphoid cancer *incidence* rates and applying linear low-dose extrapolation resulted in a preferred lifetime extra lymphoid cancer unit risk estimate of 2.9×10^{-3} per $\mu g/m^3$ (5.3×10^{-3} per ppb), as cancer incidence estimates are generally preferred over mortality estimates.

Breast cancer incidence risk estimates were calculated directly from the data from a breast cancer incidence study of the same occupational cohort (Steenland et al., 2003). Using the same life-table approach, the lower spline segment from a two-piece linear spline model, and linear low-dose extrapolation, a unit risk estimate of 8.1×10^{-4} per $\mu g/m^3$ (1.5×10^{-3} per ppb) was obtained for breast

[1]The NIOSH study (Steenland et al., 2004; Steenland et al., 2003) was judged to be a "high-quality" study based on the attributes discussed in Section 3.1 and in Section A.2.8 of Appendix A, including availability of individual worker exposure estimates from a high-quality exposure assessment, cohort study design, large size, inclusion of males and females, adequate follow-up, absence of any known confounding exposures, and use of internal comparisons. The breast cancer incidence study using the subcohort of female workers with interviews had the additional attribute of investigating and controlling for a number of breast cancer risk factors (Steenland et al., 2003).

[2]Conversion equation: 1 ppm = 1,830 $\mu g/m^3$.

cancer incidence. A unit risk estimate for breast cancer mortality was also calculated from the cohort mortality data; however, the incidence estimate is preferred over the mortality estimate.

Combining the incidence risk estimates for the two cancer types resulted in a total cancer unit risk estimate of 3.3×10^{-3} per $\mu g/m^3$ (6.1×10^{-3} per ppb).[3]

Unit risk estimates (for total cancer) were also derived from the three chronic rodent bioassays for EtO reported in the literature. These estimates, ranging from 2.2×10^{-5} per $\mu g/m^3$ to 4.6×10^{-5} per $\mu g/m^3$, are about two orders of magnitude lower than the estimate based on human data. The Agency takes the position that human data, if adequate data are available, provide a more appropriate basis than rodent data for estimating population risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from rodents to humans are avoided. Although there is a sizeable difference between the rodent-based and the human-based estimates, the human data are from a large, high-quality study, with EtO exposure estimates for the individual workers and little reported exposure to chemicals other than EtO. Therefore, the estimates based on the human data are the preferred estimates for this assessment.

Because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and as there are no chemical-specific data from which to assess early-life susceptibility, increased early-life susceptibility should be assumed, according to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*—hereinafter referred to as "EPA's *Supplemental Guidance*" (U.S. EPA, 2005b). This mode-of-action-based assumption of increased early-life susceptibility supersedes the assumption of age independence under which the human-data-based estimates presented above were derived. Thus, using the same approach and exposure-response models as for the estimates discussed above but initiating exposure in the life-table analysis at age 16 instead of at birth, adult-exposure-only unit risk estimates were calculated for lymphoid cancer incidence and breast cancer incidence under an alternate assumption that relative risk is independent of age for adults, which represent the life stage pertaining to the occupational cohort data which were used for the exposure-response modeling. These adult-exposure-only unit risk estimates were then rescaled to a 70-year basis for use in the standard age-dependent adjustment factors (ADAF) calculations and risk estimate calculations involving less-than-lifetime exposure scenarios. The resulting adult-based unit risk estimates were 2.6×10^{-3} per $\mu g/m^3$ (4.8×10^{-3} per ppb) for lymphoid cancer incidence, 7.0×10^{-4} per $\mu g/m^3$ (1.3×10^{-3} per ppb) for breast cancer incidence in females, and 3.0×10^{-3} per $\mu g/m^3$ (5.5×10^{-3} per ppb) for both cancer types combined. The adult-based unit risk estimates, which were derived under an assumption of increased early-life susceptibility, supersede those presented earlier that were derived under the assumption that RR is independent of age. When using the adult-based unit risk estimates to

[3]The method used to derive the total cancer unit risk estimate involves estimating an upper bound on the sum of the maximum likelihood estimates of risk; see Section 4.1.3.

estimate extra cancer risks for a given exposure scenario, the standard ADAFs should be applied, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b). Applying the ADAFs to obtain a full lifetime total cancer unit risk estimate yields 5.0×10^{-3} per $\mu g/m^3$ (9.1×10^{-3} per ppb), and the commensurate lifetime chronic (lower-bound) exposure level of EtO corresponding to an increased cancer risk of 10^{-6} is 2×10^{-4} $\mu g/m^3$ (1×10^{-4} ppb).

The primary sources of uncertainty in the unit risk estimates derived from the human data include the retrospective exposure assessment conducted for the epidemiology study, the exposure-response modeling of the epidemiological data, and the low-dose extrapolation.[4] Although there are uncertainties in the unit risk estimate, confidence in the estimate is relatively high. First, confidence in the hazard characterization of EtO as "carcinogenic to humans," which is based on strong epidemiological evidence supplemented by other lines of evidence, is high. Second, the unit risk estimate is based on human data from a large, high-quality epidemiology study with individual worker exposure estimates. Retrospective exposure estimation is an inevitable source of uncertainty in this type of epidemiology study; however, the NIOSH investigators put extensive effort into addressing this issue by developing a state-of-the-art regression model to estimate unknown historical exposure levels using variables, such as sterilizer size, for which historical data were available. In addition, the two-piece spline models used in this assessment to model the supralinear exposure-response relationships are considered to provide a reasonable basis for the derivation of unit risk estimates. Finally, the use of linear low-exposure extrapolation is strongly supported by the conclusion that EtO carcinogenicity has a mutagenic mode of action.

Confidence in the unit risk estimate is particularly high for the breast cancer component, which is based on over 200 incident cases for which the investigators had information on other potential breast cancer risk factors. The selected model for the breast cancer incidence data provided a good global fit as well as a good local fit in the lower exposure range of greatest relevance for the derivation of a unit risk estimate. The actual unit risk might be higher or lower; however, considering the continuous-exposure linear model as a lower bound for the supralinear exposure-response relationship suggests that while a unit risk estimate for breast cancer incidence that is up to fourfold lower is plausible, unit risk estimates lower than that are considered unlikely from the available data. Sensitivity analyses for lag time, inclusion of covariates, knot, upper-bound estimation approach, use of the full incidence cohort, and inclusion of only invasive cancers for the breast cancer background rates in the life-table indicate that the unit risk estimate is not highly influenced by these factors, with comparison unit risk estimates differing by at most 40%.

There is somewhat less, although still relatively high in general, confidence in the lymphoid cancer component of the unit risk estimate because it is based on fewer events (53 lymphoid cancer

[4]See Section 4.1.4 for additional discussion of these and other sources of uncertainty in the unit risk estimates.

deaths); incidence risk was estimated from mortality data; and the exposure-response relationship is exceedingly supralinear, complicating the exposure-response modeling and model selection to a greater extent than for breast cancer incidence. The actual unit risk might be higher or lower than that from the selected model, and there were no clear upper or lower bounds for the apparent exposure-response relationship provided by other models. Sensitivity analyses for lag time, knot, and upper-bound estimation approach, indicate that the unit risk estimate for lymphoid cancer is more influenced by these factors than was the estimate for breast cancer incidence. Comparison unit risk estimates from the sensitivity analyses ranged from about 50% of the preferred unit risk estimate to about 3-times that estimate. While there is less confidence in the lymphoid cancer unit risk estimate than in the breast cancer unit risk estimate, the lymphoid cancer estimate is considered a reasonable estimate from the available data, and overall, there is relatively high confidence in the total cancer unit risk estimate.

The unit risk estimate is intended to provide a reasonable upper bound on cancer risk. The estimate was developed for environmental exposure levels (it is considered valid for exposures up to about 40 $\mu g/m^3$ [20 ppb]) and is not applicable to higher-level exposures, such as may occur occupationally, which appear to have a different exposure-response relationship. However, occupational exposure levels of EtO are of concern to EPA when EtO is used as a pesticide (e.g., sterilizing agent or fumigant). Therefore, this document also presents estimates of extra risk for the two cancer types for a range of occupational exposure scenarios (see Section 4.7). Maximum likelihood estimates of the extra (incidence) risk of lymphoid cancer and breast cancer combined for the range of occupational exposure scenarios considered (i.e., 0.1 to 1 ppm 8-hour TWA for 35 years) ranged from 0.037 to 0.11; upper bound estimates ranged from 0.081 to 0.22. The uncertainty associated with the extra risk estimates for occupational exposure scenarios is less than that associated with the unit risk estimates for environmental exposures, and the overall confidence in the extra risk estimates for occupational exposure is high. The extra risk estimates are derived for occupational exposure scenarios that yield cumulative exposures well within the range of the exposures in the NIOSH study. Moreover, the NIOSH study is a study of sterilizer workers who used EtO for the sterilization of medical supplies or spices (Steenland et al., 1991); thus, the results are directly applicable to workers in these occupations, and these are among the occupations of primary concern to EPA.

Table 1-1 provides a summary of the major findings in this assessment.

Table 1-1. Summary of major findings

Hazard conclusions	
Hazard characterization	The weight of evidence from epidemiological studies and supporting information is sufficient to conclude that ethylene oxide is carcinogenic to humans.
Mode of action	The weight of evidence is sufficient to conclude that ethylene oxide carcinogenicity has a mutagenic mode of action.
Unit risk estimates (for environmental exposures)[a]	
Basis	**Inhalation unit risk estimate[a] (per µg/m^3)[b]**
Full lifetime unit risk estimate[c]	
Total cancer risk based on human data[d]—lymphoid cancer incidence and breast cancer incidence in females	**5.0×10^{-3}**
Adult-based unit risk estimates[e]	
Total cancer risk based on human data[d]—lymphoid cancer incidence and breast cancer incidence in females	**3.0×10^{-3}**
Lymphoid cancer incidence in both sexes based on human data	2.6×10^{-3}
Breast cancer incidence in females based on human data	7.0×10^{-4}
Total cancer incidence risk estimate from rodent data (female mouse)	4.6×10^{-5}
Extra risk estimates for occupational exposure scenarios (see Section 4.7)	
Maximum likelihood estimates of the extra risk of lymphoid cancer and breast cancer combined for the range of occupational exposure scenarios considered (i.e., 0.1 to 1 ppm 8-hr TWA for 35 yr)[f]	0.037−0.11
Upper bound estimates of the extra risk of lymphoid cancer and breast cancer combined for the range of occupational exposure scenarios considered (i.e., 0.1 to 1 ppm 8-hr TWA for 35 yr)[f]	**0.081−0.22**

[a]These unit risk estimates are not intended for use with continuous lifetime exposure levels above about 40 µg/m^3. See Section 4.7 for risk estimates based on occupational exposure scenarios. Preferred estimates are in bold.
[b]To convert unit risk estimates to (ppm)$^{-1}$, multiply the (µg/m^3)$^{-1}$ estimates by 1,830 (µg/m^3)/ppm.
[c]Because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and because of the lack of chemical-specific data, EPA assumes increased early-life susceptibility and recommends the application of ADAFs, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), for exposure scenarios that include early-life exposures. For the full lifetime (upper bound) unit risk estimate presented here, ADAFs have been applied, as described in Section 4.4.
[d]Technically, this unit risk estimate reflects the total (upper bound) cancer risk to females and not to the general population because the breast cancer risk estimate only applies to females. As a practical matter for regulatory purposes, however, females comprise roughly half the general population and this unit risk estimate enables risk managers to evaluate the individual risk for this substantial population group. For the purposes of estimating numbers of cancer cases attributable to specific exposure levels, e.g., for benefits analyses, it would be more appropriate to use the cancer-specific unit risk estimates (or central tendency estimates), taking sex into account.

Table 1 1. Summary of major findings (continued)

[e]These (upper bound) unit risk estimates are intended for use in ADAF calculations and less-than-lifetime adult exposure scenarios (U.S. EPA, 2005b). Note that these are not the same as the unit risk estimates derived directly from the human data in Section 4.1 under the assumption that RRs are independent of age. See Section 4.4 for the derivation of the adult-based unit risk estimates.

[f]Technically, these sums would reflect the total cancer risk to females and not a mixed-sex workforce because the breast cancer risk estimate only applies to females. As a practical matter for regulatory purposes, however, females typically comprise a substantial proportion of the sterilizer workforce and summing these extra risk estimates enables risk managers to evaluate the individual risk for this substantial workforce group. In a situation in which the workforce of concern is comprised predominantly of males, it might be appropriate to use a sex-weighted sum of the extra risks from the two cancer types (see Section 4.7 for the cancer-specific extra risk estimates).

2. INTRODUCTION

Chapter 2 provides background information for the assessment, including:

- A brief overview about EtO exposure.
- The purpose of the assessment.
- A history of the development of the assessment and its external reviews.
- A summary of literature search approaches (see Section 2.1)
- A discussion of consistency of the assessment with 2011 NRC recommendations (see Section 2.2)

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 certain other items and as a fumigating agent for spices. The largest sources of human exposure are in occupations involving contact with the gas in plants that manufacture or use EtO and in hospitals that sterilize medical equipment. EtO can also be inhaled by residents living near production or sterilizing/fumigating facilities. Based on EPA's 2005 National-scale Air Toxics Assessment (NATA) data, the average environmental exposure concentration from all sources (including concentrations near known sources) in the United States is 0.0062 $\mu g/m^3$; the average background concentration excluding concentrations near known sources of EtO is 0.0044 $\mu g/m^3$ (NATA 2005 data, http://www.epa.gov/ttn/atw/nata2005/tables.html).

EPA offices with an interest in EtO include the Office of Air and Radiation and the Office of Pesticide Programs. The Office of Air and Radiation has an interest because EtO is 1 of the 188 hazardous air pollutants listed in the 1990 Clean Air Act Amendments. The Office of Pesticide Programs has an interest in both environmental and occupational exposures resulting from the sterilization uses of EtO because EPA is responsible for pesticide labeling and registration decisions under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

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) (CASRN 75-21-8). 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 cancer 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 $\mu g/m^3$ 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). United States 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* (U.S. EPA, 2012), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000), *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, 2006b).

An external review draft of this carcinogenicity assessment (U.S. EPA, 2006a) was peer reviewed by a panel of EPA's Science Advisory Board (SAB) in 2007 (SAB, 2007). See Appendix H for a summary and EPA's disposition of SAB and public comments on the 2006 external review draft. In response to comments from that SAB review, EPA conducted extensive new exposure-response modeling of certain epidemiologic data. In addition, EPA updated the assessment to reflect new literature through May 2013 (Appendix J). In July 2013, EPA released a revised draft for public comment (U.S. EPA, 2013a, b), and that draft assessment was discussed at EPA's December 2013 IRIS Bimonthly Public Meeting. Appendix K contains EPA's responses to the public comments that were received on the July 2013 draft. A further revised external review draft (U.S. EPA, 2014a, b) was reviewed by another panel of the SAB in 2014−2015 (SAB, 2015), primarily to receive comments on the expanded exposure-response modeling of the epidemiologic data. See Appendix I for EPA's disposition of SAB comments on the 2014 draft. Finally, EPA has further updated the assessment to reflect new literature through July 2016; this new literature did not substantively impact the conclusions of the assessment (Appendix J).

2.1. LITERATURE IDENTIFICATION

The literature search strategy first employed for this assessment 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, and references were added after the first external peer review in response to the reviewers' and public comments.

The cutoff date for literature inclusion into the main body of this carcinogenicity assessment was 30 June 2010. At that time, the analyses and text were largely completed, with the exception of a few focused issues which remained for discussion and review.

In preparation for the second external peer review, a well-documented systematic literature search was conducted for the time frame from January 2006 to May 2013 to ensure that no major studies

were missed from the time of the first external review draft in 2006 until the cutoff date and to determine if any significant new studies had been published since the cutoff date that might alter the findings of the assessment. This systematic literature search is described in Section J.1 of Appendix J. Based on this search, 56 references were identified as potentially relevant to the EtO assessment. None of the new studies were judged to impact the assessment's major conclusions. Nonetheless, two new studies of high pertinence to the assessment were identified, and these studies are reviewed in Section J.2 of Appendix J for transparency and completeness. Furthermore, one of the studies is a follow-up study of an epidemiology study already included in the assessment, and this follow-up study is also discussed in the main body of the assessment. Reviews of an additional two new studies of high pertinence were added to Appendix J to address public comments received in October and December of 2013 (see Section J.3); these studies similarly did not impact the assessment's major conclusions.

For this current assessment, another well-documented systematic literature search was conducted using the same approach as in the earlier search (see Section J.1 of Appendix J), this time for the time frame from May 2013 through August 2016. Based on this search, 17 references were identified as potentially relevant to the EtO assessment and have been added to Section J.1 of Appendix J. A further two new studies of high pertinence to the assessment were identified, and these studies are reviewed in Section J.4 of Appendix J. Once again, none of the new studies were judged to impact the assessment's major conclusions.

All the references considered and cited in this document, including abstracts, can be found on the Health and Environmental Research Online (HERO) website.[5]

2.2. NRC RECOMMENDATIONS OF 2011

In 2011, the National Research Council (NRC), in their review of EPA's draft IRIS assessment of formaldehyde, provided a number of recommendations related to the IRIS program. The EtO assessment was one of a group of chemical assessments that had already completed an external peer review at the time the 2011 NRC recommendations were released. For this group of assessments, EPA focused on a subset of the short-term recommendations, such as streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Thus, the EtO assessment does not incorporate recent revisions to the IRIS assessment format recommended in the 2011 NRC recommendations (and the more recent 2014 NRC Review of the IRIS Process), such as the inclusion of a standard Preamble and the revised chapter structure, and does

[5]HERO is a database of scientific studies and other references used to develop EPA's risk assessments, which are aimed at understanding the health and environmental effects of pollutants and chemicals. HERO is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 1,000,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

not fully implement the longer-term NRC recommendations. The assessment does, however, conform to a number of the recommendations. For example, the assessment is streamlined and uses tables, figures, and appendices to increase transparency and clarity. In addition, the assessment is structured to have separate hazard identification and dose-response sections, and the update to the literature search was conducted using systematic literature search approaches. Furthermore, consistent with the goal that assessments should provide a scientifically sound and transparent evaluation of the relevant scientific literature and presentation of the analyses performed, the assessment contains an expanded discussion on the rationales for study evaluation and selection, as well as for other key assessment decisions.

For general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

3. HAZARD IDENTIFICATION

Chapter 3 provides an evaluation of the evidence regarding the carcinogenicity of EtO.

Major findings of Chapter 3:

1. The weight of evidence from epidemiological studies and supporting information is sufficient to conclude that EtO is "carcinogenic to humans."

2. The weight of evidence is sufficient to conclude that EtO carcinogenicity has a mutagenic mode of action.

This chapter presents the evidence considered in the hazard identification of EtO carcinogenicity and the hazard characterization resulting from the weight-of-evidence evaluation. Section 3.1 summarizes the human evidence (a more detailed discussion of the human cancer studies is presented in Appendix A). Section 3.2 describes the evidence from laboratory animal studies. Section 3.3 discusses supporting evidence, in particular evidence regarding the genotoxicity of EtO. Section 3.4 provides the mode-of-action analysis for EtO carcinogenicity. To conclude the chapter, Section 3.5 presents the hazard characterization for EtO carcinogenicity and a discussion of life stages and populations with potentially increased susceptibility.

3.1. EVIDENCE OF CANCER IN HUMANS

The literature from 1985, the year of EPA's previous health assessment document on EtO (U.S. EPA, 1985), to the present contains numerous epidemiological studies of the carcinogenic effects of EtO in occupational cohorts; some of these cohorts were the subject of multiple reports. The conclusions about the human evidence of carcinogenicity in this assessment are based on the following summary of those studies, which are discussed in more detail and critically reviewed in Appendix A. Table A-5 in Appendix A provides a tabular summary of the epidemiological studies, including some study details, results, and limitations. The strengths and weaknesses of these studies were evaluated individually using standard considerations in evaluating epidemiological studies. The major areas of concern are study design, exposure assessment, and data analysis. General features of study design considered include sample size and assessment of the health endpoint. For case-control studies, design considerations include representativeness of cases, selection of controls, participation rates, use of proxy respondents, and interview approach (e.g., blinding). For cohort studies, design considerations include selection of referent population (e.g., internal comparisons are generally preferred to comparisons with

an external population[6]), loss to follow-up, and length of follow-up. Exposure assessment issues include specificity of exposure (exposure misclassification), characterization of exposure (e.g., ever exposed or quantitative estimate of exposure level), and potential confounders. Analysis considerations include adjustment for potential confounders or effect modifiers and modeling of exposure-response relationships.

Two primary sources of exposures to EtO are production facilities and sterilization operations. There are two types of production facilities (IARC, 1994b):

1. Those using the older chlorohydrin process, where ethylene is reacted with hypochlorous acid and then with calcium oxide to make EtO (this method produces unwanted 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 was 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 in Great Britian (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

[6]Internal comparisons are considered superior to external comparisons in occupational epidemiology studies because internal comparisons help control for the healthy worker effect and other factors that might be more comparable within a study's worker population than between the workers and the general population.

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, four leukemias were observed (1.7 expected) and nine 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.

A series of retrospective mortality studies of about 2,000 male workers who were assigned to operations that used or produced EtO in either of two Union Carbide Corporation (UCC) chemical production facilities in West Virginia (Valdez-Flores et al., 2010; Swaen et al., 2009; Teta et al., 1999; Benson and Teta, 1993; Teta et al., 1993; Greenberg et al., 1990) have been published. EtO was produced at these facilities until 1971, after which it was imported to the facilities. For EtO production, the chlorohydrin process was used from 1925 to 1957, and the direct oxidation process was used from 1937 to 1971 (during overlapping years, both processes were in use). The cohort was observed from 1940 through 1978 in the original study (Greenberg et al., 1990), through 1988 in the Teta et al. (1993), Benson and Teta (1993), and Teta et al. (1999) studies, and through 2003 in the Swaen et al. (2009) and Valdez-Flores et al. (2010) studies. A large-scale industrial hygiene survey and monitoring of EtO concentrations was carried out in 1976, at which time EtO was in use at the facilities but no longer in production.

Greenberg et al. (1990) found elevated but not statistically significant risks of pancreatic cancer (SMR = 1.7) and leukemia (SMR = 2.3) (each based on seven cases) in the entire cohort; most of the cases occurred in the chlorohydrin production unit (note that the chlorohydrin production unit produced primarily ethylene chlorohydrin, which is used in chlorohydrin-based EtO production, but this unit is not where chlorohydrin-based EtO production took place). Limitations of this study included multiple exposures to many different chemicals in the facility through the years and lack of EtO exposure measurements prior to 1976. Three categories of exposure were established for analysis—low, intermediate, and high—based on a qualitative characterization of the potential for EtO exposure. The number of workers in each exposure category was not reported. No significant findings of a dose-response relationship were discernible. No quantitative estimates of individual exposure were made in this study, and no latency analysis was conducted (average follow-up was 20 years). Furthermore, EtO is not the only chemical to which the observed excesses in cancer mortality could be attributed.

A follow-up study (Teta et al., 1993) that extended the observation of this cohort (excluding the 278 chlorohydrin production unit workers, who reportedly had low EtO exposures) for an additional 10 years to 1988 found no significant risk of total cancer; there was a slight trend in the risk of leukemia with increasing duration of assignment to departments using or processing EtO, but it was not significant ($p = 0.28$) and was based on only five cases. The average follow-up was 27 years, and at least 10 years had elapsed since first exposure for all workers. The same problems of exposure ascertainment exist for this study as for that of Greenberg et al. (1990), and furthermore, the follow-up did not update work histories for the workers after 1978. EtO production at the plants was discontinued before 1978, as noted by Teta et al. (1993); however, according to Greenberg et al. (1990), certain nonproduction areas had "intermediate" potential for EtO exposure, although estimates of exposure levels suggest that the levels would also be lower during the update period [<1 ppm 8-hour time-weighted average (TWA), according to Teta et al. (1993)]. It appears from the Greenberg et al. (1990) publication that the high potential exposure group was reserved for EtO production workers, and according to Teta et al. (1993), there were only 425 EtO production workers in the cohort. Of these, only 118 worked in the chlorohydrin-based production process, where exposures were reportedly highest. Essentially, the study did not support the earlier studies of cancer in EtO workers; however, it was limited by low statistical power and a crude exposure assessment and, thus, is not very informative regarding whether exposure to EtO is causally related to cancer.

In a parallel follow-up study through 1988 of only the chlorohydrin production employees, Benson and Teta (1993) found that pancreatic cancer and lymphohematopoietic cancer cases continued to accumulate and that the SMRs were statistically significant for pancreatic cancer (SMR = 4.9; Obs = 8, $p < 0.05$) and for lymphohematopoietic cancer (SMR = 2.9; Obs = 8, $p < 0.05$). These investigators interpreted these excesses as possibly due to ethylene dichloride, a byproduct in the chlorohydrin process. Again, this small study of only 278 workers was limited by the same problems as the Greenberg et al. (1990) study and the Teta et al. (1993) study. No individual estimates of exposure are available and the workers were potentially exposed to many different chemicals (see Table A-5 in Appendix A). Furthermore, the chlorohydrin production unit was reportedly considered a low potential EtO exposure department. Hence this study has little weight in determining the carcinogenicity of EtO.

In a later analysis, Teta et al. (1999) fitted Poisson regression dose-response models to the UCC data (followed through 1988 and excluding the chlorohydrin production workers) and to data (followed through 1987) from a study by the National Institute for Occupational Safety and Health (NIOSH) (described below). Because Teta et al. (1999) did not present risk ratios for the cumulative exposure categories used to model the dose-response relationships, the only comparison that can be made between the UCC and NIOSH data is based on the fitted models. These models are almost identical for leukemia, but for the lymphoid category, the risk—according to the fitted model for the UCC data—decreased as a function of exposure, whereas the risk for the modeled NIOSH data increased as a

function of exposure. However, the models are based on small numbers of cases (16 [5 UCC, 11 NIOSH] for leukemia; 22 [3 UCC, 19 NIOSH] for lymphoid cancers), and no statistics are provided to assess model goodness-of-fit or to compare across models. In any event, this analysis is superseded by the more recent analysis by the same authors (Valdez-Flores et al., 2010) of the results of more recent follow-up studies of these cohorts (see below).

Swaen et al. (2009) studied the same UCC cohort identified by Teta et al. (1993), i.e., without the chlorohydrin production workers, but extended the cohort enumeration period from the end of 1978 to the end of 1988, identifying 167 additional workers, and conducted mortality follow-up of the resulting cohort of 2,063 male workers through 2003. Work histories were also extended through 1988 (exposures after 1988 were considered negligible compared to earlier exposure levels). Swaen et al. (2009) used an exposure assessment based on the qualitative categorizations of potential EtO exposure in the different departments developed by Greenberg et al. (1990) and time-period exposure estimates from Teta et al. (1993). This exposure assessment was relatively crude, based on just a small number of department-specific and time-period-specific categories, and with exposure estimates for only a few of the categories derived from actual measurements (see Appendix A.2.20 for details).

At the end of the 2003 follow-up, 1,048 of the 2,063 workers had died (Swaen et al., 2009). The all-cause mortality SMR was 0.85 (95% CI = 0.80, 0.90) and the cancer SMR was 0.95 (95% CI = 0.84, 1.06). None of the SMRs for specific cancer types showed any statistically significant increases. In analyses stratified by hire date (pre- [inclusive] or post-1956), the SMR for leukemia was elevated but not statistically significant (1.51; 95% CI 0.69, 2.87) in the early-hire group, based on nine deaths. In analyses stratified by duration of employment, no trends were apparent for any of the lymphohematopoietic cancers, although in the 9+ years of employment subgroup, the SMR for NHL was nonsignificantly increased (1.49; 95% CI 0.48, 3.48), based on five deaths. In SMR analyses stratified by cumulative exposure, no trends were apparent for any of the lymphohematopoietic cancers and there were no notable elevations for the highest cumulative exposure category. Note that only 27 lymphohematopoietic cancer deaths (including 12 leukemias and 11 NHLs) were observed in the cohort.

Swaen et al. (2009) also did internal Cox proportional hazards modeling for some disease categories (all-cause mortality, leukemia mortality, and lymphoid cancer [NHL, lymphocytic leukemia, and myeloma] mortality [17 deaths]), using cumulative exposure as the exposure metric. These analyses showed no evidence of an exposure-response relationship. Alternate Cox proportional hazard analyses and categorical exposure-response analyses of the UCC data conducted by Valdez-Flores et al. (2010) for a larger set of cancer endpoints similarly reported an absence of any exposure-response relationships. Each of these cancer analyses, however, relies on small numbers of cases and a crude exposure assessment, where there is a high potential for exposure misclassification.

In a study of 2,658 male workers at eight chemical plants in West Germany where EtO is produced (manufacturing process not stated), Kiesselbach et al. (1990) found slightly increased SMRs for cancers of the stomach, esophagus, and lung. A latency analysis was done only for stomach cancer and total mortality. The investigators considered 71.6% of the cohort to be "weakly" exposed; only 2.6% were "strongly exposed." No data were provided to explain how these exposure categories were derived. The workers were followed for a median 15.5 years. Without additional information on exposure to EtO, this study is of little help at this time in evaluating the carcinogenicity of EtO.

NIOSH conducted an industry-wide study of 18,254 workers (45% male and 55% female) in 14 plants where EtO was used (Steenland et al., 2004; Stayner et al., 1993; Steenland et al., 1991). Most of the workers were exposed while sterilizing medical supplies and treating spices and in the manufacture and testing of medical sterilizers. Individual exposure estimates were derived for workers from 13 of the 14 plants. The procedures for selecting the facilities and defining the cohort are described in Steenland et al. (1991), and the exposure model and verification procedures are described in Greife et al. (1988) and Hornung et al. (1994). Briefly, a regression model was developed, allowing the estimation of exposure levels for time periods, facilities, and operations for which industrial hygiene data were unavailable. The data for the model consisted of 2,700 individual time-weighted exposure values for workers' personal breathing zones, acquired from 18 facilities between 1976 and 1985. The data were divided into two sets, one for developing the regression model and the second for testing it. Seven out of 23 independent variables tested for inclusion in the regression 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. (See Appendix A, Section A.2.8, for more details on the NIOSH exposure assessment and its evaluation.) Results of the original follow-up study through 1987 are presented in Steenland et al. (1991) and Stayner et al. (1993). The cohort averaged 26.8 years of follow-up in the extended follow-up study through 1998, and 16% of the cohort had died (Steenland et al., 2004).

The overall SMR for cancer was 0.98, based on 860 deaths (Steenland et al., 2004). The SMR for (lympho)hematopoietic cancer was 1.00, based on 79 cases. Exposure-response analyses, however, revealed exposure-related increases in hematopoietic cancer mortality risk, although the effect was primarily in males, when analyzed by sex. In categorical life-table analysis, men with >13,500 ppm-days of cumulative exposure had an SMR of 1.46 (Obs = 13). In internal Cox regression analyses (i.e., analyses in which the referent population is within the cohort) with exposure as a continuous variable, statistically significant trends in males for all hematopoietic cancer ($p = 0.02$) and for "lymphoid" cancers (NHL, lymphocytic leukemia, and myeloma; $p = 0.02$) were observed using log

cumulative exposure (ppm-days) with a 15-year lag.[7] In internal categorical analyses, statistically significant odds ratios (ORs) were observed in the highest cumulative exposure quartile (with a 15-year lag) in males for all hematopoietic cancer (OR = 3.42; 95% CI = 1.09−10.73) and "lymphoid" cancer (OR = 3.76; 95% CI = 1.03−13.64). The exposure metrics of duration of exposure, average concentration, and maximum (8-hour TWA) concentration did not predict the hematopoietic cancer results as well as did the cumulative exposure metric.

Although the overall SMR for female breast cancer was 0.99, based on 102 deaths, the NIOSH mortality follow-up study reported a significant excess of breast cancer mortality in the highest cumulative exposure quartile using a 20-year lag period compared to the U.S. population (SMR = 2.07; 95% CI = 1.10−3.54; Obs = 13). Internal exposure-response analyses also noted a significant positive trend for breast cancer mortality using the log of cumulative exposure and a 20-year lag time (p = 0.01). In internal categorical analyses, a statistically significant OR for breast cancer mortality was observed in the highest cumulative exposure quartile with a 20-year lag (OR = 3.13; 95% CI = 1.42−6.92).

In summary, although the overall external comparisons did not demonstrate increased risks, the NIOSH investigators found significant internal exposure-response relationships between exposure to EtO and cancers of the hematopoietic system, as well as breast cancer mortality. (As stated earlier, internal comparisons are considered superior to external comparisons in occupational epidemiology studies because internal comparisons help control for the healthy worker effect and other factors that might be more comparable within a study's worker population than between the workers and the general population.) Exposures to other chemicals in the workplace were believed to be minimal or nonexistent. This study is the most useful of the epidemiologic studies in terms of carrying out a quantitative dose-response assessment. It possesses more attributes than the others for performing risk analysis (e.g., good-quality estimates of individual exposure, lack of exposure to other chemicals, and a large and diverse cohort of workers).

It should be noted that Steenland et al. (2004) used Cox regression models, which are log-linear relative rate models, thus providing some low-dose sublinear curvature for doses expressed in terms of cumulative exposure. However, the best-fitting dose-response model for both male lymphoid cancers and male all hematopoietic cancers was for dose expressed in terms of log cumulative exposure, indicating supralinearity of the low-dose data. Supralinearity of the dose-response data was also indicated by the categorical exposure results. This is in contrast to the reported results of Kirman et al. (2004) based on the Teta et al. (1999) analysis combining the 1993 UCC leukemia data with the 1993 NIOSH leukemia data, which are claimed by the authors to provide empirical evidence supporting a quadratic dose-response relationship. The 2004 NIOSH dose-response data for hematopoietic cancers

[7]The sex difference is not statistically significant, however, and the trends for both sexes combined are also statistically significant [p = 0.01 and p = 0.02, respectively; see Tables D-31 and D-48 in Appendix D].

clearly do not provide empirical evidence in support of a quadratic dose-response relationship. On the contrary, the NIOSH data suggest a supralinear dose-response relationship in the observable range.

Wong and Trent (1993) investigated the same cohort as Steenland et al. (1991) but added 474 new unexplained subjects and increased the follow-up period by 1 year. They incremented the total number of deaths by 176 and added 392.2 more expected deaths. The only positive finding was a statistically significantly increased risk of NHL among men (SMR = 2.5; Obs = 6; $p < 0.05$). However, there was a deficit risk of NHL among women. For breast cancer, there was no trend of increasing risk by duration of employment or by latency. This study has major limitations, not the least of which is a lack of detailed employment histories, making it impossible to quantify individual exposures and develop dose-response relationships. Furthermore, the addition of more than twice as many expected deaths as observed deaths makes the analysis by the authors questionable.

Valdez-Flores et al. (2010) conducted alternative Cox proportional hazards modeling and categorical exposure-response analyses using data from the UCC cohort (Swaen et al., 2009), the NIOSH cohort (Steenland et al., 2004) and the two cohorts combined, analyzing the sexes both separately and together. These investigators reported that they found no evidence of exposure-response relationships for cumulative exposure with either the Cox model or categorical analyses for all of the cohort/endpoint data sets examined (endpoints included all lymphohematopoietic cancers, lymphoid cancers, and female breast cancer, the latter in the NIOSH cohort only). Valdez-Flores et al. (2010) did observe statistically significant increases in response rates in the highest exposure quintile relative to the lowest exposure quintile for lymphohematopoietic and lymphoid cancers in males in the NIOSH cohort, consistent with the categorical results of Steenland et al. (2004), as well as a statistically significant increase in the highest exposure quintile for lymphoid cancers in males and females combined in the NIOSH cohort, consistent with the results in Appendix D. Because the exposure assessment conducted for the UCC cohort is much cruder (see above and Appendix A.2.20), especially for the highest exposures, than the NIOSH exposure assessment (which was based on a validated regression model; see Appendix A.2.8), EPA considers the results of exposure-response analyses of the combined cohort data to have greater uncertainty than those from analyses of the NIOSH cohort alone, despite the additional cases contributed by the UCC cohort (e.g., the UCC cohort contributes 17 cases of lymphoid cancer to the 53 from the NIOSH cohort). Furthermore, Valdez-Flores et al. (2010) did not use any log cumulative exposure models, and these were the models that were statistically significant in the Steenland et al. (2004) analyses, consistent with the apparent supralinearity of the NIOSH exposure-response data. See Appendix A.2.20 for a more detailed discussion of the Valdez-Flores et al. (2010) analyses and how they compared with the Steenland et al. (2004) analyses.

In a mortality study of 1,971 male chemical workers in Italy, 637 of whom were licensed to handle EtO but not other toxic gases, Bisanti et al. (1993) reported statistically significant excesses of hematopoietic cancers (SMR = 7.1, Obs = 5, $p < 0.05$). The study was limited by the lack of exposure

measurements and by the young age of the cohort. Although this study suggests that exposure to EtO leads to a significant excess of hematopoietic cancer, the lack of personal exposure measurements and the fact that members were potentially exposed to other chemicals in the workplace lessen the study's usefulness for establishing the carcinogenicity of EtO.

Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991) studied cancer incidence in 2,170 Swedish workers (861 male and 1,309 female) in two medical sterilizing plants. They determined concentrations in six job categories and estimated cumulative exposures for each worker. They found hematopoietic cancers in 6 individuals versus 3.4 expected (SMR = 1.8) and a nonsignificant doubling in the risk when a 10-year latency period was considered. Even though the cohort was young, the follow-up time was short (median 11.8 years), and only a small fraction of the workers was highly exposed, the report is suggestive of an association between EtO exposure and hematopoietic cancers. The risk of breast cancer was less than expected, although with such short follow-up, the total numbers of cases was small (standardized incidence ratio [SIR] = 0.5, Obs = 5).

More recently, a follow-up study of the Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991) cohort was published, with an additional 16 years of follow-up (Mikoczy et al., 2011).[8] For lymphohematopoietic cancers, nonsignificant increases in SMRs and SIRs were reported and the internal incidence analysis showed no exposure-related association, although this analysis is relatively uninformative for these cancers, given the small number of cases (five cases in each of the two highest exposure quartiles and seven cases in the referent group of workers with cumulative exposures below the median), the generally low estimated cumulative exposures, and the absence of an unexposed referent group. For breast cancer incidence (41 cases), SIRs were nonsignificantly decreased, both with and without a 15-year induction period. Internal analyses resulted in statistically significant increases in the incidence rate ratios for the two highest cumulative exposure quartiles as compared to the 50% of workers with cumulative exposures below the median, despite having a low-exposed rather than an unexposed referent group.

In a large chemical manufacturing plant in Belgium (number of employees not stated), Swaen et al. (1996) performed a nested case-control study of Hodgkin lymphoma to determine whether a cluster of 10 cases in the active male work force was associated with any particular chemical. They found a significant association for benzene and EtO. This study is limited by the exclusion of inactive workers and the potential confounding effect of other chemicals besides EtO, and it is not useful for quantitative dose-response assessment.

Olsen et al. (1997) studied 1,361 male employees working in the ethylene and propylene chlorohydrin production and processing areas located within the EtO and propylene oxide production

[8]This follow-up study was published after the general cut-off date for literature inclusion in this assessment and is reviewed in detail in Section J.2.2 of Appendix J. However, as it is a follow-up of an earlier study and as, with the additional follow-up, it provides important corroborating evidence, the study is also briefly mentioned here.

plants at four Dow Chemical Company sites in the United States. Although these investigators found a nonsignificant positive trend between duration of employment as chlorohydrin workers and lymphohematopoietic cancer (Obs = 10), they concluded that there was no appreciable risk in these workers, in contrast to the findings of Benson and Teta (1993). The small cohort size and the lack of data on EtO exposures limit the usefulness of this study in inferring risks due to EtO.

Ambroise et al. (2005) studied cancer mortality in a small cohort of 181 male municipal pest-control workers in France, 140 of whom were exposed to EtO, along with a wide variety of other chemicals, between 1979 and 1994. Because of the small cohort size and limited follow-up, few deaths were observed or expected for individual cancer sites (e.g., only one lymphohematopoietic cancer death was observed – 1 leukemia vs. 0.23 expected), and the site-specific data were reported only for the full cohort and not just the EtO-exposed workers; thus, this study was not considered further.

Norman et al. (1995) studied 1,132 workers (204 male and 928 female) in a medical sterilizing plant in the United States. In the women, there was a significant excess incidence of breast cancer (SIR = 2.6, Obs = 12, $p < 0.05$); no other cancer sites were elevated. The risk of breast cancer was not noted to be excessive in the few previous studies where adequate numbers of females were included and analyzed for breast cancer; however, only one of these studies was also an incidence study. The follow-up time was too short to draw meaningful conclusions at this time. This study lacks the power to determine whether risks for cancers other than breast cancer are statistically significantly elevated. It has no information regarding historical exposure and some subjects with breast cancer had worked for less than 1 month.

Tompa et al. (1999) reported a cluster of eight breast cancers and eight other cancers in 98 nurses exposed to EtO in a hospital in Hungary; however, the expected number of cases cannot be identified.

The NIOSH investigators used the NIOSH cohort to conduct a study of breast cancer incidence and exposure to EtO (Steenland et al., 2003). The researchers identified 7,576 women from the initial cohort who had been employed in the commercial sterilization facilities for at least 1 year (76% of the original cohort). Breast cancer incidence was determined from interviews (questionnaires), death certificates, and cancer registries. Interviews were obtained for 5,139 women (68% of the study cohort). The main reason for nonresponse was inability to locate the study subject (22% of cohort). The average duration of exposure for the cohort was 10.7 years. For the full study cohort, 319 incident breast cancer cases were identified, including 20 cases of carcinoma in situ. Overall, the SIR was 0.87 (0.94 excluding the in situ cases) using Surveillance, Epidemiology, and End Results (SEER) reference rates for comparison. Results with the full cohort are expected to be underestimated, however, because of case under-ascertainment in the women without interviews. A significant exposure-response trend was observed for SIR across cumulative exposure quintiles, using a 15-year lag time ($p = 0.002$). In internal Cox regression analyses, with exposure as a continuous variable, a significant trend for breast cancer incidence was obtained for log cumulative exposure with a 15-year lag ($p = 0.05$), taking age, race, and

year of birth into account. Using duration of exposure, lagged 15 years, provided a slightly better fit (p = 0.02), while models with cumulative (nontransformed), maximum or average exposure did not fit as well. In the Cox regression analysis with categorical exposures and a 15-year lag, the top cumulative exposure quintile had a statistically significant OR for breast cancer incidence of 1.74 (95% CI = 1.16−2.65).

In the subcohort with interviews, 233 incident breast cancer cases were identified. Information on various risk factors for breast cancer was also collected in the interviews, but only parity and breast cancer in a first-degree relative turned out to be important predictors of breast cancer incidence. In internal analyses with continuous exposure variables, the model with duration of exposure (lagged 15 years) again provided the best fit (p = 0.006). Both the cumulative exposure and log cumulative exposure models also yielded significant regression coefficients with a 15-year lag (p = 0.02 and p = 0.03, respectively), taking age, race, year of birth, parity, and breast cancer in a first-degree relative into account. In the Cox regression analysis with categorical exposures and a 15-year lag, the top cumulative exposure quintile had a statistically significant OR of 1.87 (95% CI = 1.12−3.10).

Steenland et al. (2003) suggest that their findings are not conclusive of a causal association between EtO exposure and breast cancer incidence because of inconsistencies in exposure-response trends, possible biases due to nonresponse, and an incomplete cancer ascertainment. Although that conclusion seems appropriate, those concerns do not appear to be major limitations. As noted by the authors, it is not uncommon for positive exposure-response trends not to be strictly monotonically increasing, conceivably due to random fluctuations or imprecision in exposure estimates. Furthermore, the consistency of results between the full study cohort, which is less subject to nonresponse bias, and the subcohort with interviews, which should have full case ascertainment, alleviates some of the concerns about those potential biases.

In a study of 299 female workers employed in a hospital in Hungary where EtO sterilizers were used, Kardos et al. (2003) observed 11 cancer deaths, including 3 breast cancer deaths, compared with slightly more than 4 expected total cancer deaths. Site-specific expected deaths are not available in this study, so RR estimates cannot be determined. However, the observation of 3 breast cancer deaths, with at most 4.4 (with Hungarian national rates as the referent) total cancer deaths expected, is indicative of an increased risk of breast cancer,[9] and this characterization is supported by the reference of Major et al. (2001) to a cluster of breast cancer cases in female nurses at the same hospital.

[9]Hungarian age-standardized female cancer mortality rates reported by the International Agency for Research on Cancer (http://eu-cancer.iarc.fr/country-348-hungary.html,en) suggest that the ratio of breast cancer deaths to total cancer deaths in Hungarian females is about 0.16 (28.0/100,000 breast cancer mortality rate versus 180.0/100,000 total cancer mortality rate). Although a comparison of this general population ratio with the ratio of 0.68 for breast cancer to total cancer mortality in the Kardos et al. (2003) study is necessarily crude because the general population ratio is not based on the age-standardized rates that would correspond to the age distribution of the person-time of the women in the study, which are unknown, the large

3.1.1. Conclusions Regarding the Evidence of Cancer in Humans

Most of the human studies suggest a possible increased risk of lymphohematopoietic cancers and female breast cancer, but the total weight of the epidemiological evidence does not provide conclusive proof of causality. Of the seven relevant criteria of causality envisioned by Hill (1965), temporality, coherence, biological plausibility, and analogy are clearly satisfied. There is also evidence of consistency in the response and of a dose-response relationship (biological gradient). On the other hand, most of the relative risk estimates are not large in magnitude, so the evidence of strength is more limited. See Section 3.5.1 for a more detailed discussion of the Hill criteria as applied to the EtO database.

The large NIOSH cohort of workers at 14 sterilization plants around the country provides the strongest evidence of carcinogenicity (Steenland et al., 2004; Stayner et al., 1993; Steenland et al., 1991). A statistically significant positive trend was observed in the risk of lymphohematopoietic neoplasms with increasing (log) cumulative exposure to EtO, although the results for this model were reported only for 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 (summarized briefly in Section 3.1 and Table 3-1; see also Appendix A for more details, in particular Table A-5 for a summary of study results and limitations). 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-5 in Appendix A).

In addition, the NIOSH studies have reported increases in the risk of both breast cancer mortality and incidence in women (Steenland et al., 2004; Steenland et al., 2003). Other studies have also reported increases in the risk of breast cancer in women exposed to EtO at commercial sterilization plants (Steenland et al., 2004; Steenland et al., 2003; Norman et al., 1995) as well as in Hungarian hospital workers exposed to EtO (Kardos et al., 2003). In two other studies where exposure to EtO would be expected to have occurred among female employees, no elevated risks were seen (Coggon et al., 2004; Hagmar et al., 1991) or breast cancer results were not reported (Hogstedt, 1988; Hogstedt et al., 1986). However, these latter studies had far fewer cases to analyze than did the NIOSH studies, they did not have individual exposure estimates, and they relied on external comparisons (see Table 3-2 for a brief summary and Table A-5 in Appendix A for more details). The Steenland et al. (2004) and Steenland et

difference between the ratios (0.68 for the study versus 0.16 for the general population) indicates an increased risk of breast cancer in the study.

al. (2003) 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.

Table 3-1. Epidemiological studies of ethylene oxide and human cancer—lymphohematopoietic cancer results[a]

Study/population/ industry	Number of subjects	Lymphohematopoietic cancer results				Comments
Hogstedt (1988) and Hogstedt et al. (1986). Sterilizers, production workers, Sweden.	709 (539 men, 170 women)	*Cancer deaths* leukemia (ICD-8 204-207); lymphohematopoietic (ICD-8 200-208)	*Observed* 7 9	*Expected* 0.8 2.0	*SMR (95% CI)* 9.2 (3.7, 19)[b] 4.6 (2.1, 8.7)[b]	Insufficient follow-up; 12.0% of cohort had died (85 deaths). Exposure to other chemicals.
Coggon et al. (2004). Update of Gardner et al. (1989). Sterilizing workers in eight hospitals and users in four companies, Great Britain.	2,876 (1,864 men, 1,012 women)	*Cancer deaths* leukemia (ICD-9 204-208) leukemia (definite or continual exposure) NHL (ICD-9 200 + 202) lymphohematopoietic (ICD-9 200-208)	*Observed* 5 5 7 17	*Expected* 4.6 2.6 4.8 12.9	*SMR (95% CI)* 1.1 (0.35, 2.5) 1.9 (0.62, 4.5)[b] 1.5 (0.58, 3.0)[b] 1.3 (0.77, 2.1)[b]	Short follow-up; 19.6% of cohort had died (565 deaths). Exposure to other chemicals.
Kiesselbach et al. (1990). Production workers (methods unspecified) from eight chemical plants in West Germany.	2,658 men	*Cancer deaths* leukemia (ICD-9 204-208) lymphohematopoietic (ICD-9 200-208)	*Observed* 2 5	*Expected* 2.35 5	*SMR (95% CI)* 0.85 (0.10, 3.1) 1.0 (0.32, 2.3)	Insufficient follow-up; 10.1% of cohort had died (268 deaths). Exposure to other chemicals.
Benson and Teta (1993). Follow-up of only the chlorohydrin-exposed employees from Greenberg et al. (1990) cohort. Production workers at a chemical plant in West Virginia.	278 men	*Cancer deaths* leukemia and aleukemia lymphosarcoma and reticulosarcoma lymphohematopoietic (ICD NS)	*Observed* 4 1 8	*Expected* 1.14 0.50 2.72	*SMR (95% CI)* 3.5 (0.96, 8.9) 2.0 (0.05, 11) 2.9 (1.3, 5.8)	EtO exposures reported to be low in the chlorohydrin process. Exposure to other chemicals. Very small cohort; thus, small numbers of specific cancers despite long follow-up (52.9% had died; 147 deaths).

Table 3-1. Epidemiological studies of ethylene oxide and human cancer—lymphohematopoietic cancer results[a] (continued)

Study/population/ Industry	Number of subjects	Lymphohematopoietic cancer results	Comments
Swaen et al. (2009). Update of Teta et al. (1993) [Greenberg et al. (1990) cohort minus all chlorohydrin-exposed employees] plus cohort enumeration extended an additional 10 years, adding 167 workers. Production workers and users at two chemical plants in West Virginia.	2,063 men	*Cancer deaths* — *Observed* — *Expected* — *SMR (95% CI)* leukemia — 11 — 11.8 — 0.93 (0.47, 1.7) leukemia (in workers hired before 1956) — 9 — NR — 1.5 (0.69, 2.9) NHL — 12 — 11.5 — 1.05 (0.54, 1.8) lymphohematopoietic (ICD NS) — 27 — 30.4 — 0.89 (0.59, 1.3) *Internal Cox regression analyses:* No statistically significant trends were observed for lymphoid (17 deaths) or leukemia (11 deaths) cancer categories for continuous cumulative exposure.	Small cohort; long follow-up time (50.8% had died; 1,048 deaths). Crude exposure assessment, especially for the early time periods. Exposure to other chemicals.
Steenland et al. (2004). Update of Steenland et al. (1991), Stayner et al. (1993). Sterilizers of medical equipment and spices; and manufacturers and testers of medical sterilization equipment, in 14 plants in the United States.	18,254 (45% male, 55% female)	*Cancer deaths* — *Observed* — *Expected* — *SMR (95% CI)* leukemia (ICD-9 204-208) — 29 — NR — 0.99 (0.71, 1.36) NHL (ICD-9 200+202) — 31 — NR — 1.00 (0.72, 1.35) lymphohematopoietic (ICD-9 200-208) — 79 — NR — 1.00 (0.79, 1.24) *Internal Cox regression analyses:* "lymphoid" cancers (ICD-9 200, 202, 203, 204): OR = 3.0 (p = 0.046) in highest cumulative exposure group, with 15-yr lag; significant regression coefficient for continuous log cumulative exposure (p = 0.02). lymphohematopoietic cancer (ICD-9 200-208): OR = 2.96 (p = 0.03) in highest cumulative exposure group, with 15-yr lag; significant regression coefficient for continuous log cumulative exposure (p = 0.009).	Large cohort; thus, substantial number of deaths (2,852) despite short follow-up (15.6% had died). High-quality exposure assessment. No evidence of exposure to other occupational carcinogens. No increase in lymphohematopoietic cancer risk with increase in exposure in women. Results from internal Cox regression analyses for both sexes combined from Sections D.3 and D.4 of Appendix D.
Bisanti et al. (1993). Chemical workers licensed to handle EtO and other toxic chemicals, Italy.	1,971 men	*Cancer deaths* — *Observed* — *Expected* — *SMR (95% CI)* leukemia (ICD-9 204-208) — 2 — 1.0 — 1.9 (0.23, 7.0) lymphosarcoma and reticulosarcoma (ICD-9 200) — 4 — 0.6 — 6.8 (1.9, 17) lymphohematopoietic (ICD-9 200-208) — 6 — 2.4 — 2.5 (0.91, 5.5) *In group only licensed to handle EtO (n = 637):* leukemia — 2 — 0.3 — 6.5 (0.79, 23) lymphosarcoma and reticulosarcoma — 3 — 0.2 — 17 (3.5, 50) lymphohematopoietic — 5 — 0.7 — 7.0 (2.3, 16)	Insufficient follow-up; 3.9% of cohort had died (76 deaths). Exposure to other chemicals.

Table 3-1. Epidemiological studies of ethylene oxide and human cancer—lymphohematopoietic cancer results[a] (continued)

Study/population/ Industry	Number of subjects	Lymphohematopoietic cancer results	Comments
Mikoczy et al. (2011). Update of Hagmar et al. (1995) and Hagmar et al. (1991). Two plants that produced disposable medical equipment, Sweden.	2,171 (862 men, 1,309 women)	*Cancer cases* — *Observed* — *Expected* — *SIR (95% CI)* leukemia (ICD-7 204-205) — 5 — 3.58 — 1.40 (0.45, 3.26) NHL (ICD-7 200 + 202) — 9 — 6.25 — 1.44 (0.66, 2.73) lymphohematopoietic (ICD-7 200-209) — 18 — 14.4 — 1.25 (0.74, 1.98) *In internal analyses of lymphohematopoietic cancer:* — *IRR (95% CI)* 0−0.13 ppm-yr (*n* = 1,039; 7 cases) — 1.00 0.14−0.21 ppm-yr (*n* = 486; 5 cases) — 1.17 (0.36, 3.78) ≥ 0.22 ppm-years (*n* = 495; 5 cases) — 0.92 (0.28, 3.05)	Small, young cohort (171 deaths; 203 cancer cases). Estimated cumulative exposures were generally low. There was no unexposed referent group for internal analyses.
Norman et al. (1995). Sterilizers of medical equipment and supplies that were assembled at this plant, New York.	1,132 (204 men, 928 women)	*Cancer cases* — *Observed* — *Expected* — *SIR (95% CI)* leukemia (ICD NS) — 1 — 0.54 — 1.85 (0.05, 10)[b]	Short follow-up period and small cohort (only 28 cancer cases).
Swaen et al. (1996). Nested case-control study; cases and controls from a large chemical production plant, Belgium.	10 cases of Hodgkin lymphoma (3 exposed; 7 confirmed) and 200 controls; all male	*Cancer* — *OR (95% CI)* Hodgkin lymphoma (ICD 201) — 8.5 (1.4, 40)	Hypothesis-generating study to investigate a cluster of Hodgkin lymphomas observed at a chemical plant. Exposure to other chemicals.
Olsen et al. (1997). Four EtO production plants (chlorohydrin process) in three states.	1,361 men	*Cancer deaths* — *Observed* — *Expected* — *SMR (95% CI)* leukemia (ICD-8 204-207) — 2 — 3.0 — 0.67 (0.08, 2.4) lymphosarcoma and reticulosarcoma (ICD-8 200) — 1 — 1.1 — 0.91 (0.02, 5.1) lymphohematopoietic (ICD-8 200-209) — 10 — 7.7 — 1.3 (0.62, 2.4)	Short follow-up and small cohort; 22.0% had died; 300 deaths. Exposure to other chemicals.

Table 3-1. Epidemiological studies of ethylene oxide and human cancer—lymphohematopoietic cancer results[a] (continued)

Study/population/ Industry	Number of subjects	Lymphohematopoietic cancer results	Comments
Kardos et al. (2003). Female workers from pediatric clinic of hospital in Eger, Hungary.	299 women	1 lymphoid leukemia death; expected number not reported.	Short follow-up period and small cohort (11 cancer deaths). Possible exposure to natural radium, which permeates the region.

[a]Extracted from Table A-5 of Appendix A, with addition of some summary results (e.g., SMRs); see Table A-5 and Appendix A for more study details.
[b]Calculated by EPA assuming Poisson distribution.
ICD NS: ICD codes not specified; NR: not reported.

Table 3-2. Summary of epidemiological results on EtO and female breast cancer (all sterilizer workers)[a]

Study	Number of women	Breast cancer results	Comments
Hogstedt et al. (1986) and Hogstedt (1988) Swedish incidence and mortality study	170	Not reported	Eight deaths (seven from cancer) had occurred among the women; breakdown by cancer type not reported.
Coggon et al. (2004) Great Britain mortality study	1,011 women hospital workers	*Exposure category* — *Observed* — *Expected* — *SMR (95% CI)* Continual — 5 — 7.2 Intermittent — 0 — 0.7 Unknown — 6 — 5.2 ALL — 11 — 13.1 — 0.84 (0.42, 1.51)	11 breast cancer deaths. 14% of the cohort of 1,405 (including males) hospital workers had died.
Steenland et al. (2004) U.S. mortality study	9,908	SMR in highest quartile of cumulative exposure (with 20-yr lag) = 2.07 ($p < 0.05$). Significant Cox regression coefficient for log cumulative exposure (20-yr lag) ($p = 0.01$).	103 breast cancer deaths.
Steenland et al. (2003) U.S. breast cancer incidence study; nested within Steenland et al. (2004) cohort	7,576 employed for ≥1 yr; 5,139 with interviews	*Full cohort results*: Cox regression analysis OR = 1.74 (95% CI: 1.16, 2.65) for highest cumulative exposure quintile (15-yr lag). $p = 0.05$ for regression coefficient with log cumulative exposure (15-yr lag). *Subcohort results*: Cox regression analysis OR = 1.87 (95% CI: 1.12, 3.10) for highest cumulative exposure quintile (15-yr lag). $p = 0.02$ for regression coefficient with cumulative exposure (15-yr lag); $p = 0.03$ with log cumulative exposure (15-yr lag).	319 cases in full cohort. 233 cases in subcohort with interviews.
Mikoczy et al. (2011). Update of Hagmar et al. (1995) and Hagmar et al. (1991). Swedish cancer incidence study	1,309	41 cases vs. 50.9 expected SIR = 0.81 (95% CI: 0.58, 1.09). *In internal analyses:* — *IRR (95% CI)* 0–0.13 ppm-years ($n = 615$; 10 cases) — 1.00 0.14–0.21 ppm-years ($n = 287$; 14 cases) — 2.76 (1.20, 6.33) ≥ 0.22 ppm-years ($n = 295$; 17 cases) — 3.55 (1.58, 7.93)	41 cases.

Table 3-2. Summary of epidemiological results on EtO and female breast cancer (all sterilizer workers)[a] (continued)

Study	Number of Women	Breast Cancer Results	Comments
Norman et al. (1995) U.S. cancer incidence study	928	SIRs ranged from 1.72 (95% CI: 0.99, 3.00) to 2.40 (95% CI: 1.32, 4.37) depending on calendar year of follow-up, assumptions about completeness of follow-up, and reference rates used.	12 cases.
Kardos et al. (2003) Hungarian mortality study	299	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; 3 were breast cancer deaths, i.e., 3 breast cancer deaths vs. ~4.3 total deaths expected. Although the expected number of breast cancer deaths was not reported, the number of breast cancer deaths observed for the total deaths expected is indicative of an increased risk of breast cancer (see footnote 9 in Section 3.1).	Three breast cancer deaths.

[a]Extracted from Table A-5 of Appendix A; see Table A-5 and Appendix A for more study details, and also Table 3-1 above.

In summary, the largest database of evidence pertaining to a cancer risk from human exposure to EtO is for cancers of the lymphohematopoietic system. Increases in the risk of lymphohematopoietic cancer are present in most of the studies, manifested as an increase in leukemia and/or cancer of the lymphoid tissue. The few studies that fail to demonstrate any increased risks of cancer do not have those strengths of study design that give confidence to the reported lack of an exposure-related effect. The evidence of lymphohematopoietic cancer is strongest in the one study (the NIOSH study) that appears to possess the fewest limitations. In this large study, a significant dose-response relationship was evident with cumulative exposure to EtO. However, this effect was observed primarily in males and the magnitude of the effect was not large. Similarly, in most of the other studies, the increased risks are not great, and other chemicals in some of the workplaces cannot be ruled out as possible confounders. Thus, the findings of increased risks of lymphohematopoietic cancer in the NIOSH and other studies cannot conclusively be attributed to exposure to EtO.

There is also strong evidence of an elevated risk of breast cancer from exposure to EtO in a few studies. The clearest evidence again comes from the large NIOSH studies, which found positive exposure-response relationships for both breast cancer incidence and mortality (319 incident breast cancer cases; 103 breast cancer deaths). In addition, a recent follow-up study of a Swedish cohort of sterilizer workers reported significant increases in the incidence rate ratios in the highest two cumulative exposure quartiles compared to the workers with cumulative exposures below the median. Of the four other studies that included females, none approached the size of the NIOSH studies in terms of breast cancer data—the study with the next largest breast cancer database had only 12 cases. Nonetheless, two of the four other studies were supportive of an increased risk of breast cancer.

3.2. EVIDENCE OF CANCER IN LABORATORY ANIMALS

The International Agency for Research on Cancer (IARC) monograph (IARC, 1994b) has summarized the rodent studies of carcinogenicity, and Health Canada (2001) has used this information to derive levels of concern for human exposure. EPA concludes that the IARC summary of the key studies is valid and is not aware of any animal cancer bioassays that have been published since 1994. The Ethylene Oxide Industry Council (EOIC, 2001) also reviewed the same studies and did not cite additional studies. The qualitative results are described here and the incidence data are tabulated in the unit risk derivation section of this document.

One study of oral administration in rats has been published; there are no oral studies in mice. Dunkelberg (1982) administered EtO in vegetable oil to groups of 50 female Sprague-Dawley rats by gastric intubation twice weekly for 150 weeks. There were two control groups (untreated and oil gavage) and two treated groups (7.5 and 30 mg/kg-day). A dose-dependent increase in the incidence of malignant tumors in the forestomach was observed in the treated groups (8/50 and 31/50 in the low- and high-dose groups, respectively). Of the 39 tumors, 37 were squamous cell carcinomas, and metastases

to other organs were common in these animals. This study was not evaluated quantitatively because oral risk estimates are beyond the scope of this document.

One inhalation assay was reported in mice (NTP, 1987) and two inhalation assays were reported in rats [(Lynch et al., 1984a; Lynch et al., 1984b) in males; (Garman et al., 1986, 1985; Snellings et al., 1984) in both males and females]. In the National Toxicology Program (NTP) mouse bioassay (NTP, 1987), groups of 50 male and 50 female $B6C3F_1$ mice were exposed to EtO via inhalation at concentrations of 0, 50, and 100 ppm for 6 hours per day, 5 days per week, for 102 weeks. Mean body weights were similar for treated and control animals, and there was no decrease in survival associated with treatment. A concentration-dependent increase in the incidence of tumors at several sites was observed in both sexes. These data are summarized in Table 3-3. Males had carcinomas and adenomas in the lung. Females had carcinomas and adenomas in the lung, malignant lymphomas, adenocarcinomas in the uterus, and adenocarcinomas in the mammary glands. The NTP also reports that both sexes had dose-related increased incidences of cystadenomas of the Harderian glands, but these are benign lesions and are not considered further here.

In the Lynch et al. (Lynch et al., 1984a; Lynch et al., 1984b) bioassay in male Fischer 344 (F344) rats, groups of 80 animals were exposed to EtO via inhalation at concentrations of 0, 50, and 100 ppm for 7 hours per day, 5 days per week, for 2 years. Mean body weights were statistically significantly decreased in both treated groups compared with controls ($p < 0.05$). Increased mortality was observed in the treated groups, and the increase was statistically significant in the 100-ppm exposure group ($p < 0.01$). Lynch et al. (1984a) suggest that survival was affected by a pulmonary infection alone and in combination with EtO exposure. Concentration-dependent increases in the incidence of mononuclear cell leukemia in the spleen, peritoneal mesothelioma in the testes, and glioma in the brain were observed (see Table 3-4). The fact that the increased incidence of mononuclear cell leukemia was statistically significant in the low-exposure group, but not in the high-exposure group, is probably attributable to the increased mortality in the high-exposure group. The increased incidence in just the terminal kill rats in the 100-ppm group was statistically significant compared with controls.

Table 3-3. Tumor incidence data in National Toxicology Program Study of $B6C3F_1$ mice NTP (1987)[a] and exposure-response modeling results[b]

Gender/tumor type	EtO concentration (time-weighted average)[c]			EC_{10} (LEC_{10})[d], (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[e]	6.94 (4.51)	2.22×10^{-2}
Females					
Lung adenomas plus Carcinomas	2/44	5/44	22/49[f]	14.8 (9.12)	1.1×10^{-2}
Malignant Lymphoma	9/44	6/44	22/49[g]	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[i]	1/44	8/44[g]	6/49	9.69 (5.35)	1.87×10^{-2}

[a]Incidence data were adjusted by EPA by eliminating the animals that died prior to the occurrence of the first tumor or prior to 52 wk, whichever was earlier.
[b]Statistical analyses and exposure-response modeling were conducted by EPA.
[c]Adjusted by EPA to continuous exposure from experimental exposure conditions of 6 hr/d, 5 d/wk; 1 ppm = 1.83 mg/m^3.
[d]Calculated by EPA using Tox_Risk program.
[e]$p < 0.01$ (pairwise Fisher's exact test).
[f]$p < 0.001$ (pairwise Fisher's exact test).
[g]$p < 0.05$ (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 1/1,077 for inhalation (air) female $B6C3F_1$ mice fed the NIH-07 diet.
[i]Highest dose was deleted in order to fit a model to the dose-response data.

Table 3-4. Tumor incidence data in Lynch et al. (1984a; 1984b) study of male F344 rats and exposure-response modeling results

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 by EPA to continuous exposure from experimental exposure conditions of 7 hr/d, 5 d/wk; 1 ppm = 1.83 mg/m^3.
[b]Calculated by EPA 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 bioassay conducted by Snellings et al. (1984), 120 male and 120 female F344 rats in each sex and dose group were exposed to EtO via inhalation at concentrations of 0 (two control groups of 120 rats of each sex were used), 10, 33, and 100 ppm for 6 hours per day, 5 days per week, for 2 years, with scheduled kills at 6 (10 rats per group), 12 (10 rats per group), and 18 (20 rats per group) months. Significant decreases in mean body weight were observed in the 100-ppm exposure group in males and in the 100-ppm and 33-ppm exposure groups in females.

During the 15th month of exposure, an outbreak of viral sialodacryoadenitis occurred, resulting in the deaths of 1—5 animals per group. Snellings et al. (1984) claim that it is unlikely that the viral outbreak contributed to the EtO-associated tumor findings. After the outbreak, mortality rates returned to preoutbreak 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 3-5. Note that these investigators observed the same types of tumors (splenic leukemia and peritoneal mesothelioma) seen by Lynch et al. (1984a) and Lynch et al. (1984b). 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. (1984) report that when male rats with unscheduled deaths were included in the analysis of peritoneal mesotheliomas, it appeared that EtO exposure was associated with earlier tumor occurrence, and a mortality-adjusted trend analysis yielded a significant positive trend ($p < 0.005$). In later publications describing brain tumors (Garman et al., 1986, 1985), both males and females had a concentration-dependent increased incidence of brain tumors (see Table 3-5). Garman et al. (1986) and Garman et al. (1985) report incidences including all rats from the 18- and 24-month kills and all rats found dead or killed moribund. The earliest brain tumors were observed in rats killed at 18 months.

3.2.1. Conclusions Regarding the Evidence of Cancer in Laboratory Animals

In conclusion, EtO causes cancer in laboratory animals. After inhalation exposure to EtO, statistically significant increased incidences of cancer have been observed in both rats and mice, in both males and females, and in multiple tissues (lung, mammary gland, uterus, lymphoid cells, brain, tunica vaginalis testis). In addition, one oral study in rats has been conducted, and a significant dose-dependent increase in carcinomas of the forestomach was reported.

3.3. SUPPORTING EVIDENCE

3.3.1. Metabolism and Kinetics

Information on the kinetics and metabolism of EtO has been derived primarily from studies conducted with laboratory animals exposed via inhalation, although some limited data from humans have been identified. Details are available in several reviews (Fennell and Brown, 2001; Csanady et al., 2000; Brown et al., 1998; Brown et al., 1996).

Following inhalation, EtO is absorbed efficiently into the blood and rapidly distributed to all organs and tissues. EtO is metabolized primarily by two pathways (see Figure 3-1): (1) hydrolysis to ethylene glycol (1,2-ethanediol), with subsequent conversion to oxalic acid, formic acid, and carbon dioxide; and (2) glutatione conjugation and the formation of *S*-(2-hydroxyethyl)cysteine and N-acetylated derivatives (WHO, 2003). From the available data, the route involving conjugation with glutathione appears to predominate in mice; in larger species (including humans), the conversion of EtO is primarily via hydrolysis through ethylene glycol. Because EtO is an epoxide capable of reacting directly with cellular macromolecules, both pathways are considered to be detoxifying.

Table 3-5. Tumor incidence data in Snellings et al. (1984) and Garman et al. (1985) reports on F344 rats[a] and exposure-response modeling results[b]

Gender/tumor type	Concentration (time-weighted average)[c]				EC_{10} (LEC_{10})[e] (mg/m^3)	Unit risk (0.1/LEC_{10}) (per mg/m^3)
	0 ppm[d]	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%)[f]	9/51 (18%)	12/39[g] (32%)	9/30[g] (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[g] (13%)	22.3 (11.6)	8.66×10^{-3}
Primary brain tumors	1/181 (0.55%)	1/92 (1.1%)	5/85[g] (5.9%)	7/87[h] (8.1%)	36.1 (22.3)	4.5×10^{-3}
Females						
Splenic mononuclear cell leukemia	11/116 (9.5%)	11/54[g] (21%)	14/48[h] (30%)	15/26[i] (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[g] (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]Statistical analyses and exposure-response modeling were conducted by EPA.
[c]Adjusted by EPA to continuous exposure from experimental exposure conditions of 6 hr/d, 5 d/wk; 1 ppm = 1.83 mg/m^3.
[d]Results for both control groups combined.
[e]Calculated by EPA using Tox_Risk program.
[f]Numbers in parentheses indicate percentage incidence values.
[g]$p < 0.05$ (pairwise Fisher's exact test).
[h]$p < 0.01$ (pairwise Fisher's exact test).
[i]$p < 0.001$ (pairwise Fisher's exact test).

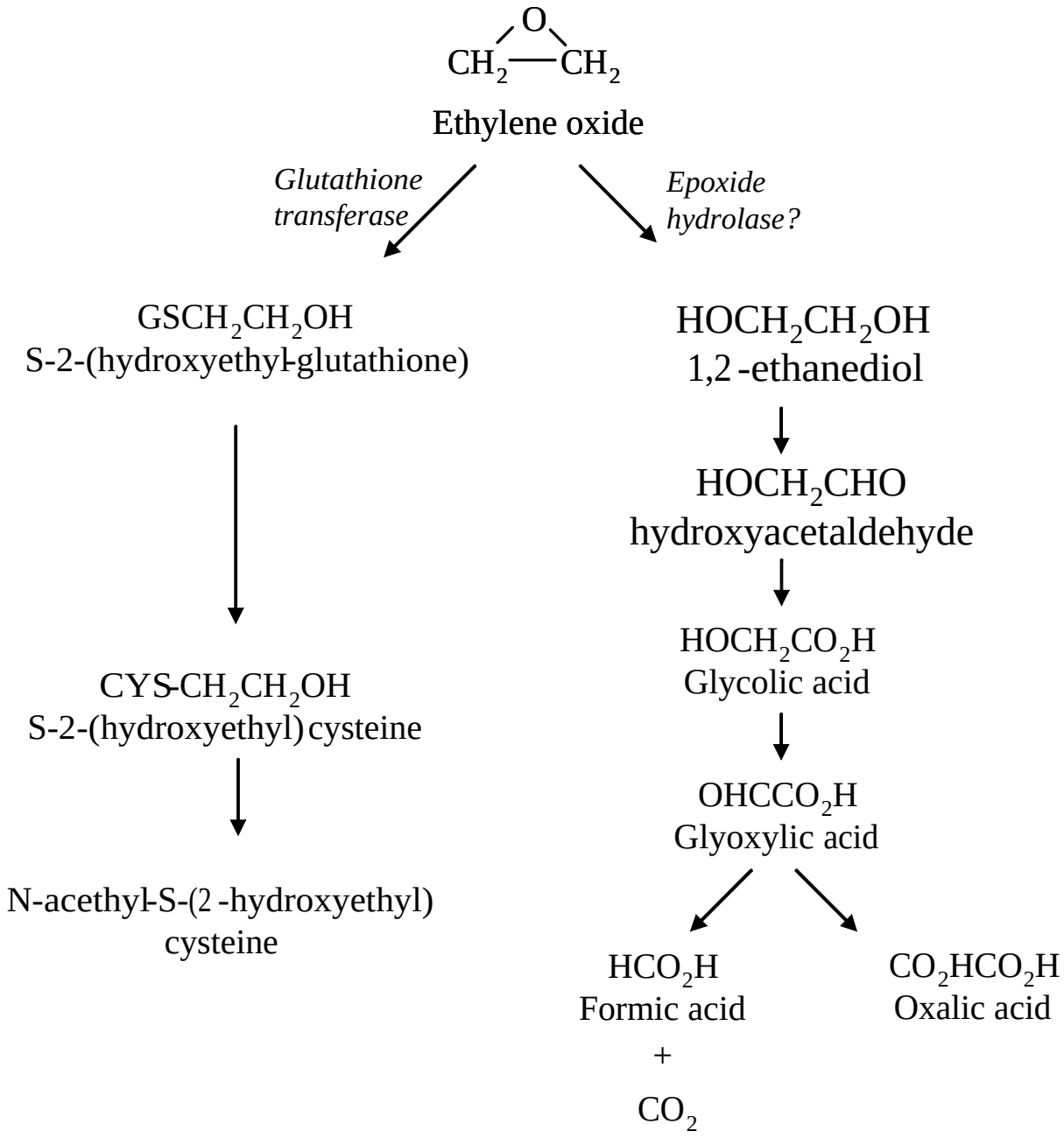


Figure 3-1. Metabolism of ethylene oxide.

Among rodent species, there are clear quantitative differences in metabolic rates. The rate of clearance of EtO from the blood, brain, muscle, and testes was measured by Brown et al. (1998) and Brown et al. (1996). Clearance rates were nearly identical across blood and other tissues. Following a 4-hour inhalation exposure to 100 ppm EtO in mice and rats, the average blood elimination half-lives ranged from 2.4 to 3.2 minutes in mice and 11 to 14 minutes in rats. The elimination half-life in humans is 42 minutes (Filser et al., 1992), and the half-life in salt water is 4 days (IARC, 1994b).

In a more detailed study in mice, Brown et al. (1998) measured EtO concentrations in mice after 4-hour inhalation exposures at 0, 50, 100, 200, 300, or 400 ppm. They found that blood EtO concentration increased linearly with inhaled concentrations of less than 200 ppm, but above 200 ppm the blood concentration increased more rapidly. In addition, glutathione levels in liver, lung, kidney, and testes decreased as exposures increased above 200 ppm. The investigators interpreted this, along with other information, to mean that at low concentrations the metabolism and disappearance of EtO is primarily a result of glutathione conjugation, but at higher concentrations, when tissue glutathione begins to be depleted, the elimination occurs via a slower nonenzymatic hydrolysis process, leading to a greater-than-linear increase in blood EtO concentration.

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

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

3.3.2. Protein Adducts

EtO forms DNA (see Section 3.3.3.1) and hemoglobin adducts within tissues throughout the body (Walker et al., 1992a; Walker et al., 1992b). Formation of hemoglobin adducts has been used as a measure of exposure to EtO. The main sites of alkylation are cysteine, histidine, and the N-terminal valine; however, for analytical reasons, the N-(2-hydroxyethyl)valine adduct is generally preferred for measurements (Walker et al., 1990). Walker et al. (1992b) reported measurements of this hemoglobin adduct and showed how the concentration of the adducts changes according to the dynamics of red blood cell turnover. Walker et al. (1992b) measured hemoglobin adduct formation in mice and rats

exposed to 0, 3, 10, 33, 100, and 300 (rats only) ppm of EtO (6 hours/day, 5 days/week, for 4 weeks). Response was linear in both species up to 33 ppm; at 100 ppm, the slope had increased. The exposure-related decrease in glutathione concentration in liver, lung, and other tissues observed by Brown et al. (1998) in mice is a plausible explanation for the increasing rate of hemoglobin adduct formation at higher exposures.

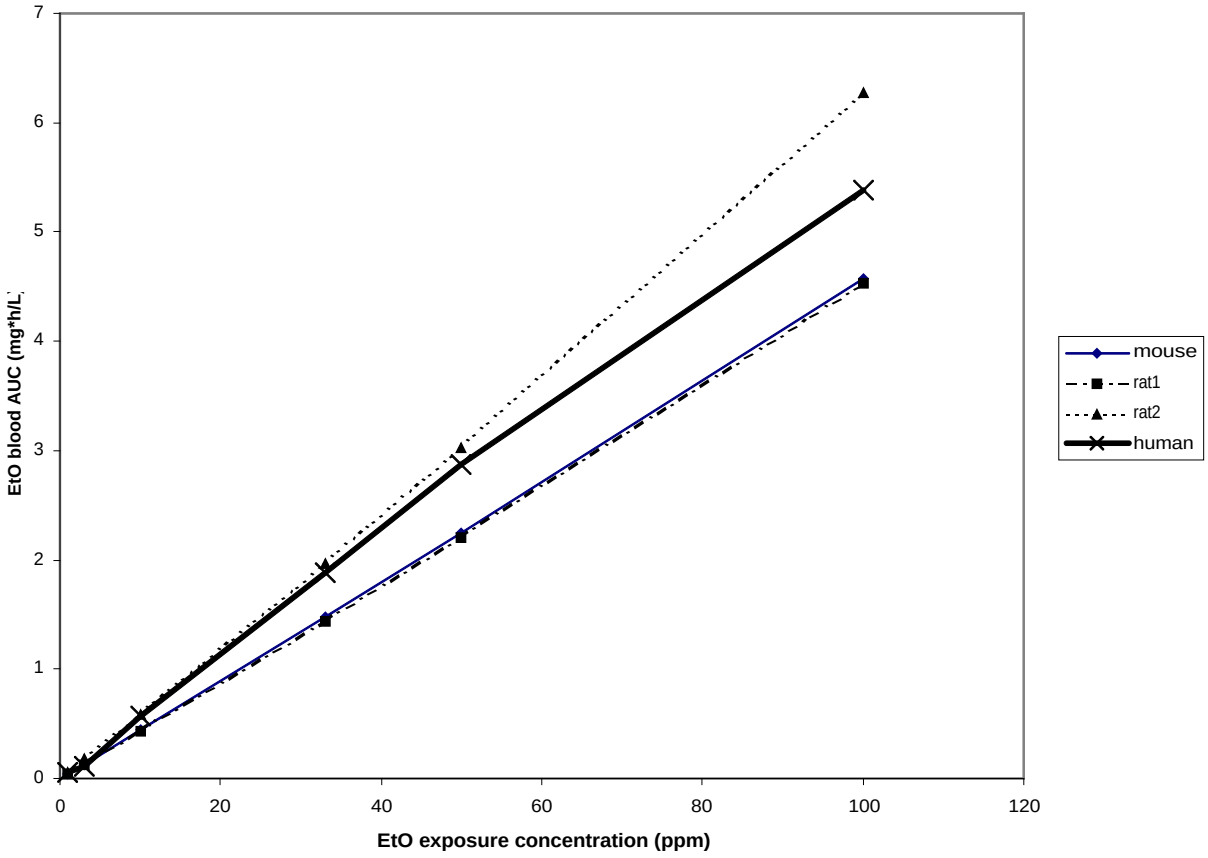


Figure 3-2. Simulated blood AUCs for EtO following a 6-hour exposure to EtO from the rat, mouse, and human PBPK models of Fennell and Brown (2001); based on data presented in Fennell and Brown (2001). (Rat1 and rat2 results use different values for pulmonary uptake.)

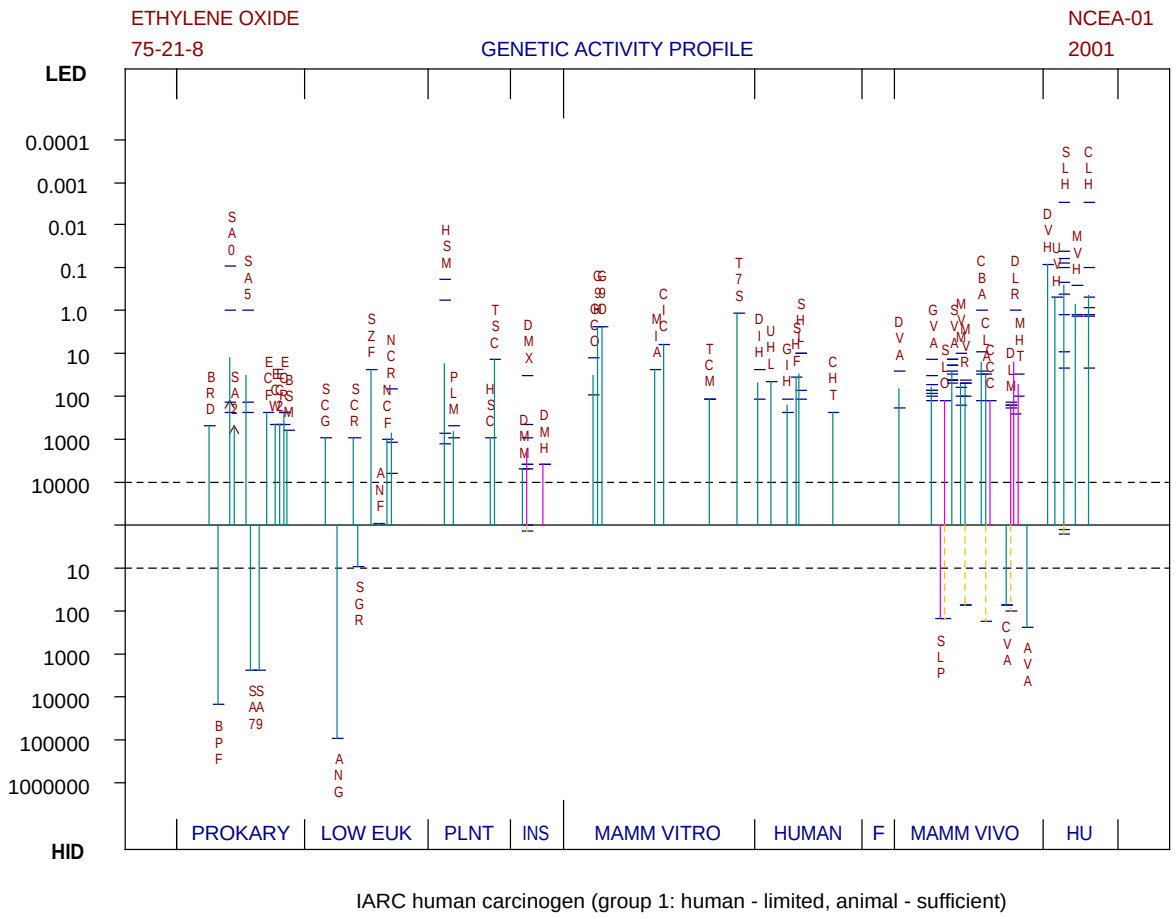
In humans, hemoglobin adducts can be used as biomarkers of recent exposure to EtO (IARC, 2008; Boogaard, 2002; IARC, 1994b), and several studies have reported exposure-response relationships between hemoglobin adduct levels and EtO exposure levels [e.g., (van Sittert et al., 1993; Schulte et al., 1992)].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

exposure). However, Föst et al. (1991) noted that human erythrocytes showed marked interindividual differences in the amounts of EtO bound to hemoglobin, and Yong et al. (2001) reported that levels of N-(2-hydroxyethyl)valine were approximately twofold greater in persons with a *GSTT1*-null genotype than in those with positive genotypes. Endogenous EtO (see Section 3.3.3.1) also contributes to hemoglobin adduct levels, making it more difficult to detect the impacts of low levels of exogenous EtO exposure. In addition, Walker et al. (1993) reported that hemoglobin adducts in mice and rats were lost at a greater rate than would be predicted by the erythrocyte life span and suggested that EtO exposure can reduce the lifespan of erythrocytes.

Together, the rodent studies on EtO-hemoglobin adduct levels and on blood EtO concentrations and tissue glutathione levels (see Section 3.3.1) support the hypothesis that decreasing capacity for rapid detoxification (i.e., tissue glutathione depletion) results in greater than linear EtO blood content and protein adduct levels as exposure concentrations exceed 100 ppm.

3.3.3. Genotoxicity

Since the first report of EtO induction of sex-linked recessive lethals in Drosophila (Rapoport, 1948), numerous papers have reported positive genotoxic activity in biological systems, spanning a wide 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, DNA adducts, 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. (IARC, 2008; Kolman et al., 2002; Thier and Bolt, 2000; Natarajan et al., 1995; Preston et al., 1995; IARC, 1994b; Dellarco et al., 1990; Ehrenberg and Hussain, 1981)]. This review briefly summarizes the evidence of the genotoxic potential of EtO, focusing primarily on more recent studies that provide information on the mode of action of EtO (see Appendix C for more details from some individual studies).



[This is an updated version of the figure in IARC (1994b).]
See Appendix B for list of references.

Figure 3-3. Display of 203 data sets, including bacteria, fungi, plants, insects, and mammals (in vitro and in vivo), measuring the full range of genotoxic endpoints.

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 (Beranek, 1990; Golberg, 1986; Warwick, 1963). 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 (N3-HEA), and O6-hydroxyethylguanine (O^6-HEG), in the ratios 200:8.8:1 (although in vivo the ratio may be closer to 300:1:1, see below); two other peaks, suspected of representing other adenine adducts, were also observed at levels well below that of N7-HEG.

While the N7-HEG adducts are abundant, their mutagenic potential may be minimal, as adducts in this position are unlikely to interfere with the hydrogen-bonding involved in DNA base-pairing and can be rapidly depurinated (Boysen et al., 2009). Imidazole ring-opening of N7-HEG could result in stable, potentially mutagenic lesions (Solomon, 1999); however, EtO-induced N7-HEG ring-opening has not specifically evaluated in vivo (IARC, 2008). While less abundant, N3-HEA adducts may inhibit DNA replication by interacting with the minor groove of the DNA helix, which could lead to strand scission at the replication fork, and O^6-HEG adducts are thought to be highly promutagenic as they can directly interfere with nucleotide base-pairing, typically resulting in thymine incorporation (Mazon et al., 2009). At present, both the identity of the responsible adduct(s) and the mechanism(s) by which such DNA adducts may induce mutations are unknown. Possible mechanisms include DNA misrepair and enzymatic or chemical depurination followed by the insertion of the incorrect base [typically an adenine; see, e.g., IARC (2008); Houle et al. (2006); Tates et al. (1999)], although the levels of apurinic sites were not increased in rats following EtO exposure [Rusyn et al. (2005); see also Section 3.4]. Such lesions could also lead to the formation of DNA single-strand breaks and, subsequently, to chromosomal damage (see Section 3.3.3.3).

In addition to exposures from external sources, EtO is produced endogenously through the cytochrome P450-mediated conversion of ethylene (Törnqvist, 1996), which itself is produced during normal physiological processes (see Section C.7 of Appendix C). Such processes reportedly include oxidation of methionine and hemoglobin, lipid peroxidation of fatty acids, and metabolism of intestinal bacteria [reviewed in (Thier and Bolt, 2000; IARC, 1994a)]. While the percentage of endogenous ethylene converted to EtO is not known, only ~3% of exogenous ethylene was converted to EtO in workers exposed to 0.3 ppm (Törnqvist et al., 1989), and 3,000 ppm ethylene induced ~$1/10^{th}$ of the 7-HEVal hemoglobin or N7-HEG DNA adduct levels as did 100 ppm EtO in various rat tissues (Rusyn et al., 2005), suggesting that exogenous ethylene exposure is unlikely to contribute significantly to the effects associated with exposure to exogenous EtO in humans or rodents.

Endogenous production of EtO contributes significantly to background levels of DNA adducts, making it difficult to detect the impacts of low levels of exogenous EtO exposure on DNA adduct levels. For example, in DNA extracted from the lymphocytes of unexposed individuals, mean background levels of N7-HEG ranged from 2 to 8.5 pmol/mg DNA (Bolt, 1996). Using sensitive detection techniques and an approach designed to separately quantify both endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO treatment in rats, Marsden et al. (2009) reported

increases in exogenous adducts in DNA of spleen and liver at the lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days) and statistically significant linear dose-response relationships ($p < 0.05$) for exogenous adducts in all three tissues examined (spleen, liver, and stomach), although the authors caution that some of the adduct levels induced at low EtO concentrations are below the limit of accurate quantitation (for further discussion of the dose-response relationships, see Section 4.5). Note that the whole range of doses studied by Marsden et al. (2009) lies well below the dose corresponding to the lowest LOAEL from an EtO cancer bioassay (see Section C.7 of Appendix C). Marsden et al. (2009) also observed significant increases in endogenous N7-HEG adduct formation in the liver and spleen, but not the stomach, at the two highest doses (0.05 and 0.1 mg/kg), suggesting that, in addition to direct adduct formation via alkylation, exogenous EtO can induce endogenous N7-HEG adduct production or inhibit removal indirectly, and in a tissue-specific manner. Marsden et al. (2009) hypothesized that this indirect adduct formation by EtO results from the induction of ethylene generation under conditions of oxidative stress, although tissue ethylene levels were not measured directly.

In experiments with rats and mice exposed via inhalation to EtO at concentrations of 0, 3, 10, 33, 100, or 300 (rats only) ppm for 6 hours per day, 5 days per week, for 4 weeks, Walker et al. (1992a) measured N7-HEG adducts in the DNA of lung, brain, kidney, spleen, liver, and testes. At 100 ppm, the adduct levels for all tissues except testes were similar (within a factor of 3), despite the fact that not all of these tissues are targets for toxicity. The study's data on the persistence of the DNA adducts indicate that DNA repair rates differ in different tissues. Although Walker et al. (1992a) suggested that N7-HEG adducts are likely to be removed by depurination forming apurinic/apyrimidinic (AP) sites in DNA, a later study from the same group showed that EtO-induced DNA damage is repaired without accumulation of AP sites or involving base excision repair (Rusyn et al., 2005). In rats exposed to 300 ppm, steady-state levels of O^6-HEG adducts were detected in both target (brain, spleen) and nontarget (lung, kidney) tissues, and N3-HEA adducts were observed in the spleen (no other tissues evaluated); these adducts were not detectable in control animals and were present in the exposed rats at levels ~250—300 times lower than the N7-HEG levels (Walker et al., 1992a).[10]

Two studies provide evidence of N7-HEG DNA adduct formation in human populations occupationally exposed to EtO, one reporting a modest increase in white blood cells (van Delft et al.,

[10] In a study published after the cutoff date for literature inclusion and described in more detail in Section J.4.1 of Appendix J, Zhang et al. (2015) exposed male B6C3F$_1$ mice to 0, 100 or 200 ppm EtO for 6 hours/day, 5 days/week, for 12 weeks and examined the lungs for DNA adducts using more sensitive techniques than those used by Walker et al. (1992a). The Zhang et al. (2015) study supports the identification of the O^6-HEG adduct as a direct product of EtO reactivity and adds coherence to the available database by observing an exposure-related increase in lung O^6-HEG levels at lower concentrations than previously evaluated (i.e., 100 – 200 ppm vs. 300 ppm), quantification in another rodent species (i.e., mice vs. rats), and even detection in the majority of unexposed lung samples (3/5), suggesting that endogenous EtO may be responsible for a low background level of this potentially mutagenic DNA adduct.

1994) and the other a four- to fivefold increase in granulocytes (Yong et al., 2007) compared to unexposed controls. However, these differences were not statistically significant due to high interindividual variation in adduct levels.

The results from the available studies reporting DNA adduct formation or gene mutation frequency in humans and/or laboratory animals following exogenous EtO exposure are summarized in Table 3-6.

Table 3-6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
N7-HEG DNA ADDUCTS							
Intraperitoneal exposure							
Marsden et al. (2009)	4 (4)	Rats/F344	Spleen†	3 days; killed 4 hr after last dose	0, 0.0001, 0.0005, 0.001*, 0.005*, 0.01*, 0.05*, 0.1*	+	Dose-dependent and linear increases in exogenous N7-HEG adduct levels in all tissues; endogenous N7-HEG levels in liver and spleen increased in the two highest dose groups, but did not change in the stomach at any dose.
			Liver		0, 0.0001, 0.0005, 0.001, 0.005*, 0.01*, 0.05*, 0.1*	+	
			Stomach		0, 0.0001, 0.0005*, 0.001*, 0.005*, 0.01*, 0.05*, 0.1*	+	
Marsden et al. (2007)	3 (3)		Liver, heart, colon	1 day; killed 6 hr post-treatment	0, 0.01, 0.1*, 0.5*, 1*	+	Dose-dependent increases in N7-HEG adduct levels in a tissue-specific manner; adduct levels in liver significantly higher than those in heart or colon at 0.1 and 1 mg/kg.
			Spleen† Liver, lung, stomach, heart, kidney	3 days; killed 2, 4, 8, or 10 hr after last dose	0, 0.1*, 1*	+	Dose-dependent increases in N7-HEG adduct levels in a tissue-specific manner; adduct levels did not accumulate with three doses compared to a single dose in the same study.
Inhalation Exposure							
Yong et al. (2007)	58 (6)	Humans	Granulocytes†	4.7−6.5 yr	0.003−0.36 (8-hr TWA)	–	Considerable interindividual variation in N7-HEG adduct levels reported.
van Delft et al. (1994)	42 (29)	Humans	WBC†	NR	2−5	–	Increase in N7-HEG adduct levels was observed in EtO-exposed persons but was not statistically significant.
Wu et al. (1999)	2–5 (8-16)	Rats/ F344	Brain†, spleen† Liver, lung	4 wk; 6 hr/d, 5 d/wk	0, 3*, 10*, 33* or 100*	+	Dose-dependent and linear increases in N7-HEG adduct levels; lung levels > brain levels > liver levels > spleen levels at the highest dose.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Walker et al. (1992a)	10 (10)	Rats/F344	Brain†, spleen† Lung	4 wk; 6 hr/d, 5 d/wk	0, 3, 10*, 33* ,100* also 300* from Walker et al. (1990)	+	Dose-dependent increases in N7-HEG in all tissues.
	3 (3) tissues from Walker et al. (1990)		Brain†, spleen† Lung, kidney	1–4 wk; 6 hr/d, 5 d/wk	0, 300*	+	Significant increase in O^6-HEG levels observed in all tissues; levels reached steady-state by two weeks.
	3 (3) tissues from Walker et al. (1990)		Spleen	4 wk; 6 hr/d, 5 d/wk; killed 0, 1, 3, or 5 days after exposure	0, 300*	+	N3-HEA levels, evaluated in spleen only, were elevated immediately and 1 day after exposure; could not be detected 3 and 5 days after exposure.
van Sittert et al. (2000)	1-4 (1-2)	Rat/ Lewis	Liver	4 wk; 6 hr/d, 5 d/wk; killed 5, 21, 35, or 49 days after exposure	0, 50*, 100*, 200*	+	Dose-dependent increases in N7-HEHG adducts at 5 days post-exposure, which persisted up to 35 days post-exposure; at 49 days after exposure (200 ppm group only), adduct levels had decreased almost to background levels.
Rusyn et al. (2005)	3–4 (3–4)	Rats/F344	Brain†, spleen† Liver	1, 3, or 20 days; 6 hr/d, 5 d/wk; killed 2 h after exposure as well as 6, 24, and 72 h after 1-day exposure	0, 100*	+	Duration-dependent increases in N7-HEG adduct levels; increased rapidly over the first few days, and then more slowly up to 20 days; brain levels > spleen levels > liver levels at 20 days; loss of adducts initially faster in spleen compared to brain and liver.
Walker et al. (2000)	7 (7)	Rats/F344	Spleen†	4 wk; 6 hr/d, 5 d/wk	0, 200*	+	Significant increase in N7-HEG adducts in exposed versus control group.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Walker et al. (1990)	4−5 (4–5)	Rats/F344	WBC† Brain†, spleen† Liver, lung, kidney, testis	1, 3, 7, 14, 28 days; 6 hr/d, 5 d/wk	0, 300*	+	Duration-dependent increase in N7-HEG adducts in all tissues; brain levels > lung levels > WBC levels > spleen levels > kidney levels > liver levels > testis levels.
			WBC† Brain†, spleen† Liver, lung, kidney, testis	4 wk; 6 hr/d, 5 d/wk; killed 1-10 days after exposure	0, 300*	+	Duration-dependent increase in N7-HEG adduct in all tissues; loss of N7-HEG initially faster in WBC and spleen compared to other tissues.
			Brain†, spleen† Lung, kidney	1, 3, 7, 14 days; 6 hr/d, 5 d/wk	0, 500*	+	Duration-dependent increase in N7-HEG adducts in all tissues; brain levels > lung levels > spleen levels > kidney levels.
Wu et al. (1999)	4–7 (8-9)	Mice/ B6C3F1	Brain†, spleen†, lung† Liver	4 wk; 6 hr/d, 5 d/wk	0, 3*, 10*, 33* or 100*	+	Dose-dependent and linear increases in N7-HEG adducts in a tissue-specific manner (lung levels > brain levels > spleen levels > liver levels, at 100 ppm).
Walker et al. (1992a)	4 (4)	Mice/ B6C3F1	Brain†, spleen†, lung†	4 wk; 6 hr/d, 5 d/wk	0, 10*, 33*, 100*	+	Dose-dependent increases in N7-HEG adducts similarly in all tissues; DNA adduct levels 2−3 times lower than identical tissues in concurrently exposed rats.
MUTATIONS							
Intraperitoneal Exposure							
Tates et al. (1999)	4 (4)	Rats/Lewis	Splenic lymphocytes†	1 day; evaluated 35 or 42 days post-exposure	0, 10*, 20*, 40*, 80*	+	*Hprt* mutations: dose-dependent increase in MFs only 35 days post-exposure.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
	8 (5–6)			1 day; evaluated 19 or 32 days post-exposure	0, 20*, 40*, 80*	+	*Hprt* mutations: dose-dependent increase in MFs only 32 days post-exposure.
Walker and Skopek (1993)	4 (4)	Mice/B6C3F1 pups	Splenic T Cells	5 consecutive days	30*, 60*, 90*, 120* mg/kg	+	*Hprt* mutations: dose-dependent increase in MF in exon 3 of *hprt* gene.
Walker and Skopek (1993)	3 (3)	Mice/B6C3F1 pups	Splenic T Cells	2, 6, or 9 doses given every other day	100* mg/kg	+	*Hprt mutations*: dose-dependent increase in MF in exon 3 of *hprt* gene; 11 base-pair substitutions (4 AT and 2 GC transversions, 3 AT and 2 GC transitions) and seven +1 frameshift mutations in a run of six consecutive G bases reported.
Generoso et al. (1980)	50–75 (25)	Mice/T-stock	Germ cells from males mated post-exposure to (SEC x C57BL)F1 females	5 wk; once daily, 5 d/wk	0, 30*, 60* mg/kg	+	*Heritable translocation*: dose-dependent increase in frequency of translocation heterozygotes
Generoso et al. (1980)	12 (12)	Mice/ (101 × C3H)F1	Germ cells from males mated post-exposure to either of T-stock, (SEC × C57BL)F1, (101 × C3H)F1, or (C3H × C57BL)F1 females	Single injection	0, 150* mg/kg	+	*Dominant lethal mutations*: all four stocks were positive for DLM during 4.5–7.5 days post-treatment.
In Drinking Water							

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Tates et al. (1999)	4–5 (4–5)	Rats/Lewis	Splenic lymphocytes†	30 days; analyzed 6, 19, 33, 41 and 44 days after last exposure	88*, 220*, 440*	+	*Hprt* mutations: dose-dependent increase in MFs, with expression maximum 19, 33 and 41 days after exposure, for the respective doses.
Inhalation Exposure							
Tates et al. (1995)	7 (7)	Humans	PBLs†	<5 yr	<0.005–0.02	–	*HPRT* mutations: cytogenetic effects (SCEs and MN) also were negative (see Table 3-8); would have needed 50 subjects/group to detect a 50% increase in MF.
				>15 yr	<0.005–0.01	–	
Major et al. (2001)	Budapest hospital nurses: 9 (14)	Humans	PBLs†	4 yr	2.7 (2.7−10.9)	–	*HPRT* mutation and variant frequency: VF in both Eger EtO-exposed group and controls was higher than both Budapest groups, industrial and historical controls. Cytogenetic effects (CAs and/or SCEs) were positive [see Table 3-8, Major et al. (1996)].
	Eger hospital nurses: 27 (10)			15 yr	5.4 (2.7−82)	–	
Tates et al. (1991)	Factory Workers: 15 (15)	Humans	PBLs†	3–27 yr (mean, 12 yr)	17–33	+	*HPRT* mutations: 60% increase in MF in factory workers was statistically significant, but 55% increase in MF in hospital workers was not.
	Hospital workers: 9 (8)			2–6 yr (mean, 4 yr)	20–25	–	
van Sittert et al. (2000)	6–8 (10)	Rat Lewis	Splenic lymphocytes†	4 wk; 6 hr/d, 5 d/wk	0, 50, 100, 200*	+	*Hprt* mutations: MF analyzed 21 or 22 days post-exposure.
Tates et al. (1999)	8 (1–2)	Rats/Lewis	Splenic lymphocytes†	4 wk; 6 hr/d, 5 d/wk	0, 50*, 100*, 200*	+	*Hprt* mutations: dose-dependent increases in MF; in 200 ppm concentration group, MF 1.4-fold higher than background levels in controls.
Walker et al. (2000)	7 (7)	Rats/F344	Splenic T Cells†	4 wk; 6 hr/d, 5 d/wk	0, 200*	+	*Hprt* mutations: ~sixfold higher in exposed animals compared to unexposed controls.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Embree et al. (1977)	15 (10)	Rats/Long-Evans	Germ cells from males mated post-exposure to female rats	4 hr[d]	1000*	+	Dominant lethal mutations: mutagenic index positive for first 5 wk of mating
Recio et al. (2004)	4–5 (5)	Mice/B6C3F1 *lacI* (TG)	Bone marrow[†]	12 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	*LacI* mutations: at 200 ppm, significant increase in AT→TA transversion mutations (25.4%) vs. controls (1.4%).
	4–6 (6)			24 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	
	6 (6)			48 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100*, 200*	+	
Sisk et al. (1997)	4 (4)	Mice/B6C3F1 *lacI* (TG)	Bone marrow[†]	4 wk; 6 hr/d, 5 d/wk	0, 50, 100, 200	–	*LacI* mutations: Negative 2 or 8 wk post-treatment.
Walker et al. (1997)	7–9 (5)	Mice/B6C3F1 *lacI* (TG)	Splenic lymphocytes[†]	4 wk; 6 hr/d, 5 d/wk	0, 50*, 100*, 200*	+	*Hprt* mutations: dose-dependent increase in MFs.
			Thymic lymphocytes[†]		0, 50, 100*, 200*	+	*Hprt* mutations: dose-dependent increase in MFs; MF increased at 2 hr after treatment reaching a peak at 2 wk post-exposure in 200 ppm group.
Sisk et al. (1997)	4 (4)	Mice/B6C3F1 *lacI* (TG)	Spleen[†]	4 wk; 6 hr/d, 5 d/wk	0, 50, 100, 200	–	*LacI* mutations: Negative 2 or 8 wk post-treatment.
Walker et al. (2000)	7 (7)	Mice/B6C3F1	Splenic T Cells	4 wk; 6 hr/d, 5 d/wk	0, 200*	+	*Hprt* mutations: ~fivefold higher than controls; necropsied 8 wk post-exposure
Sisk et al. (1997)	4 (4)	Mice/B6C3F1 *lacI* (TG)	Lung[†]	4 wk; 6 hr/d, 5 d/wk	0, 50, 100, 200*	+	*LacI* mutations: MF positive at the highest dose compared to control 8 wk post-treatment.
Recio et al. (2004)	5 (5)	Mice/B6C3F1 *lacI* (TG)	Testes	12 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	*LacI* mutations: no increases in any one specific type of mutation observed at any concentration.
	5 (6)			24 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	
	6 (6)			48 wk; 6 hr/d, 5 d/wk	0, 25*, 50*, 100*, 200	+	

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Sisk et al. (1997)	4 (4)	Mice/B6C3F1 *lacI* (TG)	Germ cells	4 wk; 6 hr/d, 5 d/wk	0, 50, 100, 200	–	*LacI* mutations: Negative 2 or 8 wk post-treatment.
NTP (1987); Hong et al. (2007)	50 (50)	Mice/B6C3F1	108 spontaneous lung tumors, and 23 EtO-induced lung tumors†	102 wk; 6 hr/d, 5 d/wk	0, 50*, 100*	+	*Kras* mutations (codons 12, 13 and 61): detected in 100% of EtO-induced tumors vs. 25% of spontaneous tumors; 91% mutations in EtO-induced tumors were codon 12 Gly → Val vs.1% in spontaneous tumors (primarily codon 12 Gly → Asp).
			27 spontaneous HG tumors, and 21 EtO-induced HG tumors†		0, 50*, 100*	+	*Kras* mutations (codons 12, 13 and 61): detected in 86% of all EtO-induced tumors vs. 7% of spontaneous tumors; mutations in EtO-induced tumors were primarily codon 12 Gly → Cys and codon 13 Gly → Arg vs. spontaneous tumors codon 61 Gln → Leu.
			6 EtO-induced uterine carcinomas† (no spontaneous tumors)		0, 50*, 100*	+	*Kras* mutations (codons 12, 13 and 61): detected in 83% of all EtO-induced tumors; mutations in EtO-induced tumors were primarily codon 13 Gly → Gly.
NTP (1987); Houle et al. (2006)	50 (50)	Mice/B6C3F1	19 spontaneous mammary carcinomas, and 12 EtO-induced mammary carcinomas†	102 wk; 6 hr/d, 5 d/wk	0, 50*, 100*	+	*p53* mutations (exons 5−8): P53 mutations were induced in a dose-dependent manner, detectable in 67% of all EtO-induced tumors vs. 58% of spontaneous tumors; p53 protein expression was induced in a dose-dependent manner.
						+	*Hras* mutations (codon 61): detectable in 33% of EtO-induced tumors vs. 26% of spontaneous tumors; 75% of EtO-induced tumors bearing H*ras* mutations contained concurrent *p53* mutations vs. 40% of spontaneous tumors.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Generoso et al. (1990)	29–44 (29−45)	Mice/(C3Hx101) F1	Germ cells from males mated post exposure to either T-stock females or (SEC × C57BL)F1	8.5 wk; 6 hr/d, 5 d/wk for 6 wk, then daily for 2.5 wk	0, 165, 204*, 250*, 300*	+ (DLM)	Increases in both DLM and HT levels were dose-dependent; higher frequencies of translocations recovered in males mated to T-stock females.
					0, 165*, 204*, 250*, 300*	+ (HT)	
Lewis et al. (1986)	1,891 (1,348) progeny	Mice/ DBA/2J	Germ cells from males mated post exposure to untreated C57BL/6J females	From 7, 24, or 28 wk; 6 hr/d, 5 d/wk	0, 200*	+ (DVEE)	Progeny selected for evaluation based upon external appearance deviating from expected F_1 phenotype; eight such variants were conceived from exposed mice, while only one was observed from concurrent controls. Authors estimated MF = $212/10^5$ progeny.
Generoso et al. (1983)	36–58 (36)	Mice/ (101 × C3H)F1	Germ cells from males mated post-exposure to (C3H × C57BL)F1 females	2 or 11 wk; 6 hr/d, 5 d/wk	0, 255*	+	Dominant lethal effects: Duration-dependent increase in % dominant lethals.
Generoso et al. (1986)	16 (16)	Mice/ (101 × C3H)F1	Germ cells from males mated post-exposure to (C3H × C57BL)F1 females	4 days; 6 hr/d	0, 300*, 400*, 500*	+	Dominant lethal effects: dose-related nonlinear increase.

Table 3 6. Dose-response results for EtO-induced DNA adducts and mutations in humans and laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/Strain	Tissue(s) assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Results	Comments[c]
Generoso et al. (1986)	16 (16)	Mice/ (101 × C3H)F1	Germ cells from males mated post-exposure to females	6, 3 and 1.5 hr for the different exposure groups, respectively	0, 300*, 600*, 1200*	+	Dominant lethal effects: dose-related linear increase.

'+' = positive (statistically significant in exposed vs. controls); '–' = negative (statistically no difference between exposed and controls).
*Concentrations or doses reported by the study authors as associated with effects that were significantly significant at $p < 0.05$ compared with the corresponding control group.
[†]Tissues associated with tumor formation in that species following chronic exposure to EtO.
[a]Data sorted in the order of endpoint (adduct or mutation), route of exposure, species, tissue (for mutations), dose, and duration.
[b]The doses for inhalation are in ppm and for all other routes in mg/kg body weight.
[c]Authors' conclusions.
[d]Treated males mated with untreated females for one week and changed to new set of females every week for 10 wk.
N7-HEG, N7-hydroxyethyl guanine; O6-HEG, O6-hydroxyethyl adenine; N3-HEA, N3-hydroxyethyl adenine; ND, not determined; MF, mutation frequency; VF, variant frequency; hprt, hypoxanthine phosphoribosyl transferase; lac, lactose; i.p., intraperitoneal; NTP, National Toxicology Program; PBLs, peripheral blood lymphocytes; WBC, white blood cells; Harderian Gland, HG; TG, transgenic; DLM, dominant lethal mutation; HT, heritable translocation; DVEE, dominant visible and electrophoretically expressed mutations.

3.3.3.2. *Point Mutations*

EtO has consistently yielded positive results in in vitro mutation assays from bacteriophage, bacteria, fungi, yeast, insects, plants, and mammalian cell cultures (including human cells). For example, EtO induces single base pair deletions and base substitutions in the *HPRT* gene in human diploid fibroblasts (Kolman and Chovanec, 2000; Lambert et al., 1994; Bastlová et al., 1993) in vitro. The results of in vivo studies on the mutagenicity of EtO have also been consistently positive following ingestion, inhalation, or injection [e.g., Tates et al. (1999)]; these studies are summarized in Table 3-6. Increases in the frequency of mutations in genes such as *Hprt* and *LacI*, which are experimentally convenient to evaluate as surrogate markers of cancer-associated mutagenesis but unlikely to be directly involved in cellular transformation themselves (Albertini, 2001), have been observed in T-lymphocytes (*Hprt* locus) (Walker et al., 1997) and in bone marrow and testes (*LacI* locus) (Recio et al., 2004) from transgenic mice exposed to EtO via inhalation at concentrations similar to those in carcinogenesis bioassays with this species (NTP, 1987). At somewhat higher concentrations than those used in the carcinogenesis bioassays (200 ppm, but for only 4 weeks), increases in the frequency of gene mutations have also been observed in the lungs of transgenic mice (*LacI* locus) (Sisk et al., 1997) and in T-lymphocytes of rats (*Hprt* locus) (van Sittert et al., 2000; Tates et al., 1999). In in vivo studies with male mice, EtO also causes heritable mutations and other effects in germ cells (Generoso et al., 1990; Lewis et al., 1986).

In a study of 12 mammary gland carcinomas in EtO-exposed $B6C3F_1$ mice from the 1987 NTP bioassay (NTP, 1987) and 19 mammary gland carcinomas in control $B6C3F_1$ mice from various NTP bioassays from the same time period, Houle et al. (2006) measured mutation frequencies in exons 5−8 of the *Trp53* tumor suppressor gene (homologous to the human *TP53* gene) and in codon 61 of the *Hras* proto-oncogene. Mutation frequencies in the mammary carcinomas of EtO-exposed mice were only slightly increased over frequencies in spontaneous mammary carcinomas (33% of the carcinomas in the EtO-exposed mice had *Hras* mutations versus 26% of spontaneous tumors; 67% of the carcinomas in the EtO-exposed mice had *Trp53* mutations versus 58% of spontaneous tumors); however, the tumors in the EtO-exposed mice exhibited distinctly different mutational spectra in the *Trp53* and *Hras* genes, compared to the spontaneous tumors, and more commonly displayed concurrent mutations of the two genes (Houle et al., 2006). The mutational spectra reported by Houle et al. (2006) in both *Trp53* and *Hras* indicate that purine bases (i.e., guanine and adenine) were the predominant targets for mutations in tumors of EtO-exposed mice, while the majority of mutations in spontaneous tumors involved pyrimidine bases (primarily cytosine) (see Section 3.4.1.3 for further discussion of these mutations). Furthermore, Houle et al. (2006) detected about sixfold higher levels of p53 protein expression in the mammary carcinomas of EtO-exposed mice than in spontaneous mammary carcinomas, and there was an apparent dose-response relationship between EtO exposure level and both p53 protein expression and *Trp53* gene mutation (three of the seven tumors in the 50-ppm exposure group and all five tumors in the

100-ppm group had increased protein expression; also, three *Trp53* gene mutations were found in the seven tumors in the 50-ppm exposure group and nine were found in the five tumors in the 100-ppm group).

Some of the same investigators conducted a similar study of *Kras* mutations (evaluating codons 12, 13 and 61) in lung, Harderian gland, and uterine tumors in EtO-exposed $B6C3F_1$ mice from the NTP bioassay (Hong et al., 2007). Substantial increases were observed in *Kras* mutation frequencies in the tumors from the EtO-exposed mice. *Kras* mutations were reported in 100% of the lung tumors from EtO-exposed mice versus 25% of spontaneous lung tumors (108 NTP control animal tumors, including 8 from the EtO bioassay), in 86% of Harderian gland tumors from EtO-exposed mice versus 7% of spontaneous Harderian gland tumors (27 NTP control animal tumors, including 2 from the EtO bioassay), and in 83% of uterine tumors from EtO-exposed mice (there were no uterine tumors in control mice in the 1986 NTP bioassay and none were examined from other control animals). Furthermore, a specific *Kras* mutation, a G → T transversion in codon 12, was nearly universal in lung tumors from EtO-exposed mice (21/23) but rare in lung tumors from control animals (1/108). Other specific mutations were also predominant in the Harderian gland and uterine tumors, but too few *Kras* mutations were available in spontaneous Harderian gland tumors, and no spontaneous uterine tumors were examined; thus, meaningful comparisons of mutation incidence between tumors in EtO-exposed mice and spontaneous tumors could not be made for these sites. While the uterine carcinomas in EtO-exposed mice contained *Kras* mutations resulting from C → T transitions, the primary mutations reported in the lung and Harderian gland tumors of exposed mice resulted from G → C and G → T transversions, consistent with purine nucleotides being the predominant target for the *Hras* and *Trp53* mutagenesis in the aforementioned mammary gland carcinomas from EtO-exposed mice (Houle et al., 2006). Interestingly, codon 12 *Kras* mutations were the most prevalent *Kras* mutations in lung tumors of EtO-exposed mice (23/1/1 mutations in codons 12/13/61, respectively), while codon 13 mutations were more frequent in Harderian gland tumors and uterine carcinomas (9/16/5 and 0/5/not determined, respectively), suggesting that some tissue-specific factors may regulate the formation and resolution of EtO-induced genotoxicity, or facilitate the neoplastic progression of clones bearing specific mutations over others. Overall, these data strongly suggest that EtO-induced mutations in proto-oncogenes (*Kras*, *Hras*) and tumor-suppressor genes (Tr*p53*) play a role in EtO-induced carcinogenesis in multiple tissues.

Only a few studies have investigated gene mutations in people occupationally exposed to EtO. In one study, *HPRT* mutant frequency in peripheral blood lymphocytes was measured in a group of 9 EtO-exposed hospital workers, a group of 15 EtO-exposed factory workers, and their respective controls (Tates et al., 1991). EtO exposure scenarios suggest higher exposures in the factory workers, and this is supported by the measurement of higher hemoglobin adduct levels in those workers. *HPRT* mutant frequencies were increased by 55% in the hospital workers, but the increase was not statistically significant. In the factory workers, a statistically significant increase of 60% was reported. In a study of

workers in an EtO production facility (Tates et al., 1995), *HPRT* mutations were measured in three exposed groups and one unexposed group (seven workers per group). No significant differences in mutant frequencies were observed between the groups; however, the authors stated that about 50 subjects per group would have been needed to detect a 50% increase.

Major et al. (2001) measured *HPRT* mutations in female nurses employed in hospitals in Eger and Budapest, Hungary. This study and an earlier study measuring effects on chromosomes (see Section 3.3.3.3) were conducted to examine a possible causal relationship between EtO exposure and a cluster of cancers (mostly breast) in nurses exposed to EtO in the Eger hospital. The Budapest hospital was chosen because there was no apparent increase in cancer among nurses exposed to EtO. Controls were female hospital workers in the respective cities, and nurses in Eger with known cancers were excluded. 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. *HPRT* variant frequencies in both controls and EtO-exposed workers in the Eger hospital were higher than either group in the Budapest hospital, but there was no significant increase among the EtO-exposed workers in either hospital when compared with the respective controls. The authors noted that the *HPRT* variant frequencies among smoking EtO-exposed nurses in Eger were significantly higher than among smokers in the Eger controls; however, the fact that the *HPRT* variant frequency was almost three times higher in nonsmokers than in smokers in the Eger hospital control group raises questions about the basis of the claimed EtO effect.

3.3.3.3. *Chromosomal Effects*

As discussed by Preston (1999) in an extensive review of the cytogenetic effects of EtO, a variety of cytogenetic assays can be used to measure induced chromosome damage. However, most of the assays commonly employed measure events that are detectable only in the first (or in some cases the second) metaphase after exposure and require DNA synthesis to convert DNA damage into a chromosomal aberration. In addition, DNA repair is operating in peripheral lymphocytes to repair induced DNA damage. Thus, for acute exposures, the timing of sampling is of great importance. For chronic studies, the endpoints measure only the most recent exposures, and if the time between last exposure and sampling is long, any induced DNA damage not converted to a stable genotoxic alteration is certain to be missed. The events measured include all types of chromosomal aberrations, micronuclei, SCE, and numerical chromosomal changes (aneuploidy). While SCEs are evidence that chromosomal damage has been successfully repaired, and are frequently evaluated as indirect biomarkers of genotoxicity, SCEs themselves are not chromosomal mutations, unlike chromosomal aberrations and micronuclei, which can both directly result from misrepaired chromosome damage. Consistent with this distinction, SCEs are not as strongly or consistently associated with carcinogenesis as chromosomal aberration or micronuclei induction (Zeiger, 2010). Stable chromosomal aberrations include reciprocal translocations, inversions, and some fraction of insertions and deletions as well as some numerical

changes. However, until the development of fluorescent in situ hybridization (FISH), chromosome banding techniques were needed to detect these types of aberrations.

In in vitro assays, EtO has consistently tested positive in studies for multiple types of chromosomal effects, including DNA strand breaks, SCEs, micronuclei, and chromosomal aberrations [e.g., see Table 11 of IARC (2008)]. Of note, Adám et al. (2005) measured the sensitivity of different human cell types to EtO-induced DNA damage using the comet assay, which measures direct strand breaks and/or DNA damage converted to strand breaks during alkaline treatment. Adám et al. (2005) reported dose-dependent increases in DNA damage in the concentration range 0−100 μM in each of the cell types examined with no notable cytotoxicity. At the lowest concentration reported (20 μM), significant increases in DNA damage were observed in lymphoblasts, lymphocytes, and breast epithelial cells, but not in keratinocytes or cervical epithelial cells, suggesting that breast epithelial cells may have increased sensitivity to EtO-induced genotoxicity compared to other nonlymphohematopoietic cell types. In addition, Godderis et al. (2006) investigated the effects of genetic polymorphisms on DNA damage induced by EtO in peripheral blood lymphocytes of 20 nonsmoking university students. No significant increases in micronuclei were observed following EtO treatment; however, dose-related increases in DNA strand breaks were seen in the comet assay. GST polymorphisms did not have a significant impact on the EtO-induced effects; however, significant increases in DNA strand breaks were associated with low-activity alleles of two DNA repair enzymes compared to wild-type alleles.

In vivo, inhalation studies in laboratory animals have demonstrated that EtO exposure levels in the range of those used in the rodent bioassays induce SCEs in several species, including rats [Table 3-7; see also Table 11 of IARC (2008)]. SCEs and micronuclei in mice have not been well studied, but the available results are generally positive for these effects [see Table 3-7; see also Table 11 of IARC (2008)]. In inhalation studies of chromosomal aberrations in mice, EtO exposure levels in the range of those used in the rodent bioassays consistently induce chromosomal aberrations, with lower exposure levels requiring longer durations of exposure (see Table 3-7). In rats, evidence for micronuclei and chromosomal aberrations from short-term exposures (≤4 weeks) to these same exposure levels is lacking. In particular, studies by van Sittert et al. (2000) and Lorenti Garcia et al. (2001) observed increases in micronuclei and chromosomal aberrations in splenic lymphocytes of rats exposed to 50, 100, or 200 ppm EtO for 6 hours/day, 5 days/week, for 4 weeks compared to levels from control rats, but the increases were not statistically significant. IARC (2008) noted, however, that "strong conclusions cannot be drawn" from these two studies because the cytogenetic analyses "were initiated 5 days after the final day of exposure, a suboptimal time, and the power of the (FISH) studies were limited by analysis of only a single chromosome and the small numbers of rats per group examined," which was 3 per exposure group in both of the studies, although numerous cells/rat were examined. In addition, the more recent study of chromosomal aberrations in mice by Donner et al. (2010) showed a clear duration effect, with no significant increases at 6 weeks of exposure to those same EtO

concentrations but with statistically significant increases in the 100 and 200 ppm groups starting at 12 weeks of exposure and a statistically significant increase at the lowest exposure level tested (25 ppm) at 48 weeks of exposure.

In humans, various studies of occupationally exposed workers have reported SCEs and other chromosomal effects associated with EtO exposure, including micronuclei and chromosomal aberrations. The genotoxicity of EtO was demonstrated in humans as early as 1979. Table 3-8 summarizes the cytogenetic effects of EtO on human exposures (see also Sections C.3−C.5 of Appendix C for more details on some of the studies).

Table 3-7. EtO-induced cytogenetic effects in laboratory animals

Study[a]	Number exposed per exposure group (number of controls)	Species/strain	Tissue assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Cytogenetic observations			Comments[c]
						CA	SCE	MN	
Intraperitoneal injection									
Farooqi et al. (1993)	4 (4)	Mice/Swiss albino	Bone marrow cells	Single injection; killed 24 hr after dosing	0, 30, 60*, 120*, 150*	+	+	+	Significant positive association between CAs, SCEs and MN and exposure concentrations.
Jenssen and Ramel (1980)	3 (3)	Mice/CBA	Bone marrow cells	Single injection; killed 24 hr after dosing	0, 50, 100, 125*, 150*, 175*	ND	ND	+	No dose-response relationship observed.
Intravenous injection									
Appelgren et al. (1978)	4–8 (10)	Rats/Sprague-Dawley	Bone marrow cells	Two injections, given 30 and 6 hr before killing	0, 100*, 150, 200 (T)	ND	ND	+	EtO toxic at the highest dose.
Appelgren et al. (1978)	4–8 (11)	Mice/NMRI	Bone marrow cells	Two injections, given 30 and 6 hr before killing	0, 50, 100*, 150*, 200*, 300 (T)	ND	ND	+	Dose-dependent increase in MN induction in bone marrow cells; EtO toxic at the highest dose
Inhalation exposure									
Lynch et al. (1984c)	9–12 (12)	Monkeys/ Cynomolgus	PBLs	2 yr; 7 hr/d, 5 d/wk	0, 50*, 100*	+	+	ND	Significant positive association between exposure concentration and SCEs, chromosome- and chromatid-type CAs.
Yager and Benz (1982)	3 (3)	Rabbits/ New Zealand white	PBLs	12 wk; 6 hr/d, 5 d/wk	0, 10, 50*, 250*	ND	+	ND	Significant positive association between SCE levels and exposure concentrations. Analyzed 1, 7, 12, 15 wk post-exposure—elevated SCE levels persisted above base-line at 15 wk post-exposure.

Yager (1987)	3 (3)	Rabbits/ New Zealand white	PBLs	40 d (6 hr/d), 20 d (6 hr/d), or 64 d (0.25 hr twice a day), 5 d/wk, for different exposure groups, respectively	0, 200*, 400*, 1500*	ND	+	ND	All treated groups significantly different in SCEs compared to controls. Cumulative exposure for all groups is same (48,000 ppm × hours)—no difference in SCEs within exposure groups.

Table 3 7. EtO-induced cytogenetic effects in laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/strain	Tissue assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Cytogenetic observations			Comments[c]
Kligerman et al. (1983)	3–4 (4)	Rats/F344	PBLs	1 day; 6 hr/d	0, 50, 150, 450*	–	+	ND	Dose-dependent trend observed for SCEs.
	3–4 (4)			3 day; 6 hr/d	0, 50*, 150*, 450*	–	+	ND	Significant positive association between SCE levels and exposure concentrations.
Preston and Abernethy (1993)	6 (6)	Rats/F344	PBLs	1, 2, 3, 4 wk; 6 hr/d, 5 d/wk	0, 150*	–	+	ND	SCE levels significantly higher than controls at all time points post-exposure. Duration-dependent (1–4 wk) increase in SCEs.
van Sittert et al. (2000)	3 (3)	Rats/Lewis	Splenic lymphocytes	4 wk; 6 hr/d, 5 d/wk		–	+	–	Significant positive association between SCE levels and exposure concentrations; analyses were initiated 5 days after the final day of exposure.
Lorenti Garcia et al. (2001)	3 (5)	Rats/Lewis	Splenic lymphocytes	4 wk; 6 hr/d, 5 d/wk	0, 50*, 100*, 200*	–	+	–	Significant positive association between SCE levels and exposure concentrations, persisting up to 3 wk post-exposure; analyses were initiated 5 days after the final day of exposure.
Ong et al. (1993)	18 (18)	Rats/F344/CR/BR	Spleen cells	3, 6, or 9 mon 6, 2, and 1 hr/d for the different exposure groups, respectively, i.e., 600 ppm-hr cumulative exposure per day, 5 d/wk	0, 100*, 300*, 600*	ND	+	ND	SCE levels significantly higher than controls for all dose-rate and duration groups and a trend of duration-dependent increase in SCE levels.
			Bone marrow cells		0, 100*, 300*, 600*	ND	+	ND	SCE levels significantly higher than controls for all dose-rates for 6 and 9 mon time points and only for the low dose-rate group at 3 mon time point. No duration-dependent increase in SCEs.
Vergnes and Pritts (1994)	10 (10)	Rats/F344	Bone marrow cells	4 wk ; 6 hr/d, 5 d/wk	0, 200*	ND	ND	+	Ethylene exposures up to 3000 ppm in the same study were negative for MN induction.

Table 3 7. EtO-induced cytogenetic effects in laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/strain	Tissue assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Cytogenetic observations			Comments[c]
Donner et al. (2010)	4–8 (4–7)	Mice/B6C3F1	PBLs	6 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	ND	ND	Significant positive association between CA levels and exposure concentrations, and significant positive association with duration after ≥12 wk at 200 ppm; reciprocal translocations significantly increased after ≥12 wk at ≥100 ppm.
				12 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100*, 200*	+	ND	ND	
				24 wk; 6 hr/d, 5 d/wk	0, 25, 50*, 100*, 200*	+	ND	ND	
				48 wk; 6 hr/d, 5 d/wk	0, 25*, 50*, 100*, 200*	+	ND	ND	
Ribeiro et al. (1987)	10 (10)	Mice/Swiss Webster	Bone marrow cells	1 day; 6 hr/d	0, 200, 400*, 600*	+	ND	ND	Significant positive association between CA levels and exposure concentrations
	10 (10)			2 wk; 6 hr/d, 5 d/wk	0, 200*, 400*	+	ND	ND	Significant positive association between CA levels and exposure concentrations
Vergnes and Pritts (1994)	10 (10)	Mice/B6C3F1	Bone marrow cells	4 wk; 6 hr/d, 5 d/wk	0, 200*	ND	ND	+	Ethylene exposures up to 3000 ppm in the same study were negative for MN induction.
Donner et al. (2010)	3–8 (4–8)	Mice/B6C3F1	Spermatogonia cells	6 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200	–	ND	ND	Only reciprocal translocations reported; total aberrations not presented. Positive trend test result at 12 wk considered equivocal.
				12 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100, 200*	(+)	ND	ND	
				24 wk; 6 hr/d, 5 d/wk	0, 25, 50, 100*, 200	+	ND	ND	
				48 wk; 6 hr/d, 5 d/wk	0, 25*, 50*, 100*, 200*	+	ND	ND	

Table 3 7. EtO-induced cytogenetic effects in laboratory animals (continued)

Study[a]	Number exposed per exposure group (number of controls)	Species/strain	Tissue assessed	Exposure duration	Exposure concentration (ppm) or dose (mg/kg)[b]	Cytogenetic observations			Comments[c]
Ribeiro et al. (1987)	10 (10)	Mice/Swiss Webster	Primary spermatocytes	1 day; 6 hr/d	0, 200, 400*, 600*	+	ND	ND	Significant positive association between CA levels and exposure concentrations.
				2 wk 6 hr/d, 5 d/wk	0, 200*, 400*	+	ND	ND	Significant positive association between CA levels and exposure concentrations.

'+' = positive (statistically significant in exposed vs. controls), '–' = nonpositive (statistically no difference between exposed and controls) as reported by authors, typically via pair-wise comparisons; '(+)' = equivocal or weakly positive result; ND, not determined.
*Doses at which incidence of reported effects were statistically significantly higher ($p < 0.05$) than the corresponding control groups.
[a]Studies arranged by intraperitoneal and other routes followed by inhalation, cancer target tissues followed by nontarget tissues, largest to smallest species, lowest dose to highest dose, and duration by lowest to highest when doses are similar.
[b]ppm for inhalation route and mg/kg for all other routes.
[c]Authors' interpretations.
MN, micronucleus; SCE, sister chromatid exchange; CA, chromosomal aberration; ppm, parts per million; T, toxic; i.p., intraperitoneal; i.v., intravenous; PBLs, peripheral blood lymphocytes.

Table 3-8. EtO-induced cytogenetic effects in humans

Study[a]	Number exposed (number of controls)	Exposure duration (yr)		Ethylene oxide level in air (ppm)		Cytogenetic observations			Comments[b,c]
		Range	Mean	Range	Mean (TWA)	CA	SCE	MN	
Karelova et al. (1987)	Sterilization unit: 22 (10)	1–8	NR	0–2.6[d]	NR	+	ND	ND	Significantly higher CAs in exposed vs. control groups. Significant difference in CAs between smokers and nonsmokers of control group, but not exposed group. However, smoking appears to increase CA levels in exposed group.
	Factory workers: 21 (20)	2–17	NR	0–4.5[d]	NR	+	ND	ND	
	Laboratory workers: 25 (20)	1–15	NR	0–4.8[d]	NR	+	ND	ND	
Tates et al. (1995)	7 (7)	<5	NR	<0.005–0.02	NR	ND	–	–	
	7 (7)	>15	NR	<0.05–0.01	NR	ND	–	–	
	7 (7)	Accidental exposure		28–429[d]	NR	ND	–	–	
Clare et al. (1985)	33 (32)	1–14	NR	0.05–8	0.01[e]	(+)	ND	ND	Positive correlation between total number of aberrations and duration of exposure.
Schulte et al. (1992)	LEG: 9 (1)	NR	NR	0.02–0.02[d]	0.02	ND	–	–	
	HEG: 12 (1)	NR	NR	0.27–1.36[d]	0.54	ND	–	–	
	LEG: 32 (8)	NR	5.1	0–0.3[d]	0.04	ND	+	–	Levels of HEVal adducts and SCEs significantly positively associated with cumulative EtO exposure after controlling for smoking exposure.
	HEG: 11 (8)	NR	9.5	0.13–0.3[d]	0.16	ND	+	–	
Sarto et al. (1991)	Preparation area: 5 (10)	0.1–4	2	<1–4.4	0.025[f]	ND	–	–	Observations in the total exposed group (sterilization + preparation workers combined) were not significantly different compared to referent group.
	Sterilization area 5 (10):	4–12	8.6	NR	0.38[f]	ND	+	–	
	Preparation area: 5 (10)	0.1–4	2	<1–4.4	0.025[f]	ND	ND	–[g]	
	Sterilization area: 5 (10)	4–12	8.6	NR	0.38[f]	ND	ND	–[g]	
Sarto et al. (1990)	9 (27)	0.5–12	5	0.025–0.38[d]	NR	ND	ND	–	
	3 (27)	Accidental exposure		NR	>0.38[h]	ND	ND	+	
Van Sittert et al. (1985)	19 (35)	1–5	NR	<0.05–8	<0.05	(+)	ND	ND	Positive correlation between number of chromosome breaks and duration of employment.
	17 (35)	6–14	NR	<0.05–8	<0.05	(+)	ND	ND	
Hansen et al. (1984)	14 (14)	NR	NR	<0.07–4.3[d]	NR	ND	–	ND	Smoking history not associated with SCE levels.

Table 3 8. EtO-induced cytogenetic effects in humans (continued)

Study[a]	Number exposed (number of controls)	Exposure duration (yr)		Ethylene oxide level in air (ppm)		Cytogenetic observations			Comments[b,c]
		Range	Mean	Range	Mean (TWA)	CA	SCE	MN	
Mayer et al. (1991)	34 (23)	NR	8[h]	<0.1–2.4[d]	<0.3	–	+	–	Levels of HEVal adducts and SCEs significantly positively associated with EtO exposure and DNA repair capacity suppressed in EtO-exposed individuals, independent of smoking history.
Sarto et al. (1984)	LEG: 22 (22)	0.6–4	3	0.2–0.5[d]	0.35	(+)	+	ND	Weak positive correlation between total CA levels and duration of exposure.
	HEG: 19 (19)	1.5–15	6.8	3.7–20[d]	10.7	+	+	ND	Significant positive correlation between SCE level and EtO exposure concentration.
Stolley et al. (1984); Galloway et al. (1986)	Site I: 13 (12)	NR	NR	0.5[d]	NR	–	–	ND	
	Site II: 22 (19)	NR	NR	5–10[d]	NR	–	+	ND	
	Site III: 25–26 (22)	NR	NR	5–20[d]	NR	+	+	ND	Significant positive correlation between SCE levels and EtO exposure concentration; SCE levels weakly positively associated with CAs.
Pero et al. (1981)	LEG (packers): 12 (5)	1–8	4	0.5–1	NR	–	ND	ND	Observations in the total exposed group (sterilization + packing workers combined) were significantly higher compared to referent group.
	HEG (sterilizers): 5 (5)	0.8–3	1.6	5–10	NR	+	ND	ND	
Popp et al. (1994)	Smokers: 11	NR	NR	0.5–417[k]	NR	ND	–	ND	High urinary concentrations of hydroxyethyl mercapturic acid present in exposed compared to controls, but not correlated with EtO exposure.
	Nonsmokers: 14	NR	NR	0.5–208[k]	NR	ND	–	ND	
Schulte et al. (1995)	Hospital workers (LEG): 28 (8)	NR	5.5	0–0.30	0.08	ND	+	–	MN levels significantly different between LEG vs. HEG, but not compared to control subjects.
	Hospital workers (HEG): 10 (NR)	NR	10.0	0.13–0.30	0.17	ND	+	–	
Tomkins et al. (1993)	47 (47)	NR	NR	NR	<1	–	–	ND	Exposed and control groups were matched for age, gender and smoking. Levels of SCE and somatic cell mutation were not associated with EtO exposure. However, SCE levels were associated with current smokers but not with former or never smokers.
Högstedt et al. (1983)	Factory I: 18 (11)	0.5–8	3.2	NR	<1	+	–	+	
	Factory I: 18 (10)	0.5–8	3.2	NR	<1	ND	ND	+[j]	

Table 3 8. EtO-induced cytogenetic effects in humans (continued)

Study[a]	Number exposed (number of controls)	Exposure duration (yr)		Ethylene oxide level in air (ppm)		Cytogenetic observations			Comments[b,c]
		Range	Mean	Range	Mean (TWA)	CA	SCE	MN	
	Factory II: 10 (9)	0.5–8	1.7	NR	<1	+	–	ND	Significant positive correlations between different cytogenetic endpoints in peripheral blood lymphocytes and bone marrow cells.
Richmond et al. (1985)	LEG: 79 (141)	1–10	NR	1–40[d]	NR	–	+	ND	Significant positive correlation between exposure duration and CA levels in HEG but not LEG.
	HEG: 50 (141)	1–10	NR	1–40[d]	NR	+	+	ND	
Sarto et al. (1987)	10 (10)	NR	NR	0–9.3[d]	1.84	ND	+	ND	Significant positive correlation between SCE level and EtO exposure concentration.
Ribeiro et al. (1994)	75 (22)	3–14	7	2–5[d]	NR	+	ND	+	
	75 (22)	3–14	7	2–5[d]	NR	ND	ND	–[g]	
Major et al. (1996)	LEG: 9 (14)	NR	4	2.7–10.9	2.7	+	–	ND	CA levels in HEG significantly higher than LEG.
	HEG: 27 (10)	NR	15	2.7–82	5.5	+	+	ND	
Yager et al. (1983)	LEG: 9 (13)	NR	0.5	NR	13[l]	ND	–	ND	
	HEG: 5 (13)	NR	0.5	NR	501[l]	ND	+	ND	
Tates et al. (1991)	Factory workers: 15 (15)	3–27	12	17–33	NR	+	+	+	Factory workers showed 17 times higher SCE frequencies compared with hospital workers.
	Hospital workers: 9 (8)	2–6	4	20–25	NR	+	+	–	
Laurent et al. (1984)	Smokers: 15 (7)	0.5–10	4.5	20–123	NR	ND	+	ND	Higher levels of SCEs reported in workers with greater duration of exposure. Effects from smoking were not found to be additive in EtO exposed smoking workers compared with non-smokers.
	Nonsmokers: 10 (15)	0.5–10	5.7	20–123	NR	ND	+	ND	
Garry et al. (1979)	12 (12)	NR	0.42	NR	36[d]	ND	+	ND	SCE levels remained elevated 8 wk post-exposure.

Table 3 8. EtO-induced cytogenetic effects in humans (continued)

Study[a]	Number exposed (number of controls)	Exposure duration (yr)		Ethylene oxide level in air (ppm)		Cytogenetic observations			Comments[b,c]
		Range	Mean	Range	Mean (TWA)	CA	SCE	MN	
Lerda and Rizzi (1992)	10 (10)	NR	3	60–69[d]	NR	+	+	ND	

'+' = positive (statistically significant in exposed vs. controls), '–' = nonpositive (statistically no difference between exposed and controls) as reported by authors, typically via pair-wise comparisons; '(+)' = some indication of an response associated with exposure, as reported by authors, typically statistically significant by one analytical methods but not others (i.e., correlation or trend test vs. pair-wise comparison), or associated with duration vs. concentration.

[a]Studies are arranged in order of increasing mean exposure concentration, when available, or by the lower end of the range when a mean is not available.

[b]Authors' interpretations.

[c]All studies analyzed in PBLs except where indicated.

[d]TWA (8-hr).

[e]Geometric mean.

[f]TWA (6.5 hr).

[g]Buccal epithelial cells.

[h]Exposed acutely from sterilizer leakage in addition to chronic exposure.

[i]Maximum years exposed.

[j]Erythroblast cells and polychromatic erythrocytes.

[k]Peak concentrations.

[l]Average 6-month cumulative exposure (mg).

ANOVA, analysis of variance; CAs, chromosomal aberrations; EtO, ethylene oxide; HEVal adduct, hydroxyethylvaline adduct; MN, micronucleus; ND, not determined; NR, not reported; PBLs, peripheral blood lymphocytes; ppm, parts per million; SCE, sister chromatid exchange; TWA, time-weighted average; LEG, low exposure group; HEG, high exposure group.

As illustrated in Table 3-8, numerous studies observed increased SCEs in occupationally exposed workers, especially for workers with the highest exposures [e.g., (Major et al., 1996; Sarto et al., 1991; Tates et al., 1991; Sarto et al., 1987)]. Several studies of occupationally exposed workers have also reported increased micronucleus formation in lymphocytes (Ribeiro et al., 1994; Tates et al., 1991), in nasal mucosal cells (Sarto et al., 1990), and in bone marrow cells (Högstedt et al., 1983), although this endpoint seems to be less sensitive than SCEs and data for workers with the higher exposures are limited. An association between increased micronucleus frequency and cancer risk has been reported in at least one large prospective general population study (Bonassi et al., 2007). In addition, chromosomal aberrations have been reported in multiple studies of workers occupationally exposed to EtO (Ribeiro et al., 1994; Tates et al., 1991; Sarto et al., 1987), especially for workers with the highest exposures. Chromosomal aberrations have been linked to an increased risk of cancer in several large prospective general population studies [e.g., (Boffetta et al., 2007; Rossner et al., 2005; Hagmar et al., 2004; Liou et al., 1999)].

3.3.3.4. *Summary*

As presented above (see Sections 3.3.3.1 and 3.3.3.2) and summarized in Table 3-9, inhalation exposure to ≥3 ppm EtO for ≤4 weeks generally increased N7-HEG DNA adduct levels in all tissues examined in a tissue-specific concentration-dependent manner in both mice and rats, with no apparent difference in sensitivity between cancer target and nontarget tissues in either rodent species. No studies measured DNA adducts levels following >4 weeks of EtO exposure. Tissue N7-HEG DNA adduct levels in control or exposed mice were 2−3 times lower than levels reported in analogous tissues from similarly exposed rats, and adduct levels in unexposed rats were approximately 10 times lower than levels in the peripheral blood cells of unexposed humans. In rats, ≤3 daily i.p. injections of doses as low as 0.0005−0.001 mg/kg (comparable to ≤0.1 ppm inhalation exposure) increased the incidence of exogenous N7-DNA adducts in a tissue-specific concentration-dependent manner. Administration of 0.1 mg/kg (comparable to ~1 ppm inhalation exposure) induced rat tissue levels of exogenous N7-HEG adducts comparable to the amount of endogenous adducts in concurrent controls, while ≥10 mg/kg induced a concentration-dependent increase in *Hprt* mutations in rat lymphocytes. Mutation frequency in the reporter genes *Hprt* and *LacI* was increased in a concentration-dependent manner primarily in lymphocytes from rats and mice exposed to concentrations associated with significant tumor induction in cancer bioassays (i.e., ≥50 ppm) for up to 48 weeks, with dominant lethal mutations and heritable translocations reported in germ cells from male mice following exposure to ≥165 ppm. In mice exposed to ≥50 ppm for 102 weeks, the frequency of novel *Trp53* and *Hras* mutations increased in mammary tumors, with greater increases reported in novel *Kras* mutations in lung, Harderian gland and uterine tumors. Furthermore, the vast majority of the mutations in tumors from EtO-exposed mice occurred at purine nucleotides (i.e., adenine and guanine), which is consistent with the formation of EtO

DNA-adducts on guanine (N7-HEG and O^6-HEG) and adenine (N3-HEA) bases in vivo. Human data on DNA adducts and mutations are more limited. In humans, N7-HEG adducts were not significantly increased in peripheral blood samples from two occupational exposure studies, while *HPRT* mutations were only observed in PBLs from a cohort of factory workers after 12 years of exposure to 17−33 ppm, and not in other workers after a shorter duration of exposure to similar concentrations, or a longer duration of exposure to much lower concentrations.

As presented above (see Section 3.3.3.3) and summarized in Table 3-10, cytogenetic effects (chromosomal aberrations, micronuclei and/or SCEs) were increased in a concentration-dependent manner after 3−730 days of exposure to ≥50 ppm EtO, frequently the lowest concentration evaluated, in various mammalian species, including nonhuman primates, rabbits, rats, and mice. In studies evaluating multiple time-points, cytogenetic effects were observed at lower concentrations following longer exposure durations in both mice and rats. Neither chromosomal aberrations nor micronuclei were induced in rats exposed to ≤450 ppm for ≤3 days or to ≤200 ppm for ≤4 weeks (there were no studies of these endpoints in rats with durations longer than 4 weeks), while positive associations were consistently reported between exposure concentration and increased lymphocyte SCE levels. In mice, chromosomal aberration levels exposed to ≤600 ppm for ≤48 weeks were positively associated with exposure concentration, and significant responses were observed at lower exposure concentrations (≥25 ppm) with longer exposure durations (48 weeks). SCE and micronucleus induction in mice likely follow a similar pattern, but insufficient exposure-response data were available for any definitive interpretation. In humans, few positive associations were reported in cohorts exposed to <0.04 ppm (mean time-weighted average), while predominantly positive associations with increased SCE formation and exposure concentration, or chromosomal aberrations and exposure duration, were reported in cohorts exposed to 0.04−0.4 ppm. Exposures ≥1 ppm were positively associated with increased incidence of micronuclei formation, as well as SCEs and chromosomal aberrations. Overall, in the majority of cohorts, the induction of chromosomal aberrations and SCEs appeared to be positively associated with both exposure concentration and duration; this association is not as clear with micronuclei formation, which was rarely evaluated in the more highly exposed populations.

Table 3-9. Summary of exposure and duration patterns for EtO-induced DNA adducts and mutations in humans and laboratory animals (inhalation studies)

DNA adducts[a]					Mutations[b]				
Positive responses in humans with occupational exposure[c]									
Exposure (ppm)	Time (yr)			Total +ve (%)[d]	Exposure (ppm)	Time (yr)			Total +ve (%)[d]
	0–5	>5–10	>10			0–5	>5–10	>10	
0–5	ND	0/2	ND	0/2 (0)	0–5	0/2	ND	0/1	0/3 (0)
>5–25	ND	ND	ND	ND	>5–25	0/1	ND	1/2	1/3 (33)
>25	ND	ND	ND	ND	> 25	ND	ND	ND	ND
Positive responses in rats[c]									
Exposure (ppm)	Time (wk)			Total +ve (%)[d]	Exposure (ppm)	Time (wk)			Total +ve (%)[d]
	0–0.15	>0.15–0.5	>0.5–4			0–12	>12–48	>48–102	
0–1	ND	ND	ND	ND	0–50	1/2	ND	ND	1/2 (50)
>1–50	ND	ND	19/19	19/19 (100)	>50–200	4/5	ND	ND	4/5 (80)
>50–500	14/14	14/14	41/49	69/77 (90)	>200–1200	1/1	ND	ND	1/1 (100)
Positive responses in mice[c]									
Exposure (ppm)	Time (wk)			Total +ve (%)[d]	Exposure (ppm)	Time (wk)			Total +ve (%)[d]
	0–0.15	>0.15–0.5	>0.5–4			0–12	>12–48	>48–102	
0–1	ND	ND	ND	ND	0–50	1/10	2/8	4/4[e]	7/22 (32)
>1–50	ND	ND	18/21	18/21 (86)	>50–200	8/19	5/10	4/4[e]	17/33 (52)
>50–100	ND	ND	7/7	7/7 (100)	>200–1200	11/11	ND	ND	11/11 (100)

ND, not determined; ppm, parts per million.

[a]Includes primarily N7-hydroxyethylguanine (N7-HEG) adducts; other adducts (e.g., O^6-hydroxyethylaguanine, 3-hydroxyethyladenine) were rarely evaluated, and when reported, were induced only in conditions also significantly increasing N7-HEG levels.

[b]Includes mutations at *Hprt* (rodents), *HPRT* (humans), *Lac* I, *p53*, dominant lethal mutations and reciprocal translocations in various tissues.

[c]The number of individual exposure groups with positive, exposure-associated results for DNA adducts or mutation endpoints, for each tissue evaluated, are expressed as a fraction of the total number of exposed groups evaluated (i.e. number of exposure groups with a positive response / total number of groups exposed) within each concentration (row) and duration (column) category, by species.

[d]The sum of the row combining groups across each exposure duration range category, by exposure concentration group, for each endpoint category, expressed as a fraction (and percentage).

[e]Tumor tissue evaluated for the presence of mutations in oncogenes *H-ras* or *Kras*, and/or the tumor suppressor gene p53.

Table 3-10. Summary of exposure and duration patterns for EtO-induced cytogenetic effects in humans and laboratory animals (inhalation studies)

Chromosomal aberrations (CA)					Sister chromatid exchanges (SCE)				Micronuclei (MN)			
Positive responses in humans with occupational exposure[a]												
Exposure (ppm)	Time (yr)			Total +ve (%)[b]	Time (yr)			Total +ve (%)[b]	Time (yr)			Total +ve (%)[b]
	0−5	>5−10	>10		0−5	>5−10	>10		0−5	>5−10	>10	
0−5	8/13	1/3	ND	9/16 (56)	3/8	6/6	0/1	9/15 (60)	2/6	1/9	0/1	3/16 (19)
>5−25	1/1	1/1	2/2	4/4 (100)	2/3	2/2	2/2	6/7 (86)	0/1	ND	1/2	1/3 (33)
>25	1/1	ND	ND	1/1 (100)	3/4	ND	ND	3/4 (75)	0/1	ND	ND	0/1 (0)
Positive responses in rats[a]												
Exposure (ppm)	Time (wk)			Total +ve (%)[b]	Time (wk)			Total +ve (%)[b]	Time (wk)			Total +ve (%)[b]
	0−4	>4−24	>24−48		0−4	>4−24	>24−48		0−4	>4−24	>24−48	
0−50	0/4	ND	ND	0/4 (0)	3/4	ND	ND	3/4 (75)	0/2	ND	ND	0/2 (0)
>50−150	0/8	ND	ND	0/8 (0)	7/8	4/4	2/2	13/14 (93)	0/2	ND	ND	0/2 (0)
>150−450	0/4	ND	ND	0/4 (0)	4/4	8/8	4/4	16/16 (100)	1/3	ND	ND	1/3 (33)
Positive responses in mice[a]												
Exposure (ppm)	Time (wk)			Total +ve (%)[b]	Time (wk)			Total +ve (%)[b]	Time (wk)			Total +ve (%)[b]
	0−4	>4−24	>24−48		0−4	>4−24	>24−48		0−4	>4−24	>24−48	
0−100	ND	4/18	6/6	10/24 (42)	ND	ND	ND	ND	ND	ND	ND	ND
>100−200	1/1	2/6	2/2	6/12 (50)	ND	ND	ND	ND	1/1	ND	ND	1/1 (100)
>200−600	6/6	ND	ND	6/6 (100)	ND	ND	ND	ND	ND	ND	ND	ND

ND, not determined; ppm, parts per million.

[a]The number of individual exposure groups with positive, exposure-associated results for chromosomal aberrations (CA), sister chromatid exchanges (SCE), or micronuclei (MN) endpoints, for each tissue evaluated, are expressed as a fraction of the total number of exposed groups evaluated (i.e. number of exposure groups with a positive response/total number of groups exposed) within each concentration (row) and duration (column) category, by species. Human studies that did not report exposure duration were omitted from the counts.

[b]The sum of the row combining groups across each exposure duration range category, by exposure concentration group, for each endpoint category, expressed as a fraction (and percentage). Endpoints for which results were equivocal or weakly positive ['(+)'] were considered negative for the counts.

In conclusion, the available data from in vitro studies, laboratory animal models, and epidemiological studies establish that EtO is a mutagenic and genotoxic agent that causes various types of genetic damage in a manner positively associated with both exposure concentration and duration, including chromosome mutations (chromosomal aberrations, micronuclei) as well as genetic mutations in proto-oncogenes (*Kras, Hras*) and the tumor suppressor *Trp53*. To the extent that they have been evaluated, similar outcomes have been reported in both exposed humans and laboratory animals. In rodents, genotoxicity is induced in germ cells, as well as in cancer target and nontarget tissues, frequently at the lowest concentrations evaluated. While N7-HEG DNA adducts appear to be the most sensitive outcome following EtO exposure, this may be an artifact of experimental design, as few rodent studies evaluated clastogenic endpoints following exposures to <50 ppm, and none evaluated exposures <25 ppm. Together, the concentration- and duration-dependent positive associations consistently observed in available laboratory animal and human studies strongly support a causal relationship between EtO exposure and genotoxicity or mutagenesis in numerous tissues.

3.4. MODE OF ACTION

EtO is an alkylating agent that has consistently been found to produce numerous genotoxic effects in a variety of biological systems ranging from bacteriophages to occupationally exposed humans. It is carcinogenic in mice and rats, inducing tumors of the lymphohematopoietic system, brain, lung, connective tissues, uterus, and mammary gland. In addition, epidemiological studies have shown an increased risk of various types of human cancers (see Table A-5 in Appendix A), in particular lymphohematopoietic and breast cancers. Target tissues for EtO carcinogenicity in laboratory animals are varied, and the cancers are not clearly attributable to any specific type of genetic alteration, which appear following similar exposure durations and concentrations in both cancer target and nontarget tissues alike. Although the precise mechanisms by which the multisite carcinogenicity in mice, rats, and humans occurs are unknown, EtO is clearly a mutagenic and genotoxic agent, as discussed in Section 3.3.3, and mutagenicity and genotoxicity are well established as playing a key role in carcinogenicity. Section 3.4.1 discusses possible mechanisms by which a mutagenic mode of action might be instrumental in EtO carcinogenesis, Section 3.4.2 briefly summarizes the limited evidence for alternative or additional modes of action, and Section 3.4.3 presents an analysis of the evidence for a mutagenic mode of action for EtO carcinogenicity under EPA's mode-of-action framework [(U.S. EPA, 2005a), Section 2.4.3].

3.4.1. Possible Mechanisms for Mutagenic Mode of Action

3.4.1.1. *General Mechanisms*

Exposure of cells to DNA-reactive agents results in the formation of carcinogen-DNA adducts. The formation of DNA adducts results from a sequence of events involving absorption of the agent,

distribution to different tissues, and accessibility of the molecular target (Swenberg et al., 1990). Alkylating agents may induce several different DNA alkylation products (Beranek, 1990) with varying proportions, depending primarily on the electrophilic properties of the agent. The predominant DNA adduct formed by EtO is N7-HEG, although other adducts, such as N3-HEA and O^6-HEG, have also been observed, in much lesser amounts (Walker et al., 1992a; Segerbäck, 1990). In addition to direct DNA adduct formation via alkylation, Marsden et al. (2009) observed an indirect effect of EtO exposure on endogenous N7-HEG adduct formation at the highest doses tested and hypothesized that exogenous EtO could also indirectly increase endogenous EtO-DNA adduct formation via oxidative stress (see also Section 3.3.3.1 and Appendix C); alternatively, it is also possible that the responsible DNA adduct removal and repair mechanisms were overwhelmed, or inhibited, although the effect of EtO exposure on DNA repair pathway activity has not been evaluated.

The various adducts are processed by different repair pathways, and the subsequent genotoxic responses elicited by unrepaired DNA adducts are dependent on a wide range of variables. While the specific adduct(s) responsible for EtO-induced mutagenesis and genotoxicity and the mechanism(s) by which this adduct(s) induces heritable genotoxic damage are unknown, the similar induction of N7-HEG adducts in both cancer target and nontarget tissues in vivo suggests that the formation of DNA adducts may not be the limiting factor in regulating EtO-induced carcinogenesis (see Sections 3.3.3.1 and 3.3.3.4).

It had been postulated that the predominant EtO-DNA adduct, N7-HEG, although unlikely to be directly promutagenic, could be subject to depurination, resulting in an apurinic site which could be vulnerable to miscoding during cell replication [e.g., Walker and Skopek (1993); see also Sectio 3.3.3.1]. However, in a study designed to test this hypothesis, Rusyn et al. (2005) failed to detect an accumulation of abasic sites in brain, spleen, and liver tissues of rats exposed to EtO. Rusyn et al. (2005) conclude that the accumulation of abasic sites is unlikely to be a primary mechanism for EtO mutagenicity, although they note that it is also possible that their assay was not sufficiently sensitive to detect small increases in abasic sites or that abasic sites are only mutagenic under conditions of rapid cell turnover, when cell replication may occur before repair of the abasic site (the tissues examined in their study were relatively quiescent). Another potential mechanism for EtO-induced mutagenicity is the direct mutagenicity of the promutagenic adducts such as O^6-HEG, although these adducts are generally considered to occur at levels too low to explain all of the observed mutagenicity (IARC, 2008). In an in vitro study, Tompkins et al. (2009) exposed plasmid DNA to a range of EtO concentrations in water and reported that only the N7-HEG adduct was detectable after exposure to EtO concentrations up to 2,000 µM; at higher EtO concentrations (≥10 mM), N1-hydroxyethyladenine and O^6-HEG adducts were also quantifiable but at much lower levels than the N7-HEG adducts. Tompkins et al. (2009) then examined the mutagenicity of these adducts in a *supF* forward mutation assay and reported that the relative mutation frequencies were statistically significantly elevated only for plasmids

exposed to these higher EtO concentrations (increases in relative mutation frequency were observed for N7-HEG adduct levels corresponding to lower EtO concentrations, however, and biologically relevant EtO-related increases in mutation frequency at these lower concentrations cannot be ruled out given the variability of the data and the limitations of the study) (see Appendix C, Sections C.1.1.2 and C.2.2, for a more detailed discussion of this study). An additional mechanism that has been suggested for EtO-induced mutagenicity is the imidazole ring-opening of N7-HEG, which can result in stable, potentially mutagenic lesions; however, EtO-induced N7-HEG ring-opening has not been corroborated in vivo (IARC, 2008).

The events involved in the formation of chromosomal damage by EtO are similarly unknown. N-alklylated bases are removed from DNA by base excision repair pathways. A review by Memisoglu and Samson (2000) notes that the action of DNA glycosylase and apurinic endonuclease creates a DNA single-strand break, which can in turn lead to DNA double-strand breaks (DSBs). DSBs can also be produced by normal cellular functions, such as during V(D)J recombination in the development of lymphoid cells or topoisomerase II-mediated cleavage at defined sites, or they can result from interference in normal cellular functions, such as the inhibition of DNA replication, or chromosome missegregation during mitosis. Furthermore, genotoxic damage in cells undergoing mitosis may induce chromosome segregation errors via selective stabilization of kinetochore-microtubule assemblies by DNA damage response proteins, providing one possible link between gene- and chromosome-level damage (Bakhoum et al., 2014). Reviews of mechanisms of DSB repair indicate that the molecular mechanisms are not fully understood (Lieber, 2010; Pfeiffer et al., 2000). These reviews provide a thorough discussion of both sources (endogenous and exogenous) of DSBs and the variety of repair pathways that have evolved to process the breaks. Although homology-directed repair generally restores the original sequence, during nonhomologous end-joining, the ends of the breaks are frequently modified by addition or deletion of nucleotides. The lack of accumulation of abasic sites observed in the Rusyn et al. (2005) study discussed above argues against a mechanism involving abasic sites as hot spots for strand breaks, although it is possible that abasic sites accumulate more readily in replicating lymphocytes, which were not examined in the study of Rusyn et al. (2005). Another postulated mechanism for EtO-induced strand breaks is via the formation of hydroxyethyl adducts on the phosphate backbone of the DNA, but this mechanism requires further study (IARC, 2008).

3.4.1.2. *Mechanisms Specific to Lymphohematopoietic Cancers*

Lymphohematopoietic malignancies, like all other cancers, are considered to be a consequence of an accumulation of genetic and epigenetic changes involving multiple genes and chromosomal alterations. Although it is clear that chromosome translocations are common features of some hematopoietic cancers, there is evidence that mutations in *TP53* or *NRAS* are involved in certain types of leukemia (U.S. EPA, 1997). It should also be noted that therapy-related leukemias exhibiting reciprocal

translocations are generally only seen in patients who have previously been treated with chemotherapeutic agents that act as topoisomerase II inhibitors (U.S. EPA, 1997). In NHL, the *BCL6* gene is frequently activated by translocations (Chaganti et al., 1998) as well as by mutations within the gene coding sequence (Lossos and Levy, 2000). Preudhomme et al. (2000) observed point mutations in the *AML1* gene in 9 of 22 patients with the M0 type (minimally differentiated acute myeloblastic leukemia) of acute myeloid leukemia (AML), and Harada et al. (2003) identified *AML1* point mutations in cases of radiation-associated and therapy-related myelodysplastic syndrome (MDS)/AML. In both reports, point mutations within the coding sequence were found in patients with normal karyotypes as well as some with translocations or other chromosomal abnormalities. Zharlyganova et al. (2008) identified *AML1* mutations in 7 of 18 radiation-exposed MDS/AML patients but in none of 13 unexposed MDS/AML cases. Other point mutations have also been identified in therapy-related MDS/AML patients, including *TP53* gene mutations after exposure to alkylating agents (Christiansen et al., 2001) and mutations in *RAS* family members and other genes in the receptor tyrosine kinase signal transduction pathway (Christiansen et al., 2005). Several models have been developed to integrate these various types of genetic alterations. One recent model suggests that the pathogenesis of MDS/AML can be subdivided into at least eight genetic pathways that have different etiologies and different biologic characteristics (Pedersen-Bjergaard et al., 2006).

A mode-of-action-motivated modeling approach based solely on chromosome translocations has been proposed by Kirman et al. (2004). The authors suggest a nonlinear dose-response relationship for EtO and leukemia, based on a consideration that "chromosomal aberrations are the characteristic initiating events in chemically induced acute leukemia and gene mutations are not characteristic initiating events." They propose that EtO must be responsible for two nearly simultaneous DNA adducts, yielding a dose-squared (quadratic) relationship between EtO exposure and leukemia risk. However, as discussed above, there is evidence that does not support the assumption that chromosomal aberrations represent the sole initiating event. In fact, these aberrations or translocations could be a downstream event resulting from genomic instability, which itself could result from genetic damage in cells undergoing mitosis (see Section 3.4.1.1.). In addition, it is not clear that acute leukemia is the lymphohematopoietic cancer subtype associated with EtO exposure; in the large NIOSH study, increases in lymphohematopoietic cancer risk were driven by increases in lymphoid cancer subtypes. Furthermore, even if two reactions with DNA resulting in chromosomal aberrations or translocations are early-occurring events in some EtO-induced lymphohematopoietic cancers, it is not necessary that both events be associated with EtO exposure (e.g., background error repair rates or exposure to other alkylating agents may be the cause). Moreover, EtO could also produce translocations indirectly by forming DNA or protein adducts that affect the normally occurring recombination activities of lymphocytes or the repair of spontaneous DSBs. Evidence suggests that human leukocytes are more sensitive to increased DNA fragmentation than are human epithelial cells following EtO exposure in

vitro (Adám et al., 2005), and increased levels of genotoxicity were noted in human leukocytes bearing polymorphisms in the *XRCC3* DSB-repair pathway component [Godderis et al. (2006); see Section C.6 in Appendix C]. Thus, broader mode-of-action considerations were not regarded as supportive of the hypothesis that the exposure-response relationship is purely quadratic.

3.4.1.3. *Mechanisms Specific to Breast Cancer*

Breast cancer is similarly considered to be a consequence of an accumulation of genetic and epigenetic changes involving multiple genes and chromosomal alterations (Ingvarsson, 1999). Again, the precise mechanisms by which EtO induces breast cancer are unknown. As discussed in Section 3.3.3.2, Houle et al. (2006) noted that the mammary gland carcinomas in EtO-exposed mice exhibited a distinct shift in the mutational spectra of the *Trp53* and *Hras* genes, compared to spontaneous tumors, and more commonly displayed concurrent mutations of the two genes. The mutational spectra reported by Houle et al. (2006) in both *Trp53* and *Hras* indicate that purine bases (i.e., guanine and adenine) were the predominant targets for mutations in tumors of EtO-exposed mice, while the majority of mutations in spontaneous tumors involved pyrimidine bases (primarily cytosine). While *HRAS* mutations in human breast cancer are rare (0.4% samples in COSMIC)[11], and tumor mutations in *RAS* genes are more frequently reported at codon 12 than 61 [codon 12 mutations were not evaluated by Houle et al. (2006)], half of the *Hras* mutations in mammary gland tumors in the EtO-exposed mice resulted from an A → T transversion, which was the only codon 61 *HRAS* mutation reported in human breast cancer. While murine *Trp53* and human *TP53* genes are highly homologous, they exhibit sequence differences in post-translational modification and intragenic suppressor sites, as well as specific critical codons, which prevent facile comparison of site-specific mutational events between species. In human breast cancers, *TP53* is the second most frequently mutated gene (23% of samples in COSMIC); at the exon level, the majority of both *TP53* mutations in human breast cancers and *Trp53* mutations in mammary gland tumors in EtO-exposed mice are similarly distributed across exons 5−8 in the DNA-binding domain, suggesting that EtO-induced tumors bear *Trp53* mutations affecting p53 function in a manner similar to the *TP53* mutations reported in human breast cancers.

As noted above, polymorphisms in a *XRCC* family gene associated with DSB repair were associated with increased DNA fragmentation in human leukocytes (see Section C.6 in Appendix C), and other DSB repair genes (e.g., *BRCA1*, *BRCA2*, *XRCC1*) are known to regulate breast cancer susceptibility (Shi et al., 2004; Hu et al., 2002), but the role of any of these pathways in mediating EtO-induced murine mammary gland or human breast cancers remains unknown. In addition, the comet assay results of Adám et al. (2005) suggest that human breast epithelial cells may have increased

[11]Catalogue of Somatic Mutations in Cancer (COSMIC), accessed 08 June, 2016 (http://cancer.sanger.ac.uk/wgs/ browse/; tissue = breast, all subtissue and histology selections set to "include all"). Out of 3,087 breast cancer tissue samples, 13 contained mutations at *HRAS*; out of 12,318 breast cancer tissue samples, 2,877 had mutations in *TP53*.

sensitivity to EtO-induced genotoxicity compared to cervical and epidermal epithelial cells (see Section C.6 in Appendix C); however, the basis for any increased sensitivity of breast epithelial cells is similarly unknown.

3.4.1.4. *Summary on Mutagenic Mode of Action*

In summary, EtO induces a variety of types of genetic damage. It directly interacts with DNA, causing concentration- and duration-dependent increases in DNA adducts, genetic mutations, including mutations in proto-oncogenes and tumor suppressor genes, and chromosome damage in various rodent tissues and human peripheral blood cells. EtO-induced genotoxicity is observed after shorter exposure durations and at lower exposure concentrations than those associated with tumor induction in both rodents and occupationally exposed humans (see Section 3.3.3.4). Depending on a number of variables, EtO-induced DNA adducts (1) may be repaired, (2) may result in a base-pair mutation during replication, or (3) may be converted to a DSB, which also may be repaired or result in unstable (micronuclei) or stable (translocation) cytogenetic damage. The available data are strongly supportive of a mutagenic mode of action involving gene mutations and chromosomal aberrations (translocations, deletions, or inversions) that critically alter the function of oncogenes or tumor suppressor genes. Although it is clear that chromosome translocations are common features of many hematopoietic cancers, there is evidence that mutations in *TP53*, *AML1*, or *NRAS* are also involved in some leukemias. The above scientific evidence along with the summarized genotoxicity evidence in Section 3.3.3.4 provide support for a mutagenic mode of action.

3.4.2. Evidence and Possible Mechanisms for Alternative Modes of Action

There are no compelling alternative or additional hypothesized modes of action for EtO carcinogenicity. For example, there is no cytotoxicity or other toxicity indicative of regenerative proliferation or some other toxicity-related mode of action. Oxidative stress has been hypothesized as a mode of action, but there is little evidentiary support for this hypothesis and the role of oxidative stress in EtO-induced carcinogenicity is speculative at this time (see Section 3.3.2 and Sections J.3.2 and J.4.1 of Appendix J, as well as the response to comment 7 in Appendix K).

3.4.3. Analysis of the Mode of Action for Ethylene Oxide Carcinogenicity under EPA's Mode-of-Action Framework

In this section, the evidence for a mutagenic mode of action for EtO carcinogenicity is analyzed under the mode of action framework in EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA (2005a), Section 2.4.3).

The *hypothesis* is that EtO carcinogenicity has a mutagenic mode of action. This hypothesized mode of action is presumed to apply to all of the tumor types.

The *key events* in the hypothesized mutagenic mode of action are (1) DNA adduct formation by EtO, which is a direct-acting alkylating agent; (2) the resulting heritable genetic damage, including DNA mutations, particularly in oncogenes and tumor suppressor genes, as well as chromosomal alterations; and (3) clonal expansion of mutated cells during later stages of cancer development; eventually resulting in (4) tumor formation. Mutagenicity is a well-established cause of carcinogenicity.

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

Consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* [U.S. EPA (2005a), Section 2.4.3], this mode-of-action analysis for a mutagenic mode of action is organized around the Hill "criteria" (or considerations) developed for the analysis of epidemiological studies (Hill, 1965). These considerations are denoted in italics in the discussion below.

Numerous studies have demonstrated that EtO forms protein and DNA adducts, in mice and rats (see Sections 3.3.2, 3.3.3.1, and 3.3.3.4), and there is incontrovertible evidence that EtO is mutagenic and genotoxic (see Sections 3.3.3.2, 3.3.3.3, and 3.3.3.4). The evidence for causal associations between the key events and tumor formation has *strength* and *consistency*. Increases in the frequency of gene mutations in reporter genes have been observed in the lung, T-lymphocytes, bone marrow, and testes of transgenic mice and in T-lymphocytes of rats exposed to EtO via inhalation at concentrations similar to those inducing tumors in the rodent carcinogenesis bioassays. In addition, in the lung, uterine, mammary gland and Harderian gland tumors from EtO-exposed mice in those bioassays, dramatic shifts toward guanine and adenine mutations have been observed in the mutational spectra of the proto-oncogenes *Hras* and *Kras*, as well as the tumor suppressor *Trp53*, consistent with the propensity of EtO to form DNA-adducts on purine bases (see Section 3.3.3.2).

Inhalation studies in laboratory animals have also demonstrated that EtO exposure levels in the range of those used in the rodent bioassays induce SCEs in several species and consistently induce chromosome aberrations in mice (see Sections 3.3.3.3 and 3.3.3.4). No inhalation studies of SCEs or micronuclei are available for mice. In rats, although SCEs are consistently observed in the available studies, the results for micronuclei formation and chromosomal aberrations following subchronic (up to 4-week) inhalation exposures to the same exposure levels as those used in the rodent bioassays have been nonpositive (see Sections 3.3.3.3 and 3.3.3.4); however, IARC (2008) has noted analytical limitations with some of these analyses (see Section 3.3.3.3). In addition, Donner et al. (2010) demonstrated a clear duration effect in mice, with chromosomal aberrations being induced at those same exposure levels only following longer exposure durations (≥12 weeks).

S*pecificity* is not expected for a multisite mutagen and carcinogen such as EtO (U.S. EPA, 2005a).

A *temporal relationship* is clearly evident, with DNA adducts, point mutations, and chromosomal effects observed in acute and subchronic assays (see Sections 3.3.3.4 and 3.2.1.1).

Dose-response relationships have been observed between EtO exposure in vivo and DNA adducts, SCEs, and *Hprt* and *Trp53* mutations (see Section 3.3.3). A mutagenic mode of action for EtO carcinogenicity also clearly comports with notions of *biological plausibility* and *coherence* because EtO is a direct-acting alkylating agent. Such agents are generally capable of forming DNA adducts, which in turn have the potential to cause genetic damage, including mutations; and mutagenicity, in its turn, is a well-established cause of carcinogenicity. This chain of key events is consistent with current understanding of the biology of cancer.

In addition to the clear evidence supporting a mutagenic mode of action in test animals, there are no other compelling hypothesized modes of action for EtO carcinogenicity. For example, there is no evidence of cytotoxicity or other cellular dysfunction indicative of regenerative proliferation, and little-to-no evidence supporting some other toxicity-related mode of action, such as oxidative stress (see Section 3.4.2).

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

The evidence discussed above demonstrates that EtO is a systemic mutagen in test animals; thus, there is the presumption that it would also be a mutagen in humans. Moreover, there is human evidence directly supporting a mutagenic mode of action for EtO carcinogenicity. Several studies of humans have reported exposure-response relationships between hemoglobin adduct levels and EtO exposure levels [e.g., van Sittert et al. (1993); Schulte et al. (1992); see Section 3.3.2], demonstrating the ability of EtO to bind covalently in systemic human cells, as it does in rodent cells. DNA adducts in EtO-exposed humans have not been well studied, and the evidence of increased DNA adducts is limited (see Sections 3.3.3.1 and 3.3.3.4). EtO has yielded positive results in in vitro mutagenicity studies of human cells (see Figure 3-3). Although the studies of point mutations in EtO-exposed humans are few and insensitive and the evidence for mutations is limited, there is clear evidence from a number of human studies that EtO causes chromosomal aberrations, SCEs, and micronucleus formation in peripheral blood lymphocytes, with some evidence of positive relationships with exposure concentration and duration (see Section 3.3.3.3 and Table 3-8).

Finally, there is strong evidence that EtO causes cancer in humans, including cancer types observed in rodent studies (i.e., lymphohematopoietic cancers and breast cancer), providing further weight to the relevance of the aforementioned genotoxic effects to the development of cancer in humans (see Sections 3.1 and 3.5.1).

In conclusion, the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity. Although oxidative stress or other processes might contribute to the development of EtO-induced cancers, the available evidence best supports a mutagenic mode of action as the primary process describing EtO carcinogenicity.

3. Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?

The mutagenic mode of action is considered relevant to all populations and lifestages. According to EPA's *Supplemental Guidance* (U.S. EPA, 2005b), there may be increased susceptibility to early-life exposures to carcinogens with a mutagenic mode of action. Therefore, because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and in the absence of chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility should be assumed and, if there is early-life exposure, the age-dependent adjustment factors should be applied, in accordance with the *Supplemental Guidance* (see Section 4.4).

In addition, as discussed in Section 3.5.2 below, people with DNA repair deficiencies or genetic polymorphisms conveying a decreased efficiency in detoxifying enzymes may have increased susceptibility to EtO-induced carcinogenicity (see Sections 3.4.1.2 and 3.4.1.3 and Section C.6 of Appendix C).

3.5. HAZARD CHARACTERIZATION

3.5.1. Characterization of Cancer Hazard

In studies of humans there is substantial evidence that EtO exposure is causally associated with lymphohematopoietic cancers and female breast cancer, but the evidence is not strong enough to be conclusive. Of the seven relevant[12] Hill "criteria" (or considerations) for causality (Hill, 1965), *temporality*, *coherence*, *biological plausibility*, and *analogy* are readily satisfied, and the other three criteria (*consistency*, *biological gradient*, and *strength of association*) are satisfied to varying degrees, as discussed below.

Temporality, the sole necessary criterion, is satisfied because the subjects of all the epidemiology studies of EtO were workers who were exposed to EtO before the cancers of interest were observed, i.e., exposure preceded the development of the disease.

The related criteria of *coherence*, *biological plausibility* and *analogy* are fulfilled by the well-established knowledge that EtO is mutagenic and genotoxic, which are common mechanistic features of many carcinogens; that EtO is carcinogenic in rodents, with lymphohematopoietic cancers being observed in both rats and mice and mammary carcinomas being observed in female mice; and that EtO is an epoxide and epoxides are capable of directly interacting with DNA and are the active metabolites of many carcinogens.

There is evidence of *consistency* between studies with respect to cancer of the lymphohematopoietic system as a whole. Most of the studies focus on examining risks associated with

[12]*Specificity* is not expected for an agent, such as EtO, which is widely distributed across tissues and is a direct-acting, multisite mutagen (U.S. EPA, 2005a), and *experimental evidence* is seldom available for human populations and is not available in the case of human exposures to EtO.

subcategories of the lymphohematopoietic system. These cancers include leukemia, Hodgkin lymphoma, NHL, reticulosarcoma, and myeloma. [Note that, with the exception of the Steenland et al. (2004) study, which includes lymphocytic leukemia in a lymphoid cancer category, the studies do not subcategorize leukemia into its distinct myeloid and lymphocytic subtypes.] In most of the studies, an enhanced risk of cancer of the lymphohematopoietic system is evident, and in some studies, it is statistically significant. The studies that do not report a significant lymphohematopoietic cancer effect have major limitations, such as small numbers of cases (from small study size and/or insufficient follow-up time), inadequate exposure information, and/or reliance on external analyses (see Table 3-1 and Table A-5 in Appendix A). Overall, about 9 of 11 studies (including only the last follow-up of independent cohorts) with adequate information to determine RR estimates reported an increased risk of lymphohematopoietic cancers or a subgroup thereof, although not all were statistically significant, possibly due to the limitations noted above (see Table 3-1 and Table A-5 in Appendix A). The large, high-quality[13] NIOSH study shows statistically significant exposure-response trends for lymphoid cancers and all lymphohematopoietic cancers [Steenland et al. (2004); see Sections D.3 and D.4 of Appendix D for results for both sexes combined]. Four other studies reported statistically significant increases in risk (Swaen et al., 1996; Benson and Teta, 1993; Bisanti et al., 1993; Hogstedt et al., 1986), although EtO exposures were reportedly low in the Benson and Teta (1993) study and the increased risks may be due to other chemical exposures. Nonsignificant increases in lymphohematopoietic cancer risk were observed in four other studies, based on small numbers of cases (Coggon et al., 2004; Olsen et al., 1997; Hagmar et al., 1995; Norman et al., 1995; Hagmar et al., 1991) [e.g., Norman et al. (1995) had only 1 case]. Only 2 of the 11 studies showed no evidence of an increase in lymphohematopoietic cancer risk (Swaen et al., 2009; Kiesselbach et al., 1990).

Regarding *consistency* in the breast cancer studies, the large, high-quality NIOSH study shows statistically significant increased risks for both breast cancer mortality [n = 103 deaths; Steenland et al. (2004)] and breast cancer incidence [n = 319 cases; Steenland et al. (2003)]. In addition, a recent follow-up study of a Swedish cohort of sterilizer workers reported significant increases in the incidence rate ratios for breast cancer in internal analyses [n = 41 cases; Mikoczy et al. (2011)]. Two other studies suggest an increased risk of breast cancer despite their small size [Norman et al. (1995), n = 12 cases; Kardos et al. (2003), n = 3 deaths]. No elevated risks were seen in the only other study reporting breast

[13]The NIOSH study (_ENREF_156; _ENREF_155) was judged to be a "high-quality" study based on the attributes discussed (Section 3.1 and Section A.2.8 of Appendix A), including availability of individual worker exposure estimates from a high-quality exposure assessment (which used a comprehensive job-exposure matrix with exposure-level estimates from a regression model that incorporated a variety of plant and production variables and that explained 85% of the variability in an independent data set of EtO measurements), cohort study design, large size, inclusion of males and females, adequate follow-up, absence of any known confounding exposures, and use of internal comparisons. The breast cancer incidence study using the subcohort with interviews had the additional attribute of investigating and controlling for several breast cancer risk factors (_ENREF_155).

cancer results; however, that study had few cases [Coggon et al. (2004), *n* = 11 deaths] (see Table 3-2 and Table A-5 in Appendix A).

There is also some evidence of dose-response relationships (*biological gradient*). In the large, high-quality NIOSH study, a statistically significant positive trend was observed in the risk of lymphohematopoietic cancers with increasing (log) cumulative exposure to EtO, although results for this model were reported only for males (Steenland et al., 2004) (the sex difference is not statistically significant, however, and the trend for both sexes combined is also statistically significant; see Tables D-31 and D-48 in Appendix D). For only two other cohorts were results for exposure-response analyses reported, probably because most cohorts had too few cases and/or lacked adequate exposure information. In the Swaen et al. (2009) study of the UCC cohort, no statistically significant trends were observed for leukemia or lymphoid cancer using a Cox proportional hazards model with cumulative exposure, a model which notably did not yield statistically significant trends in the NIOSH study, either. Similarly, no exposure-response relationship was observed for lymphohematopoietic cancers in internal analyses in the Mikoczy et al. (2011) study; however, this study was limited by a small number of cases (10 exposed cases of all lymphohematopoietic cancers) and the lack of a nonexposed referent group. For breast cancer, exposure-response analyses were reported only for the NIOSH cohort and the Swedish sterilizer worker study of Mikoczy et al. (2011), again presumably because most cohorts with female workers had too few cases and/or lacked adequate exposure information. For the NIOSH cohort, these analyses yielded clear, statistically significant trends for both breast cancer mortality (Steenland et al., 2004) and breast cancer incidence (Steenland et al., 2003) for a variety of models. The Mikoczy et al. (2011) study reported significant increases in the incidence rate ratios in the highest two cumulative exposure quartiles compared to the workers with cumulative exposures below the median, with the highest RR estimate for the highest exposure quartile.

Whereas most of the considerations are largely satisfied, as discussed above, there is little *strength* in the associations, as reflected by the modest magnitude of most of the RR estimates. For example, in the large NIOSH study, the RR estimate for lymphoid cancer mortality in the highest exposure quartile is about 3.0 and the RR estimate for breast cancer incidence in the highest exposure quintile in the subcohort with interviews is on the order of 1.9. While large RR estimates increase the confidence that an observed association is not likely due to chance, bias, or confounding, modest RR estimates, such as those observed with EtO, do not preclude a causal association (U.S. EPA, 2005a). With EtO, the modest RR estimates may, in part, reflect the relatively high background rates of these cancers, particularly of breast cancer incidence.

In addition to the Hill criteria, other factors such as *chance, bias, and confounding* are considered in analyzing the weight of epidemiological evidence. Given the consistency of the findings across studies and the exposure-response relationships observed in the largest study, none of these factors is likely to explain the associations between these cancers and EtO exposure. Coexposures to other

chemicals are expected to have occurred for workers in the chemical industry cohorts but would have been much less likely in the sterilizer worker cohorts, such as the NIOSH cohort, which reported no evidence of confounding exposures to other occupational carcinogens (Steenland et al., 1991). For breast cancer in the NIOSH subcohort with interviews (Steenland et al., 2003), other risk factors for breast cancer were assessed, and statistically significant factors were included in the exposure-response models.

In conclusion, the overall epidemiological evidence for causal associations between EtO exposure and lymphohematopoietic cancer as well as female breast cancer was judged to be strong but less than conclusive.

There is inadequate evidence for other cancer types (e.g., stomach cancer and pancreatic cancer) in the epidemiology studies.

The laboratory animal evidence for carcinogenicity is concluded to be "sufficient" based on findings of tumors at multiple sites, by both oral and inhalation routes of exposure, and in both sexes of both rats and mice. Tumor types resulting from inhalation exposure included mononuclear cell leukemia in male and female rats and malignant lymphoma and mammary carcinoma in female mice, suggesting some site concordance with the lymphohematopoietic and breast cancers observed in humans, also exposed by inhalation.

The evidence of EtO genotoxicity and mutagenicity is unequivocal. EtO is a direct-acting alkylating agent and has invariably tested positive in in vitro mutation assays from bacteriophage, bacteria, fungi, yeast, insects, plants, and mammalian cell cultures (including human cells). In mammalian cells (including human cells), EtO-induced genotoxic effects include unscheduled DNA synthesis, gene mutations, SCEs, and chromosomal aberrations. The results of in vivo genotoxicity studies of EtO have also been largely positive, following ingestion, inhalation, or injection. Increases in frequencies of gene mutations have been reported in the lung, T-lymphocytes, bone marrow, and testes of EtO-exposed mice. In particular, increases in frequencies of proto-oncogene mutations have been observed in several tumor types from EtO-exposed mice compared to spontaneous mouse tumors of the same types. Inhalation studies in laboratory animals have demonstrated that EtO exposure levels in the range of those used in the rodent bioassays induce SCEs in several species, including rats, and consistently induce chromosomal aberrations in mice (no inhalation studies of SCEs or micronuclei are available for mice). Evidence for micronuclei and chromosomal aberrations in rats from these same exposure levels in short-term studies (4 weeks or less) is lacking, although concerns have been raised about some of the negative studies. A recent mouse study showed clear, statistically significant increases in chromosomal aberrations with longer durations of exposure (≥12 weeks) to the concentration levels used in the rodent bioassays. The studies of point mutations in EtO-exposed humans are few and insensitive and the evidence for mutations is limited; however, there is clear evidence from a number of human studies that EtO causes chromosomal aberrations, SCEs, and

micronucleus formation in peripheral blood lymphocytes, and one study has reported increased levels of micronuclei in bone marrow cells in EtO-exposed workers.

Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the conclusion can be made that EtO is "carcinogenic to humans." In general, the descriptor "carcinogenic to humans" is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer. This descriptor is also appropriate when there is a lesser weight of epidemiologic evidence that is strengthened by specific lines of evidence set forth in the *Guidelines* (U.S. EPA, 2005a), which are satisfied for EtO. The lines of evidence supporting the characterization of "carcinogenic to humans" include the following: (1) there is strong, although less than conclusive on its own, evidence of cancer in humans associated with EtO exposure via inhalation, specifically, evidence of lymphohematopoietic cancers and female breast cancer in EtO-exposed workers; (2) there is extensive evidence of EtO-induced carcinogenicity in laboratory animals, including lymphohematopoietic cancers in rats and mice and mammary carcinomas in mice following inhalation exposure; (3) EtO is a direct-acting alkylating agent whose mutagenic and genotoxic capabilities have been well established in a variety of experimental systems, and a mutagenic mode of carcinogenic action has been identified in animals involving the key precursor events of DNA adduct formation and subsequent DNA damage, including point mutations and chromosomal effects; and (4) there is strong evidence that the key precursor events are anticipated to occur in humans and progress to tumors, including evidence of chromosome damage, such as chromosomal aberrations, SCEs, and micronuclei in EtO-exposed workers.

3.5.2. Susceptible Life Stages and Populations

There are no data on the relative susceptibility of children and the elderly when compared with adult workers, in whom the evidence of hazard has been gathered, but because EtO does not have to be metabolized before binding to DNA and proteins, the maturing of enzyme systems in very young children is thought not to be a predominant factor in its hazard, at least for activation. However, the immaturity of *detoxifying* enzymes in very young children may increase children's susceptibility because children may clear EtO at a slower rate than adults. As discussed in Section 3.3.1, EtO is metabolized (i.e., detoxified) primarily by hydrolysis in humans but also by glutathione conjugation. Both hydrolytic activity and glutathione-S-transferase activity apparently develop after birth (Clewell et al., 2002); thus, very young children might have a decreased capacity to detoxify EtO compared to adults. In the absence of data on the relative susceptibility associated with EtO exposure in early life, increased early-life susceptibility is assumed, in accordance with 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 (see Section 3.4).

Other than the occurrence of sex-specific cancers (e.g., breast cancer in human females, mammary and uterine carcinomas in female mice, and testicular peritoneal mesotheliomas in male rats; see Section 3.2), there is no clear sex difference in EtO-induced carcinogenicity. With the exception of the sex-specific cancers and the observation of malignant lymphomas in female but not male mice, there is no sex difference in EtO-induced cancer types in the rat and mouse bioassays. Cancer potency estimates for females are roughly 50% higher than those for males for both mice and rats (see Table 4-18 in Section 4.2.5). In humans, in the large NIOSH study (Steenland et al., 2004), the association between lymphoid cancers and EtO exposure was seen primarily in males, but the sex difference was not statistically significant (see Section D.3.3 in Appendix D).

Brown et al. (1996) reported that sex differences in EtO toxicokinetics were observed in mice but not in rats; female mice had a significantly higher steady-state blood EtO concentration after 4 hours of exposure to either 100 or 330 ppm than male mice. As noted above and discussed in Section 3.3.1, EtO is metabolized primarily by hydrolysis in humans. Mertes et al. (1985) reported no sex difference in microsomal or cytosolic epoxide hydrolase activities in human liver in vitro using benzo[*a*]pyrene 4,5-oxide or *trans*-stilbene oxide, respectively, as substrates. Using EtO as a substrate, but with far fewer subjects, Fennell and Brown (2001) reported similar values for males and females for epoxide hydrolase activity in human liver microsomes and for GSH transferase in human liver cytosolic fractions.

Because EtO is detoxified by glutathione conjugation or hydrolysis, people with genotypes conveying deficiencies in glutathione-S-transferase or epoxide hydrolase activities may be at increased risk of cancer from EtO exposure. Yong et al. (2001) measured approximately twofold greater EtO-hemoglobin adduct levels in occupationally exposed persons with a null GSTT1 genotype than in those with positive genotypes. Similarly, in a study of hospital workers, Haufroid et al. (2007) reported increased urinary excretion of a glutathione conjugate of EtO, reflecting increased detoxification of EtO, associated with a nonnull GSTT1 genotype, although the increase was not statistically significant in all the regression models tested; associations were less clear for other glutathione-S-transferase or epoxide hydrolase polymorphisms.

In addition, people with DNA repair deficiencies such as xeroderma pigmentosum, Bloom's syndrome, Fanconi anemia, and ataxia telangiectasia (Gelehrter et al., 1990) are expected to be especially sensitive to the damaging effects of EtO exposure. Paz-y-Miño et al. (2002) have recently identified a specific polymorphism in the excision repair pathway gene *hMSH2*. The polymorphism was present in 7.5% of normal individuals and in 22.7% of NHL patients, suggesting that this polymorphism may be associated with an increased risk of developing NHL.

4. CANCER DOSE-RESPONSE ASSESSMENT FOR INHALATION EXPOSURE

Chapter 4 presents the derivation of cancer unit risk estimates from human and rodent data and discusses the sources of uncertainty in these estimates.

Major findings of Chapter 4:

1. Full lifetime cancer incidence unit risk (upper bound; adjusted with age-dependent adjustment factors, see Section 4.4) estimate based on human data (lymphoid cancer and breast cancer in females): 5.0×10^{-3} per $\mu g/m^3$ (9.1 per ppm)

2. Unadjusted adult-based unit risk (upper bound) estimate for use with age-dependent adjustment factors (see Section 4.4): 3.0×10^{-3} per $\mu g/m^3$ (5.5 per ppm).

3. Upper bound estimates of the extra risk of lymphoid cancer and breast cancer incidence combined for the range of occupational exposure scenarios considered (i.e., 0.1 to 1 ppm 8-hr TWA for 35 years) (see Section 4.7): 0.081−0.22.

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. (Note that the estimates in Section 4.1 were derived under the common assumption that relative risk is independent of age. This assumption is later superseded by an assumption of increased early-life susceptibility, and it is the unit risk estimates derived under this latter assumption, which are developed in Section 4.4, that are the ultimate estimates proposed in this assessment.) 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 data sets. 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 (see Section 3.4). Section 4.5 presents conclusions about the unit risk estimates. Section 4.6 compares the unit risk estimates derived in this 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 17,530 workers in 13 sterilizing facilities with sufficient exposure information [most recent follow-up by Steenland et al. (2004) and Steenland et al. (2003)] 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. Exposure estimates, including estimates for early exposures for which no measurements were available, were determined using a regression model that estimated exposures to each individual as a function of facility, exposure category, and time period. The regression model was based on extensive personal monitoring data from 18 facilities from 1976 to 1985 as well as information on factors influencing exposure, such as engineering controls [Hornung et al. (1994); see also Section A.2.8 in Appendix A]. When evaluated against independent test data from the same set of monitoring data, the model accounted for 85% of the variation in average EtO exposure levels. The investigators were then able to estimate the cumulative exposure (ppm × 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 follow-up by Swaen et al. (2009)] described risks to Union Carbide workers exposed to EtO 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 periods when no measurements were available (1925−1973). Exposure estimation for the individual workers was based on a relatively crude exposure matrix which cross-classified three levels of exposure intensity with 4 time periods. The exposure estimates for 1974−1988 were based on measurements from air sampling at the West Virginia plants since 1976. The exposure estimates for 1957−1973 were based on measurements in a similar plant in Texas. The exposure estimates for 1940−1956 were based loosely on a “rough” estimate reported for chlorohydrin-based EtO production in a Swedish facility in the 1940s (Hogstedt et al., 1979). The exposure estimates for 1925−1939 were further conjectures based on the Swedish 1940s estimate. Thus, for the two earliest time periods (1925−1939 and 1940−1956) at least, the exposure estimates are highly uncertain. (See Section A.2.20 of Appendix A for a more detailed discussion of the exposure assessment for the Union Carbide cohort.) This is in contrast to the NIOSH exposure assessment in

which exposure estimates were based on extensive sampling data and regression modeling. In addition, the sterilization processes used by the NIOSH cohort workers were fairly constant back in time, unlike chemical production processes, which likely involved much higher and more variable exposure levels in the past. Furthermore, the Union Carbide cohort is of much smaller size and has far fewer deaths than the NIOSH cohort, it is restricted to males and so cannot be used to investigate breast cancer risk in females, and there are coexposures to other chemicals.

A third study (Hagmar et al., 1995; Hagmar et al., 1991) estimated cumulative exposures for individual sterilizer workers; however, insufficient exposure-response data are presented for the derivation of unit risk estimates. A more recent follow-up of this cohort (Mikoczy et al., 2011) provides exposure-response results based on a greater number of cases; however, in the internal analyses, incidence rate ratios were calculated by comparing the incidence rates for the two highest cumulative exposure quartiles with that for the 50% of workers with cumulative exposures below the median. Such results are not well suited to the derivation of unit risk estimates, and EPA does not have the individual data to model. Obtaining the data was not pursued because the NIOSH sterilizer worker study is much larger and has many more cases.

Table 4-1 provides a summary of the considerations taken into account in selecting the NIOSH study as the basis for the derivation of unit risk estimates. The NIOSH EtO cohort mortality data can be obtained from the Industrywide Studies Branch of NIOSH.[14]

[14]Industrywide Studies Branch; Division of Surveillance, Hazard Evaluations and Field Studies: NIOSH; Centers for Disease Control and Prevention, 4676 Columbia Parkway MS R-13,Cincinnati, Ohio 45226, telephone: 513-841-4203.

Table 4-1. Considerations used in this assessment for selecting epidemiology studies for quantitative risk estimation

Consideration	Studies	Comments
Availability of quantitative exposure estimates	1. Mikoczy et al. (2011) [latest follow-up of Swedish sterilizer cohort studies by Hagmar et al. (1995) and Hagmar et al. (1991)] 2. Swaen et al. (2009) [latest follow-up of Union Carbide Corporation (UCC) cohort] 3. Steenland et al. (2004) and Steenland et al. (2003) (latest follow-up of NIOSH cohort)	These are the only three studies with quantitative exposure estimates, which is an essential criterion for quantitative risk estimation.
Availability of exposure-response information	1. Swaen et al. (2009) 2. Steenland et al. (2004) and Steenland et al. (2003)	The grouped exposure-response results reported by Mikoczy et al. (2011) are not well suited to derivation of unit risk estimates.
Other factors affecting the utility of epidemiology studies for quantitative risk estimation	Steenland et al. (2004) and Steenland et al. (2003)	The NIOSH study [Steenland et al. (2004) and Steenland et al. (2003)] alone was selected for quantitative risk estimation, as it was judged to be substantially superior to the UCC study (Swaen et al., 2009) with respect to a number of key considerations [in particular, in order of importance: (1) quality of the exposure estimates, (2) cohort size, and (3) the absence of coexposures and the inclusion of women].

The following subsections present the derivation of unit risk estimates, defined as the lifetime risk of cancer from chronic inhalation of EtO per unit of air concentration, for lymphohematopoietic cancer mortality and incidence and for breast cancer mortality and incidence in females, based on results of the recent analyses of the NIOSH cohort.

The exposure-response models used to fit the epidemiological data are empirical "curve-fitting" models. Model selection for each cancer data set was primarily based on a preference for models of the individual-level continuous exposure data, prioritization of models that are more tuned to local behavior in the low-exposure data, and a weighing of statistical and biological considerations. All of the exposure-response models considered in this assessment are fitted treating exposure as a continuous variable except for the categorical models and the linear regressions of categorical results, which are explicitly described as such. The continuous exposure models used are members of the set of general relative hazard models, which have the form $h(t) = h_0(t) \times \varphi$, or $h(t)/h_0(t) = \varphi$, where $h(t)$ is the hazard function, $h_0(t)$ is the baseline hazard, and $\varphi(\beta, Z)$ is a function of β, the parameters to be estimated, and Z, a vector of the explanatory data from the epidemiology study [see, e.g., Langholz and Richardson (2010)]. Relative hazard models with two different functional forms were considered in this assessment, and recognizing that the hazard function ratio expresses relative risk, these are also relative risk (or,

more specifically for this assessment, relative rate [RR]) models. One is an exponential model obtained when $\varphi = e^{\beta Z}$ (Cox proportional hazards model); in this assessment, models of this form will be referred to as Cox proportional hazards or Cox regression models or log-linear models, because they are linear in (natural) log RR. The second is the linear model, or $\varphi = 1 + \beta Z$ [referred to as the linear excess relative risk model (ERR) by Langholz and Richardson (2010)]; in this assessment, models of this form will be referred to as linear models.

4.1.1. Risk Estimates for Lymphohematopoietic Cancer

4.1.1.1. *Lymphohematopoietic Cancer Results From the NIOSH Study*

Steenland et al. (2004) investigated the relationship between (any) EtO exposure and mortality from cancer at a number of sites using life-table analyses with the U.S. population as the comparison population. Categorical SMR analyses were also done by quartiles of cumulative exposure. Then, to further investigate apparent exposure-response relationships observed for (lympho)hematopoietic cancer and breast cancer, internal exposure-response analyses were conducted using Cox proportional hazards models, which, when exposure is the only variable in the model, have the form

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

where β represents the regression coefficient and X(t) is the time-dependent exposure (or some function of exposure, e.g., the natural log of exposure). Internal analyses were done two ways—with exposure as a categorical variable and with exposure as a continuous variable. A nested case-control approach was used, with age as the time variable used to form the risk sets. Risk sets were constructed with 100 controls randomly selected for each case from the pool of those surviving to at least the age of the index case. According to the authors, use of 100 controls per case has been shown to result in ORs virtually identical to the RR estimates obtained with full cohorts. Cases and controls were matched on race (white/nonwhite), sex, and date of birth (within 5 years). Exposure was the only variable in the model, so the *p*-value for the model also serves as a *p*-value for the regression coefficient, β, as well as for a test of exposure-response trend.

For lymphohematopoietic cancer mortality, Steenland et al. (2004) analyzed both all lymphohematopoietic cancers combined and a subcategory of lymphohematopoietic cancers that they called "lymphoid" cancers; these included NHL, myeloma, and lymphocytic leukemia. Their exposure-response analyses focused on cumulative exposure and (natural) log cumulative exposure, with various lag periods. Other EtO exposure metrics (duration of exposure, average exposure, and peak exposure) were also examined, but models using these metrics did not generally predict lymphohematopoietic cancer as well as models using cumulative exposure. A lag period defines an

interval before death, or end of follow-up, during which any exposure is disregarded because these exposures have likely occurred after the onset of disease. For lymphohematopoietic (and lymphoid) cancer mortality, a 15-year lag provided the best fit to the data, based on the likelihood ratio test. As is commonly done, 1 ppm × day was added to cumulative exposures in analyses using the log of cumulative exposure with a lag, to avoid taking the log of 0. For both all lymphohematopoietic and lymphoid cancers, Steenland et al. (2004) found stronger positive exposure-response trends in males and so presented the results for some of the regression models separately by sex. The apparent sex difference was not statistically significant (see Appendix D), however, and results for both sexes combined were subsequently obtained from Dr. Steenland (see Appendix D; Section 3 for lymphoid cancer, Section 4 for all lymphohematopoietic cancer). These results are presented in Table 4-2. For additional details and discussion of the Steenland et al. (2004) study, see Appendix A, and for more details about the exposure and other characteristics of the full cohort and the lymphoid cancer risk sets, see Section D.5 of Appendix D.

Table 4-2. Cox regression results for all lymphohematopoietic cancer and lymphoid cancer mortality in both sexes in the NIOSH cohort, for the models presented by Steenland et al. (2004)

Exposure variable[a]	*p*-value[b]	Coefficient (SE) (per ppm × day)	ORs by category[c] (95% CI)
All lymphohematopoietic cancer[d]			
Cumulative exposure, 15-yr lag	0.40	3.26×10^{-6} (3.49×10^{-6})	
Log cumulative exposure, 15-yr lag	0.009	0.107 (0.0418)	
Categorical cumulative exposure, 15-yr 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[e]			
Cumulative exposure, 15-yr lag	0.22	4.74×10^{-6} (3.35×10^{-6})	
Log cumulative exposure, 15-yr lag	0.02	0.112 (0.0486)	
Categorical cumulative exposure, 15-yr 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)

[a]Cumulative exposure is in ppm × days.
[b]*p*-values from likelihood ratio test.
[c]Exposure categories are 0; >0−1,199; 1,200−3,679; 3,680−13,499; ≥13,500 ppm × days.
[d]9th revision ICD codes 200−208; results based on 74 cases.
[e]NHL, myeloma, and lymphocytic leukemia (9th revision ICD codes 200, 202, 203, 204); results based on 53 cases.

Source: Additional analyses performed by Dr. Steenland (see Sections D.3 and D.4 of Appendix D).

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

The exposure-response trends for lymphohematopoietic cancers observed by Steenland et al. (2004) appear to be driven largely by the lymphoid cancers; therefore, the primary risk analyses for lymphohematopoietic cancer are based on the lymphoid cancer results. Lymphohematopoietic cancers are a diverse group of diseases with diverse etiologies, and myeloid and lymphoid cells develop from different progenitor cells; thus, there is stronger support for an etiologic role of EtO in the development of lymphoid cancers than in the development of the cancers in the aggregate all lymphohematopoietic cancer category. The consideration of NHL, (plasma cell) myeloma, and lymphocytic leukemia together as "lymphoid" cancers is consistent with the current World Health Organization classification of such cancers based on their derivation from B-cells, T-cells, and NK-cells rather than previous distinctions (Harris et al., 1999).

Nonetheless, for comprehensiveness and for the reasons listed below, risk estimates based on the all lymphohematopoietic cancer results are presented for comparison. Judging roughly from the *p*-values, the model fits do not appear notably better for lymphoid cancers than for all lymphohematopoietic cancers (see Table 4-2, *p*-values for log cumulative exposure models), and the "lymphoid" category did not include Hodgkin lymphoma, which also exhibited evidence of exposure-response trends, although based on few cases (Steenland et al., 2004). In addition, misclassification or nonclassification of tumor type is more likely to occur for subcategories of lymphohematopoietic cancer (e.g., 4 of the 25 leukemias in the analyses were classified as "not specified" and so could not be considered for the lymphoid cancer analysis).

For lymphoid cancer, the results of internal exposure-response analyses of lymphoid cancer in the NIOSH cohort (Cox regression analyses, summarized in Table 4-2) were used for predicting the extra risks of lymphoid cancer mortality from continuous environmental exposure to EtO. Extra risk is defined as

$$\text{Extra risk} = (Rx - Ro)/(1 - Ro), \qquad (4\text{-}2)$$

where Rx is the lifetime risk in the exposed population and Ro is the lifetime risk in an unexposed population (i.e., the background risk). These risk estimates were calculated using the β regression coefficients and an actuarial program (life-table analysis) that accounts for competing causes of death.[15] An inherent assumption in the Cox regression model and its application in the life-table analyses is that

[15]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.

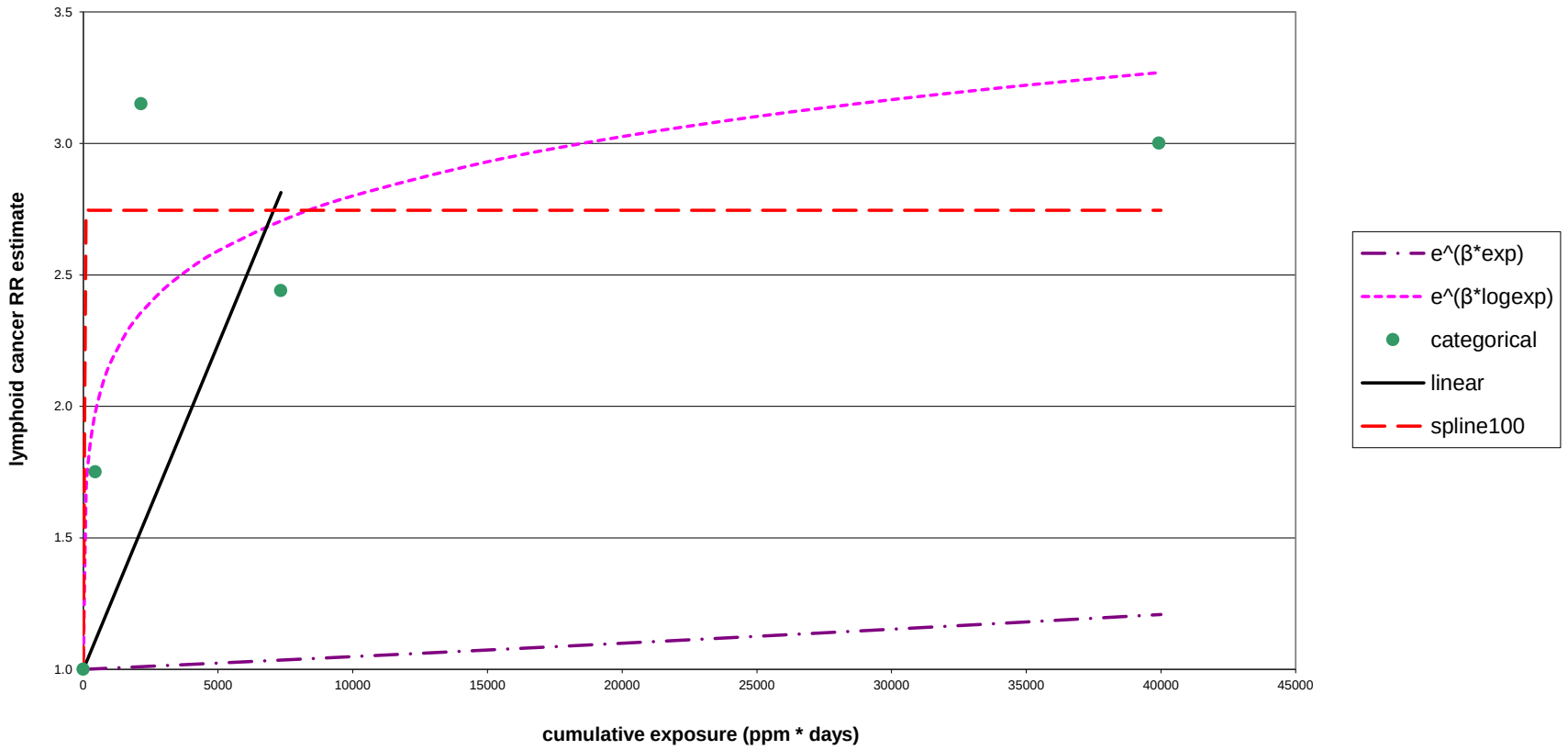
RR is independent of age. (An alternate assumption of increased susceptibility from early-life exposure to EtO, as recommended in EPA's *Supplemental Guidance* (U.S. EPA, 2005b) for chemicals, such as EtO [see Section 3.4], with a mutagenic mode of action, is considered in Section 4.4. This alternate assumption is the prevailing assumption in this assessment, based on the recommendations in the *Supplemental Guidance.* Risk estimates are first developed under the assumption of age independence, however, because that is the standard approach in the absence of evidence to the contrary or of sufficient evidence of a mutagenic mode of action to invoke the divergent assumption of increased early-life susceptibility.)

For the life-table analysis, U.S. age-specific mortality rates (for both sexes of all races combined) for all causes and for the relevant subcategories of lymphohematopoietic cancer (NHL [C82-C85 of 10th revision of the International Classification of Diseases (ICD)], multiple myeloma [C88, C90], and lymphoid leukemia [C91]) for the years 2008−2012 were obtained from the CDC WONDER Online Database (CDC, 2015). The risks were computed up to age 85 for continuous exposures to EtO beginning at birth.[16] Conversions between occupational EtO exposures and continuous environmental exposures were made to account for differences in the number of days exposed per year (240 vs. 365 days) and in the amount of EtO-contaminated air inhaled per day [10 vs. 20 m^3; U.S. EPA (1994)]. An adjustment was also made for the lag period. The reported standard errors for the regression coefficients from Table 4-2 were used to compute the 95% upper confidence limits (UCLs) for the relative rates, based on a normal approximation.

The only statistically significant Cox regression model presented by Steenland et al. (2004) for lymphoid cancer mortality was for log cumulative exposure with a 15-year lag in males (p = 0.02). This was similarly true for the analyses of lymphoid cancer using the data for both sexes (see Table 4-2). However, using the log cumulative exposure model to estimate the risks from low environmental exposures is problematic because this model, which is intended to fit the full range of occupational exposures in the study, is inherently supralinear (i.e., risk increases steeply with increasing exposures in the low exposure range and then plateaus), with the slope approaching infinity as exposures decrease towards zero, and results can be unstable for low exposures (i.e., small changes in exposure correspond to large changes in risk; see Figure 4-1). Some consideration was thus given to the cumulative exposure model, which is typically used and which is generally stable at low exposures (i.e., small changes in exposure do not correspond to large changes in risk), although the fit to these data was not statistically significant (p = 0.22). However, the Cox regression model with cumulative exposure is inherently sublinear (i.e., risk increases gradually in the low exposure range and then with increasing steepness as

[16]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 cutoff point for the life-table analysis, which uses actual age-specific mortality rates. The average lifespan for males and females combined in a life-table analysis truncated at age 85 years is about 75 years.

exposure increases) and does not reflect the apparent supralinearity of the data demonstrated by the categorical results and the superior fit of the log cumulative exposure model; thus, this model was not considered further.



e^(β*exp): Cox regression results for RR = $e^{(\beta \times \text{exposure})}$; e^(β*logexp): Cox regression results for RR = $e^{(\beta \times \ln(\text{exposure}))}$; categorical: Cox regression results for RR = $e^{(\beta \times \text{exposure})}$ with categorical exposures, plotted at the mean cumulative exposure; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); spline100: Two-piece log-linear spline model with knot at 100 ppm × days (see text). (Note that, with the exception of the categorical results and the linear regression of the categorical results, the different models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the *y*-axis. They are, however, comparable in terms of general shape.)

Source: Steenland reanalyses for males and females combined; see Appendix D (except for linear regression of categorical results, which was done by EPA).

Figure 4-1. RR estimate for lymphoid cancer vs. occupational cumulative exposure (with 15-year lag).

In a 2006 external review draft of this assessment (U.S. EPA, 2006a), which relied on the original published results of Steenland et al. (2004), EPA proposed that the best way to represent the exposure-response relationship in the lower exposure region, which is the region of interest for low-exposure extrapolation, was through the use of a weighted linear regression of the results from the Cox regression model with categorical cumulative exposure and a 15-year lag [for males only, as this was the significant finding in the published paper of Steenland et al. (2004)]. In addition, the highest exposure group was not included in the regression to alleviate some of the "plateauing" in the exposure-response relationship at higher exposure levels and to provide a better fit to the lower exposure data. Linear modeling of categorical (i.e., grouped) epidemiologic data and elimination of the highest exposure group(s) under certain circumstances to obtain a better fit of low-exposure data are both standard techniques used in EPA dose-response assessments (U.S. EPA, 2012, 2005a). An established methodology was employed for the weighted linear regression of the categorical epidemiologic data, as described by Rothman (1986) and used by others [e.g., van Wijngaarden and Hertz-Picciotto (2004)]. For the subsequent draft assessment, EPA pursued modeling the individual continuous exposure data as an alternative to modeling the published grouped data (U.S. EPA, 2014a, b). In addition, both males and females were included in the modeling of lymphohematopoietic cancer mortality. In consultation with Dr. Steenland, one of the investigators from the NIOSH cohort studies, EPA determined that an alternative way to address the supralinearity of the data when using the full continuous exposure data set (while avoiding the extreme low-exposure curvature obtained with the log cumulative exposure model) might be to use a two-piece log-linear spline model.

Spline models have been used previously for exposure-response analyses of epidemiological data (Steenland and Deddens, 2004; Steenland et al., 2001). These models are generally useful for exposure-response data such as the EtO lymphoid cancer data, for which RR initially increases with increasing exposure but then tends to plateau, or attenuate, at higher exposures. Such plateauing exposure-response relationships have been seen with other occupational carcinogens and may occur for various reasons, including the depletion of susceptible subpopulations at high exposures, mismeasurement of high exposures, or a healthy worker survivor effect (Stayner et al., 2003). No other traditional exposure-response models for continuous exposure data that might suitably fit the observed exposure-response pattern were apparent. Dr. Steenland was contracted to do the spline analyses using the full data set with cumulative exposure as a continuous variable, and his findings are included in Appendix D (see Section D.3 for lymphoid cancer, Section D.4 for all lymphohematopoietic cancer). The results of the spline analyses are presented below.

For the two-piece log-linear spline modeling approach, the Cox regression model (Equation 4-1) was the underlying basis for the splines which were fit to the lymphoid cancer exposure-response data.[17] Taking the log of both sides of Equation 4-1, log RR is a linear function of exposure (cumulative exposure is used here), and, with the two-piece log-linear spline approach, log RR is a function of two lines which join at a single point of inflection, called a "knot." The use of the two-piece spline model form is not intended to imply that an abrupt change in biological response occurs at the knot but, rather, to allow description of an exposure-response relationship in which the slope of the relationship differs notably in the low-exposure versus high-exposure regions. The shape of the two-piece log-linear spline model, in particular the slope in the low-exposure region, depends on the location of the knot. For this assessment, the knot was generally selected by evaluating different knots in increments of 100 ppm × days over some range of cumulative exposures starting at 0 and then choosing the one that resulted in the best (largest) model likelihood. The model likelihood did not change much across the different trial knots for any of the data sets, but it did change slightly, and the largest calculated likelihood was used as the basis for knot selection. For more discussion of the two-piece spline approach, see Appendix D.

For the lymphoid cancer data, the range examined for knot selection was from 0 to 15,000 ppm × days, and the largest model likelihood was observed with the knot at 100 ppm × days, although, as noted above, the model likelihood did not actually change much across the different trial knots (see Figure D-14 of Appendix D). This model yielded a very steep slope in the exposure range below the knot of 100 ppm × days (see Figure 4-1),[18] and there was low confidence in the slope, given the limited data in the low-exposure region.

A two-piece linear spline model (with a linear model, i.e., RR = 1 + β × exposure, as the underlying basis for the spline pieces) was also attempted, using the then just-published approach of Langholz and Richardson (2010) to model the individual data with cumulative exposure as a continuous variable; however, this model did not alleviate the problem of the excessively steep low-exposure spline segment (see Figure D-18 in Appendix D) and was not pursued further at that time. The Langholz and Richardson (2010) approach was also employed to model the lymphoid cancer data using linear RR models with cumulative exposure and log cumulative exposure as continuous variables; however, these linear models similarly did not alleviate the problems of the corresponding log-linear RR models (see Figure D-18 in Appendix D).

Therefore, after examining the modeling analyses, it was proposed in the subsequent SAB review draft (U.S. EPA, 2014a, b) that the weighted linear regression of the categorical results still provided the best available approach for deriving the risk estimates for lymphoid cancer. For the

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

[18]Although the log-linear spline segments appear fairly linear in the plotted range, they are not strictly linear.

weighted linear regression, the Cox regression results from the model with categorical cumulative exposure and a 15-year lag (see Table 4-2) was used, excluding the highest exposure group, as discussed above.[19] The weights used for the ORs were the inverses of the variances, which were calculated from the confidence intervals.[20] Mean and median exposures for the cumulative exposure groups were provided by Dr. Steenland (see Table D-26 of Appendix D).[21] The mean values were used for the weighted regression analysis because, under this model, the cancer response is presumed to be a linear function of cumulative exposure, which is expected to be best represented by mean exposures. If the median values had been used, a slightly larger regression coefficient would have been obtained, resulting in slightly larger risk estimates.[22] See Table 4-3 for the results obtained from the weighted linear regression and Figure 4-1 for a depiction of the resulting model.

Table 4-3. Linear regression of categorical results—modeling results for all lymphohematopoietic cancer and lymphoid cancer mortality in both sexes in the NIOSH cohort[a]

Cancer endpoint	*p*-value[b]	Coefficient (SE) (per ppm × day)
All lymphohematopoietic cancer[c]	0.08	3.459×10^{-4} (1.944×10^{-4})
Lymphoid cancer[d]	0.18	2.47×10^{-4} (1.85×10^{-4})

[a]With cumulative exposure in ppm × days as the exposure variable, with a 15-yr lag; excluding the highest exposure category.
[b]Wald *p*-values.
[c]9th revision ICD codes 200−208; results based on 74 cases.
[d]NHL, myeloma, and lymphocytic leukemia (9th revision ICD codes 200, 202, 203, 204); results based on 53 cases.

For this final assessment, EPA reassessed the modeling options. First, however, EPA revisited the issue of lag selection for the lymphoid cancer mortality data. After considering model fit for cumulative exposure with different lag periods across a larger number of models than was previously evaluated with different lags, EPA again selected 15 years as the lag period to use for the exposure-response analyses (see Section D.3.2 of Appendix D). Sensitivity of the results to choice of

[19]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 U.S. EPA in the course of its analyses.
[20]Equations for this weighted linear regression approach are presented in Rothman (1986) and summarized in Appendix F.
[21]Mean exposures for both sexes combined with a 15-year lag for the categorical exposure quartiles in Table 4-2 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.
[22]8% greater regression coefficients for both lymphoid cancer and all lymphohematopoietic cancer.

lag period is examined in Sections D.3.4 and D.3.5 of Appendix D and summarized at the end of Section 4.1.1.3).

For the final model selection, EPA had the following objectives:

1. Use the individual-level continuous exposure data.
2. Prioritize models that are more tuned to local behavior in the low-exposure data (e.g., spline models) over more global models.
3. Consider the principle of parsimony.
4. Use the same model for both environmental and occupational exposures.
5. Rely less on AIC[23].
6. Weigh models based on both biological plausibility and statistical considerations.

In light of these objectives, the linear and log-linear two-piece spline models (with a 15-year lag) were reconsidered. Two-piece spline models use the individual-level continuous exposure data, consistent with objective 1; their use for these data allows for better local fit to the low-exposure data (objective 2); the method used to preselect the knot is consistent with principles of parsimony (objective 3)[24]; and a two-piece spline model can be used for both environmental and occupational exposures (objective 4).

The linear and log-linear two-piece spline models with the maximum likelihood (and lowest AIC), both had knots at 100 ppm × days. The AICs for these linear and log-linear two-piece spline models were 461.4 and 461.8, respectively, which indicate an essentially identical global fit between the two because the SAS procedure used for the linear models consistently reported −2 log-likelihoods and AICs about 0.4 units lower than the SAS procedure used to fit the log-linear models.[25] Both model forms also had a local maximum of the likelihood (and local minimum of the AIC) at 1,600 ppm × days

[23]Akaike Information Criteria. The AIC is a measure of information loss from a dose-response model that can be used to compare a specified set of models. The AIC is defined as 2p – 2ln(L), where p is the number of estimated parameters included in the model and L is the maximized value of the likelihood function. Among a set of specified models, the model with the lowest AIC is the preferred model.

[24]See SAB (2015), page 12.

[25]For the lymphoid cancer data, SAS proc NLP consistently yielded -2LLs and AICs about 0.4 units lower than proc PHREG for the same models, including the null model, presumably for computational processing reasons. Proc NLP was used for the linear RR models, and proc PHREG was used for the log-linear RR models.

(see Figure D-14 in Appendix D), and these two models also had AICs indicating virtually identical fits.[26]

Of the two-piece spline models considered, the two-piece linear spline model with the knot at 1,600 ppm × days was selected. Although this is not the model with the lowest AIC, its AIC differs by a negligible 0.7 AIC units from the model with the lowest AIC (the two-piece linear spline model with the knot at 100 ppm × days) and its selection is consistent with the objective 5 above to rely less on AIC and is supported by additional considerations. One consideration is that the selected model has more exposed cases in the exposure range of the estimate than do the two-piece spline models with knots at 100 ppm × days. With the knot at 100 ppm × days, there are no exposed cases below the knot, and the low-exposure slope is entirely determined by the high-exposure segment and its termination at the knot. In contrast, the low-exposure slope in the two-piece linear spline model with the knot at 1600 ppm × days has 13 cases below the knot. Because EPA is interested in the low-exposure slope for unit risk estimation, the low-exposure slope provided by the selected model, which is based on more cases in the exposure range of the estimate, is preferred. This is also consistent with objective 2 above to prioritize models with more local fit in the low-exposure range.

A second consideration is that the selected model reflects a better weighing of biological and statistical considerations, consistent with objective 6 above. The two-piece spline models with the knot at 1600 ppm × days have a more gradual rise in the low-exposure region and a more plausible rise at higher exposures. This exposure-response relationship is considered more biologically realistic than the two-piece spline models with the knot at 100 ppm × days, which more closely resemble a step-function, which is not biologically probable for a complex, multistep process such as carcinogenicity in a variable human population.

Neither of the two-piece spline models with the knot at 1,600 ppm × days had a p-value <0.05; however, both were close to 0.05 ($p = 0.07$ for each model), and the significant exposure-response relationship has already been established with similar models, e.g., the two-piece spline models with the knot at 100 ppm × days and the linear and log-linear log cumulative exposure models (see Figure 4-2) (although the higher-exposure spline segments for the models with the knot at 1,600 ppm × days appear to underpredict the RRs). Of the two-piece spline models with the knot at 1,600 ppm × days, the linear spline model was preferred to the log-linear spline model because linearity is a desirable property to have in risk assessment models. For example, linear low-dose extrapolation can occur without a discontinuity between the model in the observable range and low-dose extrapolation from the point of departure, and the unit risk estimate is not dependent on the risk level chosen for determination of the

[26]An AIC of 462.1 for the linear two-piece spline model and 462.6 for the log-linear; however, for the lymphoid cancer data, SAS proc NLP (used for the linear RR models) consistently yielded -2LLs and AICs about 0.4 units lower than proc PHREG (used for the log-linear RR models) for the same models, including the null model, presumably for computational processing reasons.

point of departure, at least within the exposure range of the first spline segment for a spline model. In addition, with an overall exposure-response relationship that is supralinear, it seems contradictory to use sublinear model forms for the increments represented by the spline pieces.

Results for the various two-piece spline models considered above are presented in Table 4-4. The additional two-piece linear spline modeling, along with further sensitivity analyses, was also conducted by Dr. Steenland, under a subsequent contract to EPA, and results for the two-piece spline models are provided in more detail in Section D.3 of Appendix D). Dr. Steenland also provided results for linear and log-linear square-root transformation of cumulative exposure models, and these are also included in the current assessment for completeness (Table 4-5). These square-root transformation models were not given much consideration because, of the single-parameter models, the log-transformation models had better fits, as indicated by lower AICs (460.4 versus 462.8 for the log-linear models and 460.2 versus 461.8 for the linear models; see Section D.3 of Appendix D), and because EPA adhered to the objective to prioritize models that have a greater ability to provide a good local fit to the low-exposure range, such as two-piece spline models.

Table 4-4. Two-piece spline modeling results for lymphoid cancer and all lymphohematopoietic cancer mortality in both sexes in the NIOSH cohort[a]

Two-piece Spline Model Form	Knot (ppm × days)	*p*-value[b]	Coefficient[c] (SE[d]) (per ppm × day)
Lymphoid cancer[e]			
log-linear	100	0.047	$\beta1 = 0.0101$ (4.93×10^{-3}) $\beta2 = -0.0101$ (4.93×10^{-3})
log-linear	1,600	0.07	$\beta1 = 4.89 \times 10^{-4}$ (2.55×10^{-4}) $\beta2 = -4.86 \times 10^{-4}$ (2.6×10^{-4})
linear	100	0.046	$\beta1 = 0.0152$ (UB1 = 0.0590) $\beta2 = -0.0152$
linear	1,600	0.07	$\beta1 = 7.58 \times 10^{-4}$ $(UB1 = 2.98 \times 10^{-3})$ $\beta2 = -7.48 \times 10^{-4}$
All lymphohematopoietic cancer[f]			
log-linear	500	0.02	Low-exposure spline segment: $\beta1 = 2.01 \times 10^{-3}$ (7.73×10^{-4})

[a]With cumulative exposure in ppm × days as the exposure variable, with a 15-yr lag.
[b]*p*-values from likelihood ratio test.
[c]For the two-piece spline models, for exposures below the knot, RR = 1 + (β1 × exp); for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp – knot)).
[d]Or, for linear models of continuous exposures, the profile likelihood 95% (one-sided) upper bounds (UB).
[e]NHL, myeloma, and lymphocytic leukemia (9th revision ICD codes 200, 202, 203, 204); results based on 53 cases.
[f]9th revision ICD codes 200−208; results based on 74 cases.

Source: Additional analyses performed by Dr. Steenland (see Sections D.3 and D.4 of Appendix D).

Table 4-5. Exposure-response modeling results for lymphoid cancer[c] mortality in both sexes in the NIOSH cohort for models with square-root transformations of exposure

RR Model[a]	*p*-value[b]	Coefficient (SE) (per ppm × day)
Linear square-root transformation model	0.053	6.14×10^{-3} (--[d])
Log-linear square-root transformation model	0.08	2.83×10^{-3} (1.5×10^{-3})

[a]All with square-root transformation of cumulative exposure in ppm × days, with a 15-yr lag, as the exposure variable.
[b]*p*-values from likelihood ratio test.
[c]NHL, myeloma, and lymphocytic leukemia (9th revision ICD codes 200, 202, 203, 204); results based on 53 cases.
[d]Standard errors calculated assuming a Wald approximation are likely unrealistic for the linear RR ("ERR") models (Langholz and Richardson, 2010), and so have not been provided.

Source: Additional analyses performed by Dr. Steenland (see Section D.3 of Appendix D).

As the lymphoid cancer data set is the primary data set used for the derivation of unit risk estimates for lymphohematopoietic cancers, a summary of all the models considered for modeling the lymphoid cancer exposure-response data and the judgments made about model selection is provided in Table 4-6. See Figure 4-2 for a visual representation of the models. See Tables 4-2, 4-3, 4-4, and 4-5 and Section D.3 of Appendix D for other information about the models. As discussed above and summarized in Table 4-6, the two-piece linear spline model with the knot at 1,600 ppm × days was the preferred model for the derivation of unit risk estimates of the models considered and was thus the selected model.

The proportional hazards assumption for the selected model (two-piece linear spline model with knot at 1,600 ppm × days) was tested by evaluating the significance of an age-interaction term for each spline regression coefficient, and neither interaction term was statistically significant (Section D.3.7 of Appendix D).

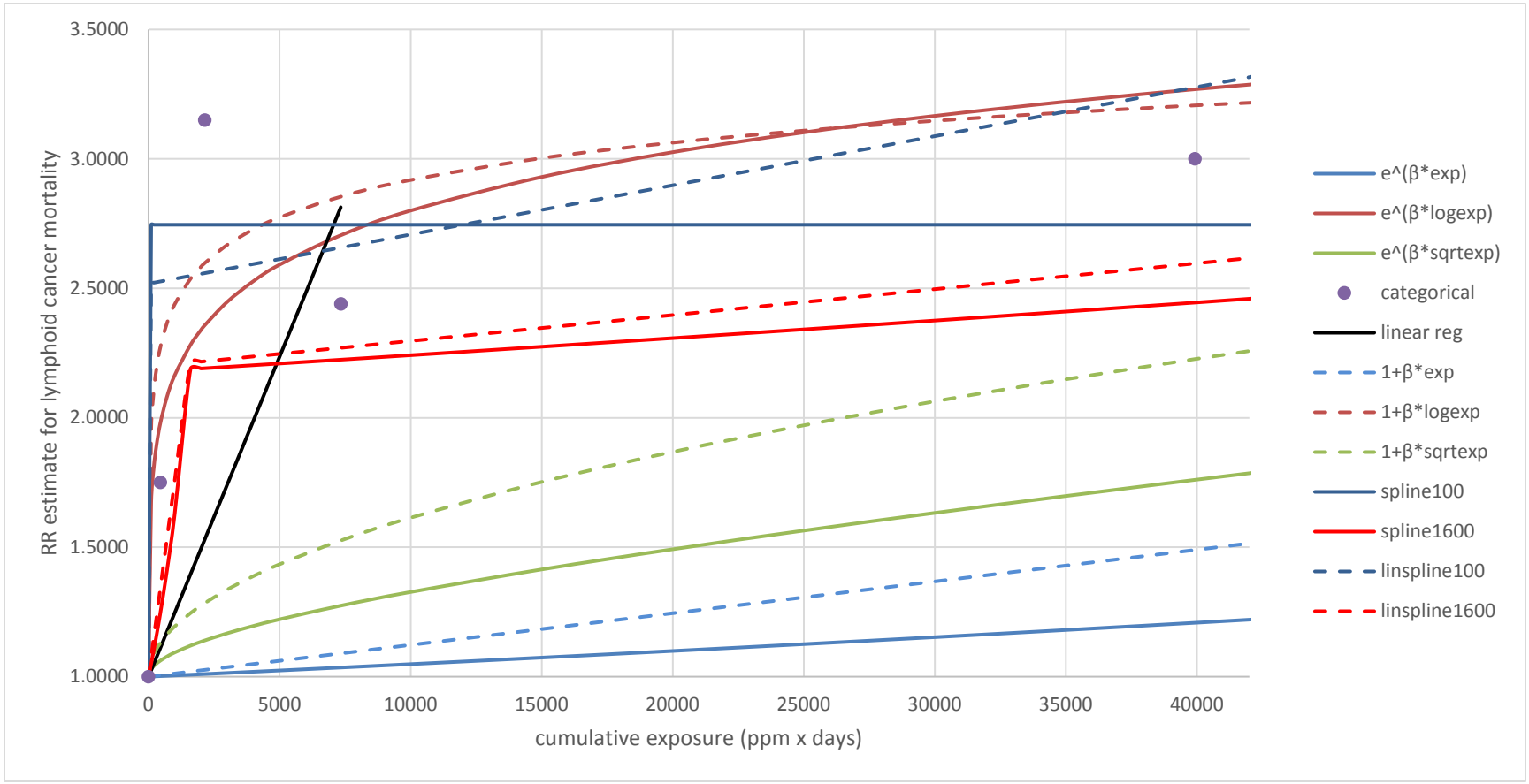
Table 4-6. Models considered for modeling the exposure-response data for lymphoid cancer mortality in both sexes in the NIOSH cohort for the derivation of unit risk estimates

Model[a]	*p*-value[b]	AIC[c]	Comments
Two-piece spline models			
Linear spline model with knot at 1,600 ppm × days	0.07	462.1	**SELECTED.** Adequate statistical and visual fit, including local fit to low-exposure range; linear model; AIC within two units of lowest AIC of models considered.
Linear spline model with knot at 100 ppm × days	0.046	461.4	Good overall statistical fit and lowest AIC of two-piece spline models, but poor local fit to the low-exposure region, with no cases below the knot.
Log-linear spline model with knot at 1,600 ppm × days	0.07	462.6	Linear model preferred to log-linear (see text above).
Log-linear spline model with knot at 100 ppm × days	0.047	461.8	Good overall statistical fit and tied for lowest AIC[c] of two-piece spline models, but poor local fit to the low-exposure region, with no cases below the knot.
Linear (ERR) models ($RR = 1 + \beta \times exposure$)			
Linear model	0.13	463.2	Not statistically significant overall fit and poor visual fit.
Linear model with log cumulative exposure	0.02	460.2	Good overall statistical fit, but poor local fit to the low-exposure region.
Linear model with square-root transformation of cumulative exposure	0.053	461.8	Borderline statistical fit, but poor local fit to the low-exposure region.
Log-linear (Cox regression) models ($RR = e^{\beta \times exposure}$)			
Log-linear model (standard Cox regression model)	0.22	464.4	Not statistically significant overall fit and poor visual fit.
Log-linear model with log cumulative exposure	0.02	460.4	Good overall statistical fit; lowest AIC[c] of models considered; low-exposure slope becomes increasingly steep as exposures decrease, and large unit risk estimates can result; preference given to the two-piece spline models because they have a better ability to provide a good local fit to the low-exposure range.
Log-linear model with square-root transformation of cumulative exposure	0.08	462.8	Not statistically significant overall fit and poor visual fit.

Table 4 6. Models considered for modeling the exposure-response data for lymphoid cancer mortality in both sexes in the NIOSH cohort for the derivation of unit risk estimates (continued)

Model[a]	*p*-value[b]	AIC[c]	Comments
Linear regression of categorical results			
Linear regression of categorical results, excluding the highest exposure quartile	0.18	--	Not statistically significant, though that is unsurprising since the approach, which is based on categorical data, has low statistical power; preference given to models that treated exposure as a continuous variable and that also provided reasonable representations of the low-exposure region.

[a]All with cumulative exposure as the exposure variable, except where noted, and with a 15-yr lag.
[b]*p*-values from likelihood ratio test, except for linear regression of categorical results, where Wald *p*-values are reported. $p < 0.05$ considered "good" statistical fit; $0.05 < p < 0.10$ considered "adequate" statistical fit if significant exposure-response relationships have already been established with similar models.
[c]AICs for linear models are directly comparable and AICs for log-linear models are directly comparable. However, for the lymphoid cancer data, SAS proc NLP consistently yielded −2LLs and AICs about 0.4 units lower than proc PHREG for the same models, including the null model, presumably for computational processing reasons, and proc NLP was used for the linear RR models. Thus, AICs for linear models are equivalent to AICs about 0.4 units higher for log-linear models. No AIC was calculated for the linear regression of categorical results.



e^(β*exp): RR = $e^{(\beta \times \text{exposure})}$; e^(β*logexp): RR = $e^{(\beta \times \ln(\text{exposure}))}$; e^(β*sqrtexp): RR = $e^{(\beta \times \text{sqrt}(\text{exposure}))}$; categorical: RR = $e^{(\beta \times \text{exposure})}$ with categorical exposures, plotted at the mean cumulative exposure; linear reg: weighted linear regression of categorical results, excluding highest exposure group (see text); spline100(1,600); 1 + β*exp: RR = 1 + β × exposure; 1 + β*logexp: RR = 1 + β × ln(exposure); 1 + β*sqrtexp: RR = 1 + β × sqrt(exposure); Two-piece log-linear spline model with knot at 100 (1,600) ppm × days (see text); linspline100(1,600): Two-piece linear spline model with knot at 100 (1,600) ppm × days (see text). (Note that, with the exception of the categorical results and the linear regression of the categorical results, the different models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the *y*-axis. They are, however, comparable in terms of general shape.)

Source: Steenland reanalyses for males and females combined; see Appendix D (except for linear regression of categorical results, which was done by EPA).

Figure 4-2. Exposure-response models for lymphoid cancer mortality vs. occupational cumulative exposure (with 15-year lag).

The modeling results for the selected two-piece linear spline model with the knot at 1,600 ppm × days for lymphoid cancer mortality in males and females combined were used with the actuarial program (life-table analysis) to estimate the exposure level (EC_x; "effective concentration") and the associated 95% lower confidence limit (LEC_x) corresponding to an extra risk of 1% ($x = 0.01$). A 1% extra risk level is commonly used for the determination of the point of departure (POD) for low-exposure extrapolation from epidemiological cancer data (except for rare cancers); higher extra risk levels, such as 10%, would be an upward extrapolation for these data. Thus, 1% extra risk was selected for determination of the POD, and, consistent with EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the LEC value corresponding to that risk level was used as the POD to derive the cancer unit risk estimates.

Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3), which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was performed. (Linear low-exposure extrapolation is also the default approach used in the absence of sufficient evidence for a nonlinear mode of action, which is also the case for EtO [Section 3.4]). The EC_{01}, LEC_{01}, and inhalation unit risk estimate calculated for lymphoid cancer mortality from the two-piece linear spline model with the knot at 1,600 ppm × days are presented in Table 4-7 (the incidence results also presented in Table 4-7 are discussed in Section 4.1.1.3 below). The resulting unit risk estimate for lymphoid cancer mortality based on the two-piece linear spline model with the knot at 1,600 ppm × days for both sexes using cumulative exposure with a 15-year lag is 1.99 per ppm (1.99×10^{-3} per ppb). This unit risk estimate is 2.3 times the unit risk estimate from the most similar alternative model, the two-piece log-linear spline model with the knot at 1,600 ppm × days (note that the EC_{01}s are similar between the two two-piece spline models with the knot at 1,600 ppm × days, with the one from the log-linear spline model just 8% greater than that from the linear spline model; however, the LEC_{01}s are more divergent [2.3-times]), and about 5.4-times the estimate from the linear regression of categorical results. EC_{01}, LEC_{01}, and unit risk estimates from the other models considered are presented for comparison only, to illustrate the differences in model behavior at the low end of the exposure-response range. As discussed above, these models were deemed unsuitable for the derivation of risks from (low) environmental exposure levels. The log-linear log cumulative exposure model, with its marked supralinear curvature in the lower exposure region, and the two-piece linear and log-linear spline models with the knot at 100 ppm × days, with their very steep low-exposure slopes, yield substantially higher unit risk estimates (17.5 to 39.4 per ppm). On the other hand, the linear model and the more gradually supralinear log-linear model with a square-root transformation, neither of which provide a statistically significant fit to the overall data, yield substantially lower unit risk estimates (0.0314 and 0.0397 per ppm, respectively).

Converting the units, the resulting unit risk estimate of 1.99 per ppm from the selected two-piece linear spline model with the knot at 1,600 ppm × days corresponds to a unit risk estimate of 1.09×10^{-3} per μg/m^3 for lymphoid cancer mortality.[27]

The life-table analysis takes into account competing risks and the occurrence of different cumulative exposures and different cause-specific background risks at different ages. A crude approximation of the general approach for obtaining EC_{01} and LEC_{01} estimates without the use of the life-table component of the analysis is presented here for illustration. In this crude approach, an estimate of the lifetime background risk of dying of lymphoid cancer is used rather than age-specific rates. For lymphoid cancer, a life-table analysis was used to obtain this lifetime background risk estimate because another source of such an estimate was not readily available. The resulting lifetime background mortality risk estimate (Ro) is 1.06%. From this and Equation 4-2, an estimate of the RR associated with a 1% extra risk can be calculated as RR = (0.99 × Ro + 0.01)/Ro = 1.93. Then, a maximum likelihood estimate (MLE) of the cumulative exposure associated with this RR can be calculated from the low-exposure spline segment from the selected two-piece linear spline model as exposure = RR – 1)/β_1. This quantity is an occupational cumulative exposure in ppm × days. To convert to environmental ppm × years, multiply by (10 m^3 breathed at work/day)/(20 m^3/day) and (240 days worked/year)/(365 days/year), as discussed earlier in Section 4.1.1.2, and then divide by 365 days/year. Because the life-table analysis is based on actual demographic rates, this crude approximation uses an average U.S. life expectancy of 80 years[28] rather than the EPA default average lifespan of 70 years, for a more appropriate comparison. With a 15-year lag, this means dividing the cumulative exposure by 65 years to get the continuous lifetime exposure level associated with a 1% extra risk (EC_{01}). The LEC_{01} is obtained using the same calculations but with the profile likelihood upper bound on beta1 in place of beta1. With beta1 of 7.58×10^{-4} per ppm × day and a profile likelihood upper bound on beta1 of 2.98×10^{-3} per ppm × day from the selected two-piece linear spline model, these calculations yield an EC_{01} of 0.0171 ppm and an LEC_{01} of 0.00434 ppm. In comparison to the estimates presented for the selected two-piece linear spline model in Table 4-7, this crude approach yields EC_{01} and LEC_{01} estimates that are both about 14% lower, which would correspond to a unit risk estimate about 16% higher.

[27]Conversion equation: 1 ppm = 1,830 μg/m^3.
[28]The overall U.S. life expectancy in 2014 was 78.8 years (Murphy et al., 2015).

Table 4-7. EC_{01}, LEC_{01}, and unit risk estimates for lymphoid cancer from various models[a,b]

Model[e]	Mortality[c]			Incidence[c,d]		
	EC_{01} (ppm)	**LEC_{01} (ppm)**	**Unit risk (per ppm)**	**EC_{01} (ppm)**	**LEC_{01} (ppm)**	**Unit risk (per ppm)**
Two-piece spline models						
Low-exposure linear spline from linear spline model with knot at 1,600 ppm × days[f]	**0.0198**	**5.03×10^{-3}**	**1.99[g]**	**7.48×10^{-3}**	**1.90×10^{-3}**	**5.26[g]**
Low-exposure linear spline from linear spline model with knot at 100 ppm × days[h]	9.87×10^{-4}	2.54×10^{-4}	39.4[g,i]	3.73×10^{-4}	9.61×10^{-5}	104[g,i]
Low-exposure log-linear spline from log-linear spline model with knot at 1,600 ppm × days[f]	0.0213	0.0114	0.877	9.92×10^{-3}	5.29×10^{-3}	1.89[g]
Low-exposure log-linear spline from log-linear spline model with knot at 100 ppm × days[h]	1.03×10^{-3}	5.73×10^{-4}	17.5[g,i]	4.80×10^{-4}	2.66×10^{-4}	37.6[g,i]
Linear models (RR = 1 + β × exposure)						
Linear model	1.22	0.318	0.0314	0.462	0.120	0.0833
linear model with log cumulative exposure	1.55×10^{-3}	--[k]	--[k]	--[k]	--[k]	--[k]
Linear model with square-root transformation of cumulative exposure	0.383	--[m]	--[m]	0.0511	--[m]	--[m]

Table 4 7. EC01, LEC01, and unit risk estimates for lymphoid cancer from various models[a,b] (continued)

Model[e]	Mortality[c]			Incidence[c,d]		
	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)
Log-linear models ($RR = e^{\beta \times exposure}$)						
Log-linear model with log cumulative exposure	6.33×10^{-3}	5.24×10^{-4}	19.1[g,i]	--[k]	--[k]	--[k]
Log-linear model with square-root transformation of cumulative exposure	0.883	0.252	0.0397	0.178	0.0509	0.196
Linear regression of categorical results						
Linear regression of categorical results[n]	0.0607	0.0272	0.368	0.0229	0.0103	0.971

[a]From lifetime continuous exposure. Unit risk = $0.01/LEC_{01}$.
[b]Models from Table 4-6 for which $p < 0.20$ for the continuous-exposure models. This criterion resulted in the omission of the log-linear model with untransformed exposure (standard Cox regression model; $p = 0.22$).
[c]Using background incidence and mortality rates from 2008−2012.
[d]Incidence estimates are presented here to facilitate comparison; they are derived in Section 4.1.1.3.
[e]All with cumulative exposure as the exposure variable, except where noted, and with a 15-yr lag.
[f]Using regression coefficient from low-exposure segment of two-piece linear or log-linear spline model with knot at 1,600 ppm × days (see text and Appendix D). Each of the EC_{01} values is at or below the value of 0.021 ppm roughly corresponding to the knot of 1,600 ppm × days [(1,600 ppm × days) × (10 m^3/20 m^3) × (240 d/365 d)/(365 d/yr × 70 yr) = 0.021 ppm] and, thus, appropriately in the range of the low-exposure segment.
[g]To obtain unit risk estimates less than 1, convert to risk per ppb (e.g., 1.99 per ppm = 1.99×10^{-3} per ppb).
[h]Using regression coefficient from low-exposure segment of two-piece linear or log-linear spline model with knot at 100 ppm × days (see text and Appendix D). Each of the EC_{01} values is below the value of 0.0013 ppm roughly corresponding to the knot of 100 ppm × days [(100 ppm × days) × (10 m^3/20 m^3) × (240 d/365 d) / (365 d/yr × 70 yr) = 0.0013 ppm] and, thus, appropriately in the range of the low-exposure segment.
[i]This unit risk estimate is not considered a good estimate of risks from (low) environmental exposure levels (see text).
[k]Estimated exposure levels were so low (<3. 6×10^{-4} ppm) that the cumulative exposures in some of the age intervals in the lifetable analysis were <1 ppm × day, resulting in ln cumulative exposures of <0 for those intervals and unreliable results for this model. 3. 6×10^{-4} ppm would yield a unit risk estimate of about 28 per ppm, so that provides a lower bound on the unit risk estimate for this model.
[m]Profile likelihood confidence bounds were not calculated for this model.
[n]Regression coefficient derived from linear regression of categorical Cox regression results from Table 4-2, dropping the highest exposure category, as described in Section 4.1.1.2. Each of the EC_{01} values is appropriately below the value of 0.090 ppm roughly corresponding to the value of about 7,000 ppm × days (see footnote f for calculation) above which the linear regression model of the categorical results does not apply (see Figure 4-1).
Results from the selected model appear in bold.

As discussed above, risk estimates based on the all lymphohematopoietic cancer results are also derived for comparison. The same methodology presented above for the lymphoid cancer results was used for the all lymphohematopoietic cancer risk estimates, except that because the risk estimates for lymphoid cancers are the preferred estimates and the estimates for all lymphohematopoietic cancers are

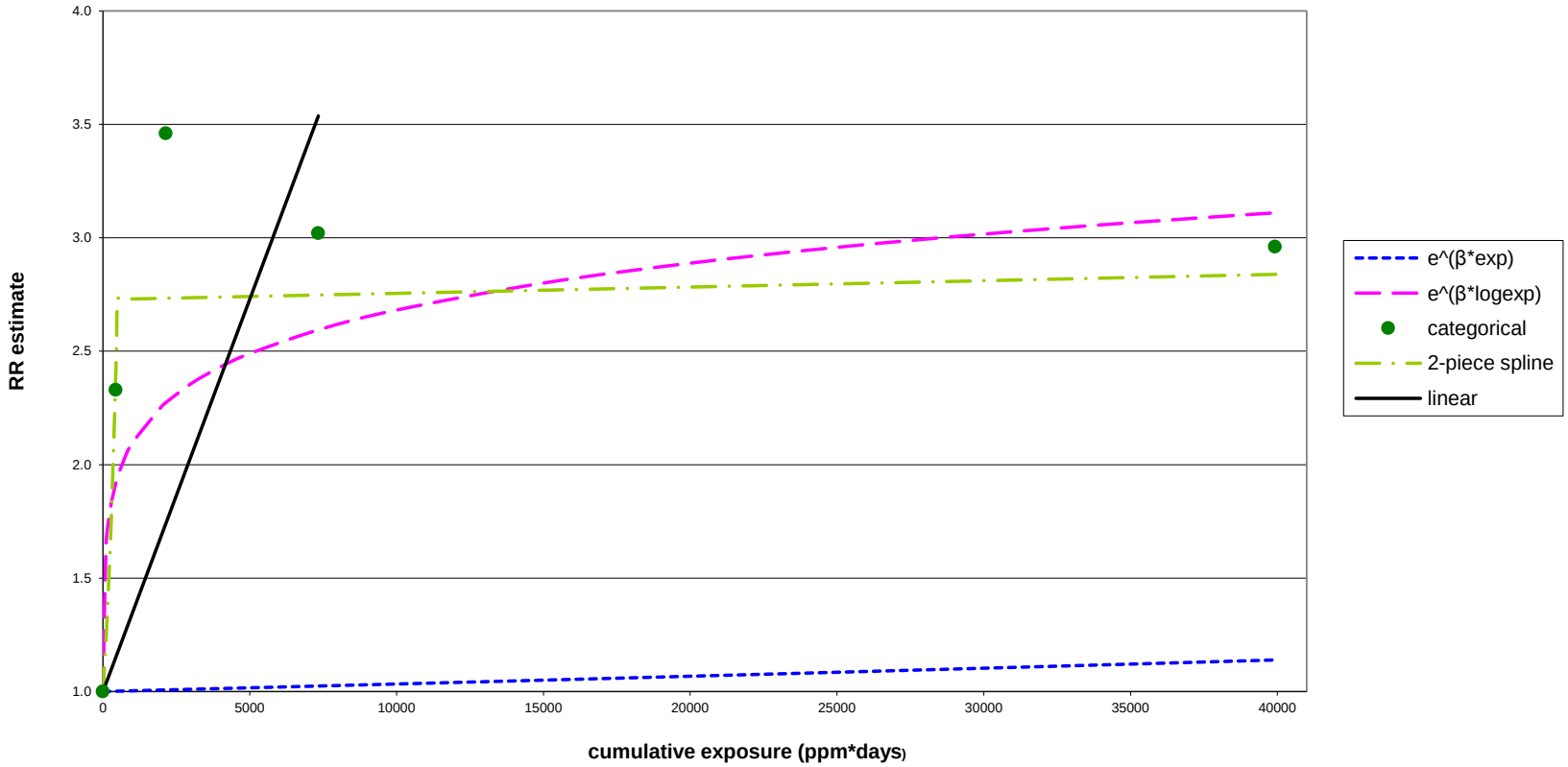
presented merely for comparison, the background mortality rates used to calculate the estimates for all lymphohematopoietic cancer were not updated in the current assessment and no additional exposure-response models were investigated. U.S. age-specific all-cause mortality rates for 2004 for both sexes of all race groups combined obtained from NCHS (Arias, 2007) were used to specify the all-cause background mortality rates in the actuarial program, and age-specific background mortality rates for all subcategories of lymphohematopoietic cancer (ICD-10 C81-C96) for the year 2004 were obtained from the NCHS Data Warehouse website (http://www.cdc.gov/nchs/datawh/statab/unpubd/mortabs.htm).

The results of Dr. Steenland's reanalyses using the Cox regression models presented in the Steenland et al. (2004) paper with data for males and females combined are presented in Table 4-2. As for lymphoid cancer and for all hematopoietic cancer in males presented in the Steenland et al. (2004) paper, the only statistically significant Cox regression model was for log cumulative exposure with a 15-year lag ($p = 0.01$). The cumulative exposure model did not provide an adequate fit to the data and is not considered further here ($p = 0.35$).

Because of the problems with the supralinear log cumulative exposure model which are discussed for the lymphoid cancers above, EPA again investigated the use of a two-piece log-linear spline model to attempt to address the supralinearity of the data while avoiding the extreme low-exposure curvature obtained with the log cumulative exposure model. For the all lymphohematopoietic cancer mortality data, the range examined for knot selection was from 0 to 7,000 ppm × days, and the largest model likelihood was obtained with the knot at 500 ppm × days (see Figure D-19 of Appendix D). See Table 4-4 and Section D.4 of Appendix D for parameter estimates and fit statistics for the two-piece spline model.[29] The linear regression model of the categorical results was also used to derive a cancer unit risk estimate for this data set.

For the weighted linear regression, the results from the Cox regression model with categorical cumulative exposure and a 15-year lag (see Table 4-2), excluding the highest exposure group, and the approach discussed above for lymphoid cancer mortality were used. See Table 4-3 for the results obtained from the weighted linear regression and Figure 4-3 for a graphical presentation of the resulting linear regression model.

[29]When the 2014 draft assessment was largely complete (U.S. EPA, 2014a,b), a few linear RR models were attempted, using the then just-published approach of Langholz and Richardson (2010) to model the individual data with cumulative exposure as a continuous variable; however, these linear models did not alleviate the problems of the corresponding log-linear RR models (see Figure D-21 in Appendix D) and have not been pursued further for the all lymphohematopoietic cancer data.



e^(β*exp): RR = $e^{(\beta \times \text{exposure})}$; e^(β*logexp): RR = $e^{(\beta \times \ln(\text{exposure}))}$; categorical: RR = $e^{(\beta \times \text{exposure})}$ with categorical exposures, plotted at the mean cumulative exposure; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); Two-piece spline: Two-piece log-linear spline model with knot at 500 ppm × days (see text). (Note that, with the exception of the categorical results and the linear regression of the categorical results, the different models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Source: Steenland reanalyses for males and females combined; see Appendix D (except for linear regression of the categorical results, which was done by EPA).

Figure 4-3. RR estimate for all lymphohematopoietic cancer vs. occupational cumulative exposure (with 15-year lag).

The EC_{01}, LEC_{01}, and inhalation unit risk estimates calculated for all lymphohematopoietic cancer mortality from the various models examined are presented in Table 4-8 (the incidence results also presented in Table 4-8 are discussed in Section 4.1.1.3 below). The unit risk estimate for all lymphohematopoietic cancer mortality based on the linear regression of the categorical results for both sexes using cumulative exposure with a 15-year lag is 0.680 per ppm and that based on the log-linear spline model (knot at 500 ppm × days) is 4.33 per ppm. For comparing with the lymphoid cancer estimates, the most comparable model is the linear regression of categorical results—the unit risk estimate for all lymphohematopoietic cancer mortality from the linear regression model of the categorical results is 85% higher than the unit risk estimate for lymphoid cancer mortality from the linear regression model (see Table 4-7). The unit risk estimate for all lymphohematopoietic cancer mortality from the log-linear spline model (knot at 500 ppm × days) is 120% higher than (i.e., 2.2 times) the unit risk estimate for lymphoid cancer mortality from the selected linear spline model (knot at 1,600 ppm × days) (see Table 4-7).

Table 4-8. EC_{01}, LEC_{01}, and unit risk estimates for all lymphohematopoietic cancer[a]

Model[d]	Mortality[b]			Incidence[b,c]		
	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk (per ppm)
Log cumulative exposure, 15-yr lag	1.40×10^{-3}	--[e]	--[e]	--[e]	--[e]	--[e]
Low-exposure log-linear spline;[f] cumulative exposure, 15-yr lag; knot at 500 ppm × days	3.77×10^{-3}	2.31×10^{-3}	4.33[g]	2.16×10^{-3}	1.32×10^{-3}	7.58[g]
Linear regression of categorical results, cumulative exposure, 15-yr lag[h]	0.0283	0.0147	0.680	0.0144	7.46×10^{-3}	1.34[g]

[a]From lifetime continuous exposure. Unit risk = $0.01/LEC_{01}$.
[b]Using background incidence and mortality rates from 2004.
[c]Incidence estimates presented here to facilitate comparison; they are derived in Section 4.1.1.3.
[d]From Dr. Steenland's analyses for males and females combined (see Appendix D), log-linear Cox regression models.
[e]Estimated exposure levels were so low ($<3.6 \times 10^{-4}$ ppm) that the cumulative exposures in some of the age intervals in the lifetable analysis were <1 ppm × day, resulting in ln cumulative exposures of <0 for those intervals and unreliable results for this model. 3.6×10^{-4} ppm would yield a unit risk estimate of about 28 per ppm, so that provides a lower bound on the unit risk estimate for this model.
[f]Using regression coefficient from low-exposure segment of two-piece log-linear spline model with knot at 500 ppm × days; see text and Appendix D. Each of the EC_{01} values is below the value of 0.0064 ppm roughly corresponding to the knot of 500 ppm × days [(500 ppm × days) × ($10\ m^3/20\ m^3$) × (240 d/365 d)/(365 d/yr × 70 yr) = 0.0064 ppm] and, thus, appropriately in the range of the low-exposure segment.
[g]For unit risk estimates below 1, convert to risk per ppb (e.g., 1.34 per ppm = 1.34×10^{-3} per ppb).
[h]Regression coefficient derived from linear regression of categorical Cox regression results from Table 4-2, as described in Section 4.1.1.2. Each of the EC_{01} values is appropriately below the value of 0.064 ppm roughly corresponding to the value of about 5,000 ppm × days (see footnote d for calculation) above which the linear regression model of the categorical results does not apply (see Figure 4-3).

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

EPA cancer risk estimates are typically derived to represent an upper bound on increased risk of cancer *incidence*, as from laboratory animal incidence data. Cancer data from epidemiologic studies are commonly mortality data, as is the case in the Steenland et al. (2004) study. For tumor sites with low survival rates, mortality-based estimates are reasonable approximations of

cancer incidence risk; however, for many lymphohematopoietic cancers, the survival rate is substantial, and incidence risk estimates are preferred by EPA (U.S. EPA, 2005a).

Therefore, another calculation was done using the same regression coefficients presented above (see Section 4.1.1.2), but with age-specific lymphoid cancer incidence rates for the relevant subcategories of lymphohematopoietic cancer (NHL, myeloma, and lymphocytic leukemia) for 2008−2012 from SEER [Howlader et al. (2014); Tables 18.7, 19.7, 13.12: both sexes, all races] in place of the lymphoid cancer mortality rates in the life-table analysis. SEER collects good-quality cancer incidence data from a variety of geographical areas in the United States. The incidence data used here are from "SEER 18," a registry of eighteen states, regions, and cities covering about 28% of the U.S. population.

The incidence risk calculation assumes that (1) lymphoid cancer incidence and mortality have the same exposure-response relationship for the relative rate of effect from EtO exposure and that (2) the incidence data are for first occurrences of primary lymphoid cancer or that relapses and secondary lymphoid cancers provide a negligible contribution. The latter assumption is probably sound; the former assumption is potentially more problematic. Because various lymphoid cancer subtypes with different survival rates are included in the categorization of lymphoid cancers, if the EtO-associated relative rates of the subtypes differ, a bias could occur, resulting in either an underestimation or overestimation of the extra risk for lymphoid cancer incidence.[30] Potential concern that the incidence risk estimates might be overestimated would come primarily from the inclusion of multiple myeloma, because that subtype has the lowest incidence:mortality ratios (and, thus, if that subtype were driving the increased mortality observed for the lymphoid cancer grouping, then including the incidence rates for the other subtypes, which have higher incidence:mortality ratios, might inflate the incidence risk estimates). Multiple myelomas, however, constitute only 25% of the lymphoid cancer cases in the cohort, and there is no evidence that multiple myeloma is driving the EtO-induced excess in lymphoid cancer mortality.[31] Thus, using the total lymphoid cancer incidence rates is not expected to result in an overestimation of the incidence risk estimates; if anything, the incidence risks would likely be diluted with the inclusion of the multiple myeloma rates. The incidence risk calculation also relies on the fact that the lymphoid

[30]Sielken and Valdez-Flores (2009) 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.2.20 for a more detailed discussion of this issue.

[31]According to data from SEER (www.seer.cancer.gov), 25% is below the proportion of multiple myeloma deaths one would expect based on age-adjusted U.S. background mortality rates of multiple myeloma, NHL, and chronic lymphocytic leukemia, and these three subtypes have the same pattern for mortality rates increasing as a function of age mostly above age 50, so the comparison with lifetime background rates is reasonable. In addition, the low proportion of multiple myeloma deaths in the lymphoid cancer subgrouping cannot be attributed to an underrepresentation of blacks, who have incidence rates of multiple myeloma more than twice those of whites (http://seer.cancer.gov/statfacts/html/mulmy.html), in the cohort because blacks comprise 16% of the cohort versus 12.3% in the U.S. population.

cancer incidence rates (more specifically, the differential rates obtained by subtracting the mortality rates from the incidence rates) are small when compared with the all-cause mortality rates.[32]

The resulting EC_{01} and LEC_{01} estimates for lymphoid cancer incidence from the various models examined are presented in Table 4-7. The unit risk estimate for lymphoid cancer incidence from the selected two-piece linear spline model with the knot at 1,600 ppm × days is 5.26 per ppm. This unit risk estimate is about 3-times the unit risk estimate from the most similar alternative model, the two-piece log-linear spline model with the knot at 1,600 ppm × days and about 5.4-times the estimate from the linear regression of categorical results. EC_{01}, LEC_{01}, and unit risk estimates from the other models considered are presented for comparison only, to illustrate the differences in model behavior at the low end of the exposure-response range. As discussed above, these models were deemed unsuitable for the derivation of risks from (low) environmental exposure levels. The highly supralinear two-piece linear and log-linear spline models with the knot at 100 ppm × days yield unit risk estimates about 20- and 7-times the preferred estimate, respectively. On the other hand, the linear model and the more gradually supralinear log-linear model with a square-root transformation, neither of which provide a statistically significant fit to the overall data, yield unit risk estimates 1.6% and 3.7% of the preferred estimate, respectively.

Overall, as discussed above, the preferred estimate for the unit risk for lymphoid cancer is the estimate of **5.26 per ppm** (2.87×10^{-3} per $\mu g/m^3$) derived, using incidence rates for the cause-specific background rates, from the two-piece linear spline model with the knot at 1,600 ppm × days. This incidence unit risk estimate is about 2.6-times the mortality-based estimate from the same model.

Sensitivity analyses were conducted to investigate the influence of lag period, knot selection, and upper-bound estimation approach on the unit risk estimates from the selected two-piece linear spline model. The sensitivity analyses are detailed in Sections D.3.5, D.3.6, and D.3.8 of Appendix D. In brief, for the two-piece linear spline model with the knot at 1,600 ppm × days, the unit risk estimates for different lag periods (0, 5, 10, and 20 years) ranged from about 48% less than (10-year lag) to about 190% greater than (i.e., 2.9-times) (no lag) the estimate for the selected model (15-year lag). Varying the knot by 1,000 ppm × days, especially in the lower direction, also changes the unit risk estimate a notable amount—the unit risk estimate with the knot at 600 ppm × days was about 210% greater than (i.e., 3.1-times), and unit risk estimate with the knot at 2,600 ppm × days was about 46% less than, the

[32] Sielken and Valdez-Flores (2009) 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.2.20, EPA disagrees. 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 (2009) 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., those "surviving" each interval without a diagnosis of breast cancer (see Section 4.1.2.3). See Appendix A.2.20 for a more detailed discussion of this issue.

unit risk estimate for the selected model (with the knot at 1,600 ppm × days). The unit risk estimate calculated using a Wald approach was 40% lower than the preferred estimate, which relied on a profile likelihood approach to estimate the upper bound on the regression coefficient.

As discussed in Section 4.1.1.2, risk estimates based on the results of Dr. Steenland's reanalyses of the all lymphohematopoietic cancer data (see Appendix D and Table 4-2) are also derived for comparison. The same methodology presented above for the lymphoid cancer incidence results was used for the all lymphohematopoietic cancer incidence risk estimates, and the same assumptions apply. Age-specific SEER incidence rates for all lymphohematopoietic cancer for the years 2000−2004 were used (Ries et al., 2007); Tables XIX, IX, XVIII, and XIII: both sexes, all races). The EC_{01}, LEC_{01}, and unit risk estimates for all lymphohematopoietic cancer incidence from the different all lymphohematopoietic cancer mortality models examined are presented in Table 4-8. The unit risk estimate for all lymphohematopoietic cancer incidence based on the linear regression of the categorical results for both sexes using cumulative exposure with a 15-year lag is 1.34 per ppm and that based on the log-linear spline model (knot at 500 ppm × days) is 7.58 per ppm. For comparing with the lymphoid cancer estimates, the most comparable model is the linear regression of categorical results—the unit risk estimate for all lymphohematopoietic cancer incidence from the linear regression model of the categorical results is 38% higher than the unit risk estimate for lymphoid cancer incidence from the linear regression model (see Table 4-7). The unit risk estimate for all lymphohematopoietic cancer incidence from the log-linear spline model (knot at 500 ppm × days) is 44% higher than the unit risk estimate for lymphoid cancer incidence from the selected linear spline model (knot at 1,600 ppm × days) (see Table 4-7).

4.1.2. Risk Estimates for Breast Cancer

4.1.2.1. *Breast Cancer Results From the NIOSH Study*

The Steenland et al. (2004) study discussed above in Section 4.1.1.1 also presents results from exposure-response analyses for breast cancer mortality in female workers. Steenland et al. (2003) present results of a breast cancer incidence study of a subcohort of the female workers from the NIOSH cohort. In addition to the analyses presented in the Steenland et al. (2003) and Steenland et al. (2004) papers, Dr. Steenland did subsequent analyses of the breast cancer mortality data set for EPA and Drs. Steenland and Deddens did additional analyses of the breast cancer incidence data set; these are discussed below and reported in Sections D.1 and D.2 of Appendix D, respectively.

Unit risk estimates are developed for both breast cancer mortality (Section 4.1.2.2) and breast cancer incidence (Section 4.1.2.3). The incidence estimates are strongly preferred over the mortality estimates for a number of reasons. First, unit risk estimates are intended to reflect cancer incidence rather than mortality; thus, incidence estimates are generally preferred over mortality estimates. Second, in the case of these specific estimates, the incidence estimates based on the subcohort of workers with

interviews are preferred because they are based on a larger number of cases than the mortality estimates and data on potential breast cancer risk factors are taken into account. Section 4.1.2.2 containing the derivation of the mortality estimates has been retained in this final assessment for completeness and for comparison with the incidence estimates; however, because of the preference for the incidence data, no further modeling of the mortality data was conducted after the 2014 external review draft, thus no additional models of the continuous exposure data were explored.

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

Results from the Cox regression models presented by Steenland et al. (2004), with some reanalyses reported by Dr. Steenland in Appendix D (see Section D.2), are summarized in Table 4-9. These models were considered for the derivation of unit risk estimates for breast cancer mortality in females from continuous environmental exposure to EtO, applying the methodologies described in Section 4.1.1.2.

Table 4-9. Cox regression results for breast cancer mortality in females in the NIOSH cohort,[a] for models presented in Steenland et al. (2004)

Exposure variable[b]	*p*-value[c]	Coefficient (SE) (per ppm × day)	ORs by category[d] (95% CI)
Cumulative exposure, 20-yr lag[e]	0.10	0.00001.22 × 10^{-5} (6.41 × 10^{-6})	
Log cumulative exposure, 20-yr lag[f]	0.02	0.084 (0.035)	
Categorical cumulative exposure, 20-yr lag[f]	0.09		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 breast cancer (ICD-9 174,175) deaths.
[b]Cumulative exposure is in ppm × days.
[c]*p*-values based on likelihood ratio test.
[d]Exposure categories are 0; >0−646; 647—2,779; 2,780−12,321; ≥12,322 ppm × days.
[e]From reanalyses in Section D.2 of Appendix D; Steenland et al. (2004) reported the Cox regression results for cumulative exposure with no lag.
[f]From Table 8 of Steenland et al. (2004).

U.S. age-specific all-cause mortality rates for 2000 for females of all race groups combined (Miniño et al., 2002) were used to specify the all-cause background mortality rates in the actuarial program (life-table analysis). The National Center for Health Statistics 1997−2001 cause-specific background mortality rates for invasive breast cancers in females were obtained from a SEER report (Ries et al., 2004). 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). 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 (i.e., small changes in exposure correspond to large changes in risk; see Figure 4-4). 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; see Section D.2 of Appendix D) in terms of the global fit to the data; however, the Cox regression model with cumulative exposure is sublinear, does not reflect the apparent supralinearity of the breast cancer mortality data, and provides a poor local fit to the lower-exposure region (see Figure 4-4).

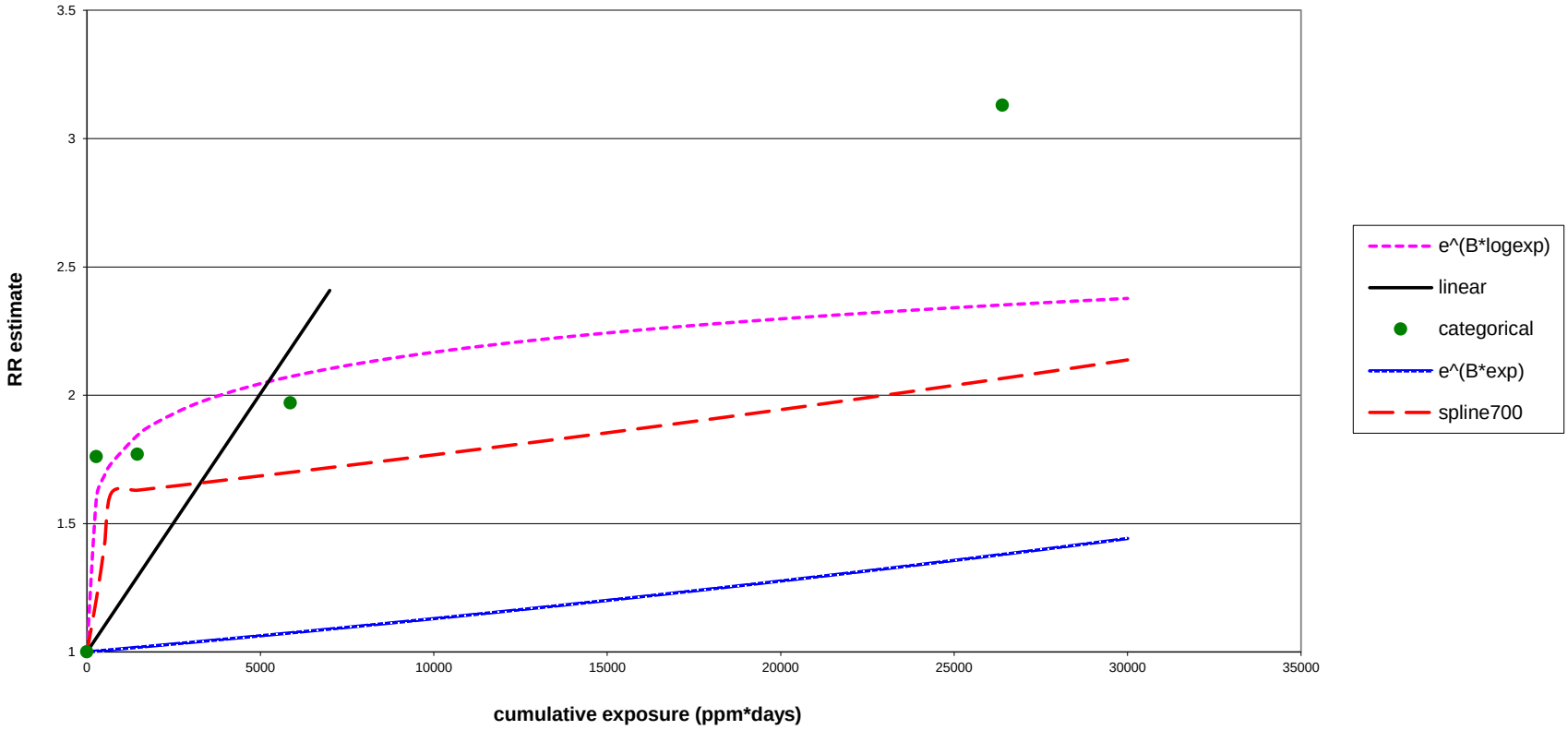
In a 2006 external review draft of this assessment (U.S. EPA, 2006a), which relied on the original published results of Steenland et al. (2004), EPA proposed that the best way to reflect the exposure-response relationship in the lower exposure region, which is the region of interest for low-exposure extrapolation, was to do a weighted linear regression of the results from the Cox regression model with categorical cumulative exposure and a 20-year lag. In addition, the highest exposure group was not included in the regression to alleviate some of the "plateauing" in the exposure-response relationship at higher exposure levels and to provide a better fit to the lower exposure data. Linear modeling of categorical epidemiologic data and elimination of the highest exposure group(s) in certain circumstances to obtain a better fit of low-exposure data are both standard techniques used in EPA dose-response assessments (U.S. EPA, 2005a).

For the weighted linear regression, the results from the Cox regression model with categorical cumulative exposure (and a 20-year lag) presented in Table 4-9 were used, excluding the highest exposure group, and the approach discussed above for the lymphoid cancers (see Section 4.1.1.2).[33] Mean and median exposures for the cumulative exposure groups were provided by Dr. Steenland (see Appendix D).[34] See Table 4-10 for the results obtained from the weighted linear regression of the categorical results and mean exposures and Figure 4-4 for a depiction of the resulting linear regression model.

However, as in Section 4.1.1.2 for the similarly supralinear lymphohematopoietic cancer data, for the subsequent draft assessment, EPA pursued modeling the individual exposure data as an alternative to modeling the published grouped data (U.S. EPA, 2014a, b). Consequently, it was determined that, using the full data set, an alternative way to address the supralinearity of the data (while avoiding the extreme low-exposure curvature obtained with the log cumulative exposure model) might

[33]Equations for this weighted linear regression approach are presented in Rothman (1986) and summarized in Appendix F.
[34]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.

be to use a two-piece spline model, and Dr. Steenland was commissioned to do the spline analyses using the full data set with cumulative exposure as a continuous variable. His findings are reported in Section D.2 of Appendix D, and the results for the breast cancer mortality analyses are summarized below. (For this final assessment, the model selection for breast cancer mortality was reconsidered in light of the objectives used for model selection for lymphoid cancer [Section 4.1.1.2] and breast cancer incidence [Section 4.1.2.3]; however, because the breast cancer mortality results are presented solely for comparison with the preferred incidence results, no additional modeling of the breast cancer mortality data was pursued and the unit risk estimates presented below were not updated to reflect more recent background mortality rates.)



e^(B*exp): Cox regression results for RR = $e^{(\beta \times \text{exposure})}$; e^(B*logexp): Cox regression results for RR = $e^{(\beta \times \ln(\text{exposure}))}$; categorical: Cox regression results for RR = $e^{(\beta \times \text{exposure})}$ with categorical exposures, plotted at the mean cumulative exposure; linear: weighted linear regression of categorical results, excluding highest exposure group (see text); spline700: Two-piece log-linear spline model with knot at 700 ppm × days (see text). (Note that, with the exception of the categorical results and the linear regression of the categorical results, the different models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Source: Steenland reanalyses with 20-year lag; see Section D.2 of Appendix D (except for linear regression of the categorical results, which was done by EPA).

Figure 4-4. RR estimate for breast cancer mortality vs. occupational cumulative exposure (with 20-year lag).

For the two-piece log-linear spline modeling approach, as described in Section 4.1.1.2 and discussed more fully in Section D.2 of Appendix D, the Cox regression model was the underlying basis for the splines which were fit to the breast cancer mortality exposure-response data (cumulative exposure, with a 20-year lag), and thus, log RR is a function of two lines that join at a single point of inflection, called a "knot." The shape of the two-piece log-linear spline model, in particular the slope in the low-exposure region, depends on the location of the knot. Knot selection was made by trying different knots over a reasonable range and choosing the one that resulted in the largest model likelihood. For the breast cancer mortality data, the range examined for knot selection was from 0 to 25,000 ppm × days, using increments of 100 ppm × days to 7,000 ppm × days and increments of 1,000 ppm × days above 7,000 ppm × days. The largest model likelihood was observed with the knot at 700 ppm × days, although, as noted above, the model likelihood did not change much across the various trial knots (see Figure D-9 of Appendix D). Parameter estimates for this model are presented in Table 4-10. The *p*-value of the two-piece spline model exceeded 0.05, although minimally ($p = 0.067$). This two-piece spline model was selected as the preferred model for breast cancer mortality primarily because it uses the individual-level exposure data and this model form is more tuned to local behavior than the other model forms considered.

The two-piece spline model with the knot at 700 ppm × days and the actuarial program (life-table analysis) were used to estimate the exposure level (EC_x) and the associated 95% lower confidence limit (LEC_x) corresponding to an extra risk of 1% ($x = 0.01$). As discussed in Section 4.1.1.2, a 1% extra risk level is a more reasonable response level for defining the POD for these epidemiologic data than 10%.

Table 4-10. Exposure-response modeling results for breast cancer mortality in females in the NIOSH cohort for models not presented by Steenland et al. (2004)

Model[a]	*p*-value[b]	Coefficient (SE) (per ppm × day)
Two-piece log-linear spline with maximum likelihood (knot at 700 ppm × days)	0.067	low-exposure spline segment: B1 = 6.88×10^{-4} (4.17×10^{-4})
Linear regression of categorical results, excluding the highest exposure quartile	0.09	2.01×10^{-4} (1.20×10^{-4})

[a]All with cumulative exposure in ppm × days as the exposure variable and with a 20-yr lag; based on 103 breast cancer deaths.
[b]*p*-values from likelihood ratio test, except for linear regression of categorical results, where Wald *p*-value is reported.

Source: Additional analyses performed by Dr. Steenland (see Section D.2 of Appendix D), except for the linear regression of the categorical results, which was performed by EPA.

Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3), which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was performed. (Linear low-exposure extrapolation is also the default approach used in the absence of sufficient evidence for a nonlinear mode of action, which is also the case for EtO [Section 3.4].) The EC_{01}, LEC_{01}, and inhalation unit risk estimates calculated for breast cancer mortality from the two-piece spline model with the knot at 700 ppm × days are presented in Table 4-11, along with estimates from some of the other models for comparison. The resulting unit risk estimate for breast cancer mortality based on the two-piece spline model with the knot at 700 ppm × days using cumulative exposure with a 20-year lag is 2.12 per ppm. This unit risk estimate is about 4-times the unit risk estimate from the linear regression of the categorical results. The standard Cox regression cumulative exposure model, with its extreme sublinearity in the lower exposure region, yields a substantially lower unit risk estimate (<2% of that from the selected model), while the log cumulative exposure Cox regression model, with its extreme supralinearity and steep slope in the lower exposure region, would have resulted in a much higher unit risk estimate, had it been possible to reliably calculate one. Converting the units, the unit risk estimate of 2.12 per ppm for breast cancer mortality from the two-piece spline model with the knot at 700 ppm × days corresponds to a unit risk estimate of 1.16×10^{-3} per $\mu g/m^3$.

This unit risk estimate for breast cancer mortality is slightly higher than the unit risk estimate derived for breast cancer incidence below (Section 4.1.2.3), which is contrary to expectations. Confidence is higher in the breast cancer incidence estimate, suggesting that this mortality estimate may be an overestimate of the unit risk for breast cancer mortality.

Table 4-11. 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-yr lag[b]	1.12×10^{-3}	--[c]	--[c]
Cumulative exposure, 20-yr lag[d]	0.530	0.285	0.0351[e]
Low-exposure log-linear spline, cumulative exposure with knot at 700 ppm × days, 20-yr lag[f]	9.41×10^{-3}	4.71×10^{-3}	**2.12**
Categorical; cumulative exposure, 20-yr 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]Estimated exposure levels were so low (<3.6×10^{-4} ppm) that the cumulative exposures in some of the age intervals in the lifetable analysis were <1 ppm × day, resulting in ln cumulative exposures of <0 for those intervals and unreliable results for this model. 3.6×10^{-4} ppm would yield a unit risk estimate of about 28 per ppm, so that provides a lower bound on the unit risk estimate for this model.
[d]From Dr. Steenland's reanalyses (see Table D-22 of Appendix D), Cox regression model.
[e]This unit risk estimate is not considered a good estimate of risks from (low) environmental exposure levels (see text).
[f]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 D-21 of Appendix D. The EC_{01} value is below the value of 0.009 ppm roughly corresponding to the knot of 700 ppm × days [(700 ppm × days) × (10 m^3/20 m^3) × (240 d/365 d)/(365 d/yr × 70 yr) = 0.0013 ppm] and, thus, appropriately 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. The EC_{01} value is appropriately below the value of 0.064 ppm roughly corresponding to the value of about 5,000 ppm × days (see footnote f for calculation) above which the linear regression model of the categorical results does not apply (see Figure 4-4).

4.1.2.3. *Prediction of Lifetime Extra Risk of Breast Cancer Incidence*

As discussed in Section 4.1.1.3, risk estimates for cancer incidence are preferred to estimates for cancer mortality, especially for cancer types with good survival rates, such as breast cancer. In the case of female breast cancer in the NIOSH cohort, there is a corresponding incidence study (Steenland et al., 2003) with exposure-response results for breast cancer incidence, so one can estimate cancer incidence risks directly rather than estimate them from mortality data. The incidence study used a (sub)cohort of 7,576 (76%) of the female workers from the original cohort. Cohort eligibility for the incidence study was restricted to the female workers who had been employed at 1 of the 13 plants with exposure estimates for at least 1 year, owing to cost considerations and the greater difficulties in locating workers with short-term employment. Interviews were sought from all the women in the incidence study cohort or their next-of-kin (18% of the cohort had died). Completed interviews were obtained for 5,139 (68%) of the 7,576 women in the cohort. The investigators also attempted to acquire breast cancer incidence

data for the cohort 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 cohort (n = 7,576) and the subcohort of women with interviews (n = 5,139). For additional details and discussion of the Steenland et al. (2003) study, see Section A.2.16 of 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 of the breast cancers 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 (Steenland et al., 2003). 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 (Steenland et al., 2003). Results of the Cox regression analyses for the cumulative and log cumulative exposure models, with 15-year lags, are shown in Table 4-12. Cumulative exposure is the preferred basis for cancer unit risk estimates. 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 the duration models are only marginally better than those with cumulative exposure. In addition, cumulative exposure with no lag was considered less biologically realistic than cumulative exposure with the 15-year lag because some lag period would be expected for the development of breast cancer. For this final assessment, EPA revisited the issue of lag selection for the breast cancer incidence data. After considering model fit for cumulative exposure with different lag periods across a larger number of models than was previously evaluated with different lags, EPA again selected 15 years as the lag period to use for the exposure-response analyses (see Section D.1.2 of Appendix D). Sensitivity of the results to choice of lag period is examined in Sections D.1.5 and D.1.6 of Appendix D and summarized at the end of this section (Section 4.1.2.3).

Table 4-12. Cox regression results for breast cancer incidence in females from the NIOSH cohort, for the models presented by Steenland et al. (2003)[a,b]

Cohort	Exposure variable[c]	Coefficient (SE) (per ppm × day), *p*-value[d]	ORs by category[e] (95% CI)
Full incidence study cohort *n* = 7,576 319 cases	Cumulative exposure, 15-yr lag	5.4×10^{-6} (3.5×10^{-6}), $p = 0.12$	
	Log cumulative exposure, 15-yr lag	0.037 (0.019), $p = 0.05$	
	Categorical cumulative exposure, 15-yr 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-yr lag	9.5×10^{-6} (4.1×10^{-6}), $p = 0.02$	
	Log cumulative exposure, 15-yr lag	0.050 (0.023), $p = 0.03$	
	Categorical cumulative exposure, 15-yr lag	--[f]	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 yr of birth (quartiles). Subcohort models include cumulative exposure, categorical variables for yr of birth (quartiles), breast cancer in first-degree relative, and parity.
[c]Cumulative exposure is in ppm × days.
[d]*p*-values for exposure variable from Wald test, as reported by Steenland et al. (2003).
[e]Exposure categories are 0, >0−647, 647−2,026, 2,026−4,919, 4,919−14,620, >14,620 ppm × days.
[f]*p*-value for the addition of the categorical exposure variables = 0.11 (email dated 5 March 2010 from Kyle Steenland, Emory University, to Jennifer Jinot, EPA).

Source: Tables 4 and 5 of Steenland et al. (2003).

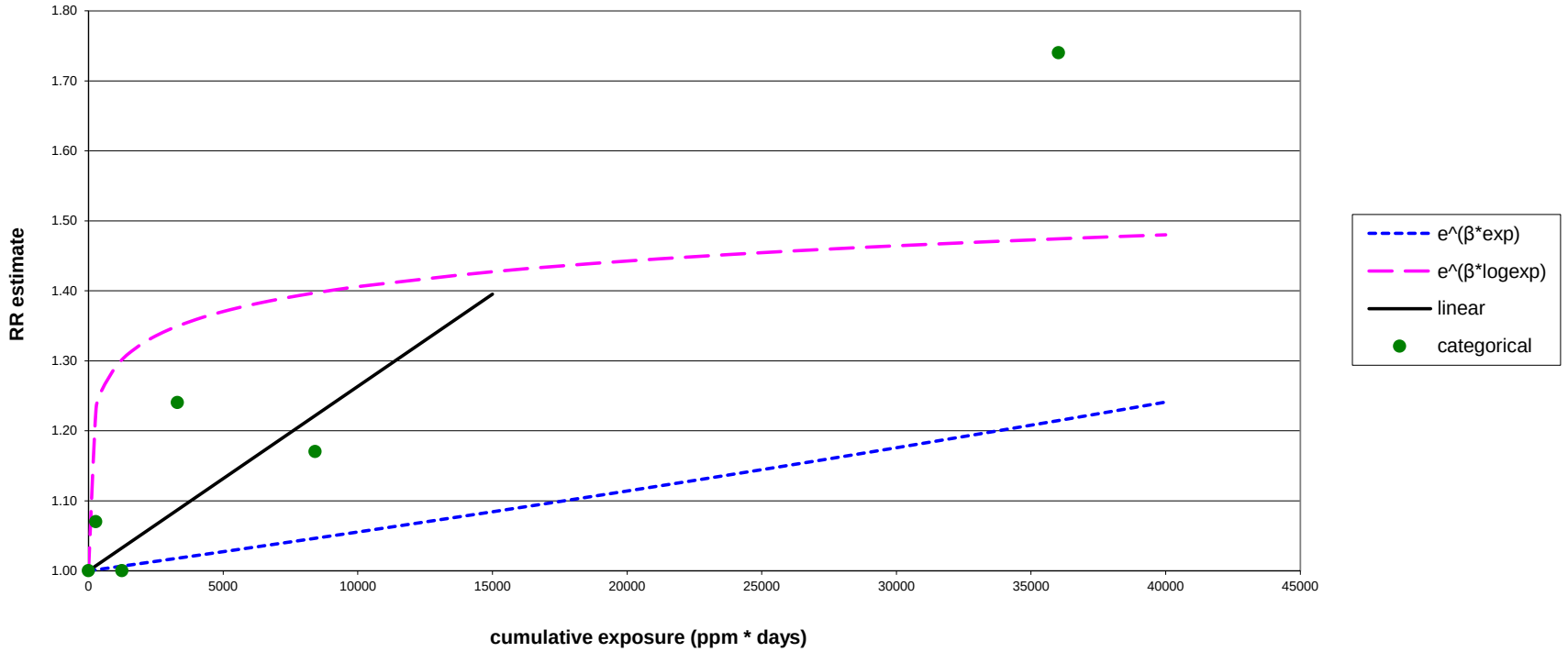
Although risk estimates based on the full cohort results are calculated for comparison, the preferred estimates are those based on the subcohort with interviews because the subcohort should have more complete case ascertainment and has additional information available on potential breast cancer confounders.

For the actuarial program (life-table analysis), U.S. age-specific all-cause mortality rates for 2008−2012 for females of all race groups combined (CDC, 2015) were used to specify the all-cause background mortality rates. Because breast cancer incidence rates (more specifically, the differential rates obtained by subtracting the mortality rates from the incidence rates) are not negligible compared to all-cause mortality rates, the all-cause mortality rates in the life-table analysis (used to calculate the population at risk) were adjusted to reflect women dying *or* being diagnosed with breast cancer in a

given age interval. 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 2008−2012 mortality rates for invasive breast cancer (ICD-10 50) in females were obtained from a SEER report (Howlader et al., 2014). The SEER report also provided SEER-18 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 progress to invasive tumors. Thus, the primary risk calculations in this assessment use the sum of invasive and in situ breast cancer incidence rates for the cause-specific background rates. Comparison calculations were performed using just the invasive breast cancer incidence rates for the cause-specific rates; this issue is further discussed in Section 4.1.3 on sources of uncertainty. 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 in Section 4.1.1.2 above.

For breast cancer incidence in both the full cohort (see Figure 4-5) and the subcohort with interviews (see Figure 4-6), the low-exposure categorical results suggest a more linear low-exposure exposure-response relationship than that obtained with either the continuous variable log cumulative exposure (supralinear) or cumulative exposure (sublinear) Cox regression models. Thus, as with the lymphohematopoietic cancer and the breast cancer mortality results above, EPA proposed in the 2006 Draft Assessment (U.S. EPA, 2006a), which relied on the original published results of Steenland et al. (2003), that the best way to reflect the data in the lower exposure region, which is the region of interest for low-exposure extrapolation, was to do a weighted linear regression of the results from the model with categorical cumulative exposure (with a 15-year lag). In addition, the highest exposure group was not included in the regression to provide a better fit to the lower-exposure data (The RR estimates for the highest exposure quintiles suggest somewhat supralinear exposure-response relationships for both the full cohort and the subcohort with interviews, and supralinearity is evidenced in the subcohort with interviews by the strong influence of the top 5% of cumulative exposures on dampening the slope of the [cumulative exposure] Cox regression model [see Section D.1 and Figure D-4 of Appendix D]. Moreover, there is more uncertainty in using the mean cumulative exposure to represent the range of exposures in a highest exposure categorical group because such groups contain a wider range of exposures; for example, for the subcohort with interviews, the highest exposure quintile contains exposures ranging from about 14,500 ppm × days to over 250,000 ppm × days). Linear modeling of categorical (i.e., grouped) epidemiologic data and elimination of the highest exposure group(s) under certain circumstances to obtain a better fit of low-exposure data are both standard techniques used in EPA dose-response assessments (U.S. EPA, 2012, 2005a). However, as in Section 4.1.1.2 for the

lymphohematopoietic cancer data, for the subsequent draft assessment, EPA explored additional analyses using the individual data rather than relying on the published grouped data.



e^(β*exp): $RR = e^{(\beta \times exposure)}$; e^(β*logexp): $RR = e^{(\beta \times \ln(exposure))}$; categorical: $RR = e^{(\beta \times exposure)}$ with categorical exposures, plotted at the mean cumulative exposure; linear: weighted linear regression of categorical results, excluding highest exposure group (see text). (Note that, with the exception of the categorical results and the linear regression of the categorical results, the various models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Source: Steenland et al. (2003) (except for linear regression of the categorical results, which was done by EPA).

Figure 4-5. RR estimate for breast cancer incidence in full cohort vs. occupational cumulative exposure (with 15-year lag).

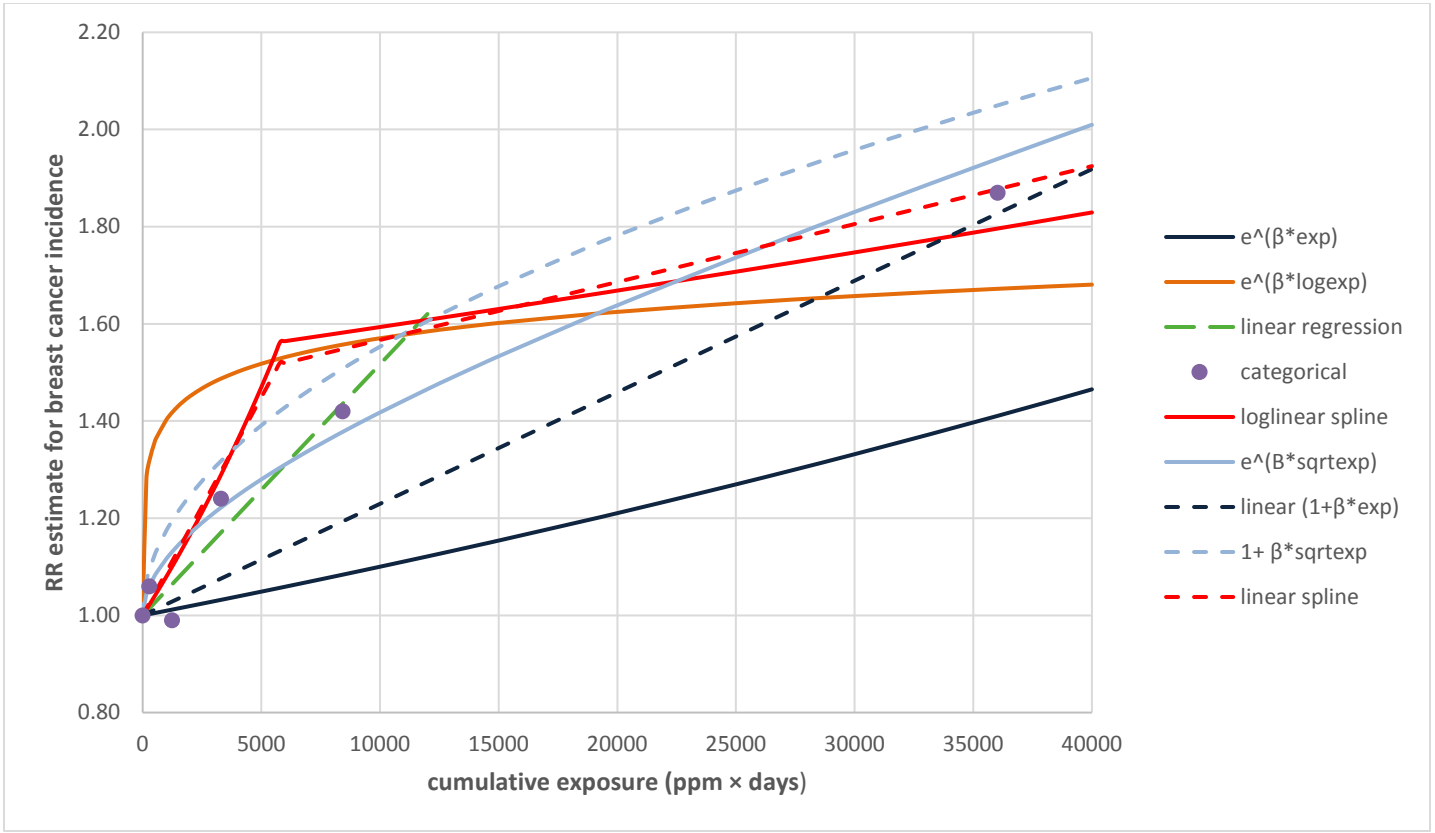


Figure 4-6. RR estimate for breast cancer incidence in subcohort with interviews vs. occupational cumulative exposure (with 15-year lag).

e^(β*exp): $RR = e^{(\beta \times exposure)}$; e^(β*logexp): $RR = e^{(\beta \times \ln(exposure))}$; categorical: $RR = e^{(\beta \times exposure)}$ with categorical exposures, plotted at the mean cumulative exposure; e^(β*sqrtexp): $RR = e^{(\beta \times sqrt(exposure))}$; linear regression: weighted linear regression of categorical results, excluding highest exposure group (see text); log-linear spline: 2-piece log-linear spline model, with knot at 5,800 ppm × days (see text); linear spline: 2-piece linear spline model, with knot at 5,750 ppm × days (see text); linear: $RR = 1 + \beta \times exposure$; 1 + β*sqrtexp: $RR = 1 + \beta \times sqrt(exposure)$. All models except for the categorical model and the linear regression of the categorical results treat exposure as a continuous variable. (Note that, with the exception of the categorical results and the linear regression of the categorical results, the various models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Sources: Steenland et al. (2003) except for 2-piece spline models (see Appendix D) and the linear regression of the categorical results, which was done by EPA.

Consequently, it was determined that using the individual data, a better way to address the apparent supralinearity of the data (while avoiding the extreme low-exposure curvature imposed by the log cumulative exposure Cox regression model) might be to use a two-piece spline model, and Dr. Steenland was commissioned to do the spline analyses. His findings are reported in Appendix D (see Section D.1), and the results for the breast cancer incidence analyses are summarized below. Note that, for the two-piece spline analyses, only the data from the subcohort with interviews and for the invasive and in situ breast cancers combined were analyzed, because this was the preferred data set, as discussed above. [Dr. Steenland also employed a cubic spline model as a semiparametric approach to visualize the underlying exposure-response relationship; however, this approach produces an overly complicated function for an empirical model, as opposed to a biologically based model, and was not used for risk assessment purposes. In addition, Dr. Steenland investigated the use of a Cox regression model with a square-root transformation of cumulative exposure; however, this approach, though less extreme than using the log transformation of cumulative exposure, also yields a notably supralinear model, which can result in unstable low-exposure risk estimates (i.e., small changes in exposure correspond to large changes in risk; see Figure 4-6). The model results for both the cubic spline and square-root transformation models are included in Appendix D, Section D.1. The cubic spline is not considered further here, and the square-root transformation model was not considered further in the 2014 draft assessment but was reexamined for the current assessment. EPA chose to pursue the development of two-piece spline models to attempt to avoid the problem of unstable risk estimates from supralinear curvature in the low-exposure region because these models provide a more general and systematic approach to modeling supralinear exposure-response data, as opposed to using random, arbitrary power-transformations of the exposure variable. The SAB panel that reviewed the 2014 draft assessment (U.S. EPA, 2014a, b) supported EPA's use of two-piece spline models, recommending prioritizing models that allow "more local fits in the low exposure range", such as spline models (SAB, 2015).]

For the two-piece log-linear spline modeling approach, as described in Section 4.1.1.2 and discussed more fully in Appendix D, the Cox regression model was the underlying basis for the splines which were fit to the breast cancer incidence exposure-response data (cumulative exposure is used here, with a 15-year lag), and, thus, log RR is a function of two lines which join at a single point of inflection, called a "knot." The shape of the two-piece spline model, in particular the slope in the low-exposure region, depends on the location of the knot. The knot was selected by evaluating different knots from 100 to 15,000 ppm × days in increments of 100 ppm × days and then choosing the one that resulted in the best (largest) model likelihood. The model likelihood did not actually change much across the different trial knots (see Figure D-1 of Appendix D), but it did change slightly, and a knot of 5,800 ppm × days was chosen for the breast cancer incidence data based on the largest likelihood. The two-piece log-linear spline model with this knot provided a statistically significant fit to the data

(p = 0.01 for the addition of the exposure terms; see Table D-8 in Appendix D), as well as a good visual fit (see Figure 4-6).

A two-piece linear spline model was also fitted, using the approach of Langholz and Richardson (2010), who describe methods for fitting nonlog-linear relative hazard models, or "excess relative risk (ERR)" models. This two-piece linear spline model is similar to the log-linear spline model discussed above; however, for the linear spline model, the underlying exposure-response function for the splines is a linear model (i.e., RR = (1 + β × exposure) × exp(Σ(β × covariates)), where β are the parameters being estimated, exposure is modeled linearly, and the nonexposure covariates are modeled multiplicatively). [For this final assessment, EPA conducted further analyses. The breast cancer incidence data, which contain protected personal information on the study participants and are not available to the public, were no longer available to Dr. Steenland, who is no longer at NIOSH. Thus, EPA arranged with NIOSH to undertake the new breast cancer incidence analyses, and revised and extended linear exposure-response analyses were conducted by Dr. James Deddens of NIOSH, who was also one of the coauthors of the Steenland et al. (Steenland and Deddens, 2004; Steenland et al., 2003) studies of the NIOSH cohort of EtO sterilizer workers. The details and comprehensive results of Dr. Deddens' analyses are summarized in Section D.1 of Appendix D.] In the case of the two-piece linear spline model, the knot was obtained by considering possible knots up to 10,000 ppm × days in increments of 500 ppm × days and then interpolating where appropriate.

A knot of 5,750 ppm × days yielded the largest likelihood (see Figure D-1 of Appendix D) for the two-piece linear spline model. The two-piece linear spline model with this knot provided a statistically significant fit to the data (p = 0.003; p = 0.014 for the addition of the exposure terms), as well as a good visual fit (see Figure 4-6). This model had essentially the same AIC as the log-linear spline model (1954.4 vs. 1954.5).[35] See Table 4-13 and Section D.1 of Appendix D for parameter estimates and fit statistics for the two spline models. Of the two spline models, the two-piece linear spline model was selected as the preferred model for the unit risk estimates for breast cancer incidence primarily because linearity is a desirable property to have in risk assessment models. For example, linear low-dose extrapolation can occur without a discontinuity between the model in the observable range and low-dose extrapolation from the point of departure, and the unit risk estimate is not dependent on the risk level chosen for determination of the point of departure, at least within the exposure range of the first spline segment for a spline model. In addition, with an overall exposure-response relationship that is supralinear, it seems contradictory to use sublinear model forms for the increments represented by the spline pieces.

[35]For the breast cancer incidence data, SAS proc NLMIXED was used for the linear models and proc PHREG was used for the log-linear models, and the discrepancies that were observed in AIC values between the linear and log-linear models for the lymphoid cancer data (see footnote 25) were not apparent; thus, these AICs are directly comparable.

For comparison, linear RR (ERR) models with cumulative exposure, log cumulative exposure, and a square root transformation of cumulative exposure as continuous variables were also investigated using the approach of Langholz and Richardson (2010), and these models all fit better than the corresponding log-linear models, based on AIC (see Table 4-14 and Section D.1 of Appendix D). The linear and log-linear square root exposure models had marginally lower AICs than the two-piece linear spline model; however, the two-piece linear spline model is preferred, for the reasons discussed above and in Table 4-14, including the greater flexibility of spline models, which allows more local fit in the low exposure range. Risk estimates based on the linear models with cumulative exposure and with the square-root transformation of cumulative exposure are developed for comparison, but the linear model with the log transformation of cumulative exposure had an inferior fit to that of the linear model with the square-root transformation (AIC of 1956.8 vs. 1952.5; see Table D-2 in Appendix D) and was not considered further. For more details of the breast cancer incidence exposure-response modeling, see Section D.1 of Appendix D.

Table 4-13. Exposure-response modeling results for breast cancer incidence in females from the NIOSH cohort for models not presented by Steenland et al. (2003)

Model[a]	*p*-value[b]	Coefficient[e] (SE[f]) (per ppm × day)
Full incidence study cohort[c]		
Linear regression of categorical results, excluding the highest exposure quintile	0.33	2.64×10^{-5} (2.69×10^{-5})
Subcohort with interviews[d]		
Two-piece log-linear spline (knot at 5,800 ppm × days)	0.01	Low-exposure spline segment: B1 = 7.70×10^{-5} (3.17×10^{-5}) B2 = -7.24×10^{-5}
Two-piece linear spline (knot at 5,750 ppm × days)	0.01	Low-exposure spline segment: B1 = 8.98×10^{-5} (UB1 = 1.84×10^{-4}[e]) B2 = -7.79×10^{-5}
Cox regression with square root cumulative exposure	0.006	3.49×10^{-3} (1.18×10^{-3})
Linear	0.01	2.30×10^{-5} (UB = 4.67×10^{-5}[e])
Linear with square root cumulative exposure	0.004	5.53×10^{-3} (UB = 1.07×10^{-2}[e])
Linear regression of categorical results, excluding the highest exposure quintile	0.16	5.17×10^{-5} (3.69×10^{-5})

[a]All with cumulative exposure in ppm × days as the exposure variable and with a 15-yr lag.
[b]*p*-value for addition of exposure variables from likelihood ratio test, except for the linear regressions of categorical results, where Wald *p*-values are reported.
[c]319 breast cancer cases.
[d]233 breast cancer cases.
[e]For the two-piece spline models, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × (exp – knot)).
[f]Or, for linear models of continuous exposure, the profile likelihood 95% (one-sided) upper bound (UB)

Source: Additional analyses performed by Dr. Steenland and Dr. Deddens (see Section D.1 of Appendix D), except for the linear regressions of categorical results, which were performed by EPA using the equations of Rothman (1986) presented in Appendix F.

Risk estimates based on the original linear regression analyses of the categorical results are also presented for comparison. For the approach of using a weighted linear regression of the results from the Cox regression model with categorical cumulative exposure (and a 15-year lag), excluding the highest exposure group, the weights used for the ORs were the inverses of the variances, which were calculated from the confidence intervals.[36] Mean and median exposures for the cumulative exposure groups for the full cohort were provided by Dr. Steenland (email dated April 21, 2004, from Kyle Steenland, Emory

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

University, to Jennifer Jinot, EPA).[37] The mean values were used for the weighted regression analysis because the (arithmetic) mean exposures best represent the model's linear relationship between exposure and cancer response. Differences between means and medians were not large for the females, especially for the lower four quintiles. If the median values had been used, a slightly larger regression coefficient would have been obtained[38], resulting in slightly larger risk estimates. Although the exposure values are for risk sets from the full cohort, they should be reasonably close to the values for the subcohort with interviews. See Table 4-13 for the results from the weighted linear regressions of the categorical results and Figures 4-5 and 4-6 for a depiction of the resulting linear regression models.

As the subcohort with interviews from the NIOSH incidence study cohort provides the preferred data set for the derivation of unit risk estimates for breast cancer, a summary of all the models considered for modeling the breast cancer exposure-response data from the subcohort and the judgments made about model selection is provided in Table 4-14. See Figure 4-6 for visual representations of the models. See Tables 4-12 and 4-13 and Section D.1 of Appendix D for parameter estimates, *p*-values, and other fit statistics.

To facilitate a visual comparison of the models, select models are replotted in Figure 4-7, this time against the categorical data in deciles. The linear and log-linear two-piece spline models are included, as spline models were the preferred model form due to their ability to allow more local fits in the low exposure range. Also included are the linear model with the square-root transformation of cumulative exposure, as this model had the lowest AIC of the models considered, and the linear model with untransformed cumulative exposure, because this model, being a linear model of the full continuous exposure data, is expected to provide a lower bound on the likely low-exposure slope, as the overall exposure-response relationship is supralinear. As can be seen in Figure 4-7, the spline models appear to have the best fit to the lower-exposure data, which are of the greatest interest in deriving a unit risk for estimating risk from environmental exposures. The linear square-root model imposes a supralinear curvature at low exposures, i.e., it has a low-exposure slope that becomes increasingly steep as exposures decrease; thus, a unit risk estimate derived from this model is highly dependent on the extra risk level chosen for the point of departure, and very steep low-exposure slopes and large unit risk estimates can result. It also appears from Figure 4-7 that the linear model has a poorer fit (too shallow) to the lower-exposure data than either of the two-piece spline models. This is consistent with the analysis presented in Section D.1 of Appendix D showing the strong influence of the upper tail of cumulative exposures on the results of the cumulative exposure Cox regression model. The responses in the upper tail of exposures are relatively dampened, such that when the highest 5% of exposures are

[37]Mean exposures for females with a 15-year lag for the exposure categories in Table 3 of Steenland et al. (2003) 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.

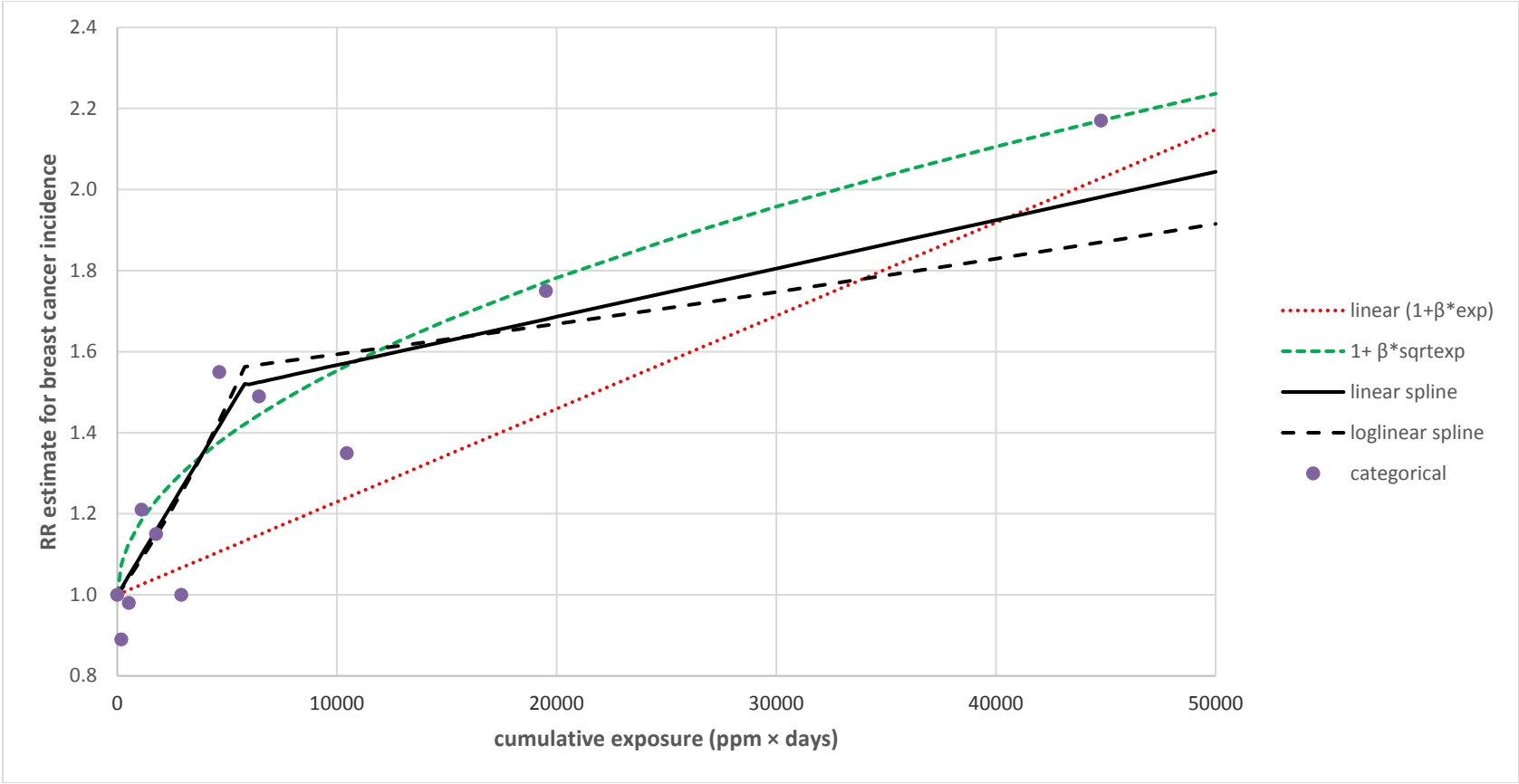
[38]8% greater regression coefficient for the subcohort, and 9% greater regression coefficient for the full cohort.

excluded, the slope of the Cox regression model is substantially increased (e.g., at 10,000 ppm × days, the RR estimate increases from about 1.1 to almost 1.5; see Figure D-4 in Appendix D). This strong influence of the upper tail of exposures would similarly attenuate the slope of the linear model. The two-piece spline models, on the other hand, are more flexible, and the influence of the upper tail of exposures would be primarily on the upper spline segment; thus, the two-piece models are able to provide a better fit to the lower-exposure data.

Table 4-14. Models considered for modeling the exposure-response data for breast cancer incidence in females in the subcohort with interviews from the NIOSH incidence study cohort for the derivation of unit risk estimates

Model[a]	AIC[b]	Comments
Two-piece spline models		
Two-piece linear spline model (knot at 5,750 ppm × days)	1954.360	**SELECTED**. Good overall statistical fit and good visual fit, including local fit to low-exposure range; linear model; AIC within two units of lowest AIC of models considered.
Two-piece log-linear spline model (knot at 5,800 ppm × days)	1954.485	Good overall statistical fit and good visual fit, including local fit to low-exposure range; preference given to the two-piece linear spline model primarily because it has the advantageous property of linearity, but it also has a marginally better statistical fit (lower AIC).
Linear (ERR) models ($RR = 1 + \beta \times exposure$)		
Linear model with square-root transformation of cumulative exposure	1952.501	Good overall statistical fit and lowest AIC; low-exposure slope becomes increasingly steep as exposures decrease, and large unit risk estimates can result; preference given to the two-piece spline models because they have a better ability to provide a good local fit to the low-exposure range.
Linear model (with untransformed cumulative exposure)	1954.526	Good overall statistical fit but poorer local fit to low-exposure range than the two-piece spline models; higher AIC than selected model.
Log-linear (Cox regression) models ($RR = e^{\beta \times exposure}$)		
Log-linear model with square-root transformation of exposure	1953.028	Good overall statistical fit; low-exposure slope becomes increasingly steep as exposures decrease, and large unit risk estimates can result; preference given to the two-piece spline models because they have a better ability to provide a good local fit to the low-exposure range.
Log-linear model with (natural) log cumulative exposure	1956.176	Good overall statistical fit but poor local fit to low-exposure range; low-exposure slope becomes increasingly steep as exposures decrease, and large unit risk estimates can result; higher AIC than selected model.
Log-linear model (standard Cox regression)	1956.675	Good overall statistical fit but poor local fit to low-exposure range (too shallow); AIC exceeds that of selected model by >2.
Linear regression of categorical results		
Linear regression of categorical results, excluding the highest exposure quintile	--[c]	Not statistically significant, though that is unsurprising since the approach, which is based on categorical data, has low statistical power; preference given to models that treated exposure as a continuous variable and that also provided reasonable representations of the low-exposure region.

[a]All with cumulative exposure as the exposure variable, except where noted, and with a 15-yr lag, and all with exposure as a continuous variable except for the linear regression of categorical results.
[b]AIC = 2p-2LL, where p = number of parameters and LL = ln(likelihood), assuming two exposure parameters for the two-piece spline models.
[c]Not calculated.



Categorical: linear model (RR = 1 + β × exposure) with categorical exposures; log-linear spline: two-piece log-linear spline model, with knot at 5,800 ppm × days (see text); linear spline: two-piece linear spline model, with knot at 5,750 ppm × days (see text); linear: RR = 1 + β × exposure; 1 + β*sqrtexp: RR = 1 + β × sqrt(exposure). All models except for the categorical model treat exposure as a continuous variable. (Note that the various models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Source: Steenland and Deddens analyses in Appendix D.

Figure 4-7. RR estimate for breast cancer incidence in subcohort with interviews vs. occupational cumulative exposure (with 15-year lag); select models compared to deciles.

In conclusion, the two-piece linear spline model with knot at 5,750 ppm × days was selected for the derivation of the unit risk estimates for breast cancer incidence. Selection of this model is consistent with the model selction objectives for this assessment (see Section 4.1.1.2). First, the model uses the individual-level exposure data. Second, the spline model is more tuned to local behavior than the other model forms considered. Third, the principle of parsimony was applied, both in the use of a preselected knot (with sensitivity analyses conducted to examine the sensitivity of the unit risk estimates to this preselection; see Section D.1.7 of Appendix D) and in the use of AICs to compare models, but without over-reliance on this statistic. Fourth, the general model shape is biologically plausible and the selected model is consistent with the data. In addition, the same model can be used to derive the estimates of extra risk for occupational exposure scenarios.

The proportional hazards assumption for the selected model (two-piece linear spline model with knot at 5,750 ppm × days) was tested by evaluating the significance of an age-interaction term for each spline regression coefficient, and neither interaction term was statistically significant (see Section D.1.9 of Appendix D).

The exposure level (EC_x) and the associated 95% lower confidence limit (LEC_x) corresponding to an extra risk of 1% ($x = 0.01$) for breast cancer incidence in females (based on invasive + in situ tumors in the subcohort with interviews) for the models discussed above were estimated using the actuarial program (life-table analysis). As noted in Section 4.1.1.2, a 1% extra risk level is a more reasonable response level for defining the POD for these epidemiologic data than a 10% level. The results are presented in Table 4-15. For comparison, the results for some models using the data from the full cohort of the breast cancer incidence study are also presented, but the subcohort with interviews is the preferred dataset.

Table 4-15. EC_{01}, LEC_{01}, and unit risk estimates for breast cancer incidence (invasive and in situ) in females from various models[a]

Model[b]	With interviews			Full cohort		
	EC_{01} (ppm)	LEC_{01}[c] (ppm)	Unit risk (per ppm)	EC_{01} (ppm)	LEC_{01}[c] (ppm)	Unit risk (per ppm)
Two-piece spline models						
Low-exposure linear spline[d]	0.0138	6.75×10^{-3}	**1.48**[e]	--[f]		
Low-exposure log-linear spline[d]	0.0155	9.27×10^{-3}	1.08[e]	--[f]		
Linear (ERR) models ($RR = 1 + \beta \times exposure$)						
Linear model with square root of cumulative exposure[g]	2.76×10^{-3}	7.42×10^{-4}	13.5[e,h]	--[f]		
Linear model (with untransformed cumulative exposure)[g]	0.0540	0.0266	0.376	--[f]		
Log-linear (Cox regression) models ($RR = e^{\beta \times exposure}$)						
Log-linear model with square root of cumulative exposure[i]	6.49×10^{-3}	2.68×10^{-3}	3.73[e]	--[f]		
Log-linear model with (natural) log cumulative exposure[j]	--[k]	--[k]	--[k]	--[k]	--[k]	--[k]
Log-linear model (standard Cox regression)[j]	0.126	0.0737	0.136[h]	0.222	0.107	0.0935[h]
Linear regression of categorical results						
Linear regression of categorical results, excluding highest exposure quintile[j,l]	0.0240	0.0110	0.909	0.0469	0.0176	0.568

[a]All-cause mortality adjusted (to dying of something other than breast cancer or developing breast cancer). Unit risk = $0.01/LEC_{01}$.
[b]All with cumulative exposure as the exposure variable, except where noted, and with a 15-yr lag, and all with exposure as a continuous variable except for the linear regression of categorical results.
[c]Confidence intervals used in deriving the LEC_{01}s were estimated employing the Wald approach for the log-linear RR models and a profile likelihood approach, which allows for asymmetric CIs, for the linear RR models (Langholz and Richardson, 2010).
[d]From low-exposure segment of two-piece spline analysis; see text and Table D-4 of Appendix D for log-linear model or Table D-10 for linear model. The EC_{01} value is below the value of 0.074 ppm roughly corresponding to a knot of 5,750 ppm × days [(5,750 ppm × days) × (10 m^3/20 m^3) × (240 d/365 d)/(365 d/yr × 70 yr) = 0.074 ppm] and, thus, appropriately in the range of the low-exposure segment.
[e]For unit risk estimates above 1, one can convert to risk per ppb (e.g., 1.75 per ppm = 1.75×10^{-3} per ppb).
[f]Not estimated; two-piece spline models, linear RR models, and log-linear RR model with square-root transformation of exposure not developed for the full cohort.
[g]From linear analyses in Section D.1.3.2 and Table D-10 of Appendix D.
[h]This unit risk estimate is not considered a good estimate of risks from (low) environmental exposure levels (see text).

Table 4 15. EC01, LEC01, and unit risk estimates for breast cancer incidence (invasive and in situ) in females from various models[a] (continued)

[i]From Table D-6 of Appenidx D.
[j]From Tables 4 and 5 of Steenland et al. (2003), Cox regression models.
[k]Estimated exposure levels were so low (<3. 6 × 10^{-4} ppm) that the cumulative exposures in some of the age intervals in the lifetable analysis were <1 ppm × day, resulting in ln cumulative exposures of <0 for those intervals and unreliable results for this model. 3. 6 × 10^{-4} ppm would yield a unit risk estimate of about 28 per ppm, so that provides a lower bound on the unit risk estimate for this model.
[l]Regression coefficient derived from linear regression of categorical results, as described in Section 4.1.2.3.

Because EtO is DNA-reactive and has direct mutagenic activity (see Section 3.3.3), which is one of the cases cited by EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) for the use of linear low-dose extrapolation, a linear low-exposure extrapolation was performed. (Linear low-exposure extrapolation is also the default approach used in the absence of sufficient evidence for a nonlinear mode of action, which is also the case for EtO [see Section 3.4].) The inhalation unit risk estimates for the different breast cancer incidence models considered are presented in Table 4-15.

As discussed above, the unit risk estimate based on the two-piece linear spline model using cumulative exposure with a 15-year lag (i.e., 1.48 per ppm, or 1.48 × 10^{-3} per ppb) is the preferred estimate for breast cancer incidence. The two-piece log-linear spline model resulted in a unit risk estimate of 1.08 per ppm, while the linear regression of categorical results yielded a unit risk estimate of 0.909 per ppm and the continuous exposure linear model produced a unit risk estimate of 0.376 per ppm; these alternate estimates are about 73%, 61%, and 25%, respectively, of the estimate based on the preferred two-piece linear spline model.

EC_{01}, LEC_{01}, and unit risk estimates from the other models examined are presented for comparison only, to illustrate the differences in model behavior at the low end of the exposure-response range. The unit risk estimates from these other models are not considered reliable estimates of risk from (low) environmental exposures because, although these models provide an adequate global fit to the overall data, they do not allow a good local fit to the low-exposure data (see Figure 4-6). As discussed above, the log-linear log cumulative exposure model and both the linear and log-linear square-root transformation models impose a low-exposure supralinear curvature that can result in inappropriately large unit risk estimates. For example, for the log-linear log cumulative exposure model, a reliable unit risk estimate could not be calculated from the 1% extra risk level, but the substantially lower EC_{01} estimate (<3. 6 × 10^{-4} ppm) from that model compared to that from the two-piece linear spline model (0.0138 ppm) indicates a much higher unit risk. The standard Cox regression cumulative exposure model, on the other hand, with its global sublinearity, has an overly shallow slope in the low-exposure range, yielding a notably higher EC_{01} estimate (0.126 ppm) than that from the two-piece linear spline model (0.0138 ppm), and a correspondingly lower unit risk estimate (0.136 per ppm vs 1.48 per ppm).

The linear untransformed exposure model might be considered a lower bound to a range of credible unit risk estimates—unit risk estimates below that value (0.376 per ppm) are considered unlikely from the available data, given that for a global linear model, the high-exposure results would dampen the low-exposure slope (as discussed above with respect to the log-linear [standard Cox regression] model [and see Section D.1 and Figure D-4 of Appendix D]). There is no clear upper bound to a range of credible unit risk estimates due to the supralinear curvature of the alternative models. The next highest unit risk estimates are 3.73 per ppm and 13.5 per ppm, from the log-linear and linear square-root transformation models, respectively, but there is no basis for considering either of those estimates an upper bound to a range of credible estimates.

As discussed above, the primary risk calculations for breast cancer incidence were based on invasive and in situ tumors in the subcohort of women with interviews, and the primary model was the two-piece linear spline model. For this assessment, the two-piece spline analyses were not performed with the full cohort. In the 2006 Draft Assessment (U.S. EPA, 2006a), however, comparison analyses were done. Using the linear regression of the categorical results, the comparable unit risk estimate for the full cohort was about 40% lower than the estimate based on the subcohort with interviews. A lower estimate from the full cohort is consistent with the expectation that there was an under-ascertainment of cases in the full cohort, as discussed above. Using the two-piece linear spline model, the corresponding unit risk estimate derived based on the subcohort results but using invasive breast cancer only for the background incidence rates was about 20% lower than the estimate based on invasive and in situ tumors, reflecting the difference between incidence rates for invasive breast cancer only and for combined in situ and invasive breast cancer.

Sensitivity analyses were also conducted to investigate the influence of lag period, inclusion of covariates, knot selection, and upper-bound estimation approach on the unit risk estimates from the selected two-piece linear spline model. The sensitivity analyses are detailed in Sections D.1.6, D.1.7, D.1.8, and D.1.10 of Appendix D. In brief, for the two-piece linear spline model with the knot at 5,750 ppm × days, the unit risk estimates for different lag periods (0, 5, 10, 15, and 20 years) ranged from about 35% less than (10-year lag) to about 21% greater than (20-year lag) the estimate for the selected model (15-year lag). (Note that the two-piece linear spline model with the knot at 5,750 ppm × days and a 20-year lag had a slightly better fit, based on log-likelihood and AIC, than the model with a 15-year lag [see Table D-12 of Appendix D].) Exclusion of covariates produced very little difference in the unit risk estimates—excluding parity and both parity and breast cancer in a 1st-degree relative changed the unit risk estimate by only about 1%. Similarly, varying the knot by 1000 ppm × days resulted in little difference in the unit risk estimates—the unit risk estimate with the knot at 4,750 ppm × days was about 14% greater than, and unit risk estimate with the knot at 6,750 ppm × days was about 11% less than, the unit risk estimate for the selected model (with the knot at 5,750 ppm × days). The unit risk estimate calculated using a Wald approach was about 3% lower

than the preferred estimate, which relied on a profile likelihood approach to estimate the upper bound on the regression coefficient.

This unit risk estimate for breast cancer incidence is slightly lower than the unit risk estimate derived for breast cancer mortality above (see Section 4.1.2.2), which is contrary to expectations. Confidence is higher in the breast cancer incidence estimate because it is based on more cases, especially in the lower-exposure region, and a better-fitting model.

The life-table analysis takes into account competing risks and the occurrence of different cumulative exposures and different cause-specific background risks at different ages. A crude approximation of the general approach for obtaining EC_{01} and LEC_{01} estimates without the use of the life-table component of the analysis is presented here for illustration. In this crude approach, an estimate of the lifetime background risk of developing breast cancer is used rather than age-specific rates. According to SEER data, this lifetime background incidence risk estimate (Ro) is 12.3%.[39] From this and Equation 4-2, an estimate of the RR associated with a 1% extra risk can be calculated as $RR = (0.99 \times Ro + 0.01)/Ro = 1.07$. Then, a maximum likelihood estimate (MLE) of the cumulative exposure associated with this RR can be calculated from the low-exposure spline segment from the selected two-piece linear spline model as exposure = $(RR - 1)/\beta_1$. This quantity is an occupational cumulative exposure in ppm × days. To convert to environmental ppm × years, multiply by (10 m^3 breathed at work/day)/(20 m^3/day) and (240 days worked/year)/(365 days/year), as discussed in Section 4.1.1.2, and then divide by 365 days/year. Because the life-table analysis is based on actual demographic rates, this crude approximation uses an average U.S. life expectancy of 80 years[40] rather than the EPA default average lifespan of 70 years, for a more appropriate comparison. With a 15-year lag, this means dividing the cumulative exposure by 65 years to get the continuous lifetime exposure level associated with a 1% extra risk (EC_{01}). The LEC_{01} is obtained using the same calculations but with the profile likelihood upper bound on beta1 in place of beta1. With beta1 of 8.98×10^{-5} per ppm × day and a profile likelihood upper bound on beta1 of 1.84×10^{-4} per ppm × day from the selected two-piece linear spline model, these calculations yield an EC_{01} of 0.0110 ppm and an LEC_{01} of 0.00538 ppm. In comparison to the estimates presented for the selected two-piece linear spline model in Table 4-15, this crude approach yields EC_{01} and LEC_{01} estimates that are both about 20% lower, which would correspond to a unit risk estimate about 26% higher.

In summary, the unit risk estimate of **1.48 per ppm** (1.48×10^{-3} per ppb) is the preferred estimate for female breast cancer risk because it is based on incidence data versus mortality data, it is based on more cases (n = 233) than the mortality estimate (n = 103), and information on personal breast cancer risk factors obtained from the interviews is taken into account. Furthermore, the two-piece linear

[39]Based on 2010−2012 data; http://seer.cancer.gov/statfacts/html/breast.html.
[40]The overall U.S. life expectancy in women in 2014 was 81.2 years (Murphy et al., 2015).

spline model, which uses the complete data set with exposure as a continuous variable, was statistically significant, had an AIC within two units of the model with the lowest AIC, and provided a good visual fit to the lower-exposure data. Converting the units, 1.48 per ppm corresponds to a unit risk estimate of 8.09×10^{-4} per $\mu g/m^3$.

4.1.3. Total Cancer Risk Estimates

According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), cancer risk estimates are intended to reflect total cancer risk, not site-specific cancer risk; therefore, an additional calculation was made to estimate the combined risk for (incident) lymphoid and breast cancers, because females would be at risk for both cancer types. The unit risk estimates for both of the individual models for these cancers were derived from linear RR models and are thus based on profile likelihood upper bound estimates of the regression coefficient (Langholz and Richardson, 2010). It was not possible to derive the total cancer unit risk estimate using a profile likelihood approach, thus, a Wald approach was employed to estimate the combined risk.

To derive a total cancer unit risk estimate, it is assumed that the cancer types are independent, which is a reasonable assumption for the cancer types involved. In addition, to employ the Wald approach, it is assumed that the risk estimates are approximately normally distributed. For breast cancer, this is reasonable, as the Wald-based estimate was only 3% less than the profile-likelihood-based unit risk estimate. For lymphoid cancer, the Wald-based estimate was 40% less than the profile-likelihood-based unit risk estimate by 40%, indicating that the total cancer unit risk estimate will also be underestimated by using the Wald approach. In fact, the total cancer unit risk estimate calculated using the Wald-based unit risk estimates and Wald standard errors (SEs) (see Sections D.1.10 and D.3.8 of Appendix D) was less than the profile-likelihood-based unit risk estimate for lymphoid cancer alone (4.03 vs. 5.26 per ppm); thus, Wald-type SEs were approximated from the profile likelihood upper bounds, and these approximated SEs were used with the profile-likelihood-based unit risk estimates in the Wald approach to derive the total cancer unit risk estimate.

Under the Wald approach, one can estimate the 95% UCL (one-sided) on the total risk as the 95% UCL on the sum of the maximum likelihood estimates (MLEs) of the risk estimates according to the formula

$$95\%\ \text{UCL} = \text{MLE} + 1.645(\text{SE}), \qquad (4\text{-}3)$$

where MLE is the MLE of total cancer risk (i.e., the sum of the individual MLEs) and the SE of the sum of the MLEs is the square root of the sum of the individual variances (i.e., the variance of the sum is the sum of the variances, and the SE is the square root of the variance). Because both models are linear in

the range around the PODs, the combining-risk calculations can be done directly from the unit risk and $0.01/EC_{01}$ estimates rather than having to do the calculations at a common exposure level near where the EC_{01} and LEC_{01} for the combined risk would be.

First, the $0.01/EC_{01}$ estimates were calculated and an EC_{01} of 4.85×10^{-3} ppm for the total cancer risk (i.e., lymphoid cancer incidence + breast cancer incidence) was estimated, as summarized in Table 4-16.

Table 4-16. Calculation of EC_{01} for total cancer risk

Cancer type	EC_{01} (ppm)	$0.01/EC_{01}$ (per ppm)	EC_{01} for total cancer risk (ppm)
Lymphoid	0.00748	1.34	--
Breast	0.0138	0.725	--
Total[a]	--	2.06	0.00485

[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 cancer risk then equals $0.01/(0.01/EC_{01})$.

Then, a unit risk estimate of 6.06 per ppm for the total cancer risk (i.e., lymphoid cancer incidence + breast cancer incidence) was derived, as shown in Table 4-17. An LEC_{01} estimate of 1.65×10^{-3} ppm for the total cancer risk can be calculated as 0.01/(6.06 per ppm).

Table 4-17. Calculation of total cancer unit risk estimate

Cancer type	Unit risk estimate[a] (per ppm)	$0.01/EC_{01}$ (per ppm)	SE[b] (per ppm)	Variance	Total cancer unit risk estimate (per ppm)	LEC_{01} for total cancer risk[e] (ppm)
Lymphoid	5.26	1.34	2.38	5.69	--	--
Breast	1.48	0.725	0.459	0.211	--	--
Total	--	2.06	(2.43)[c]	5.90	6.06[d]	0.00165

[a]Profile-likelihood-based unit risk estimates from the selected linear two-piece spline models.
[b]SE ≈ (unit risk—$0.01/EC_{01}$)/1.645; Wald-type SEs were approximated from the profile-likelihood-based unit risk estimates, i.e., profile likelihood upper bounds.
[c]The SE of the total cancer risk is calculated as the square root of the sum of the variances (next column), not as the sum of the SEs.
[d]Total cancer unit risk = 2.06 + 1.645 × 2.43.
[e]The LEC_{01} for the total cancer risk equals 0.01/(total cancer unit risk estimate).

Thus, the total cancer unit risk estimate is 6.1 per ppm (or 6.1×10^{-3} per ppb; 3.3×10^{-3} per $\mu g/m^3$). As can be seen in Table 4-17, lymphoid cancer contributes about 2/3 of the risk to the sum of the MLEs and somewhat more (between about 75 and 85%) to the total cancer unit risk estimate, i.e., to the UCL on the sum.

Recall that this is the unit risk estimate derived under the assumption that RR is independent of age (see Section 4.1.1.2). The preferred assumption of increased early-life susceptibility, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), is considered in Section 4.4.

While there are uncertainties regarding the assumption of a normal distribution of risk estimates, the resulting unit risk estimate is appropriately bounded in the roughly 1.1-fold range between the sum of the unit risk estimate for lymphoid cancer plus the $0.01/EC_{01}$ estimate for breast cancer (5.98 per ppm), which provides a lower bound for the upper bound on the sum of the individual MLEs (i.e., $0.01/EC_{01}$ estimates), and the sum of the individual 95% UCLs (i.e., unit risk estimates) (6.74 per ppm). Thus, any inaccuracy in the total cancer risk estimate resulting from the approach used to combine risk estimates across cancer types is relatively minor.

4.1.4. Sources of Uncertainty in the Human-Data-Based Cancer Risk Estimates

Discussion of the sources of uncertainty in the human-data-based unit risk estimates derived in Sections 4.1.1.2, 4.1.2.2, and 4.1.2.3 above is organized into three subsections: (1) limitations in the human database, (2) uncertainties that stem from the epidemiological study used as the basis for the unit risk estimates and its analysis, and (3) uncertainties associated specifically with the total cancer (incidence) unit risk estimate.

4.1.4.1. *Limitations in the Human Database*

The availability of suitable human data from which to derive unit risk estimates for EtO eliminates one of the primary sources of uncertainty inherent in most unit risk estimates—the uncertainty associated with interspecies extrapolation. Nonetheless, there are limitations in the human database on cancer risk from EtO exposure that contribute uncertainty to the unit risk estimates (estimates based on rodent cancer data and the sources of uncertainty pertaining to those estimates are discussed in Section 4.2 below). The sources of uncertainty related to these limitations include availability of only a single study with superior characteristics, the derivation of unit risk estimates for the general population from an occupational study, and, in the case of the lymphoid cancer (incidence) unit risk estimate, the derivation of the incidence estimate from mortality data.

Single study with superior characteristics

Three independent epidemiology studies conducted exposure-response analyses based on exposure estimates for the individual workers; however, one study, the NIOSH study (Steenland et al.,

2004; Steenland et al., 2003), was judged to be substantially superior to the other two studies (Mikoczy et al., 2011; Swaen et al., 2009) for the purposes of deriving a unit risk estimate, as discussed in Section 4.1. Although only one study was used for the unit risk estimation from human data, it is a large study that included workers from 13 different sterilization facilities in different states, decreasing the likelihood that the results are overly influenced by uncontrolled confounding related to either location or a specific facility.

Moreover, the limitations and sources of uncertainty discussed below notwithstanding, the NIOSH study is considered a high-quality study for the purposes of deriving a unit risk estimate. The NIOSH study is a large longitudinal cohort study that developed individual-worker exposure estimates using detailed employment histories and a state-of-the-art regression model for retrospectively estimating exposures based on time-period-specific plant and job variables. In addition, internal comparisons were used for estimating risk from EtO exposure, coexposures to other chemicals in the facilities were reportedly minimal, and, in the analysis of the breast cancer incidence subcohort with interviews, various nonexposure-related breast cancer risk factors were taken into account. The NIOSH study comprises a large cohort that has been followed for a sufficient length of time for cancer detection. The cohort includes 17,530 workers with exposure estimates, about 55% of whom are women, contributing a total of over 400,000 exposed person-years to the most recent follow-up (Steenland et al., 2004). The mean follow-up time was about 27 years. Thus, although the unit risk estimates are based on a single study, there is relatively high confidence in that study.

The UCC study (Swaen et al., 2009) is considered largely uninformative in terms of assessing the unit risk estimates derived from the NIOSH study because of the crude exposure assessment used in the UCC study and because of differences in the exposure-response analyses conducted [e.g., in their internal analyses, Swaen et al. (2009) only used Cox regression models in cumulative exposure, i.e., sublinear models, and may have over-adjusted by including age at hire in their models] (see Section A.2.20 of Appendix A). In addition, the Swaen et al. (2009) study was restricted to males, so there are no female breast cancer data available, and there were only 17 lymphoid cancer deaths compared to 53 in the NIOSH study.

The Mikoczy et al. (2011) study of sterilizer workers, though small, appeared to have a well done exposure assessment; however, the data reported by Mikoczy et al. (2011) were not well suited for the derivation of a unit risk estimate. Thus, crude comparison analyses were done to evaluate whether or not the exposure-response models of the NIOSH study that were used to derive unit risk estimates in this assessment gave predictions consistent with the Mikoczy et al. (2011) internal incidence ratios (IIRs) for the two highest exposure quartiles (see Section J.2.2 of Appendix J). The predicted values for lymphoid cancer were within the 95% CIs for the IIRs for lymphohematopoietic cancer reported by Mikoczy et al. (2011). The predicted values for breast cancer incidence, however, were below the lower limit of the 95% CIs for the IIRs for breast cancer, suggesting that the Mikoczy et al. (2011) results are

consistent with a higher unit risk estimate for breast cancer incidence than the one derived in this assessment. The reasons for the discrepancies are unknown, although it might be noted that a less rigorous approach was used to estimate historical exposure levels for the plants in the Mikoczy et al. (2011) study than the regression model that was developed for the NIOSH study and that there were many fewer breast cancer cases in the Mikoczy et al. (2011) study (41 incident cases [33 with ≥15 years since time of first exposure] vs. 233 cases [at least 170 with ≥15 years since time of first exposure] in NIOSH's subcohort with interviews).

High-to-low dose extrapolation

The human-based estimates are also less affected by high-dose to low-dose extrapolation than are rodent-based estimates and, thus, uncertainty from that source is less than for estimates from rodent studies. For example, the average exposure in the NIOSH cohort was more than 10 times lower than the lowest exposure level in a rodent bioassay after adjustment to continuous lifetime exposure. Nonetheless, uncertainty remains in the extrapolation from occupational exposures to lower environmental exposures.[41] Although the actual exposure-response relationship at low exposure levels is unknown, the clear evidence of EtO mutagenicity supports the linear low-exposure extrapolation that was used (U.S. EPA, 2005a). The linear low-exposure extrapolation from the 95% lower bound on the exposure level associated with the 1% extra risk level is considered to be a plausible upper bound on the risk at lower exposure levels. Actual low-exposure risks are expected to be lower, to an unknown extent.

Because of the existence of endogenous EtO (see Section 3.3.3.1), several members of the SAB panel that reviewed EPA's 2006 external review draft assessment (U.S. EPA, 2006a) felt that the exposure-response relationship for cancer at low exposures would be nonlinear and suggested that it would be consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) to

[41]Even though lifetime cumulative exposures from environmental exposure may overlap the low end of the range of lagged cumulative exposures from occupational exposure, the exposure-response model is based on the full range of the occupational exposure data and not just the data in the range of environmental exposures, which are too sparse to model on their own. Even with a two-piece spline model which gives a more local fit to the low-exposure data, there is uncertainty about the exposure-response relationship specifically in the range of environmental exposures. The point of departure (LEC_{01}) is intended to be at the low end of the "observable" range, i.e., the range of exposures for which the study might be able to detect a significant increase in risk, but it is still substantially above typical environmental exposure levels (according to EPA's 2005 National-scale Air Toxics Assessment data, the average exposure concentration of EtO from all sources [including background] in the United States is 0.0062 μg/m^3 [3.4E-6 ppm]; the average background concentration is 0.0044 μg/m^3 [2.4E-6 ppm]), and thus there is uncertainty about the low-exposure extrapolation from the points of departure (6.75E-3 ppm, or 12 μg/m^3, for breast cancer incidence and 1.9E-3 ppm, or 3.5 μg/m^3, for lymphoid cancer incidence). For lymphoid cancer, only 2 of the 13 cases below the knot (~15%) are also below the point of departure. For breast cancer, about 25% of the cases below the knot are also below the point of departure, roughly corresponding to the lowest 1.5 deciles (Table D-1 of Appendix D), but, e.g., as one can see from Table D-3 of Appendix D, the variability of the low-exposure data is such that the two lowest deciles both have RR estimates <1, and thus are not by themselves consistent with the unit risk estimate, illustrating the uncertainty that still exists in the low-exposure extrapolation.

present a nonlinear approach for "extrapolation" to lower exposures (SAB, 2007). EPA considered this suggestion but judged that the support for a nonlinear approach was inadequate. In brief, as discussed in Sections 3.1 through 3.3.3, EtO is a DNA-reactive, mutagenic, multisite carcinogen in humans and experimental species; as such, it has the hallmarks of a compound for which low-dose linear extrapolation is strongly supported under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) do provide for presenting alternate approaches when those alternatives have significant biological support; however, EPA's analysis of the arguments for using a nonlinear approach presented on page 23 and in Appendix C of the SAB report (SAB, 2007) did not find these arguments to be persuasive. The arguments posited by the SAB panel members who supported using a nonlinear approach were largely that (1) DNA adducts may show a nonlinear response when identical adducts are formed endogenously and (2) mutations do not have linear relationships with exposure but exhibit an "inflection point." However, as discussed in Section 3.3.3.1, recent data from Marsden et al. (2009) support a linear exposure-response relationship for EtO exposure and DNA adducts ($p < 0.05$) and demonstrate increases of DNA adducts from exogenous EtO exposure above those from endogenous EtO for very low exposures to exogenous EtO, providing evidence against argument (1). Moreover, Appendix C of the SAB report (SAB, 2007) presents two EtO-specific mutation data sets in support of argument (2); however, EPA's analysis of these data sets finds that they are in fact consistent with low-dose linearity. See the response to this comment under charge question 2.b in Appendix H for a more comprehensive discussion of EPA's consideration and rejection of a nonlinear approach and for the details of EPA's analysis of the two EtO mutation data sets.

EPA also considered more recent (2013) public comments proposing nonlinear modes of action for EtO carcinogenicity; however, EPA found these hypothetical modes of action to be speculative at this time (see Appendix K and Section J.3.2 of Appendix J).

Generalizability of estimates from a worker population

The NIOSH data represent an industrial worker cohort that is generally healthier than the U.S. population at large. The healthy-worker effect is often an issue in occupational epidemiology studies, but the use of internal exposure-response analyses help address this concern, at least partially. The unit risk estimates derived from the NIOSH worker cohort data could underestimate the cancer risk for the general population to an unknown extent, although the impact is expected to be relatively low for the majority of the population.

Industrial workers can also differ from the general population in factors other than health status. In terms of representing the general population, the NIOSH study cohort was relatively diverse. It contained both female (55%) and male (45%) workers, and the workers were 79% white, 16% black, and 5% "other."

The workers were, however, all adults, and a related area of uncertainty pertains to the assumption that RR is independent of age, which is a common assumption in the dose-response modeling of epidemiological data and is an underlying assumption in the Cox regression model. For the NIOSH worker cohort, the proportional hazards model assumption of RR being independent of age was tested by checking the significance of an interaction between age and cumulative exposure, and none of the models had a significant interaction term (see Sections D.1.9 and D.3.7 of Appendix D). This suggests that, for adults at least, the assumption that RR is independent of age is valid. However, the worker cohort contains no children and is uninformative on the issue of early-life susceptibility. In the absence of data on early-life susceptibility, EPA's *Supplemental Guidance* (U.S. EPA, 2005b) recommends that increased early-life susceptibility be assumed for carcinogens with a mutagenic mode of action, and the conclusion was made in Section 3.4 that the weight of evidence supports a mutagenic mode of action for EtO. Thus, in accordance with the *Supplemental Guidance*, the alternate assumption of increased early-life susceptibility is preferred as the basis for risk estimates in this assessment, and risk estimates derived under this preferred assumption, and intended for the application of ADAFs, are presented in Section 4.4.

Derivation of lymphoid cancer incidence estimates from mortality data

The study reported by Steenland et al. (2004) is a retrospective mortality study, and cancer incidence data are not available for lymphohematopoietic cancer [for breast cancer, a separate incidence study (Steenland et al., 2003) was available]. This limitation was addressed quantitatively in the life-table analysis by using incidence rates instead of mortality rates for the cause-specific background rates in order to derive unit risk estimates for lymphoid cancer incidence from the exposure-response modeling results from the mortality data. Although assumptions are made in this approach, as discussed in Section 4.1.1.3, the resulting incidence-based estimates are considered to be better estimates of cancer incidence risk than are the mortality-based estimates. The incidence unit risk estimate for lymphoid cancers is about 160% higher than (i.e., 2.6 times) the mortality-based estimate, which seems reasonable given the relatively high survival rates for lymphoid cancers (according to SEER data from 2006−2012 [www.seer.cancer.gov], 5-year survival rates are about 71% for NHL; 83% for chronic lymphocytic leukemia, which is the vast majority of the lymphocytic leukemias in adults; and 49% for multiple myeloma).

Possibility that not all potential cancer sites are reflected in the unit risk estimate

The two types of cancer identified in epidemiology studies of EtO exposure as being of concern to humans were also associated with EtO exposure in rodent studies (lymphohematopoietic cancers female mice and in male and female rats and mammary gland tumors in female mice). However, the rodent data suggest associations between EtO exposure and other tumor types as well, and although site

concordance across species is not generally assumed, it is possible that the NIOSH study, despite its relatively large size and long follow-up (mean length of follow-up was 26.8 years), had insufficient power to observe small increases in risk in certain other sites (not limited to those in which tumors were observed in rodents). For example, the tumor site with the highest potency estimate in both male and female mice was the lung. In the NIOSH study, one cannot rule out a small increase in the risk of lung cancer, which has a high background rate, thus making small increases difficult to detect.

4.1.4.2. *Sources of Uncertainty Stemming from the NIOSH Studies and Their Analyses*

Other sources of uncertainty arise from the epidemiologic studies and their analyses [Steenland et al. (2004);Steenland et al. (2003); Steenland and Deddens analyses in Appendix D], including the retrospective estimation of EtO exposures in the cohort and the exposure-response modeling of the epidemiologic data. Sources of uncertainty pertaining to the exposure-response modeling are discussed first for lymphoid cancer and then for breast cancer and include endpoint refinement, model selection, lag time, exposure metric, and potential confounding or modifying factors. Although these are common areas of uncertainty in epidemiologic studies, they were generally well addressed in the NIOSH studies.

4.1.4.2.1. Exposure estimation

Regarding exposure estimation, the NIOSH investigators conducted a detailed retrospective exposure assessment to estimate the individual worker exposures. They used extensive data from 18 facilities, including charcoal tube measurement data from 1976 to 1985, to develop and test a regression model for estimating EtO exposure levels associated with different jobs (exposure categories), facilities, and time periods (Hornung et al., 1994; Greife et al., 1988) [see also Section A.2.8 for more details about the development and evaluation of the regression model]. The model accounted for 85% of the variation in average EtO exposure levels in an independent set of test data (from the 18 facilities, six, with measurement data from 1979−1985, were randomly selected for model evaluation; the other 12 were used for model development). In addition, the modeled estimates were not highly biased nor biased in one direction when compared to the predictions of a panel of 11 industrial hygiene experts familiar with EtO levels in the sterilization industry.

The regression model was used to develop an exposure matrix stratified by time and exposure category for each facility in the cohort study. Detailed work history data for the individual workers were collected for the 1987 follow-up and used in conjunction with the facility-specific exposure matrices to derive cumulative exposure estimates for the individual workers (Steenland et al., 1991). Thus, although measurement data were not available for most of the time that the cohort was exposed (exposures started in 1938 for some workers), exposure levels for those early time periods could be estimated from the regression model based on variables for which historical data were available, e.g., plant- and year-specific sterilizer volume, which served as a surrogate measure for the amount of EtO used.

Another variable, calendar year, served as a surrogate for general improvements in work practices after the human health effects of EtO became a matter of concern in the late 1970s. This variable captured decreases in exposure after the late 1970s that were unaccounted for by the other variables. For the years before 1978, when human health effects of EtO were not a large concern, it was assumed that the other variables more fully accounted for exposure levels and the calendar year variable was fixed at the 1978 level. While this assumption is impossible to corroborate, it is reasonable, and the calendar year variable provides a means of dealing with general work practice improvements that are otherwise difficult to quantify.

For the extended follow-up through 1998 (Steenland et al., 2004; Steenland et al., 2003), the exposure assessment conducted in 1987 was not updated; however, additional information on the date last employed was obtained for those workers still employed and exposed at the time of the original work history collection for the plants still using EtO (25% of the cohort). It was then assumed that exposure for these workers continued until the date of last employment and that their exposure level stayed the same as that in their last job held at the time of the original data collection. When the investigators compared cumulative exposures estimated with and without the extended work histories, they found little difference because exposure levels were very low by the mid-1980s and, therefore, had little impact on cumulative exposure (Steenland et al., 2004; Steenland et al., 2003).

Estimated exposure levels in the NIOSH sterilizer plants are in the same rough range as those reported for the Swedish sterilizer plants (Hagmar et al., 1991), but it is difficult to compare more specifically because it is a wide range of exposure levels across different plants, time periods, and jobs. At the high end, two of the NIOSH plants had jobs with historical exposure levels as high as those estimated for the Mikoczy et al. (2011) study for the earliest time periods in that study (Hagmar et al., 1991), but most of the NIOSH plants had lower estimated exposure levels (Table J-4 of Appendix J).

In summary, EPA has relatively high confidence in the NIOSH exposure assessment because of the use of a well-validated regression model for developing exposure estimates for earlier time periods and jobs for which measurements were not available. Nonetheless, errors in retrospective exposure assignments are inevitable, and exposure estimation is a primary source of uncertainty in the unit risk estimates. Thus, the unit risk estimates based on the NIOSH study could over-predict or under-predict the true risks to an unknown extent.

4.1.4.2.2. Lymphoid cancer mortality analyses

Grouping of lymphohematopoietic cancer subtypes

With respect to the lymphohematopoietic cancer response, it is not clear exactly which lymphohematopoietic cancer subtypes are related to EtO exposure, so analyses were done for both lymphoid cancers and all lymphohematopoietic cancers (Steenland et al., 2004). The associations observed for all lymphohematopoietic cancers was largely driven by the lymphoid cancer responses, and

biologically, there is stronger support for an etiologic role for EtO in the development of the more closely related lymphoid cancers than in the development of the more diverse cancers in the aggregate all lymphohematopoietic cancer grouping; thus, the lymphoid cancer analysis is the preferred analysis for the lymphohematopoietic cancers. Unit risk estimates for all lymphohematopoietic cancer mortality and incidence are roughly 100 and 40% greater, respectively, than those for the lymphoid cancer.

Exposure-response modeling

Modeling the exposure-response relationship for lymphoid cancer is limited by the small number of cases (n = 53) and complicated by the supralinearity of the data (i.e., the response rises relatively rapidly and then plateaus). As discussed in Section 4.1.1.2, after considering multiple models, EPA selected the two-piece linear spline model with the knot at 1,600 ppm × days (cumulative exposure with a 15-year lag). This model was selected based on multiple objectives, such as less reliance on AIC, prioritization of models with more local fit in the low-exposure region, and weighing of biological and statistical considerations (see Section 4.1.1.2). Although the selected two-piece spline model was considered to be a reasonable approach for reflecting the exposure-response relationship at the lower end of the exposure range, which is of primary importance for the derivation of unit risk estimates, there is uncertainty regarding the exposure-response model. The model uncertainty is not as great, however, as might be inferred from the large range of unit risk estimates derived from the various models that were investigated (see Table 4-7). Models with better global fits than the selected model (based on AIC; Table 4-6), such as the linear and log-linear log cumulative exposure models and the two-piece spline models with knots at 100 ppm × days, had much steeper low-exposure slopes (see Figure 4-2) and correspondingly higher unit risk estimates (at a minimum, 7-times that of the selected model) (see Table 4-7); however, those models do not provide a good reflection of the exposure-response relationship in the low-exposure range. Conversely, the linear (ERR) model and the (sublinear) Cox regression model had much shallower low-exposure slopes (see Figure 4-2), providing poor local fits in that region as well as poor global fits (see Table 4-6).

Of the three models that appeared to have the best potential to provide a good fit to the low-exposure region (see Figure 4-2), the selected model had the highest unit risk estimate—about 2.3-times the unit risk estimate from the two-piece log-linear spline model with the knot at 1,600 ppm × days and 5.4-times the unit risk estimate from the linear regression of the categorical results. The EC_{01}s are similar between the two two-piece spline models with the knot at 1,600 ppm × days, with the one from the log-linear spline model just 8% greater than that from the selected linear spline model; however, the LEC_{01}s are more divergent (2.3-times) (see Table 4-7). This suggests that some of the difference in the unit risk estimates between the two spline models is more related to parameter estimate uncertainty for the linear spline model than to model uncertainty.

An inherent uncertainty in the two-piece spline models is in the selection of the knot, and the location of the knot is critical in defining the low-exposure slope. The model likelihood was used to provide a statistical basis for knot selection; although, as shown in Figure D-14 of Appendix D, the likelihood did not generally change appreciably over a range of possible knots. Ultimately, the two-piece linear spline model with the knot at 1,600 ppm × days was selected, based on multiple objectives (see Section 4.1.1.2), though this was not the model with the knot at the maximum likelihood (lowest AIC) but rather one with the knot at a local maximum likelihood. The two-piece linear spline with the knot at the maximum likelihood (100 ppm × days) yielded a unit risk estimate about 20-times that of the same model with the selected knot; however, the former model was rejected for not providing a good fit to the low-exposure region. Moreover, the difference in AIC between the two models is an insubstantial 0.7 units. Sensitivity analyses revealed that knots of ± 1,000 ppm × days from the selected knot of 1,600 ppm × days produced unit risk estimates that were about 3-times greater than that of the selected model for the knot at 600 ppm × days and about 50% lower than that of the selected model for the knot at 2,600 ppm × days (see Section D.3.6 of Appendix D).

Estimation of upper bounds

Another area of uncertainty in the unit risk estimate pertaining to the exposure-response modeling is the estimation of the 95% (one-sided) upper bound for the selected model. According to Langholz and Richardson (2010), the distribution of estimated parameters in nonlog-linear models (hazard functions), such as the linear spline model, is often not symmetrical (because beta is constrained so that the hazard cannot be less than 0) and profile likelihood confidence intervals are recommended as being more accurate than Wald-type intervals. Using a Wald approach yields a unit risk estimate about 40% lower than the preferred profile-likelihood-based estimate (see Section D.3.8 of Appendix D).

Exposure timing

A further area of uncertainty related to the exposure-response modeling is the lag period. The best-fitting models presented by Steenland et al. (2004) for lymphohematopoietic cancer mortality had a 15-year lag (lag periods of 0, 5, 10, 15, and 20 years were considered). A 15-year lag period means that exposures in the 15 years prior to death or the end of follow-up are not taken into account. After revisiting the issue of lag selection for the lymphoid cancer mortality data and considering model fit for cumulative exposure with different lag periods across a larger number of models than was previously evaluated with different lags, EPA again selected 15 years as the lag period to use for the exposure-response analyses (see Section D.3.2 of Appendix D). Sensitivity of the results to choice of lag period is examined in Section D.3.5 of Appendix D. In brief, for the two-piece linear spline model with the knot at 1,600 ppm × days, the unit risk estimates for different lag periods (0, 5, 10, and 20 years) ranged from about 48% less than (10-year lag) to about 190% greater than (i.e., 2.9-times) (no

lag) the estimate for the selected model (15-year lag). These alternative lags, however, all resulted in models with poor fits to the exposure-response data (*p*-values of 0.12, 0.23, 0.21, and 0.35 for inclusion of the exposure terms for lags of 0, 5, 10, and 20 years, respectively), with the possible exception of the unlagged model, which was considered less biologically realistic than the lagged models.

In addition, the analyses of the NIOSH investigators indicate that the regression coefficient for cumulative exposure might have decreased with increasing follow-up, suggesting that the higher exposure levels encountered by the workers in the more distant past might be having less of an impact on more recent risk. The regression coefficient for lymphoid cancers was 1.2×10^{-5} per ppm × day, for both sexes with a 10-year lag, in the 1987 follow-up (Stayner et al., 1993) versus 4.7×10^{-6} per ppm ×day, for both sexes with a 15-year lag, in the 1998 follow-up (see Steenland reanalyses in Appendix D). A similar decrease was found in the regression coefficient for cumulative exposure for all lymphohematopoietic cancers. The life-table analysis used in this dose-response assessment assumes exposure accrues over the full lifetime for the cumulative exposure metric. If, in fact, exposures in the distant past cease to have a meaningful impact on the risk of lymphohematopoietic cancers, this approach would tend to overestimate the unit risk. Thus, a comparison analysis was conducted to evaluate the impact of ignoring exposures over 55 years in the past in the life-table analysis. The actual value of such a cut point, if warranted, is unknown. A value less than 55 years might not be appropriate because exposures for some of the workers began in 1943, so any diminution of potency for past exposures occurring since 1943 is already reflected in the regression coefficient with follow-up through 1998, at least for those workers. The comparison analysis for lymphoid cancer yielded an LEC_{01} of 2.62×10^{-3} ppm and a unit risk estimate of 3.82 per ppm, which is about 27% less than the estimate obtained from the unrestricted life-table analysis. Because the appropriate cut point for excluding past exposures, if any, is unknown, the estimate from the full life-table analysis is preferred. In any event, the preferred estimate is not appreciably different from the estimate from the analysis which considered only the most recent 55 years of exposure in the life-table analysis.

Exposure metrics

In general, the ideal dose metric reflects the biologically relevant tissue dose of the active compound over time. For EtO and lymphoid cancer, the ideal dose metric is unknown. Several surrogate dose metrics (cumulative exposure, duration of exposure, maximum [8-hour TWA] exposure, and average exposure) were analyzed by Steenland et al. (2004), and cumulative exposure was the best predictor of mortality from lymphohematopoietic cancers. Cumulative exposure is considered a good measure of total exposure because it integrates exposure (levels) over time.

Confounding factors

The important potential modifying/confounding factors of age, sex, race, and calendar time were taken into account in the analysis, and the plants included in this cohort were specifically selected for the absence of any known confounding exposures (Stayner et al., 1993).

4.1.4.2.3. Breast cancer mortality analyses

With respect to the breast cancer mortality response (Steenland et al., 2004), the exposure-response modeling was based on 103 deaths. As for the lymphohematopoietic cancer responses, the exposure-response modeling is complicated by the supralinearity of the data. As discussed in Section 4.1.2.2, after considering multiple models, EPA selected the two-piece log-linear spline model with the knot at 700 ppm × days (cumulative exposure with a 20-year lag). This model was selected based on multiple objectives, such as prioritization of models fit to the individual-level data and with more local fit in the low-exposure region. The unit risk estimate from this model is about 4-times the estimate from the linear regression model of the categorical results. The Cox regression models with cumulative and log cumulative exposure indicated unit risk estimates substantially lower and higher, respectively, than the estimate from the selected model, but those models provided poor fits to the lower-exposure region.

For the lag period, the best-fitting model had a lag of 20 years, which was the longest lag period investigated. This is a commonly used lag period for solid tumors, which typically have longer latency periods than lymphohematopoietic cancers. The Steenland et al. (2004) analysis took into account age, race, and calendar time. Other risk factors for breast cancer could not be included in the mortality analysis, but many of these factors were considered in the breast cancer incidence study (Steenland et al., 2003), and the preferred breast cancer risk estimates are based on the breast cancer incidence data. As discussed below, however, inclusion of these breast cancer risk factors had little impact on the unit risk estimate for breast cancer incidence, suggesting that these factors are not confounding or modifying the exposure-response relationship for breast cancer.

The unit risk estimate for breast cancer mortality is slightly higher than the unit risk estimate derived for breast cancer incidence (see Section 4.1.2.3), which is contrary to expectations. Confidence is higher in the breast cancer incidence estimate, suggesting that the mortality estimate may be an overestimate of the unit risk for breast cancer mortality.

4.1.4.2.4. Breast cancer incidence analyses

Cohort selection

Steenland et al. (2003) conducted an incidence study for breast cancer; therefore, it was not necessary to calculate unit risk estimates for breast cancer incidence indirectly from the mortality data as was done for the lymphohematopoietic cancers. Further advantages to using the results from the

incidence study are that more cases were available for the exposure-response modeling (319 cases) and that the investigators were able to include data on potential confounders in the modeling for the subcohort with interviews (233 cases). The results for the subcohort with interviews are used for the primary breast cancer unit risk calculations because, in addition to including the data on potential confounders, the subcohort is considered to have full ascertainment of the breast cancer cases, whereas the full cohort for the incidence study has incomplete case ascertainment, as illustrated by the fact that death certificates were the only source of case ascertainment for 14% of the cases. Complete interviews were available for only 68% of the 7,576 women in the full incidence cohort, and thus, some potential exists for participation selection bias in the subcohort. There is, however, no basis for considering participation to be associated with breast cancer or EtO exposure, and the major reason for nonparticipation was a failure to locate (22% of full incidence cohort) and not lack of response (3% of cohort) or refusal to participate (7% of cohort). Unit risk estimates based on the full cohort were calculated for comparison with the subcohort estimates using the original linear regression analyses of the categorical results (U.S. EPA, 2006a). [Because of the preference for the subcohort with interviews, for which there was SAB concurrence (SAB, 2015), no further modeling of the continuous exposure data for the full cohort was done, as was done for the subcohort.] The unit risk estimate from the linear regression analysis of the categorical results based on the full cohort was about 40% lower than the corresponding estimate from the subcohort (U.S. EPA, 2006a).

Exposure-response modeling

As discussed in Section 4.1.2.3, after considering multiple models for the breast cancer incidence data in the subcohort with interviews, EPA selected the two-piece linear spline model with the knot at 5,750 ppm × days (cumulative exposure with a 15-year lag). The use of a two-piece spline model is not intended to imply that an abrupt change in biological response occurs at the knot but, rather, to allow description of an exposure-response relationship in which the slope of the relationship differs notably in the low-exposure versus high-exposure regions. The two-piece model is used here primarily for its representation of the low-exposure data, which are key for the derivation of unit risk estimates. The main uncertainty in the two-piece spline models is in the selection of the knot, and the location of the knot is critical in defining the low-exposure slope. The model likelihood was used to provide a statistical basis for knot selection; although, as shown in Appendix D (see Figure D-1), the likelihood did not change appreciably over a range of possible knots. Thus, because of the importance of knot selection, a sensitivity analysis was done to examine the impacts of selecting different knots (see Section D.1.7 of Appendix D). For the sensitivity analysis, the two-piece linear spline model was run with knots 1,000 ppm × days below and above the selected knot. For breast cancer incidence, this sensitivity analysis revealed little difference in the unit risk estimates, yielding estimates about 14%

higher than and 11% lower than, respectively, the estimate obtained with the selected knot of 5,750 ppm × days.

As can be seen in Figure 4-6 and Table 4-15, there is substantial variation in the low-exposure slopes and in the unit risk estimates from the different models considered. With the exception of the cumulative exposure and log cumulative exposure Cox regression models, which had both the poorest global fits (in terms of AIC) and local fits to the lower-exposure range of the models considered, the unit risk estimates have about a 35-fold range. At the lower end of that range, the linear cumulative exposure model is considered a lower bound on the likely low-exposure slope, given the overall supralinearity of the exposure-response data (as indicated by the apparent plateauing with the highest exposure group and evidenced by the strong influence of the top 5% of cumulative exposures on dampening the slope of the [cumulative exposure] Cox regression model [see Section D.1.3.1 and Figure D-4 of Appendix D]). At the upper end of the range, the square-root transformation models have less dramatic supralinearity than the log cumulative exposure model, and although the low-exposure curvature is largely imposed by the data at higher exposures, it is difficult to know to what extent, if any, the low-exposure slope is over-estimated by those models at the EC_{01}. The remaining models, the two two-piece spline models and the linear regression of the categorical results, gave similar results, spanning a 1.6-fold range. The selected model, which has the highest unit risk estimate in this narrow range, is the best-fitting (in terms of AIC) of the continuous exposure models represented in the range.

Although the reason for the observed supralinear exposure-response relationship is unknown, it is worth noting that the results of the Swedish sterilizer worker study reported by Mikoczy et al. (2011), though limited and consistent with a higher unit risk estimate for breast cancer incidence than that obtained from the NIOSH study results, support the general supralinear exposure-response relationship observed in the NIOSH study (see Section J.2.2 of Appendix J).

Estimation of upper bounds

Another area of uncertainty in the unit risk estimate pertaining to the exposure-response modeling is the estimation of the 95% (one-sided) upper bound for the selected model. According to Langholz and Richardson (2010), the distribution of estimated parameters in nonlog-linear models (hazard functions), such as the linear spline model, is often not symmetrical (because beta is constrained so that the hazard cannot be less than 0) and profile likelihood confidence intervals are recommended as being more accurate than Wald-type intervals. Using a Wald approach yields a unit risk estimate about 3% lower than the preferred profile-likelihood-based estimate (see Section D.1.10 of Appendix D).

Lag time

A further area of uncertainty related to the exposure-response modeling is the lag period. The best-fitting models presented by Steenland et al. (2003) for breast cancer incidence generally had a

15-year lag (lag periods of 0, 5, 10, 15, and 20 years were considered). A 15-year lag period means that exposures in the 15 years prior to diagnosis or the end of follow-up are not taken into account. After revisiting the issue of lag selection for the breast cancer incidence data and considering model fit for cumulative exposure with different lag periods across a larger number of models than was previously evaluated with different lags, EPA again selected 15 years as the lag period to use for the exposure-response analyses (see Section D.1.2 of Appendix D). Sensitivity of the unit risk estimates to choice of lag period is examined in Section D.1.6 of Appendix D. In brief, for the two-piece linear spline model with the knot at 5,750 ppm × days, the unit risk estimates for different lag periods (0, 5, 10, 15, and 20 years) ranged from about 35% less than (10-year lag) to about 21% greater than (20-year lag) the estimate for the selected model (15-year lag). Of these specific models, the model with the 20-year lag was a better-fitting model than the selected model, based on log-likelihood. The models for lags of 0, 5, and 10 years had *p*-values > 0.05 for inclusion of the exposure terms (0.11, 0.057, and 0.080, respectively).

Exposure metric

With respect to dose metrics for breast cancer incidence, models using duration of exposure provided better model fits than those using cumulative exposure (Steenland et al., 2003); however, duration is less useful for estimating unit risks and the cumulative exposure models also provided statistically significant fits to the data, thus the cumulative exposure metric was used for the quantitative risk estimates. Cumulative exposure is considered a good measure of total exposure because it integrates exposure (levels) over time. Models using peak (highest one-time exposure) or average exposure (cumulative exposure divided by duration) did not fit as well.

Confounding factors

Regarding potential confounders/modifying factors, analyses for the full cohort and the subcohort with interviews were adjusted for age, race, and calendar time. In addition, exposures to other chemicals in these plants were reportedly minimal. For the subcohort with interviews, a number of specific breast cancer risk factors were investigated, including body mass index, breast cancer in a first-degree relative, parity, age at menopause, age at menarche, socioeconomic status, and diet; however, only parity and breast cancer in a first-degree relative were determined to be important predictors of breast cancer and were included in the final models. Exclusion of these covariates produced very little difference in the unit risk estimates—excluding parity and both parity and breast cancer in a 1st-degree relative changed the unit risk estimate by only about 1%. Presumably, these covariates were not associated with exposure, and thus, though they were associated with breast cancer incidence, they did not confound the exposure-response analyses.

Endpoint definition

An area of uncertainty in the life-table analysis for breast cancer incidence pertains to the rates used for the cause-specific background rate. The regression coefficients presented by Steenland et al. (2003) represent invasive and in situ cases combined, where 6% of the cases are in situ, and the preferred unit risk estimates in this assessment are calculated similarly using background rates for invasive and in situ cases combined. The regression coefficients for invasive and in situ cases combined should be good approximations for regression coefficients for invasive cases alone; however, it is uncertain how well they reflect the exposure-response relationships for in situ cases alone. Diagnosed cases of in situ breast cancer would presumably be remedied and not progress to invasive breast cancer, so double-counting is unlikely to be a significant problem. Carcinoma in situ is a risk factor for invasive breast cancer; however, this observation is most likely explained by the fact that these two types of breast cancer have other breast cancer risk factors in common, some of which have been considered in the subcohort analysis. One might hypothesize that EtO exposure could cause a more rapid progression to invasive tumors; however, there is no specific evidence that this occurs. On the other hand, there is some indication that in situ cases in the incidence study might have been diagnosed at relatively low rates in comparison to the invasive cases. Steenland et al. (2003) reported that 6% of the cases in their study are in situ; according to the National Cancer Institute, however, ductal carcinoma in situ accounted for about 18% of newly diagnosed cases of breast cancer in 1998 (NCI, 2004).

There are several possible explanations for this difference. One is that it reflects differences in diagnosis with calendar time because the rate of diagnosis of carcinoma in situ has increased over time with increased use of mammography. Another is that the difference is partially a reflection of the age distribution in the cohort because the proportion of new cases diagnosed as carcinoma in situ varies by age. A third possible explanation is that the low proportion of in situ cases is at least partially a consequence of underascertainment of cases because in situ cases will not be reported on death certificates, although, even if all 20 in situ cases were in the subcohort with interviews, that would still be only 8.6% of the cases. In any event, this is a relatively minor source of uncertainty, and a comparison of the unit risk estimates using invasive + in situ breast cancer background rates and invasive-only background rates found that the estimate using only invasive breast cancer background rates was about 20% lower than the preferred estimate based on the invasive + in situ background rates.

Comparison with breast cancer mortality estimate

The unit risk estimate for breast cancer incidence is slightly lower than the unit risk estimate derived for breast cancer mortality, which is contrary to expectations. It is likely that the mortality estimate is an overestimate of the unit risk for breast cancer mortality. Confidence is higher in the breast cancer incidence estimate because it is based on more cases (233 cases versus 103 deaths), especially in

the lower-exposure region (e.g., 68 exposed cases with cumulative exposures < 2207 ppm × days versus 33 exposed deaths with cumulative exposures < 2780 ppm × days), and a better-fitting model.

4.1.4.3. *Uncertainties Associated with the Total Cancer Unit Risk Estimate*

To obtain the risk estimate for total cancer risk (6.1 per ppm, 6.1×10^{-3} per ppb, or 3.3×10^{-3} per $\mu g/m^3$), the preferred estimates for lymphoid cancer incidence and breast cancer incidence were combined (see Section 4.1.3). While there are uncertainties in the approach used to combine the individual estimates, the resulting unit risk estimate is appropriately bounded in the roughly 1.1-fold range between the sum of the unit risk estimate for lymphoid cancer plus the $0.01/EC_{01}$ estimate for breast cancer (5.98 per ppm), which provides a lower bound for the upper bound on the sum of the individual MLEs of risk and the sum of the individual 95% UCLs. Thus, any inaccuracy in the total cancer unit risk estimate resulting from the approach used to combine risk estimates across cancer types is relatively minor. Because the breast cancer component of the total cancer risk estimate applies only to females, the total cancer risk estimate is expected to overestimate the cancer risk to males somewhat (the preferred unit risk estimate for lymphoid cancer alone was 5.26 per ppm [or 2.87×10^{-3} per $\mu g/m^3$], which is about 87% of the total cancer risk estimate).

4.1.4.4. *Conclusion Regarding Uncertainties*

As discussed above, most of the sources of uncertainty inherent in the unit risk estimates, e.g., uncertainty about the lag times, have little potential quantitative impact on the values of the estimates. The primary sources of uncertainty—exposure estimation, model uncertainty, and low-dose extrapolation—have potentially larger impacts. Retrospective exposure estimation is an inevitable source of uncertainty in this type of epidemiology study; however, the NIOSH investigators put extensive effort into addressing this issue by developing a state-of-the-art regression model to estimate unknown historical exposure levels using variables, such as sterilizer size, for which historical data were available (see Section 4.1 and Section A.2.8 of Appendix A). Uncertainty pertaining to the exposure-response models is a particular concern with these data sets because the supralinear nature of the exposure-response relationships makes it more difficult to estimate the low-exposure slopes. Nonetheless, the two-piece spline models used in this assessment are considered to provide a reasonable basis for the derivation of unit risk estimates, especially for breast cancer incidence, for which more data were available in the lower-exposure range. Low-dose extrapolation is another inevitable source of uncertainty in the derivation of unit risk estimates; however, for EtO, the use of linear low-exposure extrapolation is strongly supported by the conclusion that EtO carcinogenicity has a mutagenic mode of action (see Section 3.4.3).

In summary, despite these uncertainties, the inhalation cancer unit risk estimate of 6.1 per ppm (or 3.3×10^{-3} per $\mu g/m^3$) for the total cancer risk from lymphoid cancer incidence and female breast

cancer incidence has the advantages of being based on human data from a large, high-quality epidemiologic study with individual exposure estimates for each worker. Furthermore, the breast cancer component of the risk estimate, which is almost 25% of the total cancer risk, is based on a substantial number of incident cases (233 total, the vast majority of which were in the exposure range below the knot of 5,750 ppm × days [at least 102 exposed and 164 total cases; see Table D-1 of Appendix D]). Thus, there is relatively high confidence in the total cancer unit risk estimate.

4.1.5. Summary of Unit Risk Estimates Derived from Human Data

Under the common assumption that RR is independent of age (which was validated over the age range of the cohort), an inhalation unit risk estimate for lymphoid cancer incidence of 5.26 per ppm (or 5.26×10^{-3} per ppb; 2.87×10^{-3} per μg/m^3) was calculated using a life-table analysis and a two-piece linear spline model for excess lymphoid cancer mortality from a high-quality occupational epidemiology study. Similarly, an inhalation unit risk estimate for female breast cancer incidence of 1.48 per ppm (or 1.48×10^{-3} per ppb; 8.09×10^{-4} per μg/m^3) was calculated using a life-table analysis and two-piece linear spline model for excess breast cancer incidence from the same high-quality occupational epidemiology study. The two-piece linear spline analysis was undertaken to address the supralinearity of the exposure-response data in the two data sets. Low-dose linear extrapolation was used, as warranted by the clear mutagenicity of EtO. An EC_{01} estimate of 0.0049 ppm, a LEC_{01} estimate of 0.0017 ppm, and a unit risk estimate of 6.1 per ppm (or 6.1×10^{-3} per ppb; 3.3×10^{-3} per μg/m^3) were obtained for the total cancer risk combined across both cancer types. Despite the uncertainties discussed above, this inhalation unit risk estimate has the advantages of being based on human data from a high-quality epidemiologic study with individual exposure estimates for each worker.

In the absence of data on early-life susceptibility, EPA's *Supplemental Guidance* (U.S. EPA, 2005b) recommends that increased early-life susceptibility be assumed for carcinogens with a mutagenic mode of action, and the conclusion was made in Section 3.4 that the weight of evidence supports a mutagenic mode of action for EtO. Thus, in accordance with the *Supplemental Guidance*, the alternate assumption of increased early-life susceptibility is preferred as the basis for risk estimates in this assessment, and risk estimates derived under this preferred assumption are presented in Section 4.4. Other than the use of the alternate assumption about early-life susceptibility, the approach used to derive the estimates presented in Section 4.4 is identical to the approach used for the estimates derived here in Section 4.1, and the comparisons made between various options and the issues and uncertainties discussed here in Section 4.1 are applicable to the estimates derived in Section 4.4.

4.2. INHALATION UNIT RISK DERIVED FROM LABORATORY ANIMAL DATA

4.2.1. Overall Approach

Lifetime animal cancer bioassays of inhaled EtO have been carried out in three laboratories, as described in Section 3.2. The data from these reports are presented in Tables 3-3 through 3-5. These studies have also been reviewed by the IARC (1994b) and Health Canada (2001). Health Canada calculated the ED_{05} for each data set using the benchmark dose methodology. The EOIC report (EOIC, 2001) tabulated only lymphatic tumors because they constituted the predominant risk.

The overall approach in this derivation is to find a unit risk for each of the bioassays—keeping data on males and females separate—from data on the incidence of all tumor types and then to use the maximum of these values as the summary measure of the unit risk from animal studies (i.e., the unit risk represents the most sensitive species and sex). The unit risk for the animals in these bioassays is converted to a unit risk in humans by first determining the continuous exposures in humans that are equivalent to the rodent bioassay exposures and then by assuming that the lifetime incidence in humans is equivalent to lifetime incidence in rodents, as is commonly accepted in interspecies risk extrapolations. For cross-species scaling of exposure levels (see Section 4.2.2 below), an assumption of ppm equivalence is used; thus, no interspecies conversion is needed for the exposure concentrations. Bioassay exposure levels are adjusted to equivalent continuous exposures by multiplying by (hours of exposure/24 hours) and by (5/7) for the number of days exposed per week. The unit risk in humans (risk per unit air concentration) is then assumed to be numerically equal to that in rodents (after adjustment to continuous exposures); the calculations from the rodent bioassay data are shown in Tables 3-3 through 3-5.

4.2.2. Cross-Species Scaling

In the absence of chemical-specific information, EPA's 1994 inhalation dosimetry methods (U.S. EPA, 1994) provide standard methods and default scaling factors for cross-species scaling. Under EPA's methodology, EtO would be considered a Category 2 gas because it is reactive and water soluble and has clear systemic distribution and effects. Dosimetry equations for Category 2 gases are undergoing EPA re-evaluation and are not being used at this time. For cross-species scaling of extrarespiratory effects, current practice is to treat Category 2 gases as Category 3 gases. For Category 3 gases, ppm equivalence is assumed (i.e., responses across species are equivalent on a ppm exposure basis), unless the air:blood partition coefficient for the experimental species is less than the coefficient for humans [U.S. EPA (1994), p. 4−61]. In the case of EtO, measured air:blood partition coefficients are 78 in the mouse (Fennell and Brown, 2001), 64 in the rat (Krishnan et al., 1992), and 61 in the human (Csanady et al., 2000); thus, ppm equivalence for cross-species scaling to humans can be assumed for extrarespiratory effects observed in mice and rats. The assumption of ppm equivalence is further supported by the PBPK modeling of Fennell and Brown (2001), who reported that simulated

blood AUCs for EtO after 6 hours of exposure to concentrations between 1 ppm and 100 ppm were similar for mice, rats, and humans and were linearly related to the exposure concentration (see Section 3.3.1 and Figure 3-2). This modeling was validated against measured blood EtO concentrations for rodents and humans. For Category 2 gases with respiratory effects, there is no clear guidance on an interim approach. One suggested approach is to do cross-species scaling using both Category 1 and Category 3 gas equations and then decide which is most appropriate. In this document, the preferred approach was to assume ppm equivalence was also valid for the lung tumors in mice because of the clear systemic distribution of EtO (e.g., see Section 3.1). Treating EtO as a Category 1 gas for cross-species scaling of the lung tumors would presume that the lung tumors are arising only from the immediate and direct action of EtO as it comes into first contact with the lung. In fact, some of the EtO dose contributing to lung tumors is likely attributable to recirculation of systemic EtO through the lung.

If one were to treat EtO as a Category 1 gas for the cross-species scaling of the lung tumor response as a bounding exercise, EPA's 1994 inhalation dosimetry methods present equations for estimating the $RGDR_{PU}$, i.e., the regional gas dose ratio for the pulmonary region, which acts as an adjustment factor for estimating human equivalent exposure concentrations from laboratory animal exposure concentrations (adjusted for continuous exposure) [U.S. EPA (1994), pp. 4−49 to 4−51]. These equations rely on parameters describing mass transport of the gas (EtO) in the extrathoracic and tracheobronchial regions for both the laboratory animal species (mouse) and humans. Without experimental data for these parameters, it seems reasonable to estimate $RGDR_{PU}$ using a simplified equation and the adjusted alveolar ventilation rates of Fennell and Brown (2001). Fennell and Brown (2001) adjusted the alveolar ventilation rates to reflect limited pulmonary uptake of EtO, a phenomenon commonly observed for highly water-soluble gases (Johanson and Filser, 1992). The adjusted ventilation rates were then used by Fennell and Brown (2001) in their PBPK modeling simulations, and good fits to blood concentration data were reported for both the mouse and human models. In this document, the adjusted alveolar ventilation rates were used to estimate the $RGDR_{PU}$ as follows:

$$RGDR_{PU} = (RGD_{PU})_m/(RGD_{PU})_h = (Q_{alv}/SA_{PU})_m/(Q_{alv}/SA_{PU})h, \quad (4\text{-}4)$$

where:

RGD_{PU} = regional gas dose to the pulmonary region,
Q_{alv} = (adjusted) alveolar ventilation rate,
SA_{PU} = surface area of the pulmonary region, and
the subscripts "m" and "h" denote mouse and human values.

Then, using adjusted alveolar ventilation rates from Fennell and Brown (2001) and surface area values from EPA [U.S. EPA (1994), p. 4−26],

$$RGDR_{PU} = [(0.78\ L/h)/(0.05\ m^2)]/[(255\ L/h)/(54.0\ m^2)] = 3.3. \quad (4\text{-}5)$$

Using this value for the $RGDR_{PU}$ would increase the human equivalent concentration about threefold, resulting in a decreased risk for lung tumors of about threefold, as a lower bound. The true value of the $RGDR_{PU}$ is expected to be between 1 and 3, and any adjustment to the lung tumor risks would still be expected to result in unit risk estimates roughly within the range of the rodent unit risk estimates derived later in Section 4.2 under the assumption of ppm equivalence.

4.2.3. Dose-Response Modeling Methods

In this document the following steps were used:

1. *Extract the incidence data presented in the original studies.* In order to crudely adjust for early mortality in the analysis of the NTP (1987) data, the incidence data have been corrected for a specific tumor type by eliminating the animals that died prior to the occurrence of the first tumor or prior to 52 weeks, whichever was earlier. It was not possible to make this adjustment with the other studies where data on individual animals were not available. With these exceptions, the tumor incidence data in Tables 3-3 through 3-5 match the original data.

2. *Fit the multistage model to the dose-response data using the Tox_Risk program.* The likelihood-ratio test was used to determine the lowest value of the multistage polynomial degree that provided the best fit to the data while requiring selection of the most parsimonious model. In this procedure, if a good fit to the data in the neighborhood of the POD is not obtained with the multistage model because of a nonmonotonic reduction in risk at the highest dose tested (as sometimes occurs when there is early mortality from other causes), that data point is eliminated and the model is fit again to the remaining data. Such a deletion was found necessary in two cases (mammary tumors in the NTP study and mononuclear cell leukemia in the Lynch study). The goodness-of-fit measures for the dose-response curves and the parameters derived from them are shown in Appendix G.

   In the NTP bioassay, where the individual animal data were available, a time-to-tumor analysis was undertaken to account for early mortality. The general model used in this analysis is the multistage Weibull model:

$$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k) \times (t - t_0)^z], \quad (4\text{-}6)$$

   where P(d,t) represents the probability of a tumor by age t (in bioassay weeks) for dose d (i.e., human equivalent exposure), and the parameter ranges are restricted as follows: $z \geq 1$,

$t_0 \geq 0$, and $q_i \geq 0$ for i = 0, 1, ..., k. The parameter t_0 represents the time between when a potentially fatal tumor becomes observable and when it causes death. The analyses were conducted using the computer software Tox_Risk version 3.5, which is based on methods developed by Krewski et al. (1983). Parameters are estimated in Tox_Risk using the method of maximum likelihood.

Tumor types can be categorized by tumor context as either fatal or incidental. Incidental tumors are those tumors thought not to have caused the death of an animal, whereas fatal tumors are thought to have resulted in animal death. Tumors at all sites were treated as incidental (although it was recognized that this may not have been the case, the experimental data are not detailed enough to conclude otherwise). The parameter t_0 was set equal to 0 because there were insufficient data to reliably estimate it.

The likelihood-ratio test was used to determine the lowest value of the multistage polynomial degree k that provided the best fit to the data while requiring selection of the most parsimonious model. The one-stage Weibull (i.e., k = 1) was determined to be the most optimal value for all the tumor types analyzed.

3. *Select the POD and calculate the unit risk for each tumor site.* The effective concentration that causes a 10% extra risk for tumor incidence, EC_{10}, and the 95% lower bound of that concentration, LEC_{10}, are derived from the dose-response model. The LEC_{10} is then used as the POD for a linear low-dose extrapolation, and the unit risk is calculated as $0.1/LEC_{10}$. This is the procedure specified in the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) for agents such as EtO that have direct mutagenic activity. See Section 3.4 for a discussion of the mode of action for EtO. Tables 3-3 through 3-5 present the unit risk estimates for the individual tumor sites in each bioassay.

4. *Develop a unit risk estimate based on the incidence of all tumors combined.* This method assumes that occurrences of tumors at multiple sites are independent and, further, that the risk estimate for each tumor type is normally distributed. Then, at a given exposure level, the MLEs of extra risk due to each tumor type are added to obtain the MLE of total cancer risk. The variances corresponding to each tumor type are added to give the variance associated with the sum of the MLEs. The one-sided 95% UCL of the MLE for the combined risk is then calculated as:

$$95\%\ UCL = MLE + 1.645(SE), \qquad (4\text{-}7)$$

where SE is the standard error and is the square root of the summed variance. (Note that as a precursor to this step, when Tox_Risk is used to fit the incidence of a single tumor type, it provides the MLE and 95% UCL of extra risk at a specific dose. The standard error in the MLE is determined using the above formula). The calculation is repeated for a few exposure levels, and the exposure yielding a value of 0.1 for the upper bound on extra risk is determined by interpolation. The unit risk is then the slope of the linear extrapolation from this POD. The results are given in Table 4-18.

Table 4-18. Upper-bound unit risks (per µg/m^3) obtained by combining tumor sites

Combination method[a]	NTP (1987) female mouse	Lynch et al. (1984b), Lynch et al. (1984a) male rat	Snellings et al. (1984)[b]	
			Male rat	Female rat
UCL 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}	–	–	–

[a]Unit risk in these methods is the slope of the straight line extrapolation from a point of departure at the dose corresponding to a value of 0.1 for the 95% upper confidence bound on total extra risk.
[b]Includes data on brain tumors from the analysis by Garman et al. (1985). See Table 3-3.
[c]UCL = 95% upper confidence bound. At a given dose, the MLE of the combined extra risk was determined by summing the MLE of risk due to each tumor type. The variance associated with this value was determined by summing over the variances due to each tumor type.
[d]Sum of values in last column of Tables 3-1 through 3-3.

4.2.4. Description of Laboratory Animal Studies

NTP (1987) exposed male and female B6C3F$_1$ mice to concentrations of 0, 50, and 100 ppm for 6 hours per day, 5 days per week, for 102 weeks. An elevated incidence of lung carcinomas was found in males, and elevated lung carcinomas, malignant lymphomas, uterine adenocarcinomas, and mammary carcinomas were found in females. These data are shown in Table 3-3.

Lynch et al. (Lynch et al., 1984a; Lynch et al., 1984b) exposed male F344 rats to 0, 50, and 100 ppm for 7 hours per day, 5 days per week, for 2 years. They found excess incidence of tumors at three sites: mononuclear cell leukemia in the spleen, testicular peritoneal mesothelioma, and brain glioma. In this study the survival in the high-dose group (19%) was less than that of controls (49%), which reduced the incidence of leukemias. In the animals in the high-dose group that survived to the termination of the experiment, the incidence of leukemias was statistically significantly higher than for controls ($p < 0.01$). The incidence data are shown in Table 3-4, uncorrected for the high-dose-group mortality. If the individual animal data were available to perform the correction, the incidence would be higher. Therefore, using these data results in an underestimate of risk.

Snellings et al. (1984) exposed male and female F344 rats to 0, 10, 33, and 100 ppm for 6 hours per day, 5 days per week, for 2 years and described their results for all sites except the brain. In two subsequent publications for the same study, (Garman et al., 1986, 1985) described the development of brain tumors in a different set of F344 rats. The Snellings et al. (1984) publication reported an elevated incidence of splenic mononuclear cell leukemia and peritoneal mesothelioma in males and an elevated incidence of splenic mononuclear cell leukemia in females. The mortality was higher in the 100-ppm groups than the other three groups for both males and females. The incidences in the animals killed

after 24 months in Snellings et al. (1984) are shown in Table 3-5. Table 3-5 also presents the brain tumor incidence data for male and female rats from the (Garman et al., 1986, 1985) publications. The brain tumor incidence was lower than that of the other tumors, particularly the splenic mononuclear cell leukemias.

4.2.5. Results of Data Analysis of Laboratory Animal Studies

The unit risks calculated from the individual site-sex-bioassay data sets are presented in Tables 3-3 through 3-5. The highest unit risk of any individual site is 3.23×10^{-5} per $\mu g/m^3$, which is for mononuclear cell leukemia in the female rats of the Snellings et al. (1984) study.

Table 4-19 presents the results of the time-to-tumor method applied to the individual animals in the NTP bioassay, compared with the results from the dose group incidence data in Table 3-3. This comparison was done for each tumor type separately. The time-to-tumor method of analyzing the individual animals results in generally higher unit risk estimates than does the analysis of dose group data, as shown in Table 4-19. The ratio is not large (less than 2.2) across the tumor types. (In the case of mammary tumors this ratio is actually less than 1. It must be noted that the incidence at the highest dose [where the incidence was substantially less than at the intermediate dose] was deleted from the analysis of grouped data, whereas it was retained in the time-to-tumor analysis. Therefore, the comparison for the mammary tumors is not a strictly valid comparison of methods.) The results also show the extent to which a time-to-tumor analysis of individual animal data increases the risk estimated from data on dose groups. It is expected that if individual animal data were available for the Lynch et al. (1984a) and Lynch et al. (1984b) and the Snellings et al. (1984) bioassays, then the time-to-tumor analysis would also result in higher estimates because both those studies also showed early mortality in the highest dose group.

The results of combining tumor types are summarized in Table 4-18. The sums of the individual unit risks tabulated in Tables 3-3 to 3-5 are given in the second row of Table 4-18.

Table 4-19. Unit risk values from multistage Weibull[a] time-to-tumor modeling of mouse tumor incidence in the NTP (1987) study

Tumor type	Unit risk, 0.1/LEC_{10} (per µg/m^3) from time-to-tumor analysis	Unit risk, 0.1/LEC_{10} (per µg/m^3) (Table 3-3)[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

[a]$P(d,t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, where d is inhaled ethylene oxide concentration in ppm, *t* is weeks until death with tumor. In all cases, $k = 1$ provided the optimal model.
[b]Incidence data modeled using multistage model without taking time to tumor into account.

Note that, as expected, they are greater than the unit risks computed from the upper bound on the sum of risks for all data sets except for the Lynch et al. (Lynch et al., 1984a; Lynch et al., 1984b) data. The reason for this exception is not known, but the differences are small. It is likely that the problem arises from the methodology used to combine the risks across tumor sites. In an attempt to be consistent with the new two-step methodology (i.e., modeling in the observable range to a POD and then doing a linear extrapolation to zero extra risk at zero exposure), the exposure concentration at which the sum of the independent tumor site risks yielded a 95% upper bound on 10% extra risk was estimated and used as the POD. Summing risks in this way results in a POD for the combined tumor risk that is different (lower) than the points of departure for each individual tumor site risk. Thus, the risk estimate for the sum is not strictly comparable to the individual risks that constitute it. These tumor-site-specific risks were based on points of departure individually calculated to correspond with a 10% extra risk. In any event, adding the upper bound risks of individual tumor sites should overestimate the upper bound of the sum, and the latter is the preferred measure of the total cancer risk because it avoids the overestimate. However, for the exceptional Lynch et al. (Lynch et al., 1984a; Lynch et al., 1984b) data, the sum of upper bounds, 3.66×10^{-5} per µg/m^3, is already an overestimate of the total risk, and this value is preferred over the anomalously high value of 4.17×10^{-5} per µg/m^3 corresponding to the upper bound on the sum of risks. The latter value is considered to be an excessive overestimate and is therefore not carried over into the summary Table 4-20. For the Snellings et al. (1984) data sets, the upper confidence bound on the sum of risks is used in the summary Table 4-20. The results of the sum-of-risks

calculations on the NTP bioassay time-to-tumor data are included in the third row of Table 4-18. The estimate for the NTP female mice is 4.55×10^{-5} per μg/m^3, which is higher than the other two measures of total tumor risk in that bioassay. This value is preferable to the other measures because it utilizes the individual animal data available for that bioassay.

Summary of results. The summary of unit risks from the five data sets is shown in Table 4-20. The data set giving the highest risk (4.55×10^{-5} per μg/m^3) is the NTP (1987) data on combined tumors in female mice. The other values are within about a factor of 2 of the highest value.

Table 4-20. Summary of unit risk estimates (per μg/m^3) in animal bioassays

Assay	Males	Females
NTP (1987), B6C3F$_1$ mice	3.01×10^{-5} [a]	4.55×10^{-5} [b]
Lynch et al. (1984b), Lynch et al. (1984a), F344 rats	3.66×10^{-5} [c]	–
Snellings et al. (1984), F344 rats	2.19×10^{-5} [d]	3.37×10^{-5} [d]

[a]From time-to-tumor analysis of lung adenomas and carcinomas, Table 4-19.
[b]Upper bound on sum of risks from the time-to-tumor analysis of the NTP data, Table 4-18.
[c]Sum of (upper bound) unit risks (see text for explanation), Table 4-18.
[d]Upper bound on sum of risks, Table 4-18.

The largest sources of uncertainty in the rodent-based unit risk estimates for EtO are interspecies extrapolation and low-dose extrapolation. Though the unit risk estimates from mouse and rat data are similar (Table 4-20), the different EtO-associated cancer sites across rodent species demonstrate species differences that are not understood and illustrate the existence of interspecies uncertainty that extends to the extrapolation of rodent-based cancer risk estimates to humans. Regarding low-dose extrapolation, the clear evidence of EtO mutagenicity supports the linear low-exposure extrapolation that was used (U.S. EPA, 2005a) (see also Section 4.1.4.1). Additional uncertainties arise from the dose-response modeling of the data in the observable range and the application of the results to potentially sensitive human populations.

4.3. SUMMARY OF INHALATION UNIT RISK ESTIMATES—NOT ACCOUNTING FOR ASSUMED INCREASED EARLY-LIFE SUSCEPTIBILITY

For both humans and laboratory animals, tumors occur at multiple sites. In humans, there was a combination of tumors having lymphohematopoietic, in particular lymphoid, origins in both sexes and breast cancer in females, and, in rodents, lymphohematopoietic tumors, mammary carcinomas, and tumors of other sites were observed. From human data, an extra cancer unit risk estimate of 2.87×10^{-3} per μg/m^3 (5.26 per ppm) was calculated for lymphoid cancer incidence, and a unit risk

estimate of 8.09×10^{-4} per μg/m^3 (1.48 per ppm) was calculated for breast cancer incidence in females. The total extra cancer unit risk estimate was 3.3×10^{-3} per μg/m^3 (6.1 per ppm) for both cancer types combined (EC_{01} = 0.0049 ppm; LEC_{01} = 0.0017 ppm). Unit risk estimates derived from the three chronic rodent bioassays for EtO ranged from 2.2×10^{-5} per μg/m^3 to 4.6×10^{-5} per μg/m^3, roughly two orders of magnitude lower than the estimates based on human data.

Adequate human data, if available, are considered to provide a more appropriate basis than rodent data for estimating human risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from rodents to humans are avoided. Although there is a sizeable difference between the rodent-based and the human-based estimates, the human data are from a large, high-quality study, with EtO exposure estimates for the individual workers and little reported exposure to chemicals other than EtO. Therefore, the total extra cancer unit risk estimate of 3.3×10^{-3} per μg/m^3 (6.1 per ppm) calculated for lymphoid cancers and breast cancer combined is the preferred estimate of those estimates, not taking assumed increased early-life susceptibility into account (estimates accounting for assumed increased early-life susceptibility are presented in Section 4.4). The unit risk estimate is intended to be an upper bound on cancer risk for use with exposures below the POD (i.e., the LEC_{01}). The unit risk estimate should not generally be used above the POD; however, in the case of this total extra cancer unit risk, which is based on cancer type-specific unit risk estimates from two linear models, the estimate should be valid for exposures up to about 0.021 ppm (38 μg/m^3), which is the minimum of the limits for the lymphoid cancer unit risk estimate (0.021 ppm; see Section 4.1.1.2) and the breast cancer unit risk estimate (0.075 ppm; see Section 4.1.2.3) dictated by the knot locations.

Because a mutagenic mode of action for EtO carcinogenicity (see Section 3.3.2) is "sufficiently supported in (laboratory) animals" and "relevant to humans," and as there are no chemical-specific data to evaluate the differences between adults and children, increased early-life susceptibility should be assumed and, if there is early-life exposure, the age-dependent adjustment factors (ADAFs) should be applied, as appropriate, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b) see Section 4.4 below for more details on the application of ADAFs).

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 (see 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), 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 of 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 data sets 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.[42] 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 mode of action (see 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. Incidence estimates are preferred over mortality estimates; thus, adult-exposure-only unit risk estimates were derived for cancer incidence for both lymphoid and breast cancers. Alternate estimates were not derived for all lymphohematopoietic cancers because lymphoid cancer was the preferred lymphohematopoietic endpoint (see Section 4.1.1.2). For each cancer endpoint, the same exposure-response model was used as that which was selected for the unit risk estimates in Section 4.1 (i.e., two-piece linear spline model with the knot at 1,600 ppm × days for lymphoid cancer and two-piece linear spline model with the knot at 5,750 ppm × days for breast cancer). The results are presented in Table 4-21 along with the unit risk

[42]In this assessment, ***adult-exposure-only unit risk estimates*** refer to estimates derived from the life-table analysis assuming exposure only for ages ≥16 years. The adult-exposure-only unit risk estimates are merely intermediate values in the calculation of adult-based unit risk estimates and should not be used in any risk calculations. ***Adult-based unit risk estimates*** refer to estimates derived after rescaling the adult-exposure-only unit risk estimates to a (70-year) lifetime, as described later in Section 4.4. The adult-based unit risk estimates are intended to be used in ADAF calculations (U.S. EPA, 2005b) for the computation of extra risk estimates for specific exposure scenarios. Note that the unit risk estimates in this section, which are derived under an assumption of increased early-life susceptibility, supersede those that were derived in Section 4.1 under the assumption that RR is independent of age.

estimates derived assuming that RR was independent of age for all ages (see Section 4.1) for comparison. As can be seen in Table 4-21, the unit risk estimates for adult-only exposures range from about 67 to about 70% of the unit risk estimates derived under the assumption of age independence across all ages.

Table 4-21. EC_{01}, LEC_{01}, and unit risk estimates for adult-only exposures*

Cancer response	EC_{01} (ppm)	LEC_{01} (ppm)	Adult-exposure-only unit risk estimate[a] (per ppm)	Lifetime-exposure unit risk estimate under assumption of age independence[b] (per ppm)
Lymphoid cancer incidence (both sexes)	0.0107	0.00271	3.69[c]	5.26[c]
Breast cancer incidence (females)	0.0206	0.0101	0.99	1.48[c]

*These are intermediate values. See Table 4-24 below for the final adult-based cancer-type-specific unit risk estimates.
[a]Unit risk estimate = $0.01/LEC_{01}$.
[b]From Tables 4-7 and 4-15 of Section 4.1.
[c]For unit risk estimates above 1, one can convert to risk per ppb (e.g., 5.26 per ppm = 5.26×10^{-3} per ppb) to obtain risk estimates below 1.

According to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) cancer risk estimates are intended to reflect total cancer risk, not site-specific cancer risk; therefore, an additional calculation was made to estimate the combined risk for (incident) lymphoid and breast cancers from adult-only exposures, because females would be at risk for both cancer types. Assuming that the tumor types are independent and that the risk estimates are approximately normally distributed, this calculation can be made as described in Section 4.1.3. First, the $0.01/EC_{01}$ for the total cancer risk (i.e., lymphoid cancer incidence + breast cancer incidence) from adult-only exposure was estimated, as summarized in Table 4-22.

Then, a unit risk estimate for the total cancer risk from adult-only exposure was derived, as shown in Table 4−23.

Table 4-22. Calculation of EC_{01} for total cancer (incidence) risk from adult-only exposure*

Cancer type	EC_{01} (ppm)	$0.01/EC_{01}$ (per ppm)
Lymphoid	0.0107	0.935
Breast	0.0206	0.485
Total[a]	--	1.42

*These are intermediate values. See Table 4-25 below for the final adult-based cancer-type-specific $0.01/EC_{01}$ estimates.
[a]The total $0.01/EC_{01}$ value equals the sum of the individual $0.01/EC_{01}$ values.

Table 4-23. Calculation of total cancer unit risk estimate from adult-only exposure*

Cancer type	Adult-exposure-only unit risk estimate (per ppm)	$0.01/EC_{01}$ (per ppm)	SE[a] (per ppm)	Variance	Adult-exposure-only total cancer unit risk estimate (per ppm)
Lymphoid	3.69	0.935	1.67	2.805	--
Breast	0.990	0.485	0.307	0.094	--
Total	--	1.42	(1.70)[b]	2.90	4.22[c]

*These are intermediate values. See Table 4-24 below for the final adult-based cancer-type-specific unit risk estimates.
[a]SE = (unit risk – $0.01/EC_{01}$)/1.645.
[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 sum of the SEs.
[c]Total cancer unit risk = 1.42 + 1.645 × 1.70.

Thus, the total cancer unit risk estimate from adult-only exposure is 4.22 per ppm (2.31×10^{-3} per $\mu g/m^3$). While there are uncertainties regarding the assumption of a normal distribution of risk estimates, the resulting unit risk estimate is appropriately bounded in the roughly threefold range between estimates based on the sum of the individual MLEs (i.e., 1.42) and the sum of the individual 95% UCLs (i.e., unit risk estimates, 4.68), or more precisely in this case, between the largest individual unit risk estimate, i.e., that for lymphoid cancer, which has most of the variance, plus the $0.01/EC_{01}$ for breast cancer (4.18) and the sum of the unit risk estimates (4.68), and thus, any inaccuracy in the total cancer risk estimate resulting from the approach used to combine risk estimates across cancer types is relatively minor.

When EPA derives unit risk estimates from rodent bioassay data, there is a blurring of the distinction between lifetime and adult-only exposures because the relative amount of time that a rodent

spends as a juvenile is negligible (<8%) compared to its lifespan. [According to EPA's *Supplemental Guidance*, puberty begins around 5−7 weeks of age in rats and around 4−6 weeks in mice (U.S. EPA, 2005b)]. Thus, when exposure in a rodent is initiated at 5−8 weeks, as in the typical rodent bioassay, and the bioassay is terminated after 104 weeks of exposure, the unit risk estimate derived from the resulting cancer incidence data is considered a unit risk estimate from lifetime exposure, except when the ADAFs were formulated and are applied, in which case the same estimate is considered to apply to adult-only exposure. Yet, when adult exposures are considered in the application of ADAFs, the adult-exposure-only unit risk estimate is prorated over the full default (average) human lifespan of 70 years, presumably because that is how adult exposures are treated when a unit risk estimate calculated in the same manner from the same bioassay exposure paradigm is taken as a lifetime unit risk estimate.

However, in humans, a greater proportion of time is spent in childhood (e.g., 16 of 70 years = 3%), and the distinction between lifetime exposure and adult-only exposure cannot be ignored when human data are used as the basis for the unit risk estimates. Thus, as described above, adult-exposure-only unit risk estimates were calculated distinct from the lifetime estimates that were derived in Section 4.1 under the assumption of age independence for all ages. In addition, the adult-exposure-only unit risk estimates need to be rescaled to a 70-year lifespan in order to be used in the ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios in the standard manner, which includes prorating even adult-based unit risk estimates over 70 years. Thus, the adult-exposure-only unit risk estimates are multiplied by 70/54 to rescale the 54-year adult period of the 70-year default lifespan to 70 years. Then, for example, if a risk estimate were calculated for a less-than-lifetime exposure scenario involving exposure only for the full adult period of 54 years, the rescaled unit risk estimate would be multiplied by 54/70 in the standard calculation and the adult-exposure-only unit risk estimate would be appropriately reproduced. Without rescaling the adult-exposure-only unit risk estimates, the example calculation just described for exposure only for the full adult period of 54 years would result in a risk estimate 77% (i.e., 54/70) of that obtained directly from the adult-exposure-only unit risk estimates, which would be illogical. The rescaled adult-based unit risk estimates for use in ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios are presented in Table 4-24. An LEC_{01} estimate for adult-based total cancer risk can be calculated as 0.01/(adult-based unit risk estimate) = 1.8×10^{-3} ppm (3.3 μg/m^3), and an EC_{01} estimate for adult-based total cancer risk can be calculated as 0.01/(the $0.01/EC_{01}$ for total cancer) × 54/70 = 5.4×10^{-3} ppm (9.9 μg/m^3).

Table 4-24. Adult-based unit risk estimates for use in ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios

Cancer response	Adult-based unit risk estimate (per ppm)	Adult-based unit risk estimate (per μg/m^3)
Preferred models[a]		
Lymphoid cancer incidence	4.78[b]	2.61×10^{-3}
Breast cancer incidence	1.28[b]	7.01×10^{-4}
Total cancer incidence	**5.47[b]**	**2.99×10^{-3}**

[a]Two-piece linear spline model with knot at 1,600 ppm × days for lymphoid cancer incidence; two-piece linear spline model with knot at 5,750 ppm × days for breast cancer incidence.
[b]For unit risk estimates above 1, convert to risk per ppb (e.g., 5.47 per ppm = 5.47×10^{-3} per ppb).

An example calculation illustrating the application of the ADAFs to the human-data-derived adult-based (rescaled as discussed above) unit risk estimate for EtO for a lifetime exposure scenario is presented below. For inhalation exposures, assuming ppm equivalence across age groups, i.e., equivalent risk from equivalent exposure levels, independent of body size, the ADAF calculation is fairly straightforward. Thus, the ADAF-adjusted lifetime total-cancer unit risk estimate is calculated as follows:

Total cancer risk from exposure to constant EtO exposure level of 1 μg/m^3 from ages 0−70 years:

Age group	ADAF	Unit risk (per μg/m^3)	Exposure conc (μg/m^3)	Duration adjustment	Partial risk
0 to <2 years	10	2.99×10^{-3}	1	2 years/70 years	8.54×10^{-4}
2 to <16 years	3	2.99×10^{-3}	1	14 years/70 years	1.79×10^{-3}
≥16 years	1	2.99×10^{-3}	1	54 years/70 years	2.31×10^{-3}
				total lifetime risk =	4.96×10^{-3}

The partial risk for each age group is the product of the values in columns 2−5 [e.g., $10 \times (2.99 \times 10^{-3}) \times 1 \times 2/70 = 8.54 \times 10^{-4}$], and the total risk is the sum of the partial risks.

This 70-year risk estimate for a constant exposure of 1 μg/m^3 is equivalent to a **lifetime unit risk estimate of 5.0×10^{-3} per μg/m^3** (9.1 per ppm, or 9.1×10^{-3} per ppb), adjusted for potential increased early-life susceptibility, assuming a 70-year lifetime and constant exposure across age groups. Note that because of the use of the rescaled adult-based unit risk estimate, the partial risk for the ≥16 years age

group is the same as would be obtained for a 1 μg/m^3 constant exposure directly from the total cancer adult-exposure-only unit risk estimate of 2.31×10^{-3} per μg/m^3 that was presented above, as it should be.

In addition to the uncertainties discussed above for the inhalation unit risk estimate, there are uncertainties in the application of ADAFs to adjust for potential increased early-life susceptibility. The ADAFs reflect an expectation of increased risk from early-life exposure to carcinogens with a mutagenic mode of action (U.S. EPA, 2005b), but they are general adjustment factors and are not specific to EtO. With respect to the breast cancer estimates, for example, evidence suggests that puberty/early adulthood is a particularly susceptible life stage for breast cancer induction (U.S. EPA, 2005b; Russo and Russo, 1999); however, EPA has not, at this time, developed alternate ADAFs to reflect such a pattern of increased early-life susceptibility, and there is currently no EPA guidance on an alternate approach for adjusting for early-life susceptibility to potential breast carcinogens.

4.5. INHALATION UNIT RISK ESTIMATES—CONCLUSIONS

For both humans and laboratory animals, tumors occur at multiple sites. In humans, there was a combination of tumors having lymphohematopoietic, in particular lymphoid, origins in both sexes and breast cancer in females, and, in rodents, lymphohematopoietic tumors, mammary carcinomas, and tumors of other sites were observed. From human data, an extra cancer unit risk estimate of 2.88×10^{-3} per μg/m^3 (5.26×10^{-3} per ppb) was calculated for lymphoid cancer incidence, and a unit risk estimate of 8.10×10^{-4} per μg/m^3 (1.48×10^{-3} per ppb) was calculated for breast cancer incidence in females, under the assumption that RR is independent of age for all ages (see Section 4.1). The total extra cancer unit risk estimate was 3.31×10^{-3} per μg/m^3 (6.06×10^{-3} per ppb) for both cancer types combined). Unit risk estimates derived from the three chronic rodent bioassays for EtO ranged from 2.2×10^{-5} per μg/m^3 to 4.6×10^{-5} per μg/m^3, roughly two orders of magnitude lower than the estimates based on human data.

Because a mutagenic mode of action for EtO carcinogenicity (see Section 3.4.1) is "sufficiently supported in (laboratory) animals" and "relevant to humans," and as there are no chemical-specific data to evaluate the differences between adults and children, increased early-life susceptibility should be assumed, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b). This assumption of increased early-life susceptibility supersedes the assumption of age independence under which the human-data-based estimates presented in the previous paragraph were derived. Thus, as described in Section 4.4, adult-exposure-only (i.e., ages ≥16 years) unit risk estimates were calculated from the human data under an alternate assumption that RR is independent of age for adults, which represent the life stage for which the data upon which the exposure-response modeling was conducted pertain. These adult-exposure-only unit risk estimates were then rescaled to a 70-year basis to derive adult-based unit risk estimates for use in the standard ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios. The resulting adult-based unit risk estimates were 2.61×10^{-3} per

$\mu g/m^3$ (4.78×10^{-3} per ppb) for lymphoid cancer incidence and 7.01×10^{-4} per $\mu g/m^3$ (1.28×10^{-3} per ppb) for breast cancer incidence in females. The adult-based total extra cancer unit risk estimate for use in ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios was 2.99×10^{-3} per $\mu g/m^3$ (5.47×10^{-3} per ppb) for both cancer types combined.

When using the adult-based unit risk estimates to estimate extra cancer risks for a given exposure scenario, the ADAFs should be applied, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b). Applying the ADAFs to obtain a full lifetime unit risk estimate yields

$$5.47/\text{ppm} \times [(10 \times 2 \text{ years}/70 \text{ years}) + (3 \times 14/70) + (1 \times 54/70)] = 9.08/\text{ppm} = 4.96 \times 10^{-3}/(\mu g/m^3). \quad (4\text{-}8)$$

Applying the ADAFs to the unit risk estimates derived from the three chronic rodent bioassays for EtO yields estimates ranging from 3.7×10^{-5} per $\mu g/m^3$ to 7.6×10^{-5} per $\mu g/m^3$, again roughly two orders of magnitude lower than the estimate based on human data.

Adequate human data, if available, are considered to provide a more appropriate basis than rodent data for estimating human risks (U.S. EPA, 2005a), primarily because uncertainties in extrapolating quantitative risks from rodents to humans are avoided. Although there is a sizeable difference between the rodent-based and the human-based estimates, the human data are from a large, high-quality study, with EtO exposure estimates for the individual workers and little reported exposure to chemicals other than EtO. Therefore, the human-based **full lifetime total extra cancer unit risk estimate of 5.0×10^{-3} per $\mu g/m^3$ (9.1×10^{-3} per ppb)** calculated for lymphoid cancers and breast cancer combined and applying the ADAFs is the preferred lifetime unit risk estimate.[43] For less-than-lifetime exposure scenarios, the human-data-derived (rescaled) adult-based unit risk estimate of 3.0×10^{-3} per $\mu g/m^3$ (5.5×10^{-3} per ppb) should be used, in conjunction with the ADAFs if early-life exposures occur.

Although there are uncertainties in this unit risk estimate—primarily related to exposure misclassification, model uncertainty, and low-dose extrapolation, as discussed in Section 4.1.4—confidence in the unit risk estimate is relatively high. First, there is high confidence in the hazard characterization of EtO as "carcinogenic to humans," which is based on strong epidemiological evidence supplemented by other lines of evidence, such as genotoxicity in both rodents

[43]Technically, this unit risk estimate reflects the total (upper bound) cancer risk to females and not to the general population because the breast cancer risk applies only to females. As a practical matter for regulatory purposes, however, females comprise roughly half the general population, and this unit risk estimate enables risk managers to evaluate the individual risk for this substantial population group. For the purposes of estimating numbers of cancer cases attributable to specific exposure levels, e.g., for benefits analyses, it would be more appropriate to use the cancer-specific unit risk estimates (or central tendency estimates), taking sex into account.

and humans (see Section 3.5.1). Second, the unit risk estimate is based on human data from a large, high-quality epidemiology study with individual worker exposures estimated using a high-quality regression model (see Section 4.1 and Section A.2.8 of Appendix A). Finally, the use of linear low-exposure extrapolation is strongly supported by the conclusion that EtO carcinogenicity has a mutagenic mode of action (see Section 3.4.1).

Confidence in the unit risk estimate is particularly high for the breast cancer component, which is based on over 200 incident cases for which the investigators had information on other potential breast cancer risk factors (see Section 4.1.2.3). The selected model for the breast cancer incidence data provided a good global fit as well as a good local fit in the lower exposure range of greatest relevance for the derivation of a unit risk estimate. The actual unit risk might be higher or lower; however, considering the continuous-exposure linear model as a lower bound for the supralinear exposure-response relationship suggests that while a unit risk estimate for breast cancer incidence that is up to fourfold lower is plausible, unit risk estimates lower than that are considered unlikely from the available data. Sensitivity analyses for lag time, inclusion of covariates, knot, upper-bound estimation approach, use of the full incidence cohort, and inclusion of only invasive cancers for the breast cancer background rates in the life-table indicate that the unit risk estimate is not highly influenced by these factors, with comparison unit risk estimates differing by at most 40% (see Section 4.1.2.3).

There is lower confidence in the lymphoid cancer component of the unit risk estimate because it is based on fewer events (53 lymphoid cancer deaths); incidence risk was estimated from mortality data; and the exposure-response relationship is exceedingly supralinear (see Figure 4-1), complicating the exposure-response modeling and model selection to a greater extent than for breast cancer incidence. The selected model had a *p*-value that minimally exceeded 0.05 ($p = 0.07$) and it was not the best-fitting spline model in terms of AIC for knot selection; however, its AIC was within two units of the lowest AIC of all the models considered and the selection of this model was consistent with the model selection objectives for this assessment (see Section 4.1.1.2), including prioritizing models providing good local fit in the lower-exposure region. The actual unit risk might be higher or lower than that from the selected model, and there were no clear upper or lower bounds for the apparent exposure-response relationship provided by other models. Sensitivity analyses for lag time, knot, and upper-bound estimation approach, indicate that the unit risk estimate for lymphoid cancer is more influenced by these factors than was the estimate for breast cancer incidence. Comparison unit risk estimates from the sensitivity analyses ranged from about 50% of the preferred unit risk estimate to about 3-times that estimate (see Section 4.1.1.3). While there is lower confidence in the lymphoid cancer unit risk estimate than in the breast cancer unit risk estimate, the lymphoid cancer estimate is considered a reasonable estimate from the available data, and overall, there is relatively high confidence in the total cancer unit risk estimate.

The unit risk estimate is intended to be an upper bound on cancer risk for use with exposures below the POD (i.e., the LEC_{01}). The unit risk estimate should not generally be used above the POD; however, in the case of this total extra cancer unit risk, which is based on cancer type-specific unit risk estimates from two linear models, the estimate should be valid for exposures up to about 0.021 ppm (38 $\mu g/m^3$), which is the minimum of the limits for the lymphoid cancer unit risk estimate (0.021 ppm: see Section 4.1.1.2) and the breast cancer unit risk estimate (0.075 ppm; see Section 4.1.2.3) dictated by the knot locations. (See Section 4.7 for risk estimates based on occupational exposure scenarios.)

Using the above full lifetime unit risk estimate of 9.1×10^{-3} per ppb (5.0×10^{-3} per $\mu g/m^3$), the (lower bound) lifetime chronic exposure level of EtO corresponding to an increased cancer risk of 10^{-6} can be estimated as follows:

$$(10^{-6})/(9.1/\text{ppm}) = 1.1 \times 10^{-7} \text{ ppm} = 1.1 \times 10^{-4} \text{ ppb} = 2 \times 10^{-4} \ \mu g/m^3. \qquad (4\text{-}9)$$

The inhalation unit risk estimate presented above, which is calculated based on a linear extrapolation from the POD (LEC_{01}), is expected to provide an upper bound on the risk of cancer incidence. For some applications, however, estimates of "central tendency" for the risk below the POD are desired. Thus, adult-based extra risk estimates per ppm for the cancer incidence responses based on linear extrapolation from the adult-exposure-only EC_{01} (i.e., $0.01/EC_{01}$), and rescaled to a 70-year basis for use in ADAF calculations and risk estimate calculations involving less-than-lifetime exposure scenarios (see Section 4.4), are reported in Table 4-25. The adult-exposure-only EC_{01}s were derived from the low-dose segments of the two-piece linear spline models for lymphoid cancer and breast cancer incidence. (Note that, for each of these models, the low-exposure extrapolated estimates are a straight linear continuation of the linear models used above the PODs, and thus, the statistical properties of the models are preserved.) These $0.01/EC_{01}$ estimates are dependent on the suitability of the models used for deriving the EC_{01} estimates as well as on the applicability of the linear low-dose extrapolation. The assumption of low-dose linearity is supported by the mutagenicity of EtO (see Section 3.4). If these (adult-based) $0.01/EC_{01}$ estimates are to be used, ADAFs should be applied if early-life exposure occurs, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b).

As can be seen by comparing the adult-based $0.01/EC_{01}$ estimates in Table 4-25 with the adult-based unit risk estimates (i.e., $0.01/LEC_{01}$ estimates) in Table 4-24, the $0.01/EC_{01}$ estimate is about 25% of the unit risk estimate for lymphoid cancer, about 50% of the unit risk estimate for breast cancer incidence, and about 33% of the unit risk estimate for total cancer incidence.

Table 4-25. Adult-based extra risk estimates per ppm based on adult-exposure-only EC_{01}s ($0.01/EC_{01}$ estimates)[a]

Cancer response	Adult-exposure-only EC_{01} (ppm)	Adult-based $0.01/EC_{01}$ (per ppm)[b,c]
Lymphoid cancer incidence (both sexes)	0.0107	1.21[d]
Breast cancer incidence (females)	0.0206	0.629
Total cancer incidence		1.84[d,e]

[a]ADAFs should be applied to the adult-based $0.01/EC_{01}$ estimates if early-life exposure occurs, in accordance with EPA's *Supplemental Guidance*.
[b]These estimates are calculated as $0.01/EC_{01}$ for the adult-exposure-only extra risk estimate per ppm rescaled to a 70-yr basis by multiplying by 70/54 (see Section 4.4).
[c]For conversion to per $\mu g/m^3$, divide by 1,830.
[d]Calculated as the sum of the individual adult-based $0.01/EC_{01}$s.
[e]For unit risk estimates above 1, convert to risk per ppb (e.g., 1.84 per ppm = 1.84×10^{-3} per ppb).

Finally, it should be noted that some investigators have posited that the high and variable background levels of endogenous EtO-induced DNA damage in the body (see Section 3.3.3.1) may overwhelm any contribution from low levels of exogenous EtO exposure (Marsden et al., 2009; SAB, 2007). It is true that the existence of these high and variable background levels may make it hard to observe statistically significant increases in risk from low levels of exogenous exposure. However, there is clear evidence of carcinogenic hazard from the rodent bioassays and strong evidence from human studies (see Section 3.5), and the genotoxicity/mutagenicity of EtO (see Section 3.4) supports low-dose linear extrapolation of risk estimates from those studies (U.S. EPA, 2005a). In fact, as discussed in Section 3.3.3.1, Marsden et al. (2009), using sensitive detection techniques and an approach designed to separately quantify endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO treatment in rats, reported (non-significant) increases in exogenous adducts in DNA of spleen and liver at the lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days, which is a very low dose compared to the LOAELs in the carcinogenicity bioassays; see Section C.7 of Appendix C). Marsden et al. (2009) also reported statistically significant linear dose-response relationships ($p < 0.05$) for exogenous adducts in all three tissues examined (spleen, liver, and stomach), although they caution that their study was not designed to test for linearity and that some of the adduct levels induced at low EtO concentrations are below the limit of accurate quantitation. Furthermore, while the contributions to DNA damage from low exogenous EtO exposures may appear "negligible" (Marsden et al., 2009) compared to those from endogenous EtO exposure, low levels of exogenous EtO may nonetheless be responsible for additional risk (above background risk) above *de minimis* risk levels, which are generally 10^{-6} to 10^{-4} for cancer. This is not inconsistent with the much higher levels of background cancer risk, to which endogenous EtO may contribute, for the two cancer types observed in the human studies—lymphoid cancers have a background lifetime incidence risk on the order of 3%, while the background lifetime incidence risk for breast cancer is on the order of 15%.[44]

Also related to the issue of endogenous EtO, Starr and Swenberg (2013) have proposed an approach for bounding the cancer risk from low levels of exogenous exposure to chemicals that also exist endogenously. In brief, Starr and Swenberg (2013) assume that all background cancer risk (P_0) for a specific cancer type is attributable to background levels (C_0) of some endogenous adduct (as a marker of exposure) of the chemical of interest [Starr and Swenberg (2013) use formaldehyde as an example] in that tissue; they then use the ratio P_0/C_0 (actually the lower bound on C_0) to estimate a linear slope for risk as a function of endogenous adduct level down to zero adducts, which they claim is a conservative (upper) bound on cancer risk from low levels of exogenous exposure (similarly expressed in terms of adduct levels). EPA disagrees that this approach necessarily yields a conservative bound on risk,

[44]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.

however, because, *even if* the adduct level is an appropriate dose metric for comparing cancer risks, the approach relies on the assumption that the dose-response relationship over the dose range of endogenous adducts is linear down to zero adducts. In contrast to this assumption, EPA considers it highly plausible that the dose-response relationship over the endogenous range is sublinear (e.g., that the baseline levels of DNA repair enzymes and other protective systems evolved to deal with endogenous DNA damage would work more effectively for lower levels of endogenous adducts), that is, that the slope of the dose-response relationship for risk per adduct would increase as the level of endogenous adducts increases. If the dose-response relationship over the endogenous range is sublinear, rather than linear as assumed by Starr and Swenberg (2013), then the approach proposed by Starr and Swenberg (2013) does not necessarily produce a conservative bound on risk (Crump et al., 2014).

See Table 4-26 for a summary of key unit risk estimates derived in this assessment. See Section 4.7 for risk estimates based on occupational exposure scenarios.

Table 4-26. Summary of key unit risk estimates from this assessment (see Section 4.7 for risk estimates based on occupational exposure scenarios)

Basis	Inhalation unit risk estimate[a] (per μg/m^3)[b]
Full lifetime unit risk estimate[c]	
Total cancer risk based on human data (NIOSH cohort of sterilizer workers)—lymphoid cancer incidence and breast cancer incidence in females (two-piece linear spline models)	**5.0×10^{-3}**
Adult-based unit risk estimates[d]	
Total cancer risk based on human data (NIOSH cohort)—lymphoid cancer incidence and breast cancer incidence in females (two-piece linear spline models)	**3.0×10^{-3}**
Lymphoid cancer incidence based on human data (NIOSH cohort)—preferred model: two-piece linear spline model	2.6×10^{-3}
Breast cancer incidence in females based on human data (NIOSH cohort)—preferred model: two-piece linear spline model	7.0×10^{-4}
Preferred total cancer incidence risk estimate from rodent data (female mouse)	4.6×10^{-5}
Range of total cancer incidence risk estimates from rodent data (mouse and rat)	2.2×10^{-5} to 4.6×10^{-5}
Adult-based 0.01/EC_{01} estimates[e]	
Lymphoid cancer incidence based on human data (NIOSH cohort)—two-piece linear spline model	6.6×10^{-4}
Breast cancer incidence in females based on human data (NIOSH cohort)—two-piece linear spline model	3.4×10^{-4}
Total cancer incidence based on human data (NIOSH cohort)	1.0×10^{-3}

[a]Technically, the values listed in this table are not all unit risk estimates as defined by EPA, but they are all potency estimates that, when multiplied by an exposure value, give an estimate of extra cancer risk. These potency estimates are not intended for use with continuous lifetime exposure levels above 38 μg/m^3. See Section 4.7 for risk estimates based on occupational exposure scenarios. Preferred estimates are in bold.

[b]To convert unit risk estimates to (ppm)$^{-1}$, multiply the (μg/m^3)$^{-1}$ estimates by 1,830 (μg/m^3)/ppm.

[c] Because the weight of evidence supports a mutagenic mode of action for EtO carcinogenicity, and because of the lack of chemical-specific data, EPA assumes increased early-life susceptibility and recommends the application of ADAFs, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b), for exposure scenarios that include early-life exposures. For the full lifetime (upper-bound) unit risk estimate presented here, ADAFs have been applied, as described in Section 4.4.

[d]These (upper-bound) unit risk estimates are intended for use in ADAF calculations and less-than-lifetime adult exposure scenarios (U.S. EPA, 2005b). Note that these are not the same as the unit risk estimates derived directly from the human data in Section 4.1 under the assumption that RRs are independent of age. Under that assumption, the key unit risk estimates were 2.9×10^{-3} per μg/m^3 for lymphoid cancer incidence, 8.1×10^{-4} per μg/m^3 for breast cancer incidence, and 3.3×10^{-3} per μg/m^3 for the combined cancer incidence risk from those two cancers. See Section 4.4 for the derivation of the adult-based unit risk estimates.

[e]These are not upper-bound risk estimates but, rather, estimates based on linear extrapolation from the EC_{01}. ADAFs should be applied if early-life exposure occurs, in accordance with EPA's *Supplemental Guidance* (U.S. EPA, 2005b).

4.6. COMPARISON WITH OTHER PUBLISHED RISK ESTIMATES

The unit risk values derived in this document are compared with other recent risk estimates presented in the published literature (see Table 4-27).

4.6.1. Unit Risk Estimates Based on Human Studies

Kirman et al. (2004) used leukemia data only and pooled data from both the Stayner et al. (1993) and the UCC studies (Teta et al., 1999; Teta et al., 1993). Based on the assumption that leukemias are due to chromosome translocations, requiring two independent events (chromosome breaks), Kirman et al. (2004) proposed that two independent EtO-induced events are required for EtO-induced leukemias and used a dose-squared model, yielding a unit risk value of 4.5×10^{-8} $(\mu g/m^3)^{-1}$ as their preferred estimate.

Table 4-27. Comparison of unit risk estimates[a]

Assessments	Data source	Inhalation unit risk estimate[b] (per µg/m^3)
Based on human data		
EPA (this document)[c]	Lymphoid cancer incidence in sterilizer workers (NIOSH cohort)[d]	7.2×10^{-4}
	Breast cancer incidence in female sterilizer workers (NIOSH cohort)[e]	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}[f]
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}[g]
Based on rodent data		
EPA (this document)[c]	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}[h]

[a]Upper-bound estimates except where footnoted to indicate that estimates are based on EC values (i.e., estimates with footnotes f and g).
[b]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. (2010) estimates, which are purported to include the ADAFs, but the ADAFs were in fact misapplied and have essentially no impact (see Appendix A.2.20).
[c]See Table 4-26 in Section 4.5 for a more complete summary of estimates from this assessment. See Section 4.7 for risk estimates for occupational exposure scenarios.
[d]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} $(\mu g/m^3)^{-1}$.
[e]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} $(\mu g/m^3)^{-1}$.
[f]Estimates based on linear extrapolation from EC0001−EC000001 obtained from the quadratic model.
[g]Estimates based on range of EC(1/million)s of 0.001−0.003 ppm obtained from the model RR = $e^{(\beta \times \text{exposure})}$ for relevant cancer endpoints.
[h]Estimates based on quadratic extrapolation model below the observable range of the data (i.e., below the LEC_{10} or LEC_{01} obtained using multistage model) with various points of departure (LEC_{01}−LEC_{000001}) for final linear extrapolation (see Section 4.4.2).

The Kirman et al. (2004) values are different from those in the current document because of the different assumptions inherent in the Kirman et al. (2004) approach and because the study used unpublished data from earlier follow-ups of the two cohorts. A key difference is that EPA uses a linear model or a two-piece linear spline model rather than a quadratic (dose-squared) model in the range of observation. Then, EPA uses a higher extra risk level (1%) for establishing the POD, whereas Kirman et al. (2004) used a risk level of 10^{-5} for their best estimate and a risk range of 10^{-4} to 10^{-6} for their range of values. The extra risk level and the corresponding POD are not critical with the linear model; however, with the quadratic model used by Kirman et al. (2004), the lower the risk level (and hence the POD), the greater the impact of the quadratic model and the lower the resulting unit risk estimates.

In addition, EPA (1) uses data for lymphoid cancers (and female breast cancers) rather than leukemias, (2) includes ages up to 85 years in the life-table analysis rather than stopping at 70 years, (3) calculates unit risk estimates for cancer incidence as well as mortality, (4) uses a lower bound as the POD rather than the maximum likelihood estimate, (5) uses the results of lagged analyses rather than unlagged analyses, and (6) uses adult-based unit risk estimates in conjunction with ADAFs (see Section 4.4) to derive the lifetime unit risk estimates.

Another key difference is that Kirman et al. (2004) relied on earlier NIOSH results (Stayner et al., 1993), whereas EPA uses the results of NIOSH's more recent follow-up of the cohort (Steenland et al., 2004). Kirman et al. (2004) claim that a quadratic dose-response model provided the best fit to the data in the observable range and that this provides support for their assumed mode of action. However, the 2004 NIOSH data for lymphohematopoietic cancers suggest a supralinear exposure-response relationship (see Section 4.1.1.2 and Figures 4-1 and 4-3), which is inconsistent with a dose-squared model. Furthermore, EPA's review of the mode of action evidence does not support the mode of action assumed by Kirman et al. (2004) (see Section 3.4).

The Valdez-Flores et al. (2010) unit risk estimates (see Table 4−27) are similarly much lower than those in the current document because of the different assumptions used. A key difference is that EPA uses a linear model or a two-piece linear spline model in the range of observation rather than an exponential model ($RR = e^{\beta \times exposure}$), which was used by Valdez-Flores et al. (2010) despite its lack of fit. Then, EPA uses a 1% extra risk level for establishing the POD for linear extrapolation, whereas Valdez-Flores et al. (2010) used a risk level of 10^{-6}. In addition, EPA (1) includes ages up to 85 years in the life-table analysis rather than stopping at 70 years, (2) calculates unit risk estimates for cancer incidence as well as mortality, (3) uses a lower bound as the POD rather than the maximum likelihood estimate, and (4) uses the results of lagged analyses rather than unlagged analyses. See Appendix A.2.20 for a more detailed discussion of the differences between the EPA and Valdez-Flores et al. (2010) analyses.

4.6.2. Unit Risk Estimates Based on Laboratory Animal Studies

Kirman et al. (2004) also used linear and dose-squared extrapolation models to derive unit risk estimates based on the rat mononuclear cell leukemia data and the mouse lymphoma data. First, they used the multistage model to calculate the LEC_{10} (LEC_{01} for the male mouse lymphoma data) for the POD from the observable range. Then, using these PODs for linear extrapolation, Kirman et al. (2004) obtained a unit risk range of 3.9×10^{-6} $(\mu g/m^3)^{-1}$ to 1.5×10^{-5} $(\mu g/m^3)^{-1}$. Alternatively, Kirman et al. (2004) used a quadratic extrapolation model below the observable range to estimate secondary points of departure (LEC_{01}−LEC_{000001}; LEC_{001}−LEC_{000001} for the male mouse) for final linear low-dose extrapolation, yielding unit risks ranging from 2.6×10^{-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 from the rodent data in this document.

4.7. RISK ESTIMATES FOR SOME OCCUPATIONAL EXPOSURE SCENARIOS

The unit risk estimates derived in the preceding sections were developed for environmental exposure levels, where maximum modeled levels are on the order of 1−2 $\mu g/m^3$ (email dated October 3, 2005, from Mark Morris, EPA, to Jennifer Jinot, EPA), i.e., roughly 0.5−1 ppb, and are not applicable to higher exposures, including some occupational exposure levels. However, occupational exposure levels of EtO are of concern to EPA when EtO is used as a pesticide (e.g., sterilizing agent or fumigant). The occupational exposure scenarios of interest to EPA include some cumulative exposures corresponding to exposure levels in the nonlinear range of some of the models (i.e., above the maximum exposure level at which the low-dose-linear unit risk estimates apply). Therefore, extra risk estimates were calculated for a number of occupational exposure scenarios of possible concern. Extra risk estimates are estimates of the extra cancer risk above background and are the same type of estimate that one gets from multiplying a unit risk estimate by an exposure level. In this case, the exposure level is used directly in the exposure-response model, thus accounting for any nonlinearities in the model above the range of exposure levels for which the linear unit risk estimate is applicable. For these occupational exposure scenarios, exposure-response models based on data from the NIOSH cohort were used in conjunction with the life-table program, as previously discussed in Section 4.1. The NIOSH cohort is especially relevant to EPA's ethylene oxide concerns because it is a cohort of sterilizer workers. A 35-year exposure occurring between ages 20 and 55 years was assumed, and exposure levels ranging from 0.1 to 1 ppm 8-hour TWA were examined (i.e., ranging from about 1,300 to 13,000 ppm × days). (Note that the current Occupational Safety and Health Administration Permissible Exposure Limit is 1 ppm [8-hour TWA].)

4.7.1. Extra Risk Estimates for Lymphoid Cancer

For lymphoid cancer mortality in both sexes, the same model of the Steenland et al. (2004) data that was selected for the derivation of the unit risk estimate was used for the estimation of the extra risks associated with the occupational exposure scenarios, consistent with the model selection objectives for this assessment (see Section 4.1.1.2). The selected model is the two-piece linear spline model with the knot at 1,600 ppm × days (cumulative exposure, with a 15-year lag) (see Section 4.1.1.2 and Section D.3 of Appendix D). While this model was considered to provide the best local fit to the data in the low-exposure region for the purposes of deriving a unit risk estimate for low-exposure extrapolation, the model appears to underestimate the risk at higher exposures of concern for the occupational exposure scenarios, as compared to models with better global fits to the full range of the data. Figure 4-2, for example, shows that the four models with the best global fits in terms of AIC (the linear and log-linear two-piece spline models with knots at 100 ppm × days and the linear and log-linear log cumulative exposure models; see Table 4-6) all indicate higher RR estimates than the selected model for the occupational exposure range of interest (1,277.5 to 12,775 ppm × days). Thus, estimates from the best-fitting[45] log cumulative exposure Cox regression model (with 15-year lag; see Section 4.1.1.2 and Section D.3 in Appendix D) are also presented for comparison. The next "best-fitting" (in terms of AIC) model across the full range of exposures is the log cumulative exposure linear model (see Section 4.1.1.2 and Section D.3.3 of Appendix D), which would yield even higher extra risk estimates than the log cumulative exposure Cox regression model across the range of occupational exposure scenarios of interest (see Figure 4-2)[46], but only the latter model is considered for comparisons here.

The extra risk results for lymphoid cancer mortality and incidence in both sexes for the selected two-piece linear spline model with the knot at 1,600 ppm × days and the log cumulative exposure Cox regression model, for comparison, are presented in Table 4-28. For the lymphoid cancer incidence estimates, the exposure-response relationship was assumed to be the same as for mortality (see Section 4.1.1.3). The models used to derive the extra risk estimates presented in Table 4-28 for lymphoid cancer for the occupational exposure scenarios are displayed in Figure 4-8 over the range of occupational cumulative exposures of interest; the categorical results are included for comparison.

[45]In terms of AIC, after accounting for the 0.4 unit discrepancy between linear and log-linear models; see footnote 25 in Section 4.1.1.2.

[46]For example, the MLEs of extra risk from the log cumulative exposure linear model would range from about 22% higher than that from the log cumulative exposure Cox regression model for the 0.1 ppm 8-hour TWA to about 5% higher for the 1 ppm 8-hour TWA.

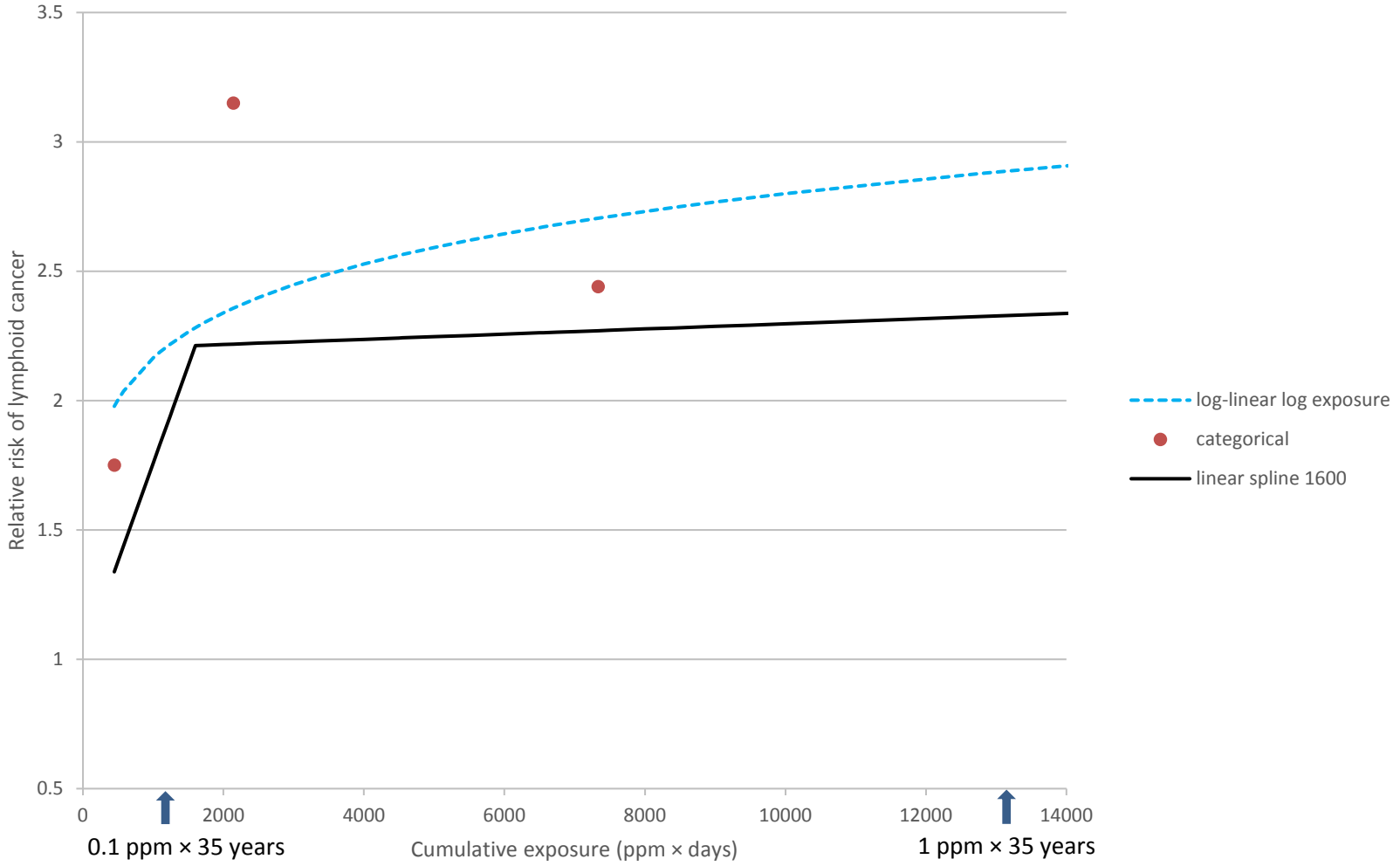
Table 4-28. Extra risk estimates for lymphoid cancer in both sexes for various occupational exposure levels[a]

8-hr TWA (ppm)	Lymphoid cancer mortality				Lymphoid cancer incidence[b]			
	Cox regression model of log cumulative exposure[c]		Linear spline model with knot at 1,600 ppm × days[d]		Cox regression model of log cumulative exposure[c]		Linear spline model with knot at 1,600 ppm × days[d]	
	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL
0.1	0.012	0.029	0.0092	0.022	0.034	0.078	0.024	**0.056**
0.2	0.014	0.035	0.012	0.029	0.039	0.092	0.033	**0.076**
0.3	0.015	0.038	0.013	0.029	0.042	0.10	0.034	**0.078**
0.4	0.016	0.041	0.013	0.030	0.044	0.11	0.035	**0.079**
0.5	0.017	0.043	0.013	0.030	0.046	0.11	0.035	**0.080**
0.6	0.017	0.045	0.013	0.030	0.047	0.12	0.036	**0.081**
0.7	0.018	0.047	0.013	0.030	0.049	0.12	0.036	**0.081**
0.8	0.018	0.048	0.013	0.030	0.050	0.13	0.037	**0.081**
0.9	0.019	0.049	0.013	0.030	0.051	0.13	0.037	**0.081**
1.0	0.019	0.051	0.014	0.030	0.052	0.13	0.037	**0.082**

[a]Assuming a 35-yr 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[47] log cumulative exposure Cox regression model for lymphoid cancer mortality in both sexes; 15-yr lag (see Appendix D; see also Section 4.1.1.2).
[d]Two-piece linear spline model with knot at 1,600 ppm × days (cumulative exposure, with 15-yr lag) (see Section 4.1.1.2).

MLE: maximum likelihood estimate; UCL: (one-sided) upper confidence limit estimate.

[47]Best-fitting nonspline model based on AIC, after accounting for the 0.4 unit difference in AICs between linear and log-linear models (see Table 4-6 and footnote 25).



Lymphoid cancer models (see Section 4.1.1.2): log cumulative exposure Cox regression model; categorical results; two-piece linear spline model with knot at 1,600 ppm × days. (Note that, with the exception of the categorical results, the various models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Figure 4-8. RR estimates for lymphoid cancer from occupational EtO exposures (with 15-year lag).

The 95% (one-sided) upper bounds for the two continuous exposure models were estimated using a Wald approach. While this approach is appropriate for the log cumulative exposure Cox regression model, a profile likelihood approach, which allows for asymmetric CIs, would have been preferred for the linear spline model (Langholz and Richardson, 2010). However, a formula for applying the profile likelihood approach in the range of the second spline segment was not available for the life-table analysis; thus, the upper bounds for the two-piece linear spline model were approximated using a Wald approach. A comparison of the 95% upper bounds on RR derived for the linear spline model using the two different approaches shows that the Wald upper bound estimates are about half-way between the MLE RR estimates and the profile likelihood upper bound estimates (see Figure D-17 in Appendix D). In the range of cumulative exposures of interest for the occupational scenarios considered in this assessment, i.e., up to 12,775 ppm × days, with a 15-year lag, the Wald-based upper bound estimates are about 67% of the profile-likelihood-based upper bound estimates.

As can be seen in Table 4-28, the extra risk estimates from the selected two-piece spline model are consistently below those of the log cumulative exposure Cox regression model. The maximum likelihood estimates (MLEs) for lymphoid cancer mortality and incidence from the log cumulative exposure Cox regression model range from about 15% higher than those from the two-piece spline model at 0.2 ppm 8-hour TWA to about 40% higher at 1 ppm 8-hour TWA. The 95% (one-sided) upper confidence limits (UCLs) on the extra risks range from about 20% higher for mortality and incidence from the log cumulative exposure Cox regression model at 0.2 ppm 8-hour TWA to about 70% higher for mortality and 60% higher for incidence at 1 ppm 8-hour TWA. As noted above, the log cumulative exposure linear model would yield even higher extra risk estimates than the log cumulative exposure Cox regression model across the range of occupational exposure scenarios of interest (see Figure 4-2). So, while the two-piece spline model was considered to provide the best local fit to the data in the low-exposure region for the purposes of deriving a unit risk estimate for low-exposure extrapolation, and is used here for the occupational extra risk estimates for the sake of consistency, following the model selection objectives for this assessment (see Section 4.1.1.2), these results confirm that the model underestimates the risks at higher exposures of concern for the occupational exposure scenarios, compared to models with better global fits to the data. This underestimation is compounded for the upper bound estimates by the use of the Wald approach rather than the more appropriate profile likelihood approach.

Finally, MLEs and Wald UCLs of extra risk for the two-piece linear spline model with the knot at 1,600 ppm × days and different lag periods (0, 5, 10, and 20 years) were calculated for the occupational exposure scenarios, for comparison with the results with the 15-year lag (see Section D.3.9 and Table D-43 of Appendix D). The MLEs ranged from about 25% of (5-year lag) to just over 80% of (no lag) the estimates for the selected model (15-year lag). The 95% (one-sided) upper bounds of extra risk ranged from about 45% of (5-year lag) to just over 5% greater than (no lag) the estimates for the

selected model. Of these models, the model with no lag was the best-fitting model after the selected model (15-year lag), based on log-likelihood (and AIC) (See Table D-38), and that is the model that had the most similar MLEs and UCLs to the selected model. The models for lags of 5, 10, and 20 years each had *p*-values > 0.20 for inclusion of the exposure terms, indicating an inadequate fit to the data.

4.7.2. Extra Risk Estimates for Breast Cancer

For *breast cancer*, incidence data were available from the NIOSH incidence study; thus, only incidence estimates were calculated. In addition to being the preferred type of cancer risk estimate, the breast cancer incidence risk estimates are based on more cases than were available in the mortality study and the incidence data (for the subcohort with interviews) are adjusted for a number of breast cancer risk factors (see Section 4.1.2.3). In terms of the incidence data, the subcohort data are preferred to the full cohort data because the subcohort data are adjusted for these potential confounders and also because the full cohort data have incomplete ascertainment of breast cancer cases.

For breast cancer incidence in the subcohort with interviews, a number of Cox regression exposure-response models from the Steenland et al. (2003) breast cancer incidence study fit almost equally well (see Section 4.1.2.3). These include a log cumulative exposure model and a cumulative exposure model, both with a 15-year lag, and a log cumulative exposure model with no lag. The latter model was not considered further because the inclusion of a 15-year lag for the development of breast cancer was considered more biologically realistic than not including a lag. Steenland et al. (2003) also provide a duration-of-exposure Cox regression model with a marginally better fit; however, models using duration of exposure are less useful for estimating exposure-related risks, and duration of exposure and cumulative exposure are correlated. Thus, only the (lagged) cumulative exposure models are considered here. The 2014 Draft Assessment (U.S. EPA, 2014a, b) provides extra risk estimates from the cumulative exposure and log cumulative exposure Cox regression models.

In the current assessment, estimates from those two models are omitted in favor of estimates from the linear cumulative exposure and square root of cumulative exposure models (with a 15-yer lag; see Section 4.1.2.3), which provide better overall fits to the data (based on lower AIC values) and better local fits to the data in the range of the exposure scenarios of interest than do those log-linear models. The two-piece linear spline model (with a 15-year lag; see Section 4.1.2.3) was also used to calculate extra risk estimates. This was the preferred model for the derivation of unit risk estimates in Section 4.1.2.3, and it is the preferred model for the derivation of the extra risk estimates for the occupational exposure scenarios presented here. Selection of the two-piece linear spline model as the preferred model is consistent with the model selection objectives for this assessment, which included prioritizing models which allow for better local fits to the low-exposure range and using the same model for deriving both the unit risk estimate and the extra risk estimates for the occupational exposure scenarios (see Section 4.1.1.2). Estimates from the other two models are provided for comparison. The

linear square-root model provided the best global fit (lowest AIC), and the linear model might be considered a lower bound on a range of credible estimates for the occupational exposure scenarios of interest (see Section 4.1.2.3).

The 95% (one-sided) upper bounds for the linear and linear square-root models were estimated using a profile likelihood approach (Langholz and Richardson, 2010), which allows for asymmetric CIs. The 95% (one-sided) upper bounds for the two-piece linear spline model were approximated using a Wald approach because a formula for applying the profile likelihood approach in the range of the second spline segment was not available for the life-table analysis. A comparison of the 95% (one-sided) upper bounds on RR derived using the two different approaches shows that the results differ little in the range of cumulative exposures of interest for the occupational scenarios considered in this assessment, i.e., up to 12,775 ppm × days, with a 15-year lag (see Figure D-8 in Appendix D). The Wald approach underestimates the upper bounds compared to the profile-likelihood approach for all exposures, but never by more than about 4%.

The extra risk estimates for breast cancer incidence in females from the linear cumulative exposure and square root of cumulative exposure models and the two-piece linear spline model for the occupational exposure scenarios of interest are presented in Table 4-29, and these models are displayed in Figure 4-9 over the corresponding range of occupational cumulative exposures.

As can be seen in Table 4-29, the extra risk estimates from the linear model are well below those of the two-piece linear spline and linear square-root models. The maximum likelihood estimates (MLEs) and 95% (one-sided) upper bounds on the extra risks from the two-piece linear spline and linear square-root models are fairly similar over much of the exposure range of interest. For the lowest exposure levels (0.1 to 0.3 ppm 8-hour TWAs), both the MLEs and UCLs are higher for the linear square-root model, but by less than twofold. For the higher exposure levels, both the MLEs and UCLs are virtually indistinguishable (differ by ≤10 %) between the linear square-root model and the two-piece linear spline model.

Table 4-29. Extra risk estimates for breast cancer incidence in females for various occupational exposure levels[a,b]

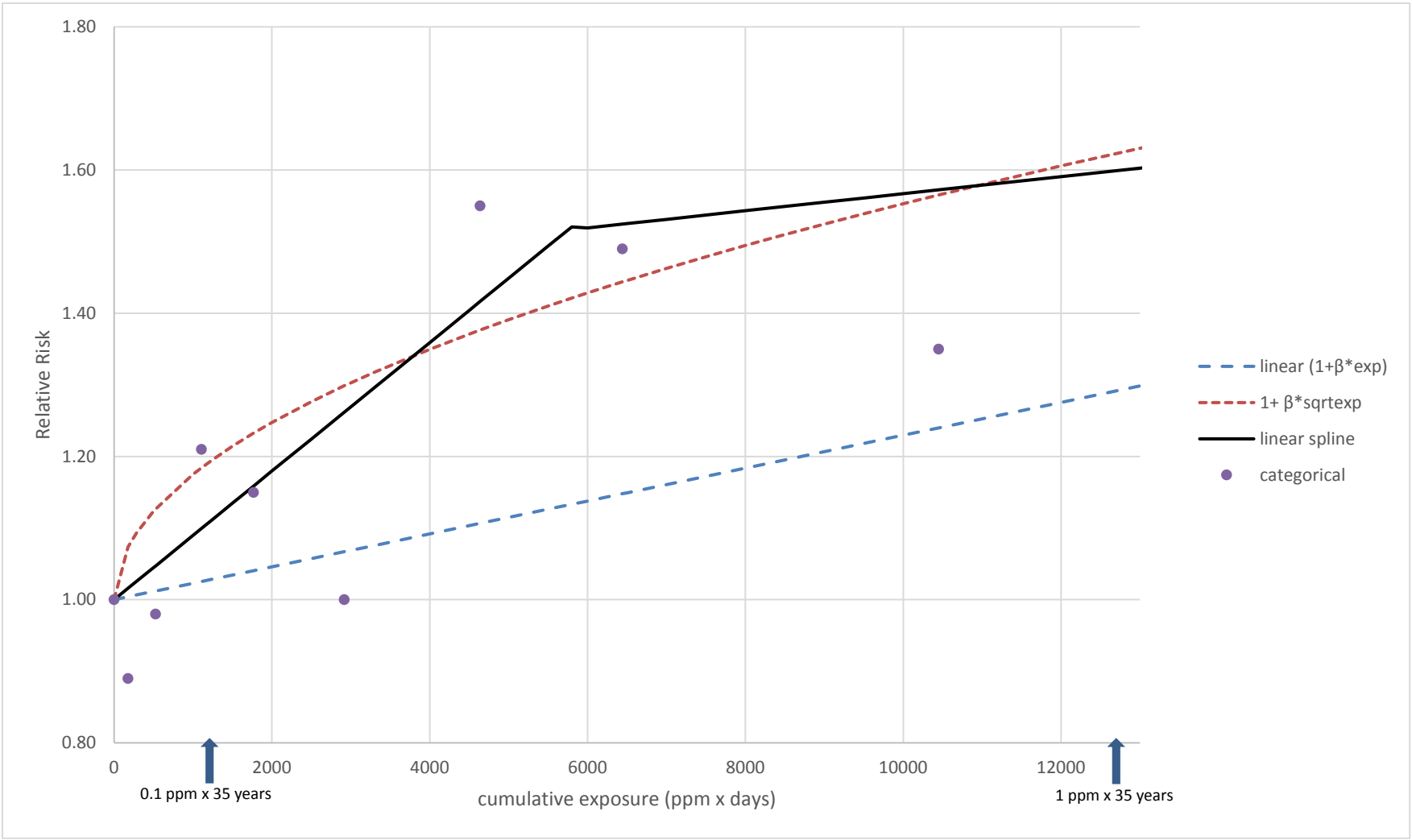
8-hr TWA (ppm)	Linear sqrt cumulative exposure model[c]		Linear model[d]		Two-piece linear spline model[e]	
	MLE	95% UCL[f]	MLE	95% UCL[f]	MLE	95% UCL[g]
0.1	0.025	0.047	0.0033	0.0067	0.013	**0.025**
0.2	0.035	0.066	0.0066	0.013	0.025	**0.050**
0.3	0.042	0.080	0.099	0.020	0.038	**0.074**
0.4	0.049	0.091	0.013	0.026	0.050	**0.097**
0.5	0.054	0.10	0.016	0.033	0.059	**0.11**
0.6	0.059	0.11	0.020	0.039	0.064	**0.12**
0.7	0.064	0.12	0.023	0.046	0.068	**0.13**
0.8	0.068	0.13	0.026	0.052	0.071	**0.13**
0.9	0.072	0.13	0.029	0.058	0.074	**0.13**
1.0	0.075	0.14	0.032	0.065	0.076	**0.14**

[a]Assuming a 35-yr 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]Linear model with square-root transformation of cumulative exposure as a continuous variable, with 15-yr lag (see Section 4.1.2.3 and Section D.1 of Appendix D)
[d]Linear model with cumulative exposure as a continuous variable, with 15-yr lag (see Section 4.1.2.3 and Section D.1 of Appendix D).
[e]Two-piece linear spline model with cumulative exposure as a continuous variable, with 15-yr lag (see Section 4.1.2.3 and Table D-10 of Appendix D for parameter values and equations). Results for occupational exposures use both spline segments; knot at 5,750 ppm × days.
[f]Confidence limits for the "one-piece" linear RR models were estimated using a profile likelihood approach (Langholz and Richardson, 2010), which allows for asymmetric CIs.
[g]Confidence limits for the two-piece linear spline model were approximated using a Wald approach (See Table D-10 of Appendix D for parameter values and equation) because a formula for using the profile likelihood approach in the range of the second spline segment was not available for the life-table analysis.

MLE: maximum likelihood estimates; UCL: (one-sided) upper confidence limit estimate.

As shown in Figure 4-9, the slope of the (continuous exposure) linear model is too shallow across the range of exposures of interest. This is consistent with the analysis presented in Section D.1 of Appendix D showing the strong influence of the upper tail of cumulative exposures on the results of the log-linear cumulative exposure (standard Cox regression) model. The responses in the upper tail of exposures are relatively dampened, such that when the highest 5% of exposures (exposures > 27,500 ppm × days, which are well in excess of the exposures corresponding to the occupational exposure scenarios considered here) are excluded, the slope of the Cox regression model is substantially increased (e.g., at 10,000 ppm × days, the RR estimate increases from about 1.1 to almost 1.5; see Figure D-4 in Appendix D). This strong influence of the upper tail of exposures would similarly attenuate the slope of the linear model, resulting in underestimation of the lower-exposure risks. The two-piece linear spline model, on the other hand, is more flexible, and the influence of the upper tail of exposures would be primarily on the upper spline segment; thus, the two-piece model is able to provide a better fit to the lower-exposure data. The linear square root of cumulative exposure model gives similar results as the two-piece linear model over the range of occupational exposures of interest.

Finally, MLEs and Wald UCLs of extra risk for the two-piece linear spline model with the knot at 5,750 ppm × days and different lag periods (0, 5, 10, and 20 years) were calculated for the occupational exposure scenarios, for comparison with the results with the 15-year lag (see Section D.1.11 and Table D-18 of Appendix D). The MLEs ranged from about 40% less than (10-year lag) to about 30% greater than (20-year lag) the estimates from the selected model (15-year lag). The 95% (one-sided) upper-bound estimates ranged from about 25% less than (10-year lag) to about 20% greater than (20-year lag) those from the selected model. The model with a 20-year lag had a slightly better fit to the data, based on log-likelihood and AIC, than the model with a 20-year lag, whereas, the models with 0-, 5-, and 10-year lags had markedly worse fits (see Table D-12 in Appendix D).



Breast cancer models (see Section 4.1.2.3): linear 2-piece spline model, with knot at 5,750 ppm × days; linear square-root cumulative exposure model; (continuous exposure) linear model; categorical results (deciles). (Note that the various models have different implicitly estimated baseline risks; thus, they are not strictly comparable to each other in terms of RR values, i.e., along the y-axis. They are, however, comparable in terms of general shape.)

Figure 4-9. RR estimates for breast cancer incidence from occupational EtO exposures (with 15-year lag).

4.7.3. Extra Risk Estimates for Total Cancer

For the *total cancer risk* combined across the two cancer types, the MLE can be obtained directly by summing the MLEs for the individual cancer types. An upper bound can be approximated by summing the 95% UCL estimates for the individual cancer types. Normally, this would tend to overestimate the corresponding 95% UCL on total cancer risk (i.e., the 95% UCL on the sum of the MLEs); however, as discussed above, because the lymphoid cancer extra risks are underestimated compared to models with better global fits to the data (based on AIC) and because the Wald approach used to approximate the upper bound estimates in place of the more accurate profile-likelihood approach underestimates the upper bounds (especially for lymphoid cancer), the overestimation is mitigated. The summed MLEs and upper bound estimates are presented in Table 4-30.[48]

Table 4-30. Extra risk estimates for total cancer incidence for various occupational exposure levels[a,b]

8-hr TWA (ppm)	Maximum likelihood estimate	Upper bound estimate
0.1	0.037	0.081
0.2	0.058	0.13
0.3	0.072	0.15
0.4	0.085	0.18
0.5	0.094	0.19
0.6	0.10	0.20
0.7	0.10	0.21
0.8	0.11	0.21
0.9	0.11	0.21
1.0	0.11	0.22

[a]Assuming a 35-yr exposure between ages 20 and 55 years.
[b]From combining results for lymphoid cancer incidence in both sexes and breast cancer incidence in females.

Comparing results in Tables 4-28 to 4-30 shows that lymphoid cancer contributes about 2/3 of the total risk at 0.1 ppm, and this contribution decreases to 1/3 by 1 ppm. In addition, one can calculate a minimum bound for what the 95% UCL on the sum of the MLEs at each exposure level would be by

[48]Technically, these sums would reflect the total cancer risk to females and not a mixed-sex workforce because the breast cancer risk estimates apply only to females. As a practical matter for regulatory purposes, females typically comprise a substantial proportion of the sterilizer workforce and summing the extra risk estimates enables risk managers to evaluate the individual risk for this substantial workforce group. In a situation in which the workforce of concern is comprised predominantly of males, it might be appropriate to use a sex-weighted sum of the extra risks from the two cancer types. For the purposes of estimating numbers of cancer cases attributable to specific exposure levels, e.g., for benefits analyses, it would be most suitable to use the cancer-specific extra risk estimates, taking sex into account.

taking the maximum of the sum of the MLE for one cancer type plus the UCL for the other cancer type. The sum of the 95% UCLs for the two cancer types (the upper bound shown in Table 4-30) exceeds this minimum upper bound by at most 33%, indicating that the overestimation incurred by approximating the 95% UCL on the total cancer risk by summing the 95% UCLs for the individual cancer types is minimal. Moreover, calculating a similar minimum upper bound using the log cumulative exposure Cox regression model for the lymphoid cancer risk estimates indicates that using one of the log cumulative exposure models with a better global fit to the lymphoid cancer data, discussed above, would alleviate all of the overestimation. In addition, the Wald approach used to approximate the upper bound estimates underestimates the more accurate profile likelihood upper bounds. Thus, the overall approach is likely underestimating the upper bounds on total risk in the range of the occupational exposure scenarios of interest.

Although there is model uncertainty, as discussed above, there is less overall uncertainty associated with the extra risk estimates for occupational exposure scenarios than with the unit risk estimates for environmental exposures, and the overall confidence in the extra risk estimates is high. The extra risk estimates are derived for occupational exposure scenarios that yield cumulative exposures well within the range of the exposures in the NIOSH study. Moreover, the NIOSH study is a study of sterilizer workers who used EtO for the sterilization of medical supplies or spices (Steenland et al., 1991); thus, the results are directly applicable to workers in these occupations, and these are among the occupations of primary concern to EPA.

4.7.4. Calculation of Extra Risk Estimates for Other Occupational Exposure Scenarios:

Some detailed guidance is provided here for calculating extra risk estimates outside of the range of occupational scenarios considered above. Note that for 35-year exposures to exposure levels between the exposure levels presented in Tables 4-28 and 4-29, e.g., 0.15 ppm, one could interpolate between the extra risk estimates presented for the closest exposure levels on either side.

4.7.4.1. *For occupational exposures with durations other than 35 years:*

Extra risk estimates for a 45-year exposure to the same exposure levels were nearly identical to those from the 35-year exposure for both lymphoid cancer in both sexes and breast cancer in females (results not shown). With exposures beginning at 20 years of age and with the 15-year lag, the assumption of an additional 10 years of exposure only negligibly affects the risks above age 70 and has little impact on lifetime risk. For exposure scenarios of 35−45 years but with 8-hour TWAs falling between those presented in the tables, one can estimate the extra risk by interpolation. For exposure scenarios with durations of exposure less than 30−35 years, one could roughly estimate extra risks by calculating the cumulative exposure and finding the extra risks for a similar cumulative exposure in Tables 4-28 and 4-29. For a more precise estimation, or for exposure scenarios of much shorter duration

or for specific age groups, one should do the calculations using a life-table analysis, as presented in Appendix E but modified for the specific exposure scenarios.

4.7.4.2. ***For occupational exposures below 0.1 ppm:***

For lymphoid cancer, the low-exposure continuation of the two-piece linear spline model presented in Table 4-28 of the assessment is recommended. For 35-year exposures, the following formulae would apply:

95% UCL on extra risk for lymphoid cancer incidence = (8-h TWA occ exp [in ppm]) × (0.056/0.1 ppm) = (8-h TWA occ exp [in ppm]) × (0.56/ppm)

MLE of extra risk for lymphoid cancer incidence = (8-h TWA occ exp [in ppm]) × (0.024/0.1ppm) = (8-h TWA occ exp [in ppm]) × (0.24/ppm)

For breast cancer, the low-exposure continuation of the two-piece linear spline model presented in Table 4-29 of the assessment is recommended. For 35-year exposures, the following formulae would apply:

95% UCL on extra risk for breast cancer incidence = (8-h TWA occ exp [in ppm]) × (0.025/0.1 ppm) = (8-h TWA occ exp [in ppm]) × (0.25/ppm)

MLE of extra risk for breast cancer incidence = (8-h TWA occ exp [in ppm]) × (0.013/0.1ppm) = (8-h TWA occ exp [in ppm]) × (0.13/ppm)

For total cancer risk, low-exposure linear extrapolation from the total cancer extra risk estimates for the 0.1 ppm 8-hour TWA exposure level presented in Table 4-30 of the assessment is recommended. Both of the underlying models are linear in the low-exposure range (e.g., at the 0.1 ppm TWA and below); thus, their sum is also linear. For 35-year exposures, the following formulae would apply:

95% UCL on extra risk for total cancer incidence ≈ (8-h TWA occ exp [in ppm]) × (0.081/0.1 ppm) = (8-h TWA occ exp [in ppm]) × (0.81/ppm)

MLE of extra risk for total cancer incidence = (8-h TWA occ exp [in ppm]) × (0.037/0.1ppm) = (8-h TWA occ exp [in ppm]) × (0.37/ppm)

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