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Evaluation of the Inhalation Carcinogenicity of Ethylene Oxide

APPENDICES

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LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2′-deoxyguanosine
ACC	American Chemistry Council
ADAF	age-dependent adjustment factor
AIC	Akaike information criterion
AIDS	acquired immune deficiency syndrome
AP	apurinic/apyrimidinic
ARASP	Advancing Risk Assessment Science and Policy
BEIR	Committee on the Biological Effects of Ionizing Radiation
CI	confidence interval
DF	degrees of freedom
EC_{01}	effective concentration (modeled) resulting in 1% extra risk
EOSA	Ethylene Oxide Sterilization Association
EPA	U.S. Environmental Protection Agency
ERR	excess relative risk
EtO	ethylene oxide
FRG	Federal Republic of Germany
GC	gas chromatography
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione S-transferase
HESG	2-hydroxyethylated glutathione
HEVal	N-(2-hydroxytheyl)valine
HOEtNU	N-(2-hydroxyethyl)-N-nitrosourea
HPLC	high–performance liquid chromatography
i.p.	intraperitoneal
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IRIS	Integrated Risk Information System
IRR	incidence rate ratio
LH	lymphohematopoietic
LL	log likelihood
LEC_{01}	95% (one-sided) lower confidence limit on the EC_{01}
LLOQ	lower limit of quantification
MF	mutant fraction
MLE	maximum likelihood estimate
MNBC	micronucleus frequencies in binucleated cells
MOA	mode of action
MS	mass spectrometry
N1-HEA	N1-(2-hydroxyethyl)adenine
N3-HEA	N3-(2-hydroxyethyl)adenine
N3-HEC	N3-(2-hydroxyethyl)cytosine
N3-HET	N3-(2-hydroxyethyl)thymine
N3-HEU	N3-(2-hydroxyethyl)uracil

N^6-HEA	N^6-(2-hydroxyethyl)adenine
N7-HEG	N7-(2-hydroxyethyl)guanine
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
O^6-HEG	O^6-(2-hydroxyethyl)guanine
OPP	Office of Pesticide Programs
OR	odds ratio
PBPK	physiologically based pharmacokinetic
POD	point of departure
RR	relative rate (i.e., rate ratio), or more generally, relative risk
SAB	Science Advisory Board
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SEER	Surveillance, Epidemiology, and End Results
SIR	standardized incidence ratio
SMR	standard mortality ratio
S_N	substitution nucleophilicity
TLC	thin-layer chromatography
TWA	time-weighted average
UCC	Union Carbide Corporation
UCL	upper confidence limit

APPENDIX A. CRITICAL REVIEW OF EPIDEMIOLOGIC EVIDENCE

[EDITORIAL NOTE: The responses to the 2007 external peer-review and public comments can be found in Appendix H, the responses to the 2013 public comments are in Appendix K, and the responses to the 2015 SAB comments are in Appendix I.]

A.1. BACKGROUND

Prompted by studies indicating that ethylene oxide (EtO) is a mutagen and that exposure to EtO produces increased chromosomal aberrations in human lymphocytes (Ehrenberg and Gustafsson, 1970; Ehrenberg and Hallstrom, 1967; Rapoport, 1948), Hogstedt and colleagues studied three small, independent cohorts of workers from Sweden. Reports on two of these cohorts (Hogstedt et al., 1984; Hogstedt et al., 1979b; Hogstedt et al., 1979a) were reviewed in the earlier health assessment document (U.S. EPA, 1985). These two small cohorts plus a third group of EtO-exposed workers from a third independent plant in Sweden were then combined and studied as one cohort (Hogstedt, 1988; Hogstedt et al., 1986). A review of this reconstituted cohort study and subsequent independent studies is presented in Section A.2.

Shortly after the third Hogstedt study was completed, another independent study of EtO-exposed employees was published (Gardner et al., 1989) on a cohort of workers from four companies and eight hospitals in Great Britain, and it was followed by a third independent study on a cohort of exposed workers in eight chemical plants from the Federal Republic of Germany (Kiesselbach et al., 1990). A follow-up study of the Gardner et al. (1989) cohort was conducted by Coggon et al. (2004).

Greenberg et al. (1990) was the first in a series of studies of workers exposed to EtO at two chemical manufacturing facilities in the Kanawha Valley (South Charleston, WV). The workers at these two facilities were studied later by Teta et al. (1993), Benson and Teta (1993), Teta et al. (1999), and Swaen et al. (2009) and became the basis for several quantitative risk assessment analyses (Valdez-Flores et al., 2010; EOIC, 2001; Teta et al., 1999).

Another independent study of EtO-exposed workers in 14 sterilizing plants from across the United States was completed by the National Institute for Occupational Safety and Health (NIOSH) (Stayner et al., 1993; Steenland et al., 1991). The Stayner et al. (1993) paper presents the exposure-response analysis performed by the NIOSH investigators. These same workers were studied again from a different perspective by Wong and Trent (1993). The NIOSH investigators then conducted a follow-up of the mortality study (Steenland et al., 2004) and a breast cancer incidence study based in the same cohort (Steenland et al., 2003). The results of the Steenland et al. (2004) and Steenland et al. (2003) analyses are the basis for the quantitative

assessment in this document, for reasons explained in the review and summary sections of this appendix as well as Section 4.1 of the main report.

Several additional studies of lesser importance have been done on EtO-exposed cohorts of workers in Sweden (Hagmar et al., 1995; Hagmar et al., 1991), Italy (Bisanti et al., 1993), Belgium (Swaen et al., 1996) and western New York State (Norman et al., 1995), and other parts of the United States (Olsen et al., 1997). These studies are discussed in the following review, but they provide limited information to the overall discussion of whether EtO induces cancer in humans.

The more important studies, which are discussed in detail in the summary, are those at two facilities in the Kanawha Valley in West Virginia (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) and at 14 sterilizing plants around the country (Steenland et al., 2004; Steenland et al., 2003; Stayner et al., 1993; Steenland et al., 1991). These studies have sufficient follow-up to analyze latent effects, and the cohorts appear to be large enough to test for small differences. In addition, exposure estimates were derived for both cohorts, and attempts were made to assess dose-response relationships.

More recently, a follow-up study of the Swedish cohort of Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991), which also had quantitative exposure estimates for the individual workers, was published (Mikoczy et al., 2011). This follow-up study was published after the general cutoff date for literature inclusion in this assessment and is reviewed in detail in Section J.2.2 of Appendix J. However, because it is a follow-up of an earlier study and, with the additional follow-up, provides important corroborating evidence, the study is also briefly mentioned here.

A.2. INDIVIDUAL STUDIES

A.2.1. Hogstedt (1988), Hogstedt et al. (1986)

Hogstedt et al. (1986) combined workers from several cohorts for a total of 733 workers, including 378 workers from two separate and independent occupational cohort mortality studies by Hogstedt et al. (1979b) and Hogstedt et al. (1979a) and 355 employees from a third EtO production plant who had not been previously examined. The combined cohort was followed until the end of 1982. The first cohort comprised employees from a small technical factory in Sweden where hospital equipment was sterilized with EtO. The second was from a production facility where EtO was produced by the chlorohydrin method from 1940 to 1963. The third was from a production facility where EtO was made by the direct oxidation method from 1963 to 1982.

In the update of the 1986 occupational mortality report (Hogstedt, 1988), the cohort inexplicably was reduced to 709 employees (539 men; 170 women). Follow-up for mortality was extended to the end of 1985. The author reported that 33 deaths from cancer had occurred, whereas only 20 were expected in the combined cohort. The excess was attributed primarily to an increased risk of stomach cancer at one plant and an increase in blood and lymphatic malignancies at all three. Seven deaths from leukemia occurred, whereas only 0.8 were expected (standard mortality ratio [SMR] = 9.2). Ten deaths from stomach cancer occurred versus only 1.8 expected (SMR = 5.46). The results tend to agree with those from clastogenic and short-term tests on EtO (Ehrenberg and Gustafsson, 1970). The authors hypothesize that the large number of positive cytogenetic studies demonstrating increased numbers of chromosomal aberrations and sister chromatid exchanges at low-level exposure to EtO indicate that the lymphatic and hematopoietic systems are particularly sensitive to the genotoxic effects of EtO. They concluded that the induction of malignancies, observed even at low-level and intermittent exposures to EtO, should be "seriously considered by industry and regulating authorities."

In Plant 1 (Hogstedt et al., 1979a) in 1977, the average air concentrations of EtO ranged from 2 to 70 ppm in the storage hall. The average 8-hour time-weighted average (TWA) concentration in the breathing zone of the employees was calculated as 20 ppm ± 10 ppm. Measured concentrations were 150 ppm on the floor outside of the sterilized boxes. Exposure levels were lower in the sterilization room.

In Plant 2 (Hogstedt et al., 1979b), EtO was produced through the chlorohydrin process. Between 1941 and 1947, levels probably averaged about 14 ppm, with occasional exposures up to 715 ppm. Between 1948 and 1963, average levels were in the range of 6 ppm to 28 ppm. After 1963, when production of EtO came to an end, levels ranged from less than 1 ppm to as much as 6 ppm.

In Plant 3 (Hogstedt et al., 1986), the 355 employees were divided into subgroups. Subgroup A had almost pure exposure to EtO. Subgroup B had principal exposure to EtO but also exposure to propylene oxide, amines, sodium nitrate, formaldehyde, and 1,2-butene oxide. Workers in the remaining subgroup C were maintenance and technical service personnel, who had multiple exposures, including EtO. Concentration levels in Plant 3 are shown in Table A-1.

Table A-1. Estimated 8-hour time-weighted average ethylene oxide exposure, Plant 3

Group	1963–1976	1977–1982
A (*n* = 128)	5–8 ppm	1–2 ppm
B (*n* = 69)	3 ppm	1 ppm
C (*n* = 158)	1–3 ppm	0.4–1.6 ppm

Source: Hogstedt et al. (1986).

In the earlier studies (Hogstedt et al., 1979b; Hogstedt et al., 1979a) of two of the plants that contributed workers to this cohort, the authors note that there was exposure to ethylene dichloride, ethylene chlorohydrin, ethylene, and small amounts of bis-(2-chloroethyl) ether, as well as other chemicals in the respective plants. Although 170 women were present in the workforce, no sex differences in risk were analyzed separately by the investigators. Of 16 patients with tumors in the two exposed cohorts, there were three cases of leukemia (0.2 expected), six cases of alimentary tract cancer, and four cases of urogenital cancer. Of the 11 cancer cases in the full-time exposed cohort, 5.9 were expected ($p < 0.05$). This study was criticized by Divine and Amanollahi (1986) for several reasons. First, they argued that the study's strongest evidence in support of a carcinogenic claim for EtO was only a "single case of leukemia" in subgroup C of Plant 3, where the workers had multiple chemical exposures; however, there were no cases in subgroups A or B of Plant 3. Hogstedt et al. (1986) countered that the expectation of leukemia in these two subgroups was 0.04 and 0.02, respectively, and that the appearance of a case could only happen if EtO had "outstanding carcinogenic properties at low levels." Divine and Amanollahi also pointed out that a study (Morgan et al., 1981) of a cohort similar to that of Plant 3 found no leukemia cases or evidence of excessive mortality. Hogstedt et al. (1986) replied that Morgan et al. (1981) stated in their paper that the statistical power of their study to detect an increased risk of leukemia was not strong.

Divine and Amanollahi (1986) also stated that the exposures to EtO were higher in Plants 1 and 2 than in Plant 3; therefore, combinations would "normally preclude comparisons among the plants for similar causes of adverse health." This potential problem could be resolved by structuring exposure gradients to analyze risk. Furthermore, they noted Plant 1 was a nonproduction facility involved in sterilization of equipment. Plant 2 used the chlorohydrin process for making EtO, and Plant 3 used the direct oxygenation process. Although these conditions are obviously different, they "are grouped together as analogous." This criticism would, in most instances, be valid only because the methods for producing EtO differ and there were differing exposures to multiple chemicals.

However, these concerns are not supported by the evidence. In all three plants the leukemia risk was elevated, even if only slightly in Plant 3. This suggests that there may have been a common exposure, possibly to EtO, endemic to all three plants that was responsible for the measured excesses. Noteworthy is the elevated risk of leukemia seen in Plant 1 (3 observed vs. 0.14 expected), where the exposures were almost exclusively to EtO in the sterilization of equipment. The argument that Plant 1 leukemias form a "chance cluster," as Shore et al. (1993) claim, and as such should be excluded from any analysis does not preclude the possibility that these cases are in reality the result of exposure to EtO. Hogstedt (1988) argues that earlier remarks by Ehrenberg and Gustafsson (1970) that EtO "constituted a potential cancer hazard" on the basis of a considerable amount of evidence other than epidemiologic should have served as a warning that the increased risk seen in Plant 1 was not necessarily a "chance cluster," and because the chlorohydrin process was not used in Plant 1, it cannot be due to exposure to a chemical in the chlorohydrin process.

A.2.2. Gardner et al. (1989)

Gardner et al. (1989) completed a cohort study of 2,876 men and women who had potential exposure to EtO. The cohort was identified from employment records at four companies that had produced or used EtO since the 1950s and from eight hospitals that have had EtO clinical sterilizing units since the 1960s. The cohort was followed to December 31, 1987. All but 1 of the 1,012 women and 394 of the men in the cohort worked at one of the hospitals. The remaining woman and 1,470 men made up the portion of the cohort from the four companies. By the end of the follow-up, 226 members (8% of the total cohort) had died versus 258.8 expected. Eighty-five cancer deaths were observed versus 76.64 expected.

No clear excess risk of leukemia (3 observed vs. 2.09 expected), stomach cancer (5 observed vs. 5.95 expected), or breast cancer (4 observed vs. 5.91 expected) was present as of the cutoff date. "Slight excesses" of deaths due to esophageal cancer (5 observed vs. 2.2 expected), lung cancer (29 observed vs. 24.55 expected), bladder cancer (4 observed vs. 2.04 expected), and non-Hodgkin lymphoma (NHL) (4 observed vs. 1.63 expected) were noted, although an adjustment made to reflect local "variations in mortality" reduced the overall cancer excess from 8 to only 3. According to the authors' published tabulations, all three leukemias identified in this study fell into the longest latent category (20 years or longer), where only 0.35 were expected. All three were in the chemical plants. This finding initially would seem to be consistent with laboratory animal evidence demonstrating excess risks of hematopoietic cancer in animals exposed to EtO. But the authors note that because other known leukemogens were present in the workplace, the excess could have been due to a confounding effect.

The hospitals began using EtO during or after 1962, whereas all of the chemical companies had handled EtO from or before 1960. In the hospitals there was occasional exposure to formaldehyde and carbon tetrachloride but few other confounding agents. On the other hand, the chemical workers were exposed to a wide range of compounds including chlorohydrin, propylene oxide, styrene, and benzene. The earliest industrial hygiene surveys in 1977 indicated that the TWA average exposures were less than 5 ppm in almost all jobs and less than 1 ppm in many. No industrial hygiene data were available for any of the facilities prior to 1977, although it is stated that peaks of exposure up to several hundred ppm occurred as a result of operating difficulties in the chemical plants and during loading and unloading of sterilizers in the hospitals. An odor threshold of 700 ppm was reported by both manufacturers and hospitals, according to the authors. The authors assumed that past exposures were somewhat higher without knowing precisely what they were. An attempt was made to classify exposures into a finite number of subjectively derived categories (definite, possible, continual, intermittent, and unknown). This exercise produced no discernable trends in risk of exposure to EtO. However, the exposure status classification scheme was so vague as to be useless for determining risk by gradient of exposure to EtO.

It is of interest that all three of the leukemia deaths entailed exposure to EtO, with very little or no exposure to benzene, according to the authors. The findings are not inconsistent with those of Hogstedt (1988) and Hogstedt et al. (1986). The possibility of a confounding effect from substances other than benzene in these chemical workers cannot be entirely ruled out. Other cancers were slightly in excess, but overall there was little increased mortality from cancer in this cohort. It is possible that if very low levels of exposure to EtO had prevailed throughout the history of these hospitals and plants, the periods of observation necessary to observe an effect may not have been long enough.

A follow-up study of this cohort conducted by Coggon et al. (2004) is discussed below.

A.2.3. Kiesselbach et al. (1990)

Kiesselbach et al. (1990) carried out an occupational cohort mortality study of 2,658 men from eight chemical plants in the Federal Republic of Germany (FRG) that were involved in the production of EtO. The method of production is not stated. At least some of the plants that were part of an earlier study by Thiess et al. (1982) were included. Each subject had to have been exposed to EtO for at least 1 year sometime between 1928 and 1981 before person-years at risk could start to accumulate. Most exposures occurred after 1950. By December 31, 1982, the closing date of the study, 268 men had died (about 10% of the total cohort), 68 from malignant neoplasms. The overall SMR for all causes was 0.87, and for total cancer, the SMR was 0.97,

based on FRG rates. The authors reported that this deficit in total mortality indicates a healthy-worker effect.

The only remarkable findings here are slightly increased risks of death from stomach cancer (14 observed vs. 10.15 expected, SMR = 1.4), cancer of the esophagus (3 observed vs. 1.5 expected, SMR = 2), and cancer of the lung (23 observed vs. 19.86 expected, SMR = 1.2). Although the authors claimed that they looked at latency, only stomach cancer and total mortality has a latency analysis included. This was accomplished by not counting the first 10 years of follow-up in the parameter "years since first exposure." This study is limited by the lack of further latency analyses at other cancer sites. The risk of stomach cancer shows only a slight nonsignificant trend upward with increasing latency. Only two leukemias were recorded versus 2.35 expected.

This is a largely unremarkable study, with few findings of any significance. No actual exposure estimates are available. The categories of exposure that the authors constructed are "weak," "medium," and "strong." It is not known whether any of these categories is based on actual measurements. No explanation of how they were derived is provided except that the authors claim that the information is available on 67.2% of the members of the cohort. If the information was based on job categories, it should be kept in mind that exposures in jobs that were classified the same from one plant to the next may have produced entirely different exposures to EtO. The tabular data regarding these exposure categories shows that only 2.4% of all members of the cohort were considered "strongly" exposed to EtO. Although 71.6% were classified as "weak," the remaining 26% were considered as having "medium" exposure to EtO.

This is largely a study in progress, and further follow-up will be needed before any definite trends or conclusions can be drawn. The authors reported that only a median 15.5 years of follow-up had passed by the end of the cutoff date, whereas the median length of exposure was 9.6 years. Before any conclusions can be made from this study, several additional years of follow-up would be needed with better characterization of exposure.

A.2.4. Greenberg et al. (1990)

Greenberg et al. (1990) retrospectively studied the mortality experience of 2,174 men who were assigned to operations that used or produced EtO in either of two Union Carbide Corporation (UCC) chemical plants in West Virginia. In 1970 and 1971, EtO production at the two plants was phased out, but EtO was still used in the plants to produce other chemicals. SMRs were calculated in comparison with the general U.S. population and the regional population. Results based on regional population death rates were found to be similar to those based on the U.S. general population. Follow-up began either on January 1, 1940, if exposure to EtO began sooner, or on the date when exposure began, if it occurred after January 1, 1940.

Follow-up ended on December 31, 1978. Note that this cohort is thus a mixture of a prevalent cohort and an incident cohort, and the prevalent part of the cohort may be especially vulnerable to bias from the healthy worker survivor effect. The healthy worker survivor effect might have occurred if workers who were employed before 1940 and who were of greater susceptibility preferentially developed a disease of interest prior to 1940 and were no longer employed when cohort enumeration began. It appears that the chemical facilities began operating in 1925, so the maximum latency for the development of a disease of interest between the time of first exposure and cohort enumeration was 15 years; however, these early (pre-1940) hires would also have had the highest EtO exposures (Swaen et al., 2009) and may thus have had short latency periods as well. The healthy worker survivor effect bias can also dampen exposure-response relationships (Applebaum et al., 2007). According to Greenberg et al. (1990), slightly over 10% of the cohort consisted of prevalent hires (223 of 2,174). This is not a large proportion, but as noted above, these early hires would also have had the highest exposures (Swaen et al., 2009). It is unknown how many workers employed before 1940 were no longer employed when cohort enumeration began. Two years of pre-1940 exposure were reportedly taken into account when categorizing the cohort into groups with ≥2 years exposure in the different potential exposure categories (see below); however, it is unclear how pre-1940 years of exposure were treated in other analyses, for example, the analyses based on duration of exposure (although presumably they were taken into account for those analyses as well).

Total deaths equaled 297 versus 375.9 expected (SMR = 0.79, $p < 0.05$). Only 60 total cancer deaths were observed versus 74.6 expected (SMR = 0.81). These deficits in mortality suggest a manifestation of the healthy-worker effect. In spite of this, nonsignificant elevated risks of cancer of the liver, unspecified and primary, (3 observed vs. 1.8 expected, SMR = 1.7), pancreas (7 observed vs. 4.1 expected, SMR = 1.7), and leukemia and aleukemia (7 observed vs. 3.0 expected, SMR = 2.3) were noted.

The authors also reported that in 1976 (3 years before the end of follow-up), an industrial hygiene survey found that 8-hour TWA EtO levels averaged less than 1 ppm, although levels as high as 66 ppm 8-hour TWA had been observed. In maintenance workers, levels averaged between 1 and 5 ppm 8-hour TWA. Because of the lack of information about exposures before 1976 (e.g., when EtO was in production), the authors developed a qualitative exposure categorization scheme with three categories of exposure (low, intermediate, and high) on the basis of the potential for exposure in each department. The number of workers in each exposure category was not reported; however, it appears from Teta et al. (1993) (see below) that only 425 workers were assigned to EtO production departments, which were apparently the only departments with high potential exposure. No significant findings of a dose-response relationship were discernable.

Except for two cases of leukemia, all the workers who died of pancreatic cancer or leukemia began their work—and hence exposure to EtO—many years before their deaths. The leukemia and pancreatic cancer deaths were concentrated in the chlorohydrin production department. Four of the seven workers who died of leukemia had been assigned to the chlorohydrin department; only 0.8 deaths (SMR = 5.0) would have been expected in this department of only 278 workers. Six of the workers who died of pancreatic cancer were assigned to the chlorohydrin department, whereas only 0.98 deaths would have been expected to occur (SMR = 6.1). All seven workers who died of leukemia, including the four in the chlorohydrin department, were listed by the authors as having only low potential exposure to EtO. In contrast, among workers ever assigned to a department in the high exposure category, no leukemia deaths and only one pancreatic cancer death occurred.

The authors hypothesized that the excesses in leukemia and pancreatic cancers were associated with production of ethylene chlorohydrin or propylene chlorohydrin, or both, in the chlorohydrin department. Some later follow-up studies (described below) were done of the cohort excluding the chlorohydrin production workers (Teta et al., 1993) and of the chlorohydrin production workers alone (Benson and Teta, 1993) to further examine this hypothesis.

A.2.5. Steenland et al. (1991)

In an industry-wide analysis by NIOSH, Steenland et al. (1991) studied EtO exposure in 18,254 workers identified from personnel files of 14 plants that had used EtO for sterilization of medical equipment, treating spices, or testing sterilizers. Each of the 14 plants (from 75 facilities surveyed) that were considered eligible for inclusion in the study had at least 400 person-years at risk prior to 1978. Within each eligible facility, at least 3 months of exposure to EtO qualified an employee for inclusion in the cohort. Employees, including all salaried workers, who were "judged never to have been exposed to EtO" on the basis of industrial hygiene surveys were excluded. Follow-up ended December 31, 1987. The cohort averaged 16 years of latency. Approximately 86% achieved the 9-year latent point, but only 8% reached the 20-year latency category. The average year of first exposure was 1970, and the average length of exposure was 4.9 years. The workers' average age at entry was not provided, nor was an age breakdown. Nearly 55% of the cohort were women.

Some 1,137 workers (6.4%) were found to be deceased at the end of the study period, upon which the underlying cause of death was determined for all but 450. If a member was determined to be alive as of January 1, 1979, but not after and no death record was found in the National Death Index through December 31, 1987, then that member was assumed to be alive for the purposes of the life-table analysis and person-years were accumulated until the cutoff date. Altogether, 4.5% of the cohort fell into this category. This procedure would tend to increase the

expected deaths and, as a consequence, potentially bias the risk ratio downward if a sizable number of deaths to such persons during this period remained undiscovered to the researchers.

In the total cohort no significantly increased risks of death from any site-specific cancer were noted. Analyses by job categories and by duration of exposure indicated no excess risks of cancer when compared with the rate in the general population. However, there was an increased trend in the risk of hematopoietic cancers, all sites, with increasing lengths of time since first exposure. After 20 years latency, the SMR was 1.76, based on 13 cases. The test for trend was significant at $p = 0.03$. For men (45%), without regard for latency, the SMR for hematopoietic cancer was a significant 1.55 ($p < 0.05$), based on 27 cases. Among men with long latency (greater than 20 years) and the longest duration of exposure (greater than 7 years) the SMR for hematopoietic cancers was 2.63, based on 7 deaths ($p < 0.05$).

The authors pointed out that the SMR for leukemia among men was 3.45, based on 5 deaths ($p < 0.05$), for deaths in the latter period of 1985 to 1987. For kidney cancer, the SMR was 3.27, based on six deaths ($p < 0.05$), after 20-years latency. The authors also reported on a significant excess risk ($p < 0.05$) of lymphosarcoma-reticulosarcoma in men (SMR = 2.6), based on seven deaths. Women had a lower nonsignificant rate. The risk of breast cancer was also nonsignificant (SMR = 0.85 based on 42 cases). The authors hypothesized that men were more heavily exposed to EtO than were women because "men have historically predominated in jobs with higher levels of exposure." However, the lack of an association between EtO exposure and lymphohematopoietic cancer in females was also observed in the exposure-response analyses of this cohort, including in the highest exposure category, performed by Stayner et al. (1993) and discussed below.

Industrial hygiene surveys indicated that sterilizer operators were exposed to an average personal 8-hour TWA EtO level of 4.3 ppm, whereas all other workers averaged only 2 ppm, based on 8-hour samples during the period 1976 to 1985. These latter employees primarily worked in production and maintenance, in the warehouse, and in the laboratory. This was during a time when engineering controls were being installed to reduce worker's exposure to EtO; earlier exposures may have been somewhat higher. The authors reported that no evidence of confounding exposure to other occupational carcinogens was documented.

The authors concluded that there was a trend toward an increased risk of death from hematopoietic cancer with increasing lengths of time since the first exposure to EtO. This trend might have been enhanced if the authors had added additional potential deaths identified from the 820 (4.5%) "untraceable" members of the cohort from 1979 to 1987. The authors felt that their results were not conclusive for the relatively rare cancers of a priori interest, based on the limited number of cases and the short follow-up. The cohort averaged 16 years of latency and 86% had at least 9 years, but only 8% reached the 20-year latent category.

Exposure-response analyses were conducted by Stayner et al. (1993) and are discussed below. More recently, a follow-up mortality study (Steenland et al., 2004) and a breast cancer incidence study (Steenland et al., 2003) of this cohort were conducted; these are also discussed below.

A.2.6. Teta et al. (1993)

In a follow-up analysis of the cohort of 2,174 male UCC workers studied by Greenberg et al. (1990), Teta and her colleagues excluded the 278 workers in the chlorohydrin unit in which Greenberg and colleagues found a high risk of leukemia and pancreatic cancer, thereby removing the potential confounding of the chlorohydrin production process. The 1,896 men in the remaining cohort were followed for an additional 10 years, through all of 1988. (Among the 278 men who were excluded because they had worked in the chlorohydrin unit, 49 had also been assigned to EtO production departments, which were considered high potential EtO exposure departments, according to Greenberg et al. (1990). Data were reportedly examined with and without the inclusion of these 49 workers with overlapping assignments; however, the results of these analyses are not fully presented.) According to Benson and Teta (1993), 112 of the 278 excluded workers were employed before 1940, reducing the prevalent part of the remaining cohort to 111 of 1,896 workers, or just under 6%. (It is unclear how pre-1940 years of exposure were treated in the analyses based on duration of exposure, although presumably they were taken into account.) The update did not include additional work histories for the study subjects. Teta et al. (1993) note that duration of assignment to an EtO production unit was not affected by the update because EtO was no longer in production at the two plants; however, assignment to EtO-using departments might have been affected, and according to Greenberg et al. (1990), some of these departments had medium EtO exposure potential.

Teta et al. (1993) reported that the average duration of exposure was more than 5 years and the average follow-up was 27 years. Furthermore, at least 10 years had elapsed since first exposure for all the workers. The reanalysis demonstrated no increased risk of overall cancer, or of leukemia, NHL, or cancers of the brain, pancreas, or stomach. The SMR for total deaths, based on comparison with mortality from the general population, was 0.79 ($p < 0.01$; observed = 431). The SMR for total cancer was 0.86 (observed = 110). No site-specific cancers were significantly elevated. Although the authors concluded that this study did not indicate any significant trends of increasing site-specific cancer risk with increasing duration of potential exposure to EtO, there appeared to be a nonsignificant increasing trend for leukemia and aleukemia ($p = 0.28$, based on five cases) as well as stomach cancer ($p = 0.13$; eight cases).

According to Greenberg et al. (1990), 8-hour TWA EtO levels averaged less than 1 ppm, based on the 1976 monitoring (after EtO production at the plants had ceased), although levels as

high as 66 ppm 8-hour TWA were reported. Teta et al. (1993) estimated that in the 1960s, exposure in the units producing EtO by direct oxidation ranged from 3 to 20 ppm 8-hour TWA, with peaks of several hundred ppm. These estimates were based on an industrial hygiene survey conducted at another UCC facility in Texas that used the same direct oxidation process as the two plants in West Virginia from which the UCC EtO cohort was taken. Ethylene oxide was also produced via the chlorohydrin process in a closed building during the years 1925 to 1957. Levels of exposure to EtO would have been higher than in the direct oxidation production process because of start-up difficulties, fewer engineering controls, less complex equipment, and the enclosed building. Employee nausea, dizziness, and vomiting were documented in the medical department in 1949. These acute effects occur in humans at exposures of several hundred ppm, according to the authors.

During the time periods under investigation, the estimated exposure ranges for departments using or producing EtO were >14 ppm from 1925 to 1939; 14 ppm from 1940 to 1956; 5–10 ppm from 1957 to 1973; and <1 ppm from 1974 to 1988, with frequent peaks of several hundred ppm in the earliest period and some peaks of similar intensity in the 1940s to mid-1950s. In the absence of monitoring data prior to 1976, these estimates cannot be confirmed. Furthermore, workers were eliminated from the analysis if they had worked in the chlorohydrin unit because it was assumed that the increased risks of leukemia and pancreatic cancer were possibly due to exposure to something in the chlorohydrin process, as conjectured by Greenberg et al. (1990). However, even when the potential confounding influence of the chlorohydrin process is removed, there remains the suggestion of a trend of an increasing risk of leukemia and aleukemia with increasing duration of exposure to EtO in the remaining cohort members ($p = 0.28$, based on 5 cases).

The authors indicated that their findings do not confirm the findings in laboratory animal studies and are not consistent with the earliest results reported among EtO workers. They also noted that they did not observe any significant trend of increasing risks of stomach cancer ($n = 8$), leukemia ($n = 5$) or cancers of the pancreas or brain and nervous system with increasing duration of exposure. No lagged exposure or latency analyses were conducted in this study.

In a later analysis, Teta et al. (1999) fitted Poisson regression dose-response models to the UCC data (Teta et al., 1993) and to the NIOSH data (Steenland et al., 1991). They reported that latency and lagging of dose did not appreciably affect the fitted models. Because Teta et al. (1999) did not present risk ratios for the categories used to model the dose-response relationships, the only comparison that could be made between the UCC and NIOSH data is based on the fitted models. The results of 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 dose, whereas the risk for the modeled NIOSH data increased as a function of dose.

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. 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 two cohorts [see discussion of the Swaen et al. (2009) study below].

A.2.7. Benson and Teta (1993)

In a companion mortality study (Benson and Teta, 1993), the remaining 278 employees who were identified by Greenberg et al. (1990) as having worked at some time in the chlorohydrin unit and who were not included in the cohort of Teta et al. (1993) were followed to the end of 1988. Note that the prevalent part (i.e., those workers first employed before the cohort enumeration date of 1 January 1940) of this reduced cohort is 112 of the 278 workers, or 40%, and therefore, the potential for bias from a healthy worker survivor effect, as discussed for the Greenberg et al. (1990) study above (see Section A.2.4), may be more pronounced in this study of the chlorohydrin unit workers. It is unknown how many chlorohydrin unit workers employed before 1940 were no longer employed when cohort enumeration began.

Altogether, 40 cancer deaths occurred versus 30.8 expected (SMR = 1.3) in the subcohort of chlorohydrin workers. In Greenberg et al. (1990), significant elevated risks of pancreatic cancer and leukemia and aleukemia occurred in only those workers assigned to the chlorohydrin process. Benson and Teta (1993) noted a significantly increased risk of pancreatic cancer (SMR = 4.9, eight observed deaths, $p < 0.05$) in the same group and a significantly increased risk of cancer in the enlarged category of lymphohematopoietic cancer (SMR = 2.9, eight observed deaths, $p < 0.05$), which included leukemia and aleukemia, after an additional 10 years of follow-up.

The authors concluded that these cancers were likely work-related and some exposure in the chlorohydrin unit, possibly to the chemical ethylene dichloride, was probably the cause. They pointed out that Greenberg et al. (1990) found that the chlorohydrin unit was likely to be a low-EtO exposure area in the West Virginia plants. The other possibility was bis-chloroethyl ether, which the authors pointed out is rated by the International Agency for Research on Cancer (IARC) as a group 3 ("not classifiable as to its carcinogenicity to humans") chemical. Circumstantial evidence seems to support the authors' contention that ethylene dichloride is the cause: IARC designated ethylene dichloride as a group 2B chemical ("possibly carcinogenic to humans"), exposure was likely heavier throughout the history of the facility, and plant medical records documented many accidental overexposures occurring to the workers who died of pancreatic cancer prior to diagnosis. However, this conclusion is disputed by Olsen et al. (1997) whose analysis is discussed later.

A.2.8. Stayner et al. (1993)

Stayner et al. (1993) provide an exposure-response analysis for the cohort study of EtO workers described by Steenland et al. (1991). Nothing was modified concerning the follow-up, cohort size, vital status, or cutoff date of the study. The exposure assessment and verification procedures were presented in Greife et al. (1988) and Hornung et al. (1994). In brief, 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. These data were divided into two sets, one for developing the regression model and the second (from six randomly selected plants) for testing it. Job titles were grouped into eight categories with similar potential for EtO exposure, and arithmetic mean exposure levels by facility, year, and exposure category were calculated from the data used for model development. The arithmetic means were logarithmically transformed, and weighted linear regression models were fitted. Seven out of 23 independent variables tested for inclusion in the model were found to be significant predictors ($p \leq 0.10$) of EtO exposure and were included in the final model (exposure category [job], type of product sterilized, sterilizer size, engineering controls [rear exhaust, aeration], days since product sterilization, and calendar year). This model predicted 85% of the variation in average EtO exposure levels in the test data. The model was also evaluated against estimates for the test data derived by a panel of 11 industrial hygienists familiar with EtO levels in the sterilization industry and provided with the values for the independent variables used in the model corresponding to the arithmetic means from the test data. The overall mean of the modeled estimates was not highly biased nor biased in one direction when compared to the overall mean exposure estimates of the individual industrial hygiene experts. Using the test data as the standard, the model estimates showed less bias (average difference) than 9 of the 11 industrial hygienists and more precision (standard deviation of the differences) than all 11. Similarly, the model outperformed the panel in terms of both bias and precision when the panel results were averaged.

Average exposure levels, including early historical exposure levels, for the exposure categories in the study plants were estimated using this industrial hygiene-based regression model. Then, the cumulative exposure for each worker was estimated by calculating the product of the average exposure in each job the worker held by the time spent in that job and then summing these over all the jobs held by that worker. This value became the cumulative exposure index for that employee and reflected the working lifetime total exposure to EtO. (Details about the exposure estimates for the cohort are presented in Section D.5 of Appendix D.)

Stayner et al. (1993) generated SMRs based on standard life-table analysis. The three categories of cumulative exposure were less than 1,200 ppm-days, 1,200 to 8,500 ppm-days, and greater than 8,500 ppm-days. Additionally, the Cox proportional hazards model was used to model the exposure-response relationship between EtO and various cancer types, using cumulative exposure as a continuous variable.

Stayner et al. (1993) noted a marginally significant increase in the risk of hematopoietic cancers, with an increase in cumulative exposure by both the life-table analysis as well as the Cox model, although the magnitude of the increased risk was not substantial. At the highest level—greater than 8,500 ppm-days of exposure—the SMR was a nonsignificant 1.24, based on 13 cases. However, 12 of these cases were in males, whereas only 6.12 were expected. Thus, in this highest exposure category, a statistically significant ($p < 0.05$) SMR of 1.96 in males was produced. This dichotomy produced a deficit in females (1 observed vs. 4.5 expected, $p < 0.05$).

The Cox analysis produced a significantly positive trend with respect to lymphoid cell tumors (combination of lymphocytic leukemia and NHL) when EtO exposures were lagged 5 years. The authors stated that these data provide some support for the hypothesis that exposure to EtO increases the risk of mortality from lymphatic and hematopoietic neoplasms. They pointed out, however, that their data do not provide evidence for a positive association between exposure to EtO and cancer of the stomach, brain, pancreas, or kidney or leukemia as a group. Breast cancer was not analyzed in this report.

This cohort was not updated with vital status information on the "untraceables" (4.5%), and cause of death information was not provided on deaths with unknown causes; thus, the cohort lacks a complete follow-up, and therefore, the risk estimates may be understated. Another potential limiting factor is the information regarding industrial hygiene measurements of EtO that were completed in the plants. According to the authors, the median length of exposure to EtO of the cohort was 2.2 years and the median exposure was 3.2 ppm. It may be unreasonable to expect any findings of increased significant risks because follow-up was too short to allow the accumulation of mortality experience (average follow-up = 16 years; only 8% of cohort had $\geq$20 years follow-up).

The authors also remind us that there is a lack of evidence for an exposure-response relationship among females or for a sex-specific carcinogenic effect of EtO in either laboratory animals or humans. In fact, the mortality rate from hematopoietic cancers among the women in this cohort was lower than that of the general U.S. population. Therefore, the contrast seen here is unusual.

The positive findings are somewhat affected by the presence in the cohort of one heavily exposed case (although the authors saw no reason to exclude it from the analysis), and there is a lack of definite evidence for an effect on leukemia as a group. Despite these limitations, the

authors believe that their data provide support for the hypothesis that exposure to EtO increases the risk of mortality from hematopoietic neoplasms.

This analysis is superseded by the more recent analysis by the same authors of the results of a more recent follow-up study of this cohort [see discussion of the Steenland et al. (2004) study below].

A.2.9. Wong and Trent (1993)

This study is a reanalysis of the same cohort that was studied by Stayner et al. (1993) and Steenland et al. (1991), with some differences. The cohort was incremented without explanation by 474 to a total of 18,728 employees and followed one more year, to the end of December 1988. This change in the cohort resulted in the addition of 176 observed deaths and 392.2 expected deaths. The finding of more than twice as many expected deaths as observed deaths is baffling. A reduced total mortality of this magnitude suggests that many deaths may have been overlooked, resulting in a further reduction of the overall SMR to a significant deficit of 0.73. Sixty additional cancer deaths were added versus 65.9 expected, for an SMR = 0.9, based on 403 total cancer deaths observed versus 446.2 expected.

The authors reported no significant increase in mortality at the cancer sites found to be of most interest in previous studies (i.e., stomach, leukemia, pancreas, brain, and breast). They also reported the lack of a dose-response relationship and correlation with duration of employment or latency. They did report a statistically significant increased risk of NHL among men (SMR = 2.47; observed = 16, expected = 6.47; $p < 0.05$) that was not dose related and a nonsignificant deficit of NHL among women (SMR = 0.32; observed = 2, expected = 6.27). The authors concluded that the increase in men was not related to exposure to EtO but could in fact have been related to the presence of acquired immune deficiency syndrome (AIDS) in the male population. When this explanation was offered in a letter to the editor (Wong, 1991) regarding the excess of NHL reported in Steenland et al. (1991), it was dismissed by Steenland and Stayner (1993) as pure speculation. Steenland and Stayner (1993) responded that most of the NHL deaths occurred prior to the AIDS epidemic, which began in the early 1980s. They also indicated that there was no reason to suspect that these working populations would be at a higher risk for AIDS than was the general population, the comparison group.

Wong and Trent (1993) also reported a slightly increased risk of cancer in other lymphatic tissue (14 observed vs. 11.39 expected). In men, the risk was nonsignificantly higher (11 observed vs. 5.78 expected). Forty-three lymphopoietic cancers were observed versus 42 expected. In men, the risk was higher (32 observed vs. 22.22 expected). Fourteen leukemia deaths were noted versus 16.2 expected. The authors did not derive individual exposure

estimates for exposure-response analysis as Stayner et al. (1993) did. Rather, they used duration of employment as a surrogate for exposure.

This study has many of the same limitations as the Stayner et al. (1993) study. The authors assumed that those individuals with an unknown vital status as of the cutoff date were alive for the purposes of the analysis, and they were unable to obtain cause-of-death information on 5% of the known deaths.

The differences between this cohort study and that of Stayner et al. (1993) are in the methods of analysis. Stayner et al. (1993) used the 9th revision of the International Classification of Diseases (ICD) to develop their site-specific cancer categories for comparison with expected cancer mortality, whereas Wong and Trent (1993) used the 8th revision. This could account for some of the differences in the observed numbers of site-specific cancers, because minor differences in the coding of underlying cause of death could lead to a shifting of some unique causes from one site-specific category to another. Furthermore, Wong and Trent (1993) did not analyze separately the category "lymphoid" neoplasms, which includes lymphocytic leukemia and NHL, whereas Stayner et al. (1993) did. Stayner et al. (1993) further developed cumulative exposure information using exposure estimates, whereas Wong and Trent (1993) used length of employment as their surrogate for exposure but did not code detailed employment histories.

Because Wong and Trent (1993) made no effort to quantify the exposures, as was the case in Stayner et al. (1993), this study is less useful in determining a exposure-response relationship. Furthermore, the assumption that a member of the cohort should be considered alive if a death indication could not be found will potentially tend to bias risk ratios downward if, in fact, a large portion of this group is deceased. In this study all untraceable persons were considered alive at the end of the follow-up; therefore, the impact of the additional person-years of risk cannot be gauged.

A.2.10. Bisanti et al. (1993)

These authors reported on a cohort mortality study of 1,971 male chemical workers licensed to handle EtO by the Italian government, whom they followed retrospectively from 1940 to 1984. Altogether, 76 deaths had occurred in this group by the end of the study period, whereas 98.8 were expected. Of those, 43 were due to cancer versus 33 expected. The cause of one death remained unknown, and 16 workers were lost to follow-up. A group of 637 individuals from this cohort was licensed to handle only EtO; the remaining 1,334 had licenses valid for handling other toxic gases as well. Date of licensing for handling EtO became the initiating point of exposure to EtO, although it is likely that some of these workers had been exposed previously to EtO. The regional population of Lombardia was used as the reference group from which comparison death rates were obtained.

Although there were excess risks from almost all cancers, one of the greatest SMRs was in the category known as "all hematopoietic cancers," where 6 observed deaths occurred when only 2.4 were expected (SMR = 2.5). In the subgroup "lymphosarcoma, reticulosarcoma" there were 4 observed deaths whereas only 0.6 were expected (SMR = 6.7, $p < 0.05$); the remaining 2 were leukemias. The authors note that five hematopoietic cancers occurred in the subgroup of workers who were licensed to handle only EtO but no other chemicals versus only 0.7 hematopoietic cancers expected (SMR = 7.1, $p < 0.05$). These deaths occurred within 10 years from date of licensing (latent period), which is consistent with the shorter latent period anticipated for this kind of cancer. According to the authors, all workers began their employment in this industry when the levels of EtO were high, although no actual measurements were available. The fact that this subgroup of workers was licensed only for handling EtO reduces the likelihood of a confounding chemical influence.

The authors concluded that the excess risk of cancer of the lymphatic and hematopoietic tissues in these particular EtO cohort members support the suggested hypothesis of a higher risk of cancer found in earlier studies, but they added that the lack of exposure information on the other industrial chemicals in the group that had a license for handling other toxic chemicals made their findings inconclusive.

This study was of a healthy young cohort, and most person-years were contributed in the latter years of observation. Many years of follow-up may be necessary in order to fully verify any trend of excess risks for the site-specific cancers of interest and to measure latent effects. Furthermore, the unusual deficit of total deaths versus expected contrasted with an excess of cancer deaths versus expected raises a question about the potential for selection bias when the members of this cohort were chosen for inclusion. Also, one of the study's major limitations is the lack of exposure data.

A.2.11. Hagmar et al. (1995) and Hagmar et al. (1991)

Cancer incidence was studied in a cohort of 2,170 EtO-exposed workers from two plants in Sweden that produced disposable medical equipment. To fit the definition for inclusion, the subjects, 1,309 women and 861 men, had to have been employed for a minimum of 12 months and some part of that employment had to have been during the period 1970–1985 in the case of one plant and 1965–1985 in the case of the other. The risk ratios were not dichotomized by sex. No records of anyone who left employment or died before January 1, 1972 in one plant and January 1, 1975 in the other were included. Expected incidence rates were generated from the Southern Swedish Regional Tumor Registries.

Because of a short follow-up period and the relative young age of the cohort, little morbidity had occurred by the end of the cutoff date of December 31, 1990. Altogether,

40 cancers occurred, compared with 46.3 expected. After 10 years latency, 22 cases of cancers were diagnosed versus 22.6 expected. However, 6 lymphohematopoietic cancers were observed versus 3.37 expected, and when latency is considered, this figure falls to 3 versus 1.51 expected. The authors pointed out that for leukemia the standard incidence ratio (SIR) is a nonsignificant 7.14, based on 2 cases in 930 subjects having at least 0.14 ppm-years of cumulative exposure to EtO and a minimum of 10 years latency. The authors believed that the results provided some minor evidence to support an association between exposure to EtO and an increased risk of leukemia. However, for breast cancer, no increase in the risk was apparent for the total cohort (SIR = 0.46; 5 cases). Even in the 10-years or more latency period, the risk was less than expected (SIR = 0.36; 2 cases).

The authors made a reasonably good attempt to determine exposure levels during the periods of employment in both plants for six job categories. Sterilizers in the years 1970–1972 were exposed to an average 40 ppm in both plants. These levels gradually dropped to 0.75 ppm by 1985–1986. Packers and developmental engineers were the next highest exposed employees, with levels in 1970–1972 of 20 to 35 ppm and by 1985–1986 of less than 0.2 ppm. During the period 1964–1966 in the older plant, EtO levels averaged 75 ppm in sterilizers and 50 ppm in packers. Peak exposures were estimated to have ranged from 500 to 1,000 ppm during the unloading of autoclaves up to 1973. The levels gradually dropped to less than 0.2 ppm in both plants by 1985–1986 in all job categories (developmental engineers, laboratory technicians, repair men, store workers, controllers, foremen, and others) except sterilizers.

These exposure estimates were verified by measurement of hydroxy ethyl adducts to N-terminal valine in hemoglobin in a sample of subjects from both plants. The adduct levels reflect the average exposure during the few months prior to the measurement of EtO. The results of this comparison were close except for sterilizers, whose air monitoring measurements were 2 to 3 times higher.

The authors pointed out two limitations in their study: a minority of subjects had a high exposure to EtO, and the follow-up (median 11.8 years) resulted in relatively few person-years at risk and was insufficient to assess the influence of a biologically relevant induction latency period. Although this study has good exposure information and the authors used this information to develop an exposure index per employee, they did not evaluate dose-response relationships that might have been present, nor did they follow the cohort long enough to evaluate morbidity. The strength of this study is the development of the cumulative exposure index as well as the absence of any potential confounding produced by the chlorohydrin process, which was a problem in workers who produced and manufactured EtO in other studies.

A follow-up study of this cohort conducted by Mikoczy et al. (2011) is discussed in Section A.2.21 below.

A.2.12. Norman et al. (1995)

These authors conducted a mortality/incidence study in a cohort of 1,132 workers, mainly women (82%), who were exposed to EtO at some time during the period July 1, 1974, through September 30, 1980. Follow-up was until December 31, 1987. Ethylene oxide was used at the study plant to sterilize medical equipment and supplies that were assembled and packaged there. This plant was selected for the study because in an earlier small study at this plant (Stolley et al., 1984) there was an indication that in a sample of workers the average number of sister chromatid exchanges was elevated over that of a control group selected from the nearby community. Cancer morbidity was measured by comparing cancers occurring in this cohort with those predicted from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program for the period 1981–1985 and with average annual cancer incidence rates for western New York for 1979–1984. Observed cancers were compared to expected cancers using this method.

Only 28 cancer diagnoses were reported in the cohort; 12 were for breast cancers. Breast cancer was the only cancer site in this study where the risk was significantly elevated, based on the SEER rates (SIR = 2.55, $p < 0.05$). No significant excesses were seen at other cancer sites of interest: leukemia (1 observed, 0.54 expected), brain (0 observed, 0.49 expected), pancreas (2 observed, 0.51 expected) and stomach (0 observed, 0.42 expected). The authors offered no explanation except chance as to why the risk of breast cancer was elevated in these workers.

In 1980, three 2-hour samples from the plant provided 8-hour TWA exposures to sterilizer operators that ranged from 50 to 200 ppm. Corrective action reduced the levels to 5 to 20 ppm.

This study has little power to detect any significant risk of cancer at other sites because morbidity was small, chiefly as a consequence of the short follow-up period. The mean number of years from the beginning of follow-up to the end of the study was 11.4 years. In fact, the authors stated that breast cancer was the only cancer site for which there was adequate power to detect an increased relative risk. Additional weaknesses in this study include no historic exposure information and too short a period of employment in some cases (<1 month) to result in breast cancer. The authors maintained that their study was inconclusive.

A.2.13. Swaen et al. (1996)

A significant cluster of 10 Hodgkin lymphoma cases in the active white male workforce of an unidentified large chemical manufacturing plant in Belgium led to a nested case-control study by Swaen et al. (1996) to determine which, if any, chemical agents within the plant may have led to the increase. By comparison with regional cancer incidence rates, the SIR for this disease was 4.97 (95% confidence interval [CI] = 2.38–9.15) over a 23-year period, from

1966 to 1992. This suggested that an occupational exposure may have produced the significant excess risk of Hodgkin lymphoma seen in these workers.

The investigators randomly selected 200 individuals from a computerized sampling frame of all men ever employed at the facility. From this list of 200, workers who were actively employed at the time of diagnosis of each case were chosen as controls. No age matching was done because the authors stated that age-specific incidence rates for Hodgkin lymphoma in the United States were relatively flat for men between ages 18 and 65. The investigators felt that a control could serve for more than one case.

Verification of the 10 cases revealed that 1 case was, in reality, a large-cell anaplastic lymphoma. Two others could not be confirmed as Hodgkin lymphoma due to the lack of tissue. The remaining seven were confirmed as Hodgkin lymphoma. In the ensuing case-control analysis, significant odds ratios (ORs) for Hodgkin lymphoma were observed for five chemicals, ammonia (6 cases, OR = 5.6), benzene (5 cases, OR = 11), EtO (3 cases, OR = 8.5), NaOH (5 cases, OR = 8), and oleum (3 cases, OR = 6.9), based on the number of cases and controls known to be exposed to the chemicals in question. This does not mean they were exposed only to the chemical in question.

The availability of exposure information made it possible to calculate cumulative exposure to the cases and controls of two chemicals, benzene and EtO. The cumulative exposure for benzene-exposed cases was 397.4 ppm-months versus an expected 99.7 ppm-months for the matched controls. This difference in cumulative exposures was not statistically significant; although, the authors noted that one case had an exceptionally high cumulative benzene exposure. Only a few studies have suggested that exposure to benzene could be related to an increase in the risk of Hodgkin lymphoma. The cumulative total exposure to EtO for the cases was 500.2 ppm-months versus 60.2 for the matched controls, which was statistically significant, the significance being due to one extreme case.

This study is limited because the authors enumerated only cases among active employees of the workforce; therefore, the distinct possibility exists that they could have missed potential cases in the inactive workers. It is possible that latent Hodgkin lymphoma cases could have been identified in the controls after the controls left active employment. However, given the many different possible exposures to the chemicals produced in the workplaces of these employees, it would be difficult to argue that either EtO or benzene could be considered solely responsible for the excess risk of Hodgkin lymphoma in this working group.

A.2.14. Olsen et al. (1997)

Olsen et al. (1997) studied 1,361 male employees of four plants in Texas, Michigan, and Louisiana who were employed a minimum of 1 month sometime during the period 1940 through

1992 in the ethylene chlorohydrin and propylene chlorohydrin process areas. These areas were located within the EtO and propylene oxide production plants. Some 300 deaths had occurred by December 31, 1992.

Plant A in Texas produced EtO beginning in 1941 and ceased production in 1967. Bis-chloroethyl ether, a byproduct of EtO continued to be produced at this plant until 1973. The plant was demolished in 1974. Plant B, which was nearby, manufactured EtO from 1951 to 1971 and then again from 1975 until 1980. This plant continues to produce propylene oxide. The Louisiana plant produced EtO and propylene oxide through the propylene chlorohydrin process from 1959 until 1970, when it was converted to propylene oxide production. The Michigan plant produced ethylene chlorohydrin and subsequently EtO beginning in 1936 and continuing into the 1950s. This plant produced propylene chlorohydrin and propylene oxide up to 1974.

The authors suggested that exposure to EtO was possible at the plants studied in this report but that exposure was unlikely in the 278 chlorohydrin unit workers who were excluded from the cohort studied by Teta et al. (1993). Unfortunately, no actual airborne measurements were reported by Olsen et al. (1997), and thus only length of employment could be used as a surrogate for exposure.

The SMR for all causes was 0.89 (300 observed). For total cancer the SMR was 0.94 (75 observed, 79.7 expected). There were 10 lymphohematopoietic cancers versus 7.7 expected (SMR = 1.3). No significantly increased risks of any examined site-specific cancer (pancreatic, lymphopoietic, hematopoietic, and leukemia) were noted even after a 25-year induction latency period, although the SMR increased to 1.44 for lymphopoietic and hematopoietic cancer. When only the ethylene chlorohydrin process was examined after 25 years latency, the SMR increased to 1.94, based on six observed deaths.

The authors concluded that there was a weak, nonsignificant, positive association with duration of employment for lymphopoietic and hematopoietic cancer with Poisson regression modeling. They stated that the results of their study provide some assurance that this cohort of ethylene chlorohydrin and propylene chlorohydrin workers has not experienced a significant increased risk for pancreatic cancer and lymphopoietic and hematopoietic cancer. They believed that this study contradicted the conclusions of Benson and Teta (1993) that ethylene dichloride, perhaps in combination with chlorinated hydrocarbons, appeared to be the causal agent in the increased risk of pancreatic cancer and hematopoietic cancer seen in that study. They pointed out that ethylene dichloride is readily metabolized and rapidly eliminated from the body after gavage or inhalation administration; therefore, they questioned whether experimental gavage studies (NCI, 1978) are appropriate for studying the effects of ethylene dichloride in humans. One study (Maltoni et al., 1980) found no evidence of tumor production in rats and mice chronically exposed to ethylene dichloride vapor concentrations up to 150 ppm for 7 hours a day.

Also, because this chemical is a precursor in the production of vinyl chloride monomer, the authors wondered why an increase in these two site-specific cancers had not shown up in studies of vinyl chloride workers. However, they believe that an additional 5 to 10 years of follow-up of this cohort would be necessary to confirm the lack of risk for the two types of cancer described above.

A.2.15. Steenland et al. (2004)

In an update of the earlier mortality studies of the same NIOSH cohort of workers exposed to EtO described by Steenland et al. (1991) and Stayner et al. (1993), an additional 11 years of follow-up were added. This increased the number of deceased to 2,852. Work history data were originally gathered in the mid-1980s. Approximately 25% of the cohort continued working into the 1990s. Work histories on these individuals were extended to the last date employed. It was assumed that these employees continued in the job they last held in the 1980s. Little difference was noted when cumulative exposure was calculated with and without the extended work histories, chiefly because the exposure levels after the mid-1980s were very low (see Section A.2.8 for a discussion of the NIOSH exposure assessment and Section D.5 of Appendix D for further characterization of the NIOSH cohort). Again, no excess risk of hematopoietic cancer was noted based on external rates. However, as in the earlier paper, exposure-response analyses reported positive trends for hematopoietic cancers limited to males ($p = 0.02$ for the log of cumulative exposure with a 15-year lag) using internal comparisons and Cox regression analysis.[1] (See Table A-2 for the categorical exposure results.)

The excess of these tumors was chiefly lymphoid (NHL, myeloma, lymphocytic leukemia) (see Table A-3), as in the earlier paper. A positive trend was also observed for Hodgkin lymphoma in males, although this was based on small numbers.

[1] Valdez-Flores et al. (2010) suggest that Steenland et al. (2004) incorrectly used one degree of freedom in their evaluation of statistical significance and that a second degree of freedom should have been included for estimating the lag. However, Steenland et al. (2004) did not estimate the lag using the likelihood; instead, they treated the lagged exposure as an alternate exposure metric.

Table A-2. Cox regression results for hematopoietic cancer mortality (15-year lag) in males

Cumulative exposure (ppm-days)	Odds ratio (95% CI)
0	1
>0–1,199	1.23 (0.32–4.73)
1,200–3,679	2.52 (0.69–9.22)
3,680–13,499	3.13 (0.95–10.37)
13,500+	3.42 (1.09–10.73)

Source: Steenland et al. (2004).

Table A-3. Cox regression results for lymphoid cell line tumors (15-year lag) in males

Cumulative exposure (ppm-days)	Odds ratio (95% CI)
0	1
>0–1,199	0.9 (0.16–5.24)
1,200–3,679	2.89 (0.65–12.86)
3,680–13,499	2.74 (0.65–11.55)
13,500+	3.76 (1.03–13.64)

Source: Steenland et al. (2004).

The hematopoietic cancer trends were somewhat weaker in this analysis than were those reported in the earlier studies of the same cohort. This is not unexpected because most of the cohort was not exposed after the mid-1980s, and the workers who were exposed in more recent years were exposed to much lower levels because EtO levels decreased substantially in the early 1980s. No association was found in females, although average exposures were only twice as high in males (37.8 ppm-years) as in females (18.2 ppm-years), and there was enough variability in female exposure estimates to expect to be able to see a similar trend if it existed. In later analyses conducted by Steenland and presented in Appendix D, the difference between the male and female results was found not to be statistically significant, and the same pattern of lymphohematopoietic cancer results observed for males by Steenland et al. (2004) was observed for the males and females combined (i.e., statistically significant positive trends for both hematopoietic and lymphoid cancers using log cumulative exposure and a 15-year lag).

This study also reports a significant excess risk of breast cancer in the highest cumulative-exposure quartile, with a 20-year lag (SMR = 2.07, 95% CI 1.1–3.54, *n* = 13) in female employees. The results using internal Cox regression analyses with a 20-year lag time produced an OR = 3.13 (95% CI 1.42–6.92) in the highest cumulative-exposure quartile. The log of cumulative exposure with a 20-year lag was found to be the best model ($p = 0.01$) for the analyses of breast cancer. As for hematopoietic cancer in males, cumulative exposure untransformed showed a weaker trend ($p = 0.16$). A breast cancer incidence study of this cohort is discussed in Steenland et al. (2003).

A.2.16. Steenland et al. (2003)

In a companion study on breast cancer incidence in women employees of the same cohort discussed in Steenland et al. (2004), the authors elaborated on the breast cancer findings in a subgroup of 7,576 women from the cohort (76% of the original cohort). They had to be employed at least 1 year and exposed while employed in commercial sterilization facilities. The average length of exposure was 10.7 years. Breast cancer incidence analyses were based on 319 cases identified via interview, death certificates, and cancer registries in the full cohort, including 20 in situ carcinomas. Interviews on 5,139 women (68% of the study cohort) were obtained (next-of-kin interviews were sought for the 18% of the cohort who were deceased); 22% could not be located. Using external referent rates (SEER), the SIR was 0.87 for the entire cohort based on a 15-year lag time. When in situ cases were excluded, the overall SIR increased to 0.94. In the top quintile of cumulative exposure, with a 15-year lag time, the SIR was 1.27 (95% CI 0.94–1.69, *n* = 48). A significant positive linear trend of increasing risk with increasing cumulative exposure was noted ($p = 0.002$) with a 15-year lag time. Breast cancer incidence was believed to be underascertained owing to incomplete response and a lack of coverage by regional cancer registries (68% were contacted directly and 50% worked in areas with cancer registries). An internal nested case-control analysis, which is less subject to concerns about underascertainment, produced a significant positive exposure-response with the log of cumulative exposure and a 15-year lag time ($p = 0.05$). The top quintile was significant with an OR of 1.74 (CI 1.16–2.65) based on all 319 cases (the entire cohort).

The authors also conducted separate analyses using the subcohort with interviews, for which there was complete case ascertainment and additional information on potential confounders. In the subcohort with interview data, the odds ratio for the top quintile equaled 1.87 (CI 1.12–3.1), based on 233 cases in the 5,139 women and controlled for with respect to parity and breast cancer in a first-degree relative. Information on other risk factors was also collected (e.g., body mass index, SES, diet, age at menopause, age at menarche, breast cancer in

a first-degree relative, and parity), but only parity and breast cancer in a first-degree relative were significant in the model. Continuous cumulative exposure, as well as the log cumulative exposure, lagged 15 years, produced *p*-values for the regression coefficient of 0.02 and 0.03, respectively, for the Cox regression model, taking into account age, race, year of birth, parity, and breast cancer in a first-degree relative.

The authors concluded that their data suggest that exposure to EtO is associated with breast cancer, but because of inconsistencies in exposure-response trends and possible biases due to nonresponse and incomplete cancer ascertainment, the case for breast cancer is not conclusive. However, monotonically increasing trends in categorical exposure-response relationships are not always the norm owing to lack of precision in the estimates of exposure. Furthermore, positive trends were observed in both the full cohort and the subcohort with interviews, lessening concerns about nonresponse bias and case underascertainment.

A.2.17. Kardos et al. (2003)

These authors reported on a study completed earlier by Muller and Bertok (1995) of cancer among 299 female workers who were employed from 1976 to 1993 in a pediatric ward at the county hospital in Eger, Hungary, where EtO gas sterilizers were used. Their observation period for cancer was begun in 1987 on the assumption that cancer deaths before 1987 were not due to EtO, based on a paper by Lucas and Teta (1996). Information about the Muller and Bertok (1995) study is unavailable because the paper is in Hungarian and no translated copy is available. Kardos and his colleagues evaluated mortality among these women and found a statistically significant excess of total cancer deaths ($n = 11$) in the period from 1987 to 1999 when compared with expected deaths generated from three different comparison populations (Hungary, $n = 4.38$; Heves County, $n = 4.03$; and city of Eger, $n = 4.28$). The SMRs are all significant at the $p < 0.01$ level. Site-specific rates were not calculated. Among the 11 deaths were 3 breast cancer deaths and 1 lymphoid leukemia death. The authors claim that their results confirm "predictions of an increased cancer risk for the Eger hospital staff." They suggest an etiological role for EtO in the excess risk. 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.[2]

[2]Hungarian age-standardized female cancer mortality rates reported by the International Agency for Research on Cancer (http://eu-cancer.iarc.fr/EUCAN/Country.aspx?ISOCountryCd=348) suggest that the ratio of breast cancer deaths to total cancer deaths in Hungarian females is about 0.14 (23.5/100,000 breast cancer mortality rate versus 163.6/100,000 total cancer mortality rate). 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; nonetheless, the large difference between the ratios (0.68 for the study versus 0.14 for the general population) indicates an increased risk of breast cancer in the study.

A.2.18. Tompa et al. (1999)

The authors reported a cluster of eight breast cancer cases and eight other malignant tumor cases that developed over a period of 12 years in 98 nurses who worked in a hospital in the city of Eger, Hungary, and were exposed to EtO. These nurses were exposed for 5 to 15 years in a unit using gas sterilizer equipment. The authors report that EtO concentrations were 5 to 150 mg/m^3. The authors state that the high breast cancer incidence in the hospital in Eger indicates a combined effect of exposure to EtO and naturally occurring radioactive tap water, possibly due to the presence of radon. This case report study is discussed further in the genotoxicity section.

A.2.19. Coggon et al. (2004)

Descriptive information about this cohort is available from the earlier study by Gardner et al. (1989). In this update, the 1,864 men and 1,012 women described in the Gardner et al. (1989) study were followed to December 31, 2000. This added 13 more years of follow-up resulting in 565 observed deaths versus 607.6 expected. For total cancer, the observed number of deaths equaled 188 versus 184.2 expected. For NHL, 7 deaths were observed versus 4.8 expected. For leukemia, 5 deaths were observed versus 4.6 expected. All 5 leukemia deaths fell into the subset with definite or continual exposure to EtO, where only 2.6 were expected. In fact, the total number of deaths classified to the lymphohematopoietic cancer category was 17 with 12.9 expected. This increased risk was not significant. When definite exposure was established, the authors found that the risk of lymphatic and hematopoietic cancer was increased with 9 observed deaths versus 4.9 expected. Deaths from leukemia were also increased in chemical workers with 4 leukemia deaths versus 1.7 expected. No increase was seen in the risk of hematopoietic cancer in the hospital sterilizing unit workers, who are mostly female. Another finding of little significance was that of cancer of the breast. Only 11 deaths were recorded in this cohort up to the cutoff date versus 13.1 expected. Because there were no female workers in the chemical industry, the results on breast cancer reflect only work in hospital sterilizing units. The researchers concluded that the risk of cancer must be low at the levels sustained by workers in Great Britain over the last 10 or 20 years.

A.2.20. Swaen et al. (2009) and Valdez-Flores et al. (2010)

Swaen et al. (2009) redefined and updated the cohort of 1,896 male UCC workers studied by Teta et al. (1993), which was itself a follow-up of the 2,174 UCC workers originally studied by Greenberg et al. (1990), excluding the 278 chlorohydrin unit workers because of potential confounding. (However, confounding by chlorohydrin production has not been established, and 49 of those excluded workers were also employed in EtO production and thus had high potential

EtO exposures.) Specifically, Swaen et al. (2009) extended the cohort enumeration period from the end of 1978 to the end of 1988 (workers hired after 1988 were not added to the cohort because they were considered to have no appreciable EtO exposure), 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 reportedly based on the qualitative categorizations of potential for EtO exposure in the different departments developed by Greenberg et al. (1990) and time-period exposure estimates from Teta et al. (1993). The exposure assessment matrix for the exposure estimates of Swaen et al. (2009) is presented in Table A-4 below. Cumulative exposures for the individual workers were estimated by multiplying the time (in months) a worker was assigned to a department by the estimated exposure level for the department and summing across the assignments.

The exposure assessment used in this study 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. For the 1974–1988 time period, based on measurements from environmental monitoring conducted in the (West Virginia) plants since 1976, exposure estimates of 1 ppm and 0.3 ppm were chosen for the high- and low-exposure-potential departments, respectively, and the average of 0.65 ppm was taken for the medium-exposure-potential departments. For the 1957–1973 time period, exposure estimates were based on measurements from an air-sampling survey of three EtO direct-oxidation production units in a UCC plant in Texas in the early 1960s (during this 1957–1973 time period, direct oxidation was the only method used for EtO production at the West Virginia plants as well). The majority of the 8-hour TWA results in these units were between 3 and 20 ppm, with levels between 5 and 10 ppm for operators. Because the West Virginia plants and equipment were much older than for the Texas facility, the high end of the range of values for operators (10 ppm) was selected as the exposure estimate for the high-exposure-potential departments, and the low end of the range (5 ppm) was selected for the low-exposure-potential departments (even though these were not EtO production departments). The average of 7.5 ppm was taken for the medium-exposure-potential departments.

Table A-4. Exposure assessment matrix from Swaen et al. (2009)—8-hour TWA exposures in ppm

Time period	Exposure potential category		
	Low (most EtO user departments)	Medium (some EtO user departments)	High (EtO production departments)
1925–1939	17	28	70
1940–1956	7	14	21
1957–1973	5	7.5	10
1974–1988	0.3	0.65	1

Source: Swaen et al. (2009).

For the 1940–1956 time period, exposure estimates were derived from "rough" estimates of exposure reported by Hogstedt et al. (1986) for a chlorohydrin-based EtO production unit in an enclosed building, as was the West Virginia chlorohydrin-based EtO production. Hogstedt et al. (1986) reportedly suggested EtO exposures were probably below 14 ppm from 1941 to 1947, although much higher levels occasionally occurred, and levels from the 1950s to 1963 averaged 5 to 25 ppm. Thus, based on these values, 14 ppm was selected as the exposure estimate for the medium-exposure-potential departments, and values 50% higher (21 ppm) and 50% lower (7 ppm) were assigned to the high- and low-exposure-potential departments, respectively. For the 1925–1939 time period, it was assumed that exposures in this earlier, start-up period would have been higher than those in the subsequent 1940–1956 time period, so the 14 ppm estimate from the medium-exposure-potential departments in the 1940–1956 time period was used as the exposure estimate for the low-exposure-potential departments for the 1925–1939 time period. Then, the same ratio of 1:2 between the low- and medium-exposure-potential departments from the 1940–1956 time period was used to obtain an estimate of 28 ppm for the medium-exposure-potential departments for the 1925–1939 time period. A factor of 5 (one-half an order of magnitude) was used between the low- and high-exposure-potential departments to obtain a highly uncertain exposure estimate of 70 ppm for the high-exposure-potential departments. Swaen et al. (2009) suggest that despite the high exposure estimates for the 1925–1939 time period, the contribution of this time period to cumulative exposure estimates is limited because only 98 workers (4.8% of the cohort) had employment histories before 1940. It appears, then, that pre-1940 employment histories may have been missing for 13 of the workers, because excluding the 112 pre-1940 chlorohydrin production workers (Benson and Teta, 1993) from the original 223 pre-1940 workers (Greenberg et al., 1990) leaves 111 pre-1940 workers in the cohort.

At the end of the 2003 follow-up, 1,048 of the 2,063 workers had died and 23 were lost to follow-up. In comparison with general population U.S. mortality rates, 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 5 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.

Internal Cox proportional hazards modeling was also done 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. Year of birth and year of hire were included as covariates in the Cox regression model, and the time variable was presumably follow-up time. Year of hire was reportedly included to adjust for potential cohort effects; however, adjusting for year of birth should have already adjusted for cohort effects, and it is unclear whether year of hire was a statistically significant factor in the regression. Furthermore, because year at hire is likely correlated with exposure (without being correlated with disease trends over time, which would have been controlled for by year of birth), including it in the regression model could overadjust and attenuate the observed exposure-related effects. These internal analyses showed no evidence of an exposure-response relationship, although, again, these analyses rely on small numbers of cases and a crude exposure assessment, with a high potential for exposure misclassification.

Swaen et al. (2009) note that one of the strengths of their study is the long average follow-up time of the workers. These authors further note that, because the UCC cohort is a much older population (50% deceased) than the NIOSH cohort (Steenland et al., 2004), the number of expected deaths is less than 3 times larger for the NIOSH cohort even though the sample size is almost 9 times larger. However, the long follow-up and aged cohort might be a limitation, as well. Because the follow-up is extended well beyond the time period of nonnegligible exposures (pre-1989) for workers still employed and, especially, beyond the highest exposures (e.g., pre-1940 or pre-1956), the follow-up is likely observing workers at the high tail end of the distribution of latency times for EtO-associated lymphohematopoietic cancers. In other words, workers that were at risk of developing lymphohematopoietic cancer as

a result of their EtO exposures would likely have developed the disease earlier. Meanwhile, having an older cohort means that the background rates of lymphohematopoietic cancers are higher, and thus, relative risks may be attenuated. Such attenuation was observed even in the younger NIOSH cohort between the 1987 follow-up (Steenland et al., 1991) and the 1998 follow-up (Steenland et al., 2004), when the follow-up was extended well beyond the period of significant EtO exposures (exposure levels were considered very low by the mid-1980s).

Swaen et al. (2009) also note that their estimate of the average cumulative exposure for the UCC cohort was more than twice the average cumulative exposure estimate for the NIOSH cohort. However, there are substantial uncertainties in the exposure assessment, especially for the early years when the highest exposures occurred. And despite the reported strengths of the Swaen et al. (2009) study in terms of follow-up, cohort age, and high exposures, a limitation of the study is the small cohort size. Based on data presented by Greenberg et al. (1990) and Benson and Teta (1993), it appears that fewer than 900 workers were hired before 1956 (1,104 of the original cohort were hired before 1960 and 233 of those were then excluded because they worked in the chlorohydrin unit) and would have been potentially exposed to the higher pre-1956 exposures levels. Moreover, according to Teta et al. (1993), only 376 workers were assigned to EtO production departments (but not the chlorohydrin unit), and these were the only departments with high exposure potential (see Table A-4). In the full cohort of 2,063 men, only 27 lymphohematopoietic (17 lymphoid) cancers were observed.

In alternate analyses of the UCC data, Valdez-Flores et al. (2010) fitted Cox proportional hazards models and conducted categorical exposure-response analyses using a larger set of cancer endpoints. These investigators also performed the same analyses using the data from the last follow-up of the NIOSH cohort (Steenland et al., 2004) and from the two cohorts combined, analyzing the sexes both separately and together. Valdez-Flores et al. (2010) 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). These investigators suggest that a review of the data from the NIOSH and UCC studies supports combining them, but it should be recognized that the exposure assessment conducted for the UCC cohort is much cruder (see above), especially for the highest exposures, than the NIOSH exposure assessment (which was based on a validated regression model; see A.2.8 above); thus, the results of exposure-response analyses of the combined cohort data are considered 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; however, as discussed

above, some of these UCC cases occur in older workers, with longer postexposure follow-up, and thus, may reflect background disease more than exposure-related disease).

Notable differences between the Steenland et al. (2004) and the Valdez-Flores et al. (2010) analyses exist. A major difference is that Valdez-Flores et al. (2010) used only cumulative exposure in the Cox regression model, so they considered only a sublinear exposure-response relationship, whereas Steenland et al. (2004) also used log cumulative exposure, which provides a supralinear exposure-response relationship model structure [e.g., see Figure 4–1, illustrating the difference between the cumulative exposure and log cumulative exposure Cox regression models ($RR = e^{\beta \times exposure}$) for the lymphoid cancers from Steenland et al. (2004)]. Valdez-Flores et al. (2010) objected to the log cumulative exposure model for a number of reasons, the primary one being that the use of log cumulative exposure forces the exposure-response relationship to be supralinear regardless of the observed data. This is correct but no different from the use of cumulative exposure imposing a *sublinear* exposure-response relationship. Moreover, Steenland et al. (2004) used log cumulative exposure specifically when the cumulative exposure Cox regression model did not yield a statistically significant fit to the exposure-response data and the categorical analyses suggested increases in risk that were more consistent with an underlying supralinear exposure-response relationship. With log cumulative exposure, Steenland et al. (2004) observed statistically significant fits to the exposure-response data for all lymphohematopoietic cancers in males, lymphoid cancers in males, and breast cancer in females, none of which yielded statistically significant fits with the cumulative exposure (sublinear exposure-response) model, supporting the apparent supralinearity of the data.[3]

Another key difference between the Steenland et al. (2004) and the Valdez-Flores et al. (2010) analyses is that Valdez-Flores et al. (2010) present results only for unlagged analyses. Valdez-Flores et al. (2010) state that their Cox regression results with different lag times were similar to the unlagged results. Because the Valdez-Flores et al. (2010) categorical results are for unlagged analyses, however, their referent groups are different from those used by Steenland et al. (2004). Valdez-Flores et al. (2010) used the lowest exposure quintile (providing there were sufficient data) as the referent group, whereas Steenland et al. (2004) used the no-exposure (lagged-out) group as the referent. Because the NIOSH cohort data have an underlying supralinear exposure-response relationship, the increased risk in the lowest exposure group is already notably elevated and using the lowest exposure quintile as a referent group would attenuate the relative risk. Nonetheless, Valdez-Flores et al. (2010) observed statistically significant increases in response rates in the highest exposure quintile relative to the lowest

[3]This pattern of findings from the NIOSH cohort data for males (i.e., statistically significant fits with log cumulative exposure but not with cumulative exposure) was replicated for both the all lymphohematopoietic cancers and the lymphoid cancers when the NIOSH data on males and females were combined (see Appendix D).

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.[4]

Although Valdez-Flores et al. (2010) found no statistically significant exposure-response relationships for any of the cohort/endpoint data sets that they analyzed using the cumulative exposure Cox regression model, these investigators derived risk estimates from the positive relationships for the purposes of comparing those estimates with the U.S. Environmental Protection Agency's (EPA's) 2006 draft risk estimates (U.S. EPA, 2006a). Valdez-Flores et al. (2010) report that their estimate of the exposure level associated with 10^{-6} risk of lymphohematopoietic cancer based on the male NIOSH cohort data is 1,500 times larger than the EPA's 2006 draft estimate (their exposure level estimate based on the NIOSH and UCC male and female data combined was a further 3 times higher). Most of the difference in magnitude between the Valdez-Flores et al. (2010) and the EPA 2006 draft estimates is attributable to the difference in the models used. The Valdez-Flores et al. (2010) estimate is based on the sublinear Cox regression model, which the EPA rejected as not providing a good representation of the low-exposure data (the EPA's 2006 draft risk estimate is based on a linear model). In addition, Valdez-Flores et al. (2010) used maximum likelihood estimates, while the EPA uses upper bounds on risk (or lower bounds on exposure). Valdez-Flores et al. (2010) also modeled down to 10^{-6} risk, whereas the EPA modeled to 10^{-2} risk and used the LEC_{01} as a point of departure (POD) for linear low-dose extrapolation. Valdez-Flores et al. (2010) suggest that PODs should be within the range of observed exposures, and they chose a 10^{-6} risk level because the corresponding exposure level was in the range of the observed occupational exposures (converted to equivalent environmental exposures). The intention of the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), however, is for the POD to be (or more specifically, to correspond to a response level) at the low end of the observable range *of responses* (i.e., a response level that might reasonably be observed to have statistical significance with respect to background responses). The underlying assumption in this approach is that one can have relative confidence in an exposure-response model in the observable range, but there is less confidence in any empirical exposure-response model for much lower exposures. The estimates also differ because Valdez-Flores et al. (2010) truncated their life-table analysis at 70 years, while the EPA uses a cutoff of 85 years.

[4]In Steenland's analyses of the NIOSH cohort data for both sexes combined, presented in Appendix D, the categorical results for all lymphohematopoietic cancers were also statistically significantly increased.

A further reason for differences between the risk estimates of Valdez-Flores et al. (2010) and the EPA's 2006 draft result is that Valdez-Flores et al. (2010) estimated mortality risks, while the EPA estimates incidence risks. In a separate publication, Sielken and Valdez-Flores (2009a) disagree with the assumption of similar exposure-response relationships for lymphohematopoietic cancer incidence and mortality used by the EPA in deriving incidence estimates and assert that the methods used by the EPA in calculating these estimates were inappropriate. Sielken and Valdez-Flores (2009a) suggest that, except at high exposure levels, the exposure-response data on all lymphohematopoietic cancers in males in the NIOSH cohort are consistent with decreases in survival time as an explanation for the apparent increases in mortality. For two of the four exposure groups, however, the best fitting survival times were 0 years, which seems improbable. Moreover, Sielken and Valdez-Flores (2009a) have not established that the excess mortality is due to decreased survival time; the data are also consistent with increased mortality resulting from increased incidence. Furthermore, the rodent bioassays show that EtO is a complete carcinogen (see Section 3.2), and the mechanistic data demonstrate that EtO is mutagenic (see Section 3.3.3), with sufficient evidence for a mutagenic mode of action (see Section 3.4). Thus, EtO can be expected to act as an initiator in carcinogenesis, and, consequently, be capable of inducing exposure-related increases in incidence. As for the methods used by the EPA in calculating the incidence estimates, the EPA used adjustments to the life-table analysis where warranted (U.S. EPA, 2006a). The EPA did not adjust the all-cause mortality rates in the lymphohematopoietic cancer analyses, because "the lymphohematopoietic cancer incidence rates are small when compared with the all-cause mortality rates" (U.S. EPA, 2006a); Section 4.1.1.3 (actually, the differential rates obtained by subtracting the mortality rates from the incidence rates) and, thus, the impact of taking into account lymphohematopoietic cancer incidence when calculating interval "survival" is negligible, as confirmed by Sielken and Valdez-Flores' own calculations, presented in their Table 2 where the "multiplier" = 1 (Sielken and Valdez-Flores, 2009a). On the other hand, for the breast cancer incidence analyses, where incidence rates (and the differentials between incidence and mortality rates) are higher, the EPA adjusted the all-cause mortality rates to take into account breast cancer incidence, effectively redefining interval "survival" (and thus the resulting population at risk) as surviving the interval without developing an incident case of breast cancer [U.S. EPA (2006a); Section 4.1.2.3]. Therefore, the concerns raised by Sielken and Valdez-Flores (2009a) about using life-table analyses to derive incidence estimates do not apply to the EPA's calculations.

Finally, the risk estimates of Valdez-Flores et al. (2010) and the EPA's 2006 draft also differ because Valdez-Flores et al. (2010), based on analyses in a separate publication by Sielken and Valdez-Flores (2009b), misinterpreted the application of the age-dependent adjustment

factors (ADAFs) such that, even though they purported to apply the factors, this application had no impact on the risk estimate. The ADAFs are default adjustment factors intended to be applied directly to the unit risk estimates (i.e., risk per unit constant exposure, or "slope factors") in conjunction with age-specific exposure level estimates (U.S. EPA, 2005b). For the purposes of applying the ADAFs, the unit risk estimate is parsed, as a proportion of an assumed 70-year lifespan, across age groups with different adjustment factors and/or exposure levels. The ADAFs were not designed to be applied in life-table analyses, as was done by Sielken and Valdez-Flores (2009b). In addition, the use of the 15-year lag in exposure in the life-table analyses does not mean that there is no risk from exposures before age 15 years, as intimated by Sielken and Valdez-Flores (2009b). Indeed, those exposures do not increase risk for cancer occurring before 15 years of age; however, they do contribute to lifetime risk. The assumption of increased early-life susceptibility that underlies the application of the ADAFs is that early-life exposure increases the *lifetime* risk of cancer, not just the risk of cancer in early life, so it is inappropriate to apply the ADAFs only to the age-specific hazard rates, as was done by Sielken and Valdez-Flores (2009b). One might conceivably incorporate the ADAFs into the life-table analysis by weighting the age-specific exposures before they are aggregated into the cumulative exposure, but such an integrated approach does not allow for the risks associated with less-than-lifetime exposure scenarios to be calculated without redoing the life-table analysis each time.

A.2.21. Mikoczy et al. (2011)

Mikoczy et al. (2011) report the results of a follow-up study of the Swedish sterilizer worker cohort investigated by Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991).[5] This update extends the follow-up period through 2006, providing an additional 16 years of follow-up (see Section J.2.2 of Appendix J for more details and discussion of this study).

For lymphohematopoietic cancers, nonsignificant increases in SMRs and SIRs were reported. For the incidence data, the internal analysis shows 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 mortality (results not shown), a "slight but nonsignificant decrease" in the SMR was reported. With a 15-year induction period included, the SMR for breast cancer was reportedly "somewhat increased." For workers with cumulative exposures above the

[5] This follow-up study was published after the general cutoff 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.

median, with a 15-year induction period, a "higher than expected" SMR, which was not statistically significant, was reported.

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 (see Table J-3 in Appendix J), despite having a low-exposed rather than an unexposed referent group.

In conclusion, the EPA found that the nonsignificant increases in SMRs and SIRs for lymphohematopoietic cancers reported in this study are consistent with an increase in lymphohematopoietic cancer risk, but overall, the study is underpowered for the analysis of lymphohematopoietic cancers and contributes little to the weight of evidence for these cancers. For breast cancer incidence, however, the statistically significant exposure-related increases in breast cancer incidence in internal analyses add support to the findings of increased risk of female breast cancer observed in the studies of NIOSH (Steenland et al., 2004; Steenland et al., 2003), Norman et al. (1995), and Kardos et al. (2003).

A.3. SUMMARY

The initial human studies by Hogstedt and colleagues (Hogstedt, 1988; Hogstedt et al., 1986; Hogstedt et al., 1979b; Hogstedt et al., 1979a), in which positive findings of leukemia and blood-related cancers suggested a causal effect, have been followed by studies that either do not indicate any increased risks of cancer or else suggest a dose-related increased risk of cancer at certain sites, chiefly cancers of the lymphohematopoietic system including leukemia, lymphosarcoma, reticulosarcoma, and NHL. More recently, an association with breast cancer has also been suggested. However, the overall epidemiological evidence is not conclusive because of inadequacies and limitations in the epidemiological database. The main effects and limitations in the epidemiological studies of EtO are presented in Table A-5.

Table A-5. Epidemiological studies of ethylene oxide and human cancer

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Sterilizers, production workers, Sweden Hogstedt (1988); Hogstedt et al. (1986)	709 (539 men, 170 women)	Plant 1: mean ≤ 20 ppm Plant 2: mean 6–28 ppm in early years, less than 6 ppm later Plant 3: mean less than 8 ppm in early years, less than 2 ppm later	33 cancer deaths vs. 20 expected 7 leukemia deaths vs. 0.8 expected (ICD-8 204–207) 9 lymphohematopoietic cancer deaths vs. 2.0 expected (ICD-8 200–208) 10 stomach cancer deaths vs. 1.8 expected	Benzene, methyl formate, bis-(2-chloroethyl) ether, ethylene, ethylene chlorohydrin, ethylene dichloride, ethylene glycol, propylene oxide, amines, butylene oxide, formaldehyde, propylene, sodium nitrate	No personal exposure information from which to estimate dose No latency analysis Mixed exposure to other chemicals
Sterilizing workers in 8 hospitals and users in 4 companies, Great Britain Gardner et al. (1989)	2,876 (1,864 men, 1,012 women)	After 1977, means ≤ 5 ppm. In earlier years, means likely higher, and peak exposures above the odor threshold of 700 ppm were reported.	3 leukemia deaths vs. 2.1 expected (ICD NS) 3 leukemia deaths vs. 0.35 expected (after 20+ years latency) 4 NHL deaths vs. 1.6 expected 5 esophageal cancer deaths vs. 2.2 expected 4 bladder cancer deaths vs. 2.04 expected 29 lung cancer deaths vs. 24.6 expected	Aliphatic and aromatic alcohols, amines, anionic surfactants, asbestos, butadiene, benzene, cadmium oxide, dimethylmine, ethylene, ethylene chlorohydrin, ethylene glycol, formaldehyde, heavy fuel oils, methanol, methylene chloride, propylene, propylene oxide, styrene, tars, white spirit, carbon tetrachloride	Insufficient follow-up Exposure classification scheme vague, making it difficult to develop dose-response gradient No exposure measurements prior to 1977, so individual exposure estimates were not made Mixed exposure to several other chemicals

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Coggon et al. (2004) Update of Gardner et al. (1989)	Same cohort followed additional 13 years	Same	5 leukemia deaths vs. 4.6 expected (ICD-9 204–208) 5 leukemia deaths vs. 2.6 expected (definite or continual exposure) 7 NHL vs. 4.8 expected (ICD-9 200 + 202) 17 lymphohematopoietic cancers vs. 12.9 expected (ICD-9 200–208) 11 breast cancers vs. 13.1 expected	Same	Same, also no latency evaluation

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Production workers (methods unspecified) from 8 chemical plants in former West Germany Kiesselbach et al. (1990)	2,658 men	No exposure information available	2 leukemia deaths vs. 2.35 expected (ICD-9 204–208) 5 lymphohematopoietic cancers vs. 5 expected (ICD-9 200–208) 14 stomach cancer deaths vs. 10.1 expected 3 esophageal cancer deaths vs. 1.5 expected 23 lung cancer deaths vs. 19.9 expected	Beta-naphthylamine, 4-amino-diphenyl, benzene, ethylene chlorohydrin, possibly alkylene oxide (ethylene oxide/propylene oxide), based on inclusion of plants that were part of a cohort study by Thiess et al. (1982).	Insufficient follow-up; few expected deaths in cancer sites of significance with which to analyze mortality Production methods not stated; information vague on what these plants do Latency analysis given only for total cancer and stomach cancer mortality Although categories of exposure are given, they are nonquantitative and are not based on actual measurements No actual measurement data are given; dose-response analysis is not possible

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Production workers and users at 2 chemical plants in West Virginia Greenberg et al. (1990)	2,174 men	Exposure prior to 1976 not known 1976 survey: average 8-hr TWA exposure levels less than 1 ppm; 1–5 ppm 8-hr TWA for maintenance workers	7 leukemia and aleukemia deaths vs. 3 expected; SMR = 2.3 (ICD NS) 2 NHL vs. 2.4 expected 9 lymphohematopoietic cancers vs. 7.5 expected 3 liver cancer deaths vs. 1.8 expected; SMR = 1.7 7 pancreatic cancer deaths vs. 4.1 expected; SMR = 1.7 Suggestion of increasing risk of stomach cancer and leukemia/aleukemia with cumulative duration of potential exposure	Acetaldehyde, acetonitrile, acrolein, aldehydes, aliphatic and aromatic alcohols, alkanolamines, allyl chloride, amines, butadiene, benzene, bis-(chloroethyl) ether, ethylene dichloride, diethyl sulphate, dioxane, epichlorhydrin, ethylene, ethylene chlorohydrin, formaldehyde, glycol ethers, methylene chloride, propylene chlorohydrin, styrene, toluidine	Low exposure levels: average 8-hr TWA exposure levels to EtO less than 1 ppm (from a 1976 survey) No actual measurements of exposure to EtO for these plants exist prior to 1976 Exposure occurred to many other chemicals, some of which may be carcinogenic Lack of quantitative estimates of individual exposure levels

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Same cohort as Greenberg et al. (1990) minus all chlorohydrin-exposed employees, followed an additional 10 years Teta et al. (1993)	1,896 men	Estimated exposure prior to 1956: 14+ ppm; after 1956: less than 10 ppm Prior to 1976, estimates were based on measurements taken at similar facilities	5 leukemia and aleukemia deaths vs. 4.7 expected (ICD NS) 2 lymphosarcoma and reticulosarcoma vs. 2.03 expected 7 lymphohematopoietic cancers vs. 11.8 expected Trend of increasing risk of leukemia and aleukemia death with increasing duration of exposure	Same (except for chemicals specific to the chlorohydrin process)	Same
Only the chlorohydrin-exposed employees from Greenberg et al. (1990) cohort, followed an additional 10 years Benson and Teta (1993)	278 men	Reported to be low exposure to EtO in the chlorohydrin process	8 lymphohematopoietic cancer deaths vs. 2.72 expected ($p < 0.05$) (ICD NS); SMR = 2.9 4 leukemia and aleukemia deaths vs. 1.14 expected 1 lymphosarcoma and reticulosarcoma vs. 0.50 expected 8 pancreatic cancer deaths vs. 1.63 expected ($p < 0.05$)	Same	Same, also very small cohort

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Same cohort as for Teta et al. (1993) followed an additional 15 years plus cohort enumeration extended to end of 1988 (an additional 10 years), adding 167 workers Swaen et al. (2009)	2,063 men	Individual exposure estimates derived from an exposure matrix based on potential EtO exposure categorizations developed by Greenberg et al. (1990) and time-period exposure estimates developed by Teta et al. (1993), which relied on measurements taken at other facilities and rough estimates for the time periods before 1974	11 leukemia deaths vs. 11.8 expected (ICD NS) 9 leukemia deaths in workers hired before 1956; SMR = 1.51 12 NHL vs. 11.5 expected 27 lymphohematopoietic cancers vs. 30.4 expected No statistically significant increases were observed for any cancer types No statistically significant trends were observed for lymphoid or leukemia cancer categories examined using Cox proportional hazards modeling	Same	Same Crude exposure assessment, especially for the early time periods Small cohort; thus, small numbers of specific cancers even though long follow-up time

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Sterilizers of medical equipment and spices; and manufacturers and testers of medical sterilization equipment, in 14 plants in the United States Steenland et al. (1991); Stayner et al. (1993)	18,254 (45% male, 55% female)	1938–1976 (estimated): 16 ppm for sterilizer operators, 5 ppm for remainder 1977–1985 (mean): 4.3 for sterilizers, 2 ppm for remainder Individual cumulative exposure estimates calculated for workers in 13 of the 14 facilities	36 lymphohematopoietic cancer deaths vs. 33.8 expected (ICD NS) 13 leukemia and aleukemia deaths vs. 13.5 expected 8 lymphosarcoma and reticulosarcoma deaths vs. 5.3 expected After 20+ years latency, SMR = 1.76 for lymphohematopoietic cancer; significant trend with increasing latency ($p < 0.03$) Significantly increasing lymphohematopoietic cancer and "lymphoid" cancer (ICD-9 200, 202, 204) risks with cumulative exposure (Cox regression model)	No identified exposures to other chemicals	Potential bias due to lack of follow-up on "untraceable" members (4.5%) of the cohort Short duration of exposure and low median exposure levels Individual exposures were estimated prior to 1976 before first industrial hygiene survey was completed Short follow-up for most members of the cohort; only 8% had attained 20 years latency Little mortality (6.4%) had occurred in this large group of employees No exposure-response relationship among female workers

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Same cohort as Steenland et al. (1991) and Stayner et al. (1993) plus 474 additional members, followed 1 more yr Wong and Trent (1993)	18,728 (45% male, 55% female)	Same as Steenland et al. (1991) and Stayner et al. (1993)	43 lymphohematopoietic cancer deaths observed vs. 42 expected (ICD-8 200–209) 18 NHL deaths vs. 12.7 expected (ICD-8 200 + 202) 14 leukemia and aleukemia deaths vs. 16.2 expected (ICD-8 204–207)	No identifiable exposures to other chemicals	All of the limitations of Steenland et al. (1991) apply here Although this group is the same as Steenland et al. (1991), an additional unexplained 474 employees were added It is questionable that one additional yr of follow-up added 392.2 expected deaths but only 176 observed deaths No effort was made to develop exposure-response data such as in Stayner et al. (1993) on the basis of individual cumulative exposure data but only on duration of employment

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Steenland et al. (2004) Update of Steenland et al. (1991) and Stayner et al. (1993)	18,254 (45% male, 55% female)	Same as Steenland et al. (1991), with extension of worker histories based on job held at end of initial exposure assessment for those still employed at end of 1991 study (25% of cohort)	79 lymphohematopoietic cancer deaths (ICD-9 200–208): SMR = 1.00 31 NHL deaths (ICD-9 200 + 202): SMR = 1.00 29 leukemia deaths (ICD-9 204–208); SMR = 0.99 In males, in internal Cox regression analyses, OR = 3.42 ($p < 0.05$) in highest cumulative exposure group, with 15-yr lag for lymphohematopoietic cancer; significant regression coefficient for continuous log cumulative exposure ($p = 0.02$) Similar results for "lymphoid" cancers (ICD-9 200, 202, 203, 204) in males For females, in internal Cox regression analyses, OR = 3.13 ($p < 0.05$) for breast cancer mortality in highest cumulative exposure group, with 20-yr lag; significant regression coefficient for continuous log cumulative exposure ($p = 0.01$)	No identified exposures to other chemicals	Potential bias due to lack of follow-up on "untraceable" members (4.5% of the cohort) Individual exposures were estimated prior to 1976 before first industrial hygiene survey was completed No increase in lymphohematopoietic cancer risk with increase in exposure in women

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Women employees from Steenland et al. (2004) employed in commercial sterilization facilities for at least 1 yr Steenland et al. (2003)	7,576 women	Same as in Steenland et al. (2004) Minimum of 1 yr	SIR = 0.87 319 cases of breast cancer SIR = 0.94 20 in situ cases excluded A positive trend in SIRs with 15-yr lag time for cumulative exposure (p = 0.002) In internal nested case-control analysis, a positive exposure-response with log of cumulative exposure with 15-yr lag; top quintile had OR = 1.74, $p < 0.05$ Similar results in subcohort of 5,139 women with interviews (233 cases)	Same as in Steenland et al. (2004), Stayner et al. (1993)	Interviews were available for only 68% of the women; thus, there is underascertainment of cancer cases in full cohort. Also, there are potential nonresponse biases in the subcohort with interviews Exposure-response trends not strictly monotonically increasing

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Chemical workers licensed to handle EtO and other toxic chemicals, Italy Bisanti et al. (1993)	1,971 men	Levels were said to be high at beginning of employment; no actual measurements were available 637 workers were licensed only to handle EtO and no other toxic chemicals	43 total cancer deaths vs. 33 expected 6 lymphohematopoietic cancer deaths vs. 2.4 expected (ICD-9 200–208) 4 lymphosarcoma and reticulosarcoma deaths vs. 0.6 expected (ICD-9 200) 2 leukemia deaths vs. 1.0 expected (ICD-9 204–208) 5 lymphohematopoietic cancer deaths vs. 0.7 expected in group licensed to handle only EtO	Toxic gases, dimethyl sulphate, methylene chloride, carbon disulphide, phosgene, chlorine, alkalic cyanides, sulfur dioxide, anhydrous ammonia, hydrocyanic acid	Lack of exposure data Insufficient follow-up for this young cohort Potential selection bias Possible earlier exposure than date of licensing would indicate

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Two plants that produced disposable medical equipment, Sweden Hagmar et al. (1995); Hagmar et al. (1991)	2,170 (861 men, 1,309 women)	1964–1966, 75 ppm in sterilizers, 50 ppm in packers 1970–1972, 40 ppm in sterilizers, 20–35 ppm in packers and engineers By 1985, levels had dropped to 0.2 ppm in all categories except sterilizers and to 0.75 ppm in sterilizers	6 lymphohematopoietic cancer cases vs. 3.37 expected (ICD-7 200–209) 2 NHL cases vs. 1.25 expected (ICD-7 200 + 202) 2 leukemia cases vs. 0.82 expected (ICD-7 204–205) Among subjects with at least 0.14 ppm-years of cumulative exposure and 10 years latency, the SIR for leukemia was 7.14, based on 2 cases 5 breast cancer cases vs. 10.8 expected (ICD-7 170)	Fluorochlorocarbons, methyl formate (1:1 mixture with EtO)	Short follow-up period; authors recommend another 10 years of follow-up Youthful cohort—few cases and fewer deaths; unable to determine significance or relationships in categories Only a minority of subjects had high exposure to EtO

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Mikoczy et al. (2011) Update of Hagmar et al. (1995) and Hagmar et al. (1991)	2,171 (862 men, 1,309 women)	Exposure levels as for Hagmar et al. (1995). For the 2,020 cohort members for whom job titles were available, the median was 0.13 ppm × years; the 75th percentile was 0.22 ppm × years; and the 90th percentile was 1.29 ppm × years	18 lymphohematopoietic cancer cases vs. 14.4 expected (ICD-7 200–209) 9 NHL cases vs. 6.25 expected (ICD-7 200 + 202) 5 leukemia cases vs. 3.58 expected (ICD-7 204–205) 41 breast cancer cases vs. 50.9 expected (ICD-7 170) In internal Poisson regression analyses of breast cancer, IRR = 2.76 (p < 0.05) in the 3rd exposure quartile and 3.55 (p < 0.05) in the highest exposure quartile, both compared to the 50% of workers with cumulative exposures below the median	Fluorochlorocarbons, methyl formate (1:1 mixture with EtO)	Still a youthful cohort (mean age 56 years), with small numbers of events for the study of the incidence and mortality of specific cancer types—203 total cancer cases (9.4%) and 171 total cancer deaths (7.9%) Estimated cumulative exposures were generally low There was no unexposed referent group; internal analyses involved comparison of responses in the top quartiles of cumulative exposure to those in the lower 50% of cumulative exposures
Sterilizers of medical equipment and supplies that were assembled at this plant, New York Norman et al. (1995)	1,132 (204 men, 928 women)	In 1980, levels were 50–200 ppm (8-hr TWA); corrective action reduced levels to less than 20 ppm	Only 28 cancers were diagnosed 1 leukemia case vs. 0.54 expected 12 breast cancer cases vs. 4.6 to 7.0 expected ($p \leq 0.05$) 2 pancreatic cancer cases vs. 0.51 expected	No other chemical exposures cited	Little power to detect any significant risk chiefly because a short follow-up period produced few cancer cases Lack of exposure data Insufficient latency analysis

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Nested case-control study; cases and controls from a large chemical production plant, Belgium Swaen et al. (1996)	10 cases of Hodgkin lymphoma (7 cases confirmed) and 200 controls; all male	Cumulative exposure to EtO in cases was 500.2 ppm-months vs. 60.2 ppm-months in controls	3 cases indicated exposure to EtO, producing an OR = 8.5 ($p < 0.05$)	Fertilizers, materials for synthetic fiber production, PVC, polystyrene, benzene, methane, acetone, ammonia, ammonium, sulfate, aniline, caprolactam, ethylene, NaOH, oleum	This was a hypothesis-generating study; the authors were not looking for EtO exposure alone but for other chemical exposures as well to explain the excess risk Only one disease—Hodgkin lymphoma—was analyzed
Four EtO production plants in 3 states using the chlorohydrin process (both ethylene and propylene) Olsen et al. (1997)	1,361 men	No actual measurements were taken	10 lymphohematopoietic cancer deaths vs. 7.7 expected (ICD-8 200–209) After 25-yr latency, SMR = 1.44, based on 6 deaths 2 leukemia and aleukemia deaths vs. 3.0 expected (ICD-8 204–207) No increase in pancreatic cancer (1 observed vs. 4.0 expected)	Bis-chloroethyl ether, propylene oxide, ethylene chlorohydrin, propylene chlorohydrin, ethylene dichloride, chlorohydrin chemicals	No actual airborne measurements of EtO or other chemicals such as ethylene dichloride were reported; only length of employment was used as a surrogate An additional 5 to 10 years of follow-up is needed to confirm the presence or lack of risk of pancreatic cancer and lymphopoietic and hematopoietic cancers

Table A-5. Epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Female workers from pediatric clinic of hospital in Eger, Hungary Kardos et al. (2003)	299 female employees	EtO sterilizing units with unknown elevated concentrations	11 cancer deaths observed compared with 4.38, 4.03, or 4.28 expected ($p < 0.01$), based on comparison populations of Hungary, Heves County, and city of Eger, respectively 1 lymphoid leukemia death 3 breast cancer deaths	No identifiable exposures to other chemicals	Underlying cause of death provided on all 11 cases but no expected deaths available by cause Possible exposure to radon, which is common in the region

ICD NS: ICD codes not specified.

Exposure information, where available, indicates that levels of EtO probably were not high in these study cohorts. If a causal relationship exists between exposure to EtO and cancer, the reported EtO levels may have been too low to produce a significant finding. Exposures in the earlier years (prior to 1970) in most of the companies, hospitals, and other facilities where EtO was made or used are believed to have been in the range of 20 ppm, with excursions many times higher, although few actual measurements are available during this period. (One exception is the environmental study by Joyner (1964), who sampled airborne levels of EtO from 1960 to 1962 in a Texas City facility owned by Union Carbide.)

Almost all actual measurements of EtO were taken in the 1970s and 1980s at most plants and facilities in the United States and Europe, and levels had generally fallen to 5 ppm and below. Some plants may have never sustained high levels of airborne EtO. Assuming that there is a true risk of cancer associated with exposure to EtO, then the risk is not evident at the levels that existed in these plants except under certain conditions, possibly due to a lack of sensitivity in the available studies to detect associated cancers at low exposures.

The best evidence of an exposure-response relationship for lymphohematopoietic cancers comes from the large, diverse NIOSH study of sterilizer workers (Steenland et al., 2004; Stayner et al., 1993; Steenland et al., 1991). This study estimated cumulative exposure (i.e., total lifetime occupational exposure to EtO) in every member of the cohort. The investigators estimated exposures from the best available data on airborne levels of EtO throughout the history of the plants and used a regression model to estimate exposures for jobs/time periods where no measurements were available. This regression model predicted 85% of the variation in average EtO exposure levels. An added advantage to this study, besides its diversity, size, and comprehensive exposure assessment, is the absence of other known confounding exposures in the plants, especially benzene.

In the follow-up of the NIOSH cohort, as in the earlier study, Steenland et al. (2004) observed no overall excess of hematopoietic cancers (ICD-9 codes 200–208). In internal analyses, however, they found a significant positive trend ($p = 0.02$) for hematopoietic cancers for males only, using log cumulative exposure and a 15-year lag, based on 37 male cases. In the Cox regression analysis using categorical cumulative exposure and a 15-year lag, a positive trend was observed and the OR in the highest exposure quartile was statistically significant (OR = 3.42; 95% CI 1.09–10.73). Similar results were obtained for the "lymphoid" category (lymphocytic leukemia, NHL, and myeloma). No evidence of a relationship between EtO exposure and hematopoietic cancers in females in this cohort was observed. In later analyses conducted by Steenland and presented in Appendix D, the difference between the male and female results was found not to be statistically significant, and the same pattern of lymphohematopoietic cancer results observed for males by Steenland et al. (2004) was observed

for the males and females combined (i.e., statistically significant positive trends for both hematopoietic [n = 74] and lymphoid [n = 53] cancers using log cumulative exposure and a 15-year lag, as well as statistically significant ORs in the highest exposure quartile for both hematopoietic and lymphoid cancers).

In the analysis by Swaen et al. (2009) of male UCC workers, the authors discussed the development of the exposure assessment matrix used in combination with worker histories to estimate cumulative exposures for each worker in West Virginia UCC cohort. The exposure matrix was based on the qualitative categorization of potential EtO exposure in the different departments developed by Greenberg et al. (1990) and the time-period exposure estimates from Teta et al. (1993). Eight-hour TWA concentrations (ppm) were estimated over four time periods (1925–1939, 1940–1956, 1957–1973, and 1974–1978) at the two facilities for three exposure-potential categories (high-, medium-, and low-exposure departments). Average exposures in the latter time period (1974–1978) were based on industrial hygiene monitoring conducted at the locations where the study subjects worked. Estimates for the earlier time periods were inferred from data on airborne exposure levels in "similar" manufacturing operations during the time periods of interest. The estimates for the 1957–1973 time period were inferred from measurements reported for the EtO production facility at Texas City studied by Joyner (1964), and the estimates for the 1940–1956 time period were inferred from "rough" estimates of exposure reported for the Swedish company described by Hogstedt et al. (1979a). Exposures for the 1925–1939 time period were assumed to be greater than for the later time periods, but the exposure estimates for this period are largely guesses.

This relatively crude exposure assessment formed the basis of the exposure-response analyses of the UCC study described in Swaen et al. (2009). Swaen et al. (2009) conducted SMR analyses for the UCC workers stratified into those hired before and after December 31, 1956; for three subgroups of employment duration; and for three subgroups of cumulative exposure. These investigators also conducted Cox proportional hazards modeling for leukemia mortality and lymphoid malignancy mortality. No statistically significant excesses in cancer risk or positive trends were reported. Despite the long follow-up of the UCC cohort, its usefulness is limited by its small size (e.g., a total of 27 lymphohematopoietic cancer deaths were observed).

Valdez-Flores et al. (2010) used the same exposure assessment to conduct further exposure-response modeling of the UCC data. These authors used the Cox proportional hazards model to model various cancer endpoints, using the UCC data, the NIOSH data (Steenland et al., 2004), or the combined data from both cohorts. Using cumulative exposure as a continuous variable, no statistically significant positive trends were observed from any of the analyses. Unlike Steenland et al. (2004), Valdez-Flores et al. (2010) rejected the log cumulative exposure model. Using cumulative exposure as a categorical variable, statistically significant increased

risks in the highest exposure quintile were reported for all lymphohematopoietic cancers and for lymphoid cancers in the NIOSH male workers, consistent with results reported by Steenland et al. (2004). Statistically significant increased risks in the highest exposure quintile were also reported for NHL in the NIOSH male workers and for lymphoid cancers and NHL in both sexes combined in the NIOSH cohort.

The many different analyses of the UCC data are weakened by the reliance on the crude exposure assessment. The NIOSH investigators, on the other hand, based their exposure estimates on a comprehensive, validated regression model. Furthermore, the NIOSH cohort was a much larger, more diversified group of workers who were exposed to fewer potential confounders.

One other study that provides cumulative exposure estimates is the incidence study by Hagmar and colleagues (Hagmar et al., 1995; Hagmar et al., 1991). The short follow-up period and relative youthfulness of the cohort produced little morbidity by the end of the study, although some support for an excess risk of leukemia and lymphohematopoietic cancer had appeared. More recently, a follow-up of this cohort by Mikoczy et al. (2011) observed nonsignificant increases in SMRs and SIRs for lymphohematopoietic cancers, consistent with an increase in lymphohematopoietic cancer risk; however, overall, the study is still underpowered for the analysis of lymphohematopoietic cancers (n = 18) and contributes little to the weight of evidence for these cancers.

In a separate analysis of the NIOSH cohort by Wong and Trent (1993), duration of exposure to EtO was used as a surrogate for exposure. These authors did not find any positive exposure-response relationships. They did observe an elevated significant risk of "NHL" in males (SMR = 2.47, $p < 0.05$), based on 16 deaths, which was not dose related or time related. However, a deficit in females remained.

Increases in the risk of hematopoietic cancers are also suggested in several other studies (Coggon et al., 2004; Olsen et al., 1997; Swaen et al., 1996; Norman et al., 1995; Bisanti et al., 1993; Gardner et al., 1989). However, in all these studies the deaths were few and the risk ratios were mostly nonsignificant except at higher estimated exposures or after long observation periods. The findings were not robust, and there were potentially confounding influences, such as exposure to benzene and/or chlorohydrin derivatives.

In those plants with no detectable risks (Norman et al., 1995; Kiesselbach et al., 1990), the cohorts were generally relatively youthful or had not been followed for a sufficient number of years to observe any effects from exposure to EtO. In the study by Olsen et al. (1997), although a slight increase in the risk of cancer of the lymphopoietic and hematopoietic system was evident, the authors stated that their study provided some assurance that working in the chlorohydrin process had not produced significantly increased risks for pancreatic cancer or

lymphopoietic or hematopoietic cancer, thus contradicting the findings of Benson and Teta (1993). This study lacks any measurement of airborne exposure to any of the chemicals mentioned and the authors indicated that an additional 5 to 10 years of follow-up would be needed to confirm the lack of a risk for the cancers described in their study.

Although the largest database pertaining to the cancer risks from EtO exposure is for lymphohematopoietic cancers, described above, more recent evidence suggests that exposure to EtO also increases the risk of breast cancer. The study by Norman et al. (1995) of women who sterilized medical equipment observed a significant twofold elevated risk of breast cancer, based on 12 cases. A study by Tompa et al. (1999) reported on a cluster of breast cancers occurring in Hungarian hospital workers exposed to EtO. In another Hungarian study of female hospital workers by Kardos et al. (2003), three breast cancers were noted out of 11 deaths reported by the authors. Although expected breast cancer deaths were not reported, the total expected deaths calculated was just slightly more than four, making this a significant finding for cancer in this small cohort. The most recent follow-up (Mikoczy et al., 2011) of the Swedish cohort of sterilizer workers originally studied by Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991) reported that the overall SMR and SIR for breast cancer were nonsignificantly decreased. However, in internal exposure-response analyses, statistically significant increases were observed in the incidence rate ratios in the highest two cumulative exposure quartiles compared to the workers with cumulative exposures below the median.

The most compelling evidence on breast cancer comes from the NIOSH cohort. In the latest update of this cohort (Steenland et al., 2004), no overall excess of breast cancer mortality was observed in the female workers; however, a statistically significant SMR of 2.07 was observed in the highest cumulative exposure quartile, with a 20-year lag. In internal Cox regression analyses, a positive exposure-response ($p = 0.01$) was observed for log cumulative exposure with a 20-year lag, based on 103 cases. Similar evidence of an excess risk of breast cancer was reported in a breast cancer incidence study of a subgroup of 7,576 female workers from the NIOSH cohort who were exposed for 1 year or longer (Steenland et al., 2003). A significant ($p = 0.002$) linear trend in SIR was observed across cumulative exposure quintiles, with a 15-year lag. In internal Cox regression analyses, there was a significant regression coefficient with log cumulative exposure and a 15-year lag, based on 319 cases. Using categorical cumulative exposure, the OR of 1.74 was statistically significant in the highest exposure quintile. In a subcohort of 5,139 women with interviews, similar results were obtained based on 233 cases, and the models for this subcohort were also able to take information on other potential risk factors for breast cancer into account. Additionally, the coefficient for continuous cumulative exposure was also significant ($p = 0.02$), with a 15-year lag.

Two other studies with female employees in the defined cohorts reported no increased risks of breast cancer due to exposure to EtO (Coggon et al., 2004; Hagmar et al., 1995; Hogstedt, 1988). However, these studies have much lower statistical power than the NIOSH studies, as evidenced by the much lower numbers of breast cancer cases that they report. The largest number of cases in these other studies is 11 cases in the Coggon et al. (2004) study. Furthermore, none of these other studies conducted internal (or external) exposure-response analyses, which are the analyses that provided the strongest evidence in the NIOSH studies and the Mikoczy et al. (2011) study.

Although the strongest evidence of a cancer risk is with cancer of the hematopoietic system and female breast cancer, there are indications that the risk of stomach cancer may have been elevated in some studies (Teta et al., 1993; Kiesselbach et al., 1990; Hogstedt et al., 1986; Hogstedt et al., 1979b); however, this increased risk attained significance only in the study by Hogstedt et al. (1979b), with 9 observed versus 1.27 expected. Shore et al. (1993) reported that this excess may have been because early workers at this plant "tasted" the chemical reaction product to assess the result of the EtO synthesis. This reaction mix would have also contained ethylene dichloride, a suspected carcinogen, and other chemicals. This increased risk of stomach cancer was not supported by analyses of intensity or duration of exposure in the remaining studies, except that Benson and Teta (1993) suggested that exposure to this chemical increased the risk of pancreatic cancer and perhaps hematopoietic cancer but not stomach cancer.

A significant risk of pancreatic cancer first reported by Morgan et al. (1981) was also reported by Greenberg et al. (1990) in their cohort of chemical workers, but only in those workers assigned to the ethylene chlorohydrin production process, where the authors reported that exposure to EtO was low. Benson and Teta (1993) attributed the increase in pancreatic cancer seen in Greenberg et al. (1990) to exposure to ethylene dichloride in the chlorohydrin process. However, Olsen et al. (1997) refuted this finding in their study. The pancreatic cancers from the study by Morgan et al. (1981) also occurred in workers in a chlorohydrin process of EtO production. The possibility that exposure to a byproduct chemical such as ethylene dichloride may have produced the elevated risks of pancreatic cancer seen in these workers cannot be ruled out.

A.4. CONCLUSIONS

Although several human studies have indicated the possibility of a carcinogenic effect from exposure to EtO, especially for lymphohematopoietic cancers and female breast cancer, the total weight of the epidemiologic evidence is not sufficient to support a causative determination. The causality factors of temporality, coherence, and biological plausibility are satisfied. There is also evidence of consistency in the human studies. When combined under the rubric

"lymphohematopoietic cancers," this loosely defined combination of blood malignancies produces a slightly elevated risk of cancer in most studies but not in all. Similarly, for breast cancer, increased risks are observed in the most of the studies with females, except for two with just a small number of cases. In addition, there is evidence of a biological gradient in the significant exposure-response relationships seen in the large, high-quality Steenland et al. (2004) study and in the Steenland et al. (2003) breast cancer incidence study and the Mikoczy et al. (2011) breast cancer incidence results.

For lymphohematopoietic cancer, the best evidence of a carcinogenic effect produced by exposure to EtO is found in the NIOSH cohort of workers exposed to EtO in 14 sterilizer plants around the country (Steenland et al., 2004; Stayner et al., 1993; Steenland et al., 1991). A positive trend in the risk of lymphohematopoietic and "lymphoid" neoplasms with increasing log cumulative exposure to EtO with a 15-year lag is evident. But there are some limitations to concluding that this is a causal relationship at this time. For example, there was a lack of dose-response relationship in females, although, as presented in Appendix D, later calculations show that the difference in response between females and males is not statistically significant and that significant increases are also observed with both sexes combined.

An elevated risk of lymphohematopoietic cancers from exposure to EtO is also apparent in several other studies. In some of these studies, confounding exposure to other chemicals produced in the chlorohydrin process concurrent with EtO may have been partially responsible for the excess risks. In other studies, where the chlorohydrin process was not present, there are no known confounding influences that would produce a positive risk of lymphohematopoietic cancer. Overall, the evidence on lymphohematopoietic cancers in humans is considered to be strong but not sufficient to support a causal association.

For breast cancer, the best evidence is again found in the NIOSH studies (Steenland et al., 2004; Steenland et al., 2003) discussed earlier, with some corroborating support from the Norman et al. (1995), Kardos et al. (2003), and Mikoczy et al. (2011) studies of breast cancer in women exposed to EtO. The risk of breast cancer was analyzed in two other studies (Coggon et al., 2004; Hogstedt, 1988), and no increase in the risk of breast cancer was found; however, these studies had far fewer cases to analyze, did not have individual exposure estimates, and relied on external comparisons. The NIOSH studies (Steenland et al., 2004; Steenland et al., 2003), 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 on internal exposure-response analyses. The authors suggest that the case is not conclusive of a causal association "due to inconsistencies in exposure-response trends and possible biases due to nonresponse and an incomplete cancer ascertainment." While these are not decisive limitations—exposure-response relationships are often not strictly monotonically increasing

across finely dissected exposure categories, and the consistency of results between the full cohort (less nonresponse bias) and the subcohort with interviews (full case ascertainment) alleviates some of the concerns about those potential biases—the evidence for a causal association between breast cancer and EtO exposure is less than conclusive at this time.

See Section 3.5 for a more detailed and comprehensive weight-of-evidence discussion.

APPENDIX B. REFERENCES FOR FIGURE 3-3

The references in this list correspond to the additional data that were added to Figure 3-3 since the IARC (1994b) genetic toxicity profile was published. See the Figure 3-3 legend for details.

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Major, J; Jakab, MG; Tompa, A. (1999) The frequency of induced premature centromere division in human populations occupationally exposed to genotoxic chemicals. Mutat Res 445(2):241–249.

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Oesch, F; Hengstler, JG; Arand, M; et al. (1995) Detection of primary DNA damage: applicability to biomonitoring of genotoxic occupational exposure and in clinical therapy. Pharmacogenetics 5 Spec No:S118–S122.

Ribeiro, LR; Salvadori, DM; Rios, AC; et al. (1994) Biological monitoring of workers occupationally exposed to ethylene oxide. Mutat Res 313:81–87.

Sisk, SC; Pluta, LJ; Meyer, KG; et al. (1997) Assessment of the in vivo mutagenicity of ethylene oxide in the tissues of B6C3F1 lacI transgenic mice following inhalation exposure. Mutat Res 391(3):153–164.

Swenberg, JA; Ham, A; Koc, H; et al. (2000) DNA adducts: effects of low exposure to ethylene oxide, vinyl chloride and butadiene. DNA Repair 464:77–86.

Tates, AD; vanDam, FJ; Natarajan, AT; et al. (1999) Measurement of HPRT mutations in splenic lymphocytes and haemoglobin adducts in erythrocytes of Lewis rats exposed to ethylene oxide. DNA Repair 431(2):397–415.

van Sittert, NJ; Boogaard, PJ; Natarajan, AT; et al. (2000) Formation of DNA adducts and induction of mutagenic effects in rats following 4 weeks inhalation exposure to ethylene oxide as a basis for cancer risk assessment. Mutat Res—Fundam Mol Mech Mutagen 447:27–48.

Vogel, EW; Nivard, MJ. (1997) The response of germ cells to ethylene oxide, propylene oxide, propylene imine and methyl methanesulfonate is a matter of cell stage-related DNA repair. Environ Mol Mutagen 29(2):124–135.

Vogel, EW; Nivard, MJ. (1998) Genotoxic effects of inhaled ethylene oxide, propylene oxide and butylene oxide on germ cells: sensitivity of genetic endpoints in relation to dose and repair status. Mutat Res 405(2):259–271.

Walker, VE; Sisk, SC; Upton, PB; et al. (1997) In vivo mutagenicity of ethylene oxide at the hprt locus in T- lymphocytes of B6C3F1 lacI transgenic mice following inhalation exposure. Mutat Res 392(3):211–222.

Walker, VE; Wu, KY; Upton, PB; et al. (2000) Biomarkers of exposure and effect as indicators of potential carcinogenic risk arising from in vivo metabolism of ethylene to ethylene oxide. Carcinogenesis 21(9):1661–1669.

APPENDIX C. GENOTOXICITY AND MUTAGENICITY OF ETHYLENE OXIDE

A summary of the available genotoxicity and mutagenicity data for ethylene oxide (EtO) is presented in Chapter 3 (see Section 3.3.3). This appendix provides further details on the available genotoxicity and mutagenicity data and on some of the studies that are briefly mentioned in Chapter 3. The genotoxic potential of EtO is a key component of the assessment of its carcinogenicity. The relationship between genotoxicity/mutagenicity and carcinogenicity is based on the observations that genetic alterations are observed in almost all cancers and that many of these alterations have been shown to play an important role in carcinogenesis. Exposure to EtO has been found to result in a number of genotoxic effects in laboratory animal studies and in studies of humans exposed in occupational settings. In particular, EtO has been shown to alter or damage genetic material in such a manner that the genetic alterations are transmissible during cell division. Evidence of genotoxicity/mutagenicity provides strong mechanistic support for potential carcinogenicity in humans (Waters et al., 1999).

Since the first report of EtO's role in inducing sex-linked recessive lethals in *Drosophila* (Rapoport, 1948), numerous papers have been published on the mutagenicity of EtO in biological systems, spanning a whole range of assay systems, from bacteriophage to higher plants and animals (see Figure 3–3 in Chapter 3). EtO, being a mono-functional alkylating agent, is DNA–reactive, capable of forming DNA adducts and inducing mutations at both the chromosome and gene levels under appropriate conditions, as evidenced in numerous in vitro and in vivo studies reviewed elsewhere (IARC, 2008; Kolman et al., 2002; Bolt, 2000; Natarajan et al., 1995; Vogel and Natarajan, 1995; Dellarco et al., 1990; Kolman et al., 1986). In prokaryotes (bacteria) and lower eukaryotes (yeasts and fungi), EtO induces DNA damage and gene mutations and conversions. In mammalian cells, EtO induces DNA adducts, unscheduled DNA synthesis, gene mutations, sister chromatid exchanges (SCEs), micronuclei, and chromosomal aberrations (IARC, 2008; Bolt, 2000; Natarajan et al., 1995; Preston et al., 1995; Dellarco et al., 1990; Walker et al., 1990; Ehrenberg and Hussain, 1981). The results of in vivo studies on the genotoxicity of EtO following ingestion, inhalation, or injection have also been consistently positive (IARC, 2008, 1994b). Furthermore, in vivo exposure to EtO-induced gene mutations in the *Hprt* locus in mouse and rat splenic T-lymphocytes and SCEs in lymphocytes from rabbits, rats, and monkeys, in bone marrow cells from mice and rats, and in rat spleen. Increases in the frequency of gene mutation in the lung and bone marrow (*LacI* locus) (Recio et al., 2004; Sisk et al., 1997) and in the *Hprt* locus in T-lymphocytes (Walker et al., 1997) in transgenic mice exposed to EtO via inhalation have been observed at concentrations similar to those in carcinogenesis bioassays (NTP, 1987). Furthermore, the frequency of *Kras* mutations

was increased in lung and Harderian gland tumors from EtO-exposed mice compared with spontaneous tumors from control mice, and the spectrum of *Kras* mutations in lung tumors arising from EtO exposure was dramatically different from that found in spontaneous tumors (Hong et al., 2007; NTP, 1987). Likewise, *Hras* and *Trp53* mutations were more frequently induced in mammary carcinomas from EtO-exposed mice, were more frequently concurrent, and expressed different mutation profiles than mammary carcinomas from control mice (Houle et al., 2006; NTP, 1987). EtO has also induced heritable mutations or effects in germ cells in rodents (Generoso et al., 1990; Lewis et al., 1986). In addition, significant increases in the frequency of SCEs and chromosomal aberrations in peripheral blood lymphocytes have been consistently reported in workers exposed to concentrations of EtO of greater than 5 ppm (TWA) [IARC (2008), and references therein]. Thus, there is consistent evidence from in vitro studies and in vivo studies of laboratory animals and occupationally exposed humans that EtO interacts with the genome. Based on these observations, exposure to EtO is considered to cause cancer through a mutagenic mode of action (see Chapter 3, Section 3.4).

The following sections provide further details on different genotoxicity test results on the mutagenic potential of EtO.

C.1. ADDUCTS

C.1.1. DNA Adducts

Covalent bonding of a chemical (direct-acting) or its electrophilic intermediates or metabolites (indirect-acting chemicals following metabolic activation) with the nucleophilic sites in DNA results in the formation of "DNA adducts," which represent the biologically effective dose of the chemical agent in question. Alkylating agents, such as EtO, are direct-acting chemical agents that can transfer alkyl groups (e.g., ethyl groups) to nucleophilic sites in DNA, alkylating the nucleotide bases. Alkylating agents are classified as S_N1-type or S_N2-type depending on the substitution nucleophilicity (S_N). The S_N1-type chemicals follow first-order kinetics (e.g., ethylnitrosourea and methylnitrosourea), while the S_N2-type agents exhibit an intermediate transition state (e.g., EtO and methyl methanesulfonate). EtO is a direct-acting S_N2 (substitution-nucleophilic-bimolecular)-type alkylating agent that forms adducts with cellular macromolecules such as proteins (e.g., hemoglobin) and DNA. The reactivity of an alkylating agent can be estimated by its Swain-Scott substrate constant (*s*-value), which ranges from 0 to 1 (Warwick, 1963). Alkylating agents such as EtO and methyl methanesulfonate, which have high *s*-values (0.96 and >0.83, respectively), target the nucleophilic centers of ring nitrogens (e.g., N7 of guanine and N3 of adenine) in DNA, while agents such as ethylnitrosourea with a low *s*-values (0.26) target the less nucleophilic centers such as O^6 of guanine. EtO has a high substrate constant favoring efficient alkylation at N7 of guanine (Beranek, 1990; Golberg, 1986; Warwick,

1963). Due to the high nucleophilicity and steric availability of the N7 of guanine, EtO predominantly forms the N7-(2-hydroxyethyl)guanine (N7-HEG) adduct, although minor 2-hydroxyethyl adducts such as those forming at the O^6 of guanine (O^6-HEG), the N1 (N1-HEA), N3 (N3-HEA), and N^6 of adenine (N^6-HEA), and the N3 of cytosine (N3-HEC), uracil (N3-HEU) and thymine (N3-HET) are found in some instances (Segerbäck, 1994).[6]

Several methods have been developed since 1988 to detect EtO-induced DNA adducts in vitro and in vivo. However, sensitivity and specificity of these methods have been a concern. These methods include immunochemical assays, fluorescence techniques, high-performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC/MS), ^{32}P-postlabeling and electrochemical detection, with varying sensitivities for detection of EtO-DNA adducts (Marsden et al., 2009; Huang et al., 2008; Tompkins et al., 2008; Marsden et al., 2007; Bolt et al., 1997; Leclercq et al., 1997; Kumar et al., 1995; Saha et al., 1995; van Delft et al., 1994; van Delft et al., 1993; Uziel et al., 1992; Bolt et al., 1988). In the following paragraphs, a brief summary of available methods is provided to aid in the discussion of the DNA adduct data.

van Delft et al. (1993) developed monoclonal antibodies against the imidazole ring of N7-alkyldeoxyguanosine, with the limits of detection being 5–10, 1–2, and 20 adducts per 10^6 nucleotides when used in the direct and competitive enzyme-linked immunosorbent assay and in immunofluorescence microscopy, respectively. Later, the same authors developed an immunoslot-blot assay with increased sensitivity that detected 0.34 N7-HEG adducts per 10^6 nucleotides (van Delft et al., 1994). Kumar et al. (1995) developed a ^{32}P-postlabeling method using thin-layer chromatography (TLC) and HPLC, which detected 0.1−1.0 fmol 7-alkylguanine adducts in rats exposed to different alkenes. Despite occasional inefficient labeling and poor recovery of adduct due to depurination, this method has potential for use in measuring human exposure to alkenes or their corresponding epoxides, as well as the endogenously formed 7-alkylguanine adducts.

Bolt et al. (1997) developed a HPLC method involving derivatization with phenylglyoxal and fluorescence detection, using 7-methylguanine as an internal standard, for measuring the physiological background of the N7-HEG adduct in DNA isolated from human blood. Using this method, the authors were able to detect N7-HEG levels in five individuals ranging between 2.1 and 5.8 pmol/mg DNA (mean 3.2). Furthermore, Leclercq et al. (1997) developed a method based on DNA neutral thermal hydrolysis, adduct micro-concentration, and HPLC coupled to

[6]For simplicity, this assessment generally uses the nomenclature and abbreviations for the nucleobase adducts; these are the same adducts encompassed in the larger deoxyribonucleoside adduct forms. Thus, for example, N3-HEA is used synonymously to refer to both the N3-(2-hydroxyethyl)adenine and the N3-(2-hydroxyethyl)-2′-deoxyadenosine (N3-HEdA) adducts.

single-ion monitoring electrospray mass spectrometry which has a detection limit of 1 fmol, reportedly allowing the detection of approximately 3 adducts/10^8 normal nucleotides. Using this method, Leclercq et al. (1997) detected a dose-response relationship for N7-HEG after exposing calf thymus DNA and blood samples to various doses of EtO. Marsden et al. (2007) used a highly sensitive LC-MS/MS assay with selected reaction monitoring that offers a limit of detection of 0.1 fmol of N7-HEG to establish background levels of N7-HEG (1.1–3.5 adducts/10^8 nucleotides) in rat tissue. Huang et al. (2008) developed an isotope-dilution online solid-phase extraction and liquid chromatography coupled with tandem mass spectrometry method with reportedly excellent accuracy, sensitivity, and specificity to analyze N7-HEG in urine samples of nonsmokers. This method also demonstrated high-throughput capacity for detecting EtO-DNA adducts and may be particularly useful for future molecular epidemiology studies of individuals with low-dose EtO exposure. Tompkins et al. (2008) used a high-performance liquid chromatography/electrospray ionization tandem mass spectrometry and reported ~8 N7-HEG adducts/10^8 nucleotides in the livers of control rats. This method was also capable of detecting the less prevalent but potentially more biologically significant N1-HEA, O^6-HEG, N^6-HEA, and N3-HEU adducts. However, these minor adducts were below the level of detection in control rat tissue DNA.

Overall, the sensitivity of EtO adduct detection depends on the method used for analysis. Hence, use of appropriate methods is important when analyzing for these adducts and will be highlighted in the following discussion.

C.1.1.1. *Detection of EtO Adducts in In Vitro and In Vivo Systems*

Numerous studies have been conducted to investigate the formation of DNA adducts following EtO exposure in a wide range of experimental models, including cell-free systems, bacteria, fungi, *Drosophila*, and other laboratory animals, as well as in exposed human subjects. The following discussion is a review of the available studies of exposure to EtO and DNA adduct formation in in vitro systems, laboratory animals, and humans (Boysen et al., 2009; Pauwels and Veulemans, 1998; Bolt et al., 1988; van Sittert and de Jong, 1985).

C.1.1.2. *In Vitro DNA Binding Studies*

The capacity of EtO to bind to DNA and form DNA adducts has been documented in a few in vitro studies. Segerbäck (1990) showed that [^{14}C]-labeled EtO reacted in vitro with calf thymus DNA to produce N7-HEG adduct as the predominant adduct, with relatively low amounts of O^6-HEG and N3-HEA adducts. The levels of N3-HEA and O^6-HEG are 4.4 and 0.5%, respectively, of the N7-HEG levels. Thus, the ratio of N7-HEG, N3-HEA, and O^6-HEG produced in vitro was 200:8.8:1, respectively. In the same study, the in vitro reaction products of

radiolabeled N-(2-hydroxyethyl)-N-nitrosourea (HOEtNU) with calf thymus DNA exhibited a higher relative amount of O^6-HEG, which was 63% of the N7-HEG formed. The difference in reactivity towards the N7 and O^6 positions in guanine by these two alkylating agents was explained by the difference in their *s*-values. EtO, with an *s*-value of 0.9, has a greater relative preference for reacting with N rather than O atoms than does HOEtNU, with an *s*-value of 0.2.

In another study, Li et al. (1992) observed that EtO in aqueous solution incubated with calf thymus DNA in vitro for 10 hours produced several 2-hydroxyethyl DNA adducts whose relative yields (nmol/mg DNA) were in the descending order: N7-HEG (330) > N3-HEA (39) > N1-HEA (28), N^6-HEA (6.2) > N3-HEC (3.1) > N3-HET (2.0) > N3-HEU (0.8). This in vitro study did not detect the O^6-HEG adduct.

More recently, Tompkins et al. (2009) treated pSP189 shuttle vector plasmid to a range of EtO concentrations in water and reported that, of the five 2-hydroxyethyl DNA adducts measurable using their LC-MS/MS analytical method, only the N7-HEG adduct was detectable at EtO concentrations up to 2,000 µM.[7] At the 10 mM concentration, the level of N7-HEG adducts was about 19 times higher than that of N1-HEA adducts and about 1,000 times higher than that of O^6-HEG adducts. At 30 mM, N3-HEU adducts were detectable, but this adduct was not quantifiable due to the lack of a suitable internal standard. Detection of the N3-HEU adduct implies that the N3-HEC adduct is also formed, as the former is the hydrolytic deamination product of the latter (Tompkins et al., 2009). No results for the N^6-HEA adduct were reported. (N3-HEA, N3-HEC, and N3-HET adducts are not measurable by their method.)

C.1.1.3. *In Vivo Studies—Laboratory Animals*

Several studies evaluated N7-HEG levels following one or a range of doses with repeated exposures of EtO given by inhalation or intraperitoneal injection in laboratory animals. Segerbäck (1983) showed that in male CBA mice exposed by inhalation to [^{14}C]-labeled EtO N7-HEG adducts are formed in spleen, testes, and liver with half-lives of 24, 20, and 12 hours, respectively.

Walker et al. (1990) conducted a time-course study to investigate the formation and persistence of N7-HEG adducts in various tissues (e.g., brain, kidney, liver, spleen, lung, and kidney) of male Fischer 344 rats exposed to one high dose of 300 ppm EtO by inhalation for 4 consecutive weeks (6 hours/day, 5 days/week) and sacrificed 1–10 days after the end of exposure. The N7-HEG adduct was detectable in both target (brain, spleen, and white blood cells) and nontarget (kidney, liver, lung, and testis) tissues with maximum levels (1.5 times

[7]The minor adducts may have been present at levels below the limits of detection, which were as follows: $0.001/10^6$ nucleotides for N7-HEG and N1-HEA; $0.016/10^6$ nucleotides for O^6-HEG; and $0.082/10^6$ nucleotides for N3-HEU (Tompkins et al., 2009).

control levels) seen in brain compared to other tissues 1 day after exposure. The similarities in N7-HEG levels in various tissues are possibly due to efficient pulmonary uptake of EtO and rapid distribution by the circulatory system. The N7-HEG adduct levels increased linearly for 3–5 days followed by a slow removal from DNA with an apparent half-life of 7 days, suggesting that the adduct was probably removed by spontaneous depurination. The calculated in vivo half-life for N7-HEG formed by EtO confirms the persistence of this adduct and is consistent with another study in rats exposed to another alkylating agent, N-nitrosomethyl-(2-hydroxyethyl)amine (Koepke et al., 1988). Walker et al. (1990) suggested that the similarity in N7-HEG formation in the target as well as nontarget tissues could also be due to factors such as cell replication, location of the adducts in the genome, and tissue susceptibility genes, which might be critical determinants quantitatively affecting tissue-specific and/or dose-response relationships.

Using fluorescence-coupled HPLC, Walker et al. (1992) measured N7-HEG levels in DNA of target and nontarget tissues from male $B6C3F_1$ mice and F344 rats exposed to 0, 3, 10, 33, 100, or 300 (rats only) ppm EtO by inhalation for 4 weeks (6 hours/day, 5 days/week). Another group of mice was exposed to 100 ppm EtO for 1, 3, 7, 14, or 28 days (5 days/week). The authors reported linear dose-response relationships for N7-HEG in rat tissues following EtO exposures between 10 and 100 ppm, with the slope increasing for exposures above 100 ppm. In mice, only exposures to 100 ppm EtO resulted in significant increase in N7-HEG levels. Walker et al. (1992) observed N7-HEG adduct levels of 2–6 pmols/mg DNA in control mice and rats, while in mice exposed to 100 ppm EtO, N7-HEG levels ranged from 17.5 ± 3.0 (testis) to 32.9 ± 1.9 (lung) pmol/mg DNA after 4 weeks of exposure. Rats and mice concurrently exposed to 100 ppm EtO for 4 weeks showed two- to threefold lower N7-HEG levels in all tissues of mice compared to rats, suggesting species differences in the susceptibility to EtO-induced genotoxicity. The half-life of N7-HEG in mouse kidney DNA was 6.9 days, and in rat brain and lung 5.4–5.8 days. The half-lives of N7-HEG adducts in DNA from other tissues of mouse and rat were 1.0–2.3 days and 2.9–4.8 days, respectively. The authors suggested that the slow linear removal of N7-HEG adducts from the DNA was mainly due to chemical depurination, while the rapid removal was due to loss by depurination and DNA repair. Rats exposed to 300 ppm EtO showed O^6-HEG adducts at a steady-state concentration of ~1 pmol/mg DNA. Based on the results from rats and mice, the authors suggested that DNA repair was saturated at the concentration of EtO used in the time-course studies and that repeated exposures to lower concentrations of EtO should lead to species- and tissue-specific differences in the levels of N7-HEG (Walker et al., 1992).

Wu et al. (1999a) analyzed DNA from liver, brain, lung, and spleen of $B6C3F_1$ mice and F344 rats for N7-HEG adducts after exposure to EtO (0, 3, 10, 33, or 100 ppm) for 4 weeks

(6 h/day, 5 days/week). The authors observed tissue- and species-specific dose-response relationships of N7-HEG adducts in the EtO-exposed animals. Mice showed linear dose-response relationships for N7-HEG adducts in liver, brain, and spleen at exposures between 3 and 100 ppm, and sublinear responses in lung between 33 and 100 ppm EtO exposure. Rats showed linear increases in adduct levels in liver and spleen DNA between 3 and 100 ppm EtO, and sublinear responses in the brain and lung between 33 and 100 ppm EtO exposure. Overall, rats and mice exposed to 3 ppm EtO showed 5.3- to 12.5- and 1.3- to 2.5-fold higher N7-HEG adducts, respectively, compared to the corresponding unexposed control animals. Thus, results from this study suggest species differences, with rats being more susceptible to adduct formation than mice, at lower levels of EtO exposure. This study also showed a clear difference in N7-HEG levels between unexposed and exposed mice at these lower exposure levels, unlike the study of Walker et al. (1992) discussed above. This difference is possibly due to the use of a highly sensitive gas chromatography high-resolution mass spectrometry assay in the Wu et al. (1999a) study.

van Sittert et al. (2000) exposed Lewis rats to 50, 100, and 200 ppm EtO by inhalation (4 weeks, 5 days/week, 6 h/day) and measured N7-HEG adducts 5, 21, 35, and 49 days after cessation of exposure. The authors used mass spectrometry following neutral thermal hydrolysis of DNA to release the N7-HEG adducts and observed a clear exposure-response relationship across the control and EtO-exposed rats. The mean levels of liver N7-HEG immediately after cessation of exposure to 50, 100, and 200 ppm were estimated by extrapolation to be 310, 558, and 1,202 adducts/10^8 nucleotides, respectively, while the mean level in control rats was 2.6 adducts/10^8 nucleotides. By 49 days postexposure, N7-HEG adducts had returned to near background levels. The N7-HEG levels in liver DNA showed a linear response between 0 and 200 ppm EtO, suggesting that detoxification and DNA repair processes were not saturated up to the highest exposure level tested. The authors observed statistically significant linear relationships between mean N7-HEG levels at "day 0" postexposure and (1) *Hprt* mutant frequencies at expression times of 21/22 and 49/50 days postexposure, (2) SCEs at 5 days postexposure, or (3) high-frequency cells measured 5 days postexposure. The authors also observed that SCEs and high-frequency cells continued to be present at 21-days postexposure and significantly correlated with N7-HEG adducts at that time. However, induction of micronuclei, chromosome breaks, or translocations did not show a dose-response relationship.

Nivard et al. (2003) showed that in male *Drosophila*, EtO exposure (2–1,000 ppm) by inhalation for 24 hours induced a linear dose-response relationship for N7-HEG adduct formation (0.15 to 105.4 adducts/10^6 nucleotides) over the entire dose range, as detected by ^{32}P-postlabeling assay. The N7-HEG adducts were undetectable in controls (i.e., below the detection limit of 1 adduct/10^8 nucleotides).

A study by Rusyn et al. (2005) tested the hypothesis that EtO exposure results in an accumulation of apurinic/apyrimidinic (AP) sites in DNA and induces changes in expression of genes involved in DNA base excision repair (BER). The authors exposed male F344 rats by inhalation to 100 ppm EtO or ethylene (40 or 3,000 ppm) for 1, 3, or 20 days (6 h/day, 5 days/week) and sacrificed them 2, 6, 24, or 72 hours after a single-day exposure. Brain and spleen were considered as target sites for EtO-induced carcinogenesis, and liver as a nontarget organ. Rusyn et al. (2005) observed a time-dependent increase in N7-HEG in brain and spleen (target organs) and liver (nontarget organ) and in N-(2-hydroxyethyl)valine (HEVal) adducts in hemoglobin. However, they could not detect any increase in AP sites in control or EtO-exposed rats for any given duration or dose of exposure. Rats exposed to EtO for 1 day showed a threefold to sevenfold decrease in expression of the DNA repair enzyme 3-methyladenine-DNA glycosylase in the brain and spleen, while rats exposed to EtO for 20 days showed increased expression of hepatic 8-oxoguanine DNA glycosylase, 3-methyladenine-DNA glycosylase, AP endonuclease, polymerase beta, and alkylguanine methyltransferase by 20–100%. Levels of brain AP endonuclease and polymerase beta were increased by <20% only in rats exposed to 3,000 ppm ethylene for 20 days. Results from this study suggest that EtO-induced DNA damage is repaired without accumulation of AP sites or involvement of the BER pathway in target organs. The authors concluded that accumulation of AP sites is not likely a primary mechanism for mutagenicity and carcinogenicity of EtO, and further suggested that minor DNA adducts such as O^6-HEG or N1-HEA are likely to be involved in mutagenicity. In fact, in a previous study from the same group (Walker et al., 1992), steady-state concentrations of O^6-HEG were reported after 4 weeks of exposure with 300 ppm EtO, a finding which warrants further investigation.[8]

Marsden et al. (2007) have shown that intraperitoneal administration of a single or three daily doses of EtO (0.01–1.0 mg/kg) induced dose-related increases in N7-HEG adduct levels in male F344 rats, except at the lowest dose (0.01 mg/kg), where N7-HEG levels were similar to endogenous levels detected in control animals. Further, they observed that N7-HEG adducts did not accumulate in rats given three daily doses of EtO.

[8] 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. (2015b) 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. (1992). The Zhang et al. (2015b) 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. Significant increases in other potentially mutagenic purine adducts (e.g., N1-HEA and N^6-HEA) were also observed.

More recently, using a dual-isotope approach combining HPLC-accelerated mass spectrometry with LC-MS/MS analysis, Marsden et al. (2009) observed linear dose-response relationships for [^{14}C]N7-HEG adducts (0.002 to 4 adducts/10^8 nucleotides) in spleen, liver, and stomach DNA of F344 rats after exposure to low, occupationally relevant concentrations of [^{14}C]EtO (0, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 mg/kg daily for 3 consecutive days, with the rats killed 4 h after the last exposure). These results suggest that by using a highly sensitive assay, it is possible to measure the N7-HEG adducts resulting from low EtO exposures above the background adduct levels.

Otteneder and Lutz (1999) reviewed the quantitative relationship between DNA adduct levels and tumor incidence in rodents that received repeated administration of EtO. The authors observed a correlation with tumor incidence when the DNA adduct levels measured at a given dose were normalized to the TD_{50} dose (the dose which results in 50% tumor incidence in a two-year study). The calculated adduct level in mice associated with the hepatocellular TD_{50} was 812 N7-HEG adducts/10^8 normal nucleotides.

C.1.1.4. *In Vivo Studies—Humans*

A few studies have examined the effect of EtO exposure on humans, particularly in occupational settings, and these have been comprehensively reviewed by Kolman et al. (2002). In that review, the authors examined the use of hemoglobin and DNA adducts as biomarkers of EtO exposure and the roles of genetic polymorphisms and confounding factors. Kolman et al. (2002) also described the genotoxic effects of EtO in mammalian cells and summarized the genotoxic and carcinogenic effects of EtO in humans. Some of the relevant studies in humans are briefly discussed below.

An immunoslot blot assay was used to analyze N7-HEG levels in white blood cell DNA from individuals exposed to EtO (2–5 ppm) and from controls (van Delft et al., 1994). The authors reported 0.1 and 0.065 N7-HEG adducts/10^6 nucleotides, respectively, in EtO-exposed individuals (n = 42) and controls (n = 29) by this method. However, these differences were not statistically significant.

In a study involving 58 sterilizer operators exposed to low and high levels of EtO (≤32 and >32 ppm-hour, respectively) and 6 nonexposed controls from different hospitals, Yong et al. (2007) examined N7-HEG adducts in granulocyte DNA. During the 4-month study, the cumulative exposure to EtO (ppm-hour) was estimated before the blood sample collection. After adjusting for cigarette smoking and other potential confounders, the mean N7-HEG adduct levels in the nonexposed, low-, and high-exposure groups were 3.8, 16.3, and 20.3 adducts/10^7 nucleotides, respectively, with considerable interindividual variation (range: 1.6–241.3 adducts/10^7 nucleotides). However, these differences in mean adduct level were not

statistically significant. The large variability across workers may reflect differences in their recent exposure patterns because granulocytes have a lifespan of less than a day. Also, the study did not find a significant correlation between the levels of N7-HEG adducts and HEVal adducts.

Mayer et al. (1991) observed an apparent suppression of DNA repair capacity in EtO-exposed individuals as measured by the DNA repair index, that is, the ratio of unscheduled DNA synthesis and N-acetoxy-2-acetylaminofluorene-DNA binding, ($p < 0.01$). In this study, 34 sterilization unit workers of a large university hospital and 23 controls working in the university library were used. Overall, this study demonstrates significant correlations between EtO-induced hemoglobin adduct levels and SCEs and the number of high frequency cells, at low levels of EtO exposure (≤1 ppm), independent of smoking history.

C.1.1.5. *DNA Adducts—Summary*

In summary, EtO predominantly forms N7-HEG adducts. Minor adducts are O^6-HEG adducts and reaction products with N1, N3, and N^6 of adenine and with N3 of cytosine, uracil and thymine in vitro. However, the minor adducts are not observed to the same extent in vivo, which may reflect a limitation in the sensitivity of the adduct assays available to date. Repeated inhalation exposure of EtO induces N7-HEG adducts in both target organs (brain, spleen, and white blood cells) and nontarget organs (kidney, liver, and lung) in rodents, with an apparent half-life of 3–6 days in rats and 1–3 days in mice (Walker et al., 1992). The dose-response relationship of N7-HEG and EtO exposure is influenced by the analytical method used, which also affects the background (endogenous) levels of adducts observed in unexposed rodents. Steady-state levels of O^6-HEG adducts (1 pmol/mg DNA) are detected in rats exposed by inhalation to high doses of EtO (300 ppm) which are ~250–300 times lower than the N7-HEG levels (Walker et al., 1992). Although N7-HEG adducts are likely to be removed by depurination forming apurinic/apyrimidinic (AP) sites, Rusyn et al. (2005) showed that DNA damage induced by exposure to EtO is repaired without accumulation of AP sites and without affecting base excision repair (BER) in target organs of Fischer rats. Only two studies are available on EtO-induced DNA adducts in human populations. Although higher levels of N7-HEG DNA adducts were observed in human white blood cells (van Delft et al., 1994) and granulocytes (Yong et al., 2007) of exposed cases compared to controls, these differences were not statistically significant, possibly due to high interindividual variability.

C.1.2. EtO-Hemoglobin Adducts

Several studies have shown that EtO-induced hemoglobin adducts (e.g., HEVal) are good biomarkers of exposure for this compound in human studies and that predicted hemoglobin adduct levels resulting from exposure to ethylene or EtO are in agreement with measured values

(Boogaard, 2002; Yong et al., 2001; Fennell et al., 2000; Tates et al., 1999; Walker et al., 1992; Britton et al., 1991). Csanády et al. (2000) found a good agreement between the predicted and measured hemoglobin adduct levels in humans. However, in rodents, hemoglobin adducts were under-predicted by a factor of 2 to 3, while DNA adduct levels were comparable, suggesting inconsistencies between the two biomarkers. Walker et al. (1993) also observed that the relationships between HEVal and N7-HEG concentrations varied with length of exposure, interval since exposure, species, and tissue, which may be due to differences in formation, persistence, repair, and chemical depurination of the DNA adduct. Thus, Walker et al. (1993) suggested that HEVal adducts do not provide accurate prediction of DNA adducts in specific tissues of humans under actual exposure conditions. In summary, HEVal adducts do not appear to be predictable markers for DNA adducts.

C.2. GENE MUTATIONS

EtO has consistently yielded positive results, at both the gene and chromosome levels, in a broad range of in vitro and in vivo mutational assays, including those performed in bacteria, fungi, yeast, insects, plants, *Drosophila*, and rodents, in both repair-deficient and proficient organisms, and in mammalian cell cultures, including cells from humans [reviewed in (IARC, 2008; Kolman et al., 2002; Bolt, 2000; Natarajan et al., 1995; Vogel and Natarajan, 1995; IARC, 1994b; Dellarco et al., 1990)]. 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)]. Increases in the frequency of gene mutations in the lung (*LacI* locus) (Sisk et al., 1997), in T-lymphocytes (*Hprt* locus) (Walker et al., 1997), and bone marrow and testes in B6C3F$_1$ *LacI* transgenic mice (Recio et al., 2004) have been observed in mice exposed to EtO via inhalation at concentrations similar to those used in the carcinogenesis bioassays (NTP, 1987), clearly documenting that EtO is a DNA-reactive mutagenic agent. Furthermore, occupational studies provide evidence for the genotoxic potential of EtO.

C.2.1. Bacterial Systems

Studies have been conducted to investigate the ability of EtO to induce gene mutations in bacterial systems. Victorin and Ståhlberg (1988) treated *Salmonella typhimurium* strain TA100 with EtO at concentrations of 1–200 ppm for 6 hours and demonstrated that EtO was mutagenic in this system. In another study, Agurell et al. (1991) compared EtO and propylene oxide (two alkylating agents) for genotoxic effectiveness in various test systems. The abilities of the two compounds to induce point mutations in *S. typhimurium* strains TA 100 and TA1535 were approximately equal. EtO induced a dose-dependent increase in the number of revertants in both tester strains. No toxic effects were observed under the conditions tested.

In contrast, Agurell et al. (1991) found EtO to be 5–10 times more effective than propylene oxide with respect to gene conversion and reverse mutation in the *Saccharomyces cerevisiae* D7 and *S. cerevisiae* RS112 strains. The greater effectiveness of EtO over propylene oxide in inducing these types of mutations was probably due to the difference in these compounds' abilities to cause strand breaks via alkylation of DNA-phosphate groups.

Mutagenicity studies of EtO have also been conducted using different *Escherichia coli* strains. Kolman (1985) investigated the influence of the *uvrB* and *umuC* genes on the induction of *LacI*-mutants and nonsense mutants by EtO in the *LacI* gene of *E. coli* and found that *uvrB* gene mutation was associated with higher mutation frequencies whereas *umuC* mutation did not significantly affect the induction of *LacI* mutations. Thus, mutations induced by EtO were enhanced by a lack of excision repair but not influenced by changes in error-prone repair. In another study by the same group of authors (Kolman and Näslund, 1987), the mutagenicity of EtO in *E. coli* B strains with different repair capacities was investigated. Deficiencies in excision repair (*uvrA*, *polA*) led to considerable increases in mutation frequency compared to the wild-type strain and strains deficient in error-prone repair (*recA*, *lexA*).

The induction of specific-locus mutations in the *adenine-3 (ad-3)* region of a two-component heterokaryon (H-12) of *Neurospora crassa* by EtO was studied by de Serres and Brockman (1995). The objective of this study was to compare EtO's mutational spectrum for induced specific-locus mutations with those of other chemical mutagens. Conidial suspensions were treated with five different concentrations of EtO (0.1–0.35%) for 3 hours. The results from these experiments showed (1) the dose-response curve for EtO-induced specific-locus mutations in the *ad-3* region was linear, with an estimated slope of 1.49 ± 0.07, and (2) the maximum forward-mutation frequency was between 10 and 100 *ad-3* mutations per 10^6 survivors. The overall data demonstrate that EtO-induced *ad-3* mutations were the result of a high percentage (96.9%) of gene/point mutations at the *ad-3A* and *ad-3B* loci.

C.2.2. Mammalian Systems

EtO has yielded positive results in virtually all in vitro mammalian cell culture systems tested, including human cells (IARC, 2008; Kolman et al., 2002; Bolt, 2000; Preston, 1999; Natarajan et al., 1995; Vogel and Natarajan, 1995; IARC, 1994b; Dellarco et al., 1990). Only select in vitro studies of human cells will be reviewed here. For reviews of other in vitro studies using mammalian cell cultures, see the aforementioned references.

C.2.2.1. *In Vitro Studies*

Single base pair deletion and base substitution (both transitions and transversions) mutations were observed in the *HPRT* gene in human diploid fibroblasts exposed to EtO

(Bastlová et al., 1993). Sequence analysis revealed that EtO induces many different kinds of *HPRT* mutations—several mutants had large *HPRT* gene deletions, a few mutants showed deletion of the entire *HPRT* gene, and other mutants had a truncated *HPRT* gene; overall, as many as 50% were large deletions. In another study by the same group of authors (Lambert et al., 1994), comparisons of the *HPRT* mutations in human diploid fibroblasts were made for three urban air pollutants (acetaldehyde, benzo[a]pyrene, and EtO). Large genomic deletions in the *HPRT* gene were observed for acetaldehyde and EtO, whereas benzo[a]pyrene induced point mutations. The authors concluded that the *HPRT* locus could be a useful target for the study of chemical-specific mutational events (Lambert et al., 1994).

The effect of EtO as a pretreatment or posttreatment to ionizing radiation was studied by Kolman and Chovanec (2000). Human diploid VH-10 fibroblasts were either preexposed to gamma rays (0.66 Gy/minute or 10 Gy/minute) and then treated with EtO (2.5 mMh) or pretreated with EtO and then exposed to gamma rays. Cell killing/cytotoxicity, DNA double-strand breakage, and mutagenicity were studied in both types of exposures. The results of the study indicate that preexposure of the cells to gamma radiation (1 Gy) followed by treatment with EtO (2.5 mMh) led to an additive interaction, irrespective of the dose rate. On the other hand, pretreatment with EtO followed by gamma ray exposure resulted in an antagonistic effect, which was most pronounced in the high-dose group (10 Gy/minute). In this group, the mutant frequency was half that of the sum of the mutant frequencies after the individual treatments. The authors suggest that one possible explanation for the difference in the results is that DNA damage induced by preexposure to gamma radiation persisted into the EtO treatment phase, and EtO might also prohibit DNA repair enzymes from operating; thus, both treatments contributed to the mutant frequency. However, when cells were exposed to gamma radiation following EtO treatment, the cells may have been able to repair, at least in part, the promutagenic lesions induced by the gamma rays.

Tompkins et al. (2009) investigated the mutagenicity of EtO-derived DNA adducts in a *supF* forward mutation assay. Aliquots of pSP189 plasmid containing the *supF* gene were exposed to various concentrations of EtO in water to induce the formation of DNA adducts. The plasmids were then transfected into human embryonic adenovirus-transformed kidney (Ad293) cells and allowed to replicate to propagate any mutations. Replicated plasmids were isolated and used to treat *E. coli* indicator bacteria under conditions in which only bacteria containing the plasmid can grow; nonmutant colonies appear dark blue and mutant colonies appear white or pale blue. Two studies were conducted: Study 1, in which the plasmid was incubated with EtO concentrations ranging from 10 to 2,000 µM at 22°C for 4 hours, and Study 2, in which the plasmid was treated under "refined" conditions optimised to produce more of the minor 2-hydroxyethyl adducts, which involved incubation of the plasmid with EtO concentrations

ranging from 10 to 100 mM at 37°C for 24 hours. For Study 1, Tompkins et al. (2009) reported that N7-HEG was the only detectable adduct of the five they measured (before transfection; see Section C.1.1.2 above), and there was no clear exposure-response relationship for the relative mutation frequency. In Study 2, N1-HEA and O^6-HEG adducts were also quantifiable, but at lower levels than the N7-HEG adduct, and there was an apparent exposure-response relationship for the relative mutation frequency for plasmids exposed to the 10 and 30 mM EtO concentrations. Plasmids exposed to higher concentrations of EtO failed to produce any *E. coli* colonies; this was attributed to excessive strand breaks in the plasmid DNA at those concentrations. For the DNA damage induced by EtO-derived adducts, this limitation in the assay imposes a short response range for the relative mutation frequency for the mutations measured by the assay—the relative mutation frequency was 5.34 for plasmids exposed to 30 mM and no *E. coli* colonies were produced with plasmids exposed to the next highest EtO concentration of 50 mM, due to excessive DNA strand breaks.

Tompkins et al. (2009) concluded that EtO is a relatively weak mutagen and that their results suggest that a certain level of total DNA adducts or of specific promutagenic adducts must be achieved before mutations become detectable above background levels. However, several methodological issues raise concerns about the interpretation of the results. For example, two solvent controls were used in the study—Solvent Control 1 was prepared in "a separate fume hood to totally exclude any possibility of [EtO] contamination" and Solvent Control 2 was prepared "alongside the [EtO] reactions." Solvent Control 1 was used as the referent group for the relative mutation frequency determinations. In two replicates, Solvent Control 2 had a relative mutation frequency of 3.0 and 2.6 compared to Solvent Control 1. If this difference reflects a real difference between the two different solvent control preparations, it raises the possibility that cross-contamination may have been a problem, and if any cross-contamination also occurred across the different EtO concentrations, it could have dampened any exposure-response relationship. In addition, if the "refined conditions" for plasmid treatment used to produce more of the minor (more directly promutagenic) adducts in Study 2, which included incubation at a temperature more comparable to mammalian body temperatures, had also been used for Study 1, a different adduct profile, and different relative mutation frequencies, might have resulted. The authors themselves acknowledged that "[in] order to categorically determine whether a threshold exists for [EtO] in this system, a more detailed examination of the dose-response relationship using the optimised reaction protocol and including more concentrations around the mutagenic range is needed" (Tompkins et al., 2009). Moreover, there is uncertainty about the generalizability of mutagenicity results from this in vitro experimental system to the mutagenicity and genotoxicity induced by EtO exposure in vivo; for example,

human embryonic adenovirus-transformed kidney cells were used for plasmid replication and mutation production, but embryonic kidneys are not a known target for EtO carcinogenesis.

C.2.2.2. *In Vivo Studies—Laboratory Animals*

The results of in vivo studies on the mutagenicity of EtO following ingestion, inhalation, or injection have also been consistently positive [e.g., Tates et al. (1999)]. For example, increases in the frequency of gene mutations in T-lymphocytes (*Hprt* locus) (Walker et al., 1997) and in bone marrow and testes (*LacI* locus) (Recio et al., 2004) have been observed in 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 lung 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). These and other key in vivo studies are discussed in more detail below.

An approach for determining mutational spectra in exon 3 of the *Hprt* gene in splenic T-lymphocytes of B6C3F$_1$ mice was developed by Walker and Skopek (1993). Mice (12 days old) were given 2, 6, or 9 single intraperitoneal (i.p.) injections of 100 mg/kg EtO every other day or 30, 60, 90, or 120 mg/kg of EtO for 5 consecutive days to achieve different cumulative doses. In mice exposed every other day, cumulative doses of 200, 600, and 900 mg/kg produced average mutant frequencies of 15×10^{-6}, 45×10^{-6}, and 73×10^{-6}, respectively, 8 weeks after dosing began. However, in mice exposed daily, cumulative doses of 150, 300, 450, and 600 mg/kg yielded average mutant frequencies of 4×10^{-6}, 8×10^{-6}, 11×10^{-6}, and 16×10^{-6}, 20 weeks after initiation of dosing. *Hprt* mutants obtained from mice exposed to 600 or 900 mg/kg EtO were isolated and analyzed for mutations, specifically in exon 3. DNA sequencing showed 11 base-pair substitutions, including 4 A-to-T and 2 G-to-C transversions as well as 3 A-to-T and 2 G-to-C transitions and seven +1 frameshift mutations in a run of six cosecutive guanine bases. The results suggested both modified guanine and adenine bases being involved in EtO-induced mutagenesis.

The same group of authors (Walker et al., 1997) studied the in vivo mutagenicity of EtO at the *Hprt* locus of T-lymphocytes following inhalation exposure of male B6C3F$_1$ *LacI* transgenic mice. Big Blue mice at 6–8 and 8–10 weeks of age were exposed to 0, 50, 100, or 200 ppm EtO for 4 weeks (6 h/day, 5 days/week). T-cells were isolated from the thymus and spleen and cultured in the presence of concanavalin A, IL-2, and 6-thioguanine. Mice were sacrificed at 2 hours, 2 weeks, and 8 weeks after exposure to 200 ppm EtO to determine a time course for the expression of *Hprt*-negative lymphocytes in the thymus. The results of this study showed that following 2 hours of exposure, the *Hprt* mutant frequency in the thymic

lymphocytes of the exposed mice was increased and reached an average maximum mutant frequency of $7.5 \pm 0.9 \times 10^{-6}$ at 2 weeks postexposure when compared to $2.3 \pm 0.8 \times 10^{-6}$ in the thymic lymphocytes of control mice. Dose-related increases in *Hprt* mutant frequency were found in thymic lymphocytes from mice exposed to 100 and 200 ppm EtO. Furthermore, a greater mutagenic efficiency (mutations per unit dose) was found at higher concentrations than at lower concentrations of EtO in splenic T-cells. The average induced mutant frequencies in splenic T-cells were 1.6, 4.6, and 11.9×10^{-6} following exposures to 50, 100, or 200 ppm EtO, respectively. For the analysis of the *LacI* mutations, lymphocytes (both B- and T-cells) were isolated from the spleen in the same animals. Two of three EtO-exposed mice at the 200 ppm exposure level demonstrated an elevated *LacI* mutant frequency. The authors suggest that these elevations were probably due to the in vivo replication of pre-existing mutants and not to the induction of new mutations associated with EtO exposure. The results of this study indicate that repeated inhalation exposures to high concentrations of EtO produce dose-related increases in mutations at the *Hprt* locus of T-lymphocytes in male *LacI* transgenic mice.

LacI mutant frequencies as a result of exposure to EtO were further investigated by Sisk et al. (1997). Male transgenic *LacI* B6C3F$_1$ mice (n = 15) were exposed to 0, 50, 100, or 200 ppm EtO for 4 weeks (6 hours/day, 5 days/week) and were sacrificed at 0, 2, or 8 weeks after the last EtO exposure. To determine the *LacI* mutant frequency, the *LacI* transgene was recovered from several tissues, including lung, spleen, germ cells, and bone marrow, selected because they were the target sites for tumor formation (particularly lung tumors and lymphomas) in chronic bioassays or germ cells. The results of this study indicate that the *LacI* mutant frequency in the lung was significantly increased at 8-weeks postexposure to 200 ppm EtO. In contrast, no significant increase in the *LacI* mutant frequencies was observed in the spleen, bone marrow, or germ cells at either 2 or 8 weeks following exposure. These results suggest that a 4-week inhalation exposure to EtO is mutagenic in lung but not in other tissues examined under similar conditions. The authors predict that the lack of mutagenic response in other tissues examined is probably because of large deletions that were either not detected or recovered in the current lambda-based shuttle vector systems. Based on the above study, the authors also suggest that the primary mechanism of EtO-induced mutagenicity in vivo is likely through the induction of deletions.

Tates et al. (1999) exposed rats to EtO via three routes: a single i.p. injection (10−80 mg/kg), ingestion of drinking water (4 weeks at concentrations of 2, 5, and 10 mM), or inhalation (50, 100, or 200 ppm for 4 weeks, 5 days/week, 6 hours/day). The goal of this study was to measure the induction of *Hprt* mutations in splenic lymphocytes using a cloning assay. Mutagenic effects of EtO following EtO administration via the three routes were compared in the *Hprt* assay based on blood doses, which were determined from HEVal adduct levels in

hemoglobin. Exposure to EtO via both injection and ingestion of drinking water led to a statistically significant dose-dependent induction of mutations (up to 2.3- and 2.5-fold increases in mutant frequency compared to background, respectively). Exposure via inhalation also caused a statistically significant increase in mutant frequency, although to a lesser extent (up to 1.4-fold over background). Plotting of the mutagenicity data for the three exposure routes against blood doses as a common denominator indicated that, at equal blood doses, the order of increased mutant frequency was i.p. injection > ingestion (drinking water) > inhalation. In the injection experiments, there was evidence for a saturation of detoxification processes at the highest doses, although such effects were not seen following subchronic administration. Taken together, the mutagenicity data from this study provide consistent results, showing that exposure to EtO gives rise to a linear dose-dependent increase in mutant frequency.

In a study by Recio et al. (2004), male Big Blue (*LacI* transgenic) B6C3F$_1$ mice were exposed to 0, 25, 50, 100, or 200 ppm EtO (6 hours per day, 5 days per week) for 12, 24, and 48 weeks. An unambiguous mutagenic response in the bone marrow was observed only after 48 weeks, with dose-related *LacI* mutant frequencies of 7.3×10^{-5}, 11.3×10^{-5}, 9.3×10^{-5}, 14.1×10^{-5}, and 30.3×10^{-5}. The mutagenic response in bone marrow is consistent with a linear exposure-response relationship, contrary to the assertion by Recio et al. (2004) which appears to be based on a misleading plotting scale. Mutant frequencies from testes (seminiferous tubules) were significantly greater than in controls at 25, 50, and 100 ppm (48-week exposure). No difference between the control and treated groups was observed in the *LacI* mutant frequency after 48 weeks of 200 ppm EtO exposure. The authors suggest that this was probably due to testicular toxicity. Furthermore, a mutation spectrum analysis of induced mutations in bone marrow indicated a decrease in mutations at G:C base pairs and an increase at A:T base pairs, exclusively in A:T to T:A transversions; however, the mutation spectrum from testes was similar to that of the untreated animals. The difference in mutation spectrum between the two tissues was probably due to differences in the repair of the DNA adducts formed.

Mutations in proto-oncogenes (*Kras, Hras*) and in the *Trp53* tumor suppressor gene have been studied in tumor tissues of several types from B6C3F$_1$ mice exposed to EtO. Hong et al. (2007) obtained tumor tissues from lung, Harderian gland, and uterus from a 2-year study (NTP, 1987) in which male and female mice were exposed to 0, 50, or 100 ppm EtO by inhalation 6 hours/day, 5 days/week and from control mice from other National Toxicology Program (NTP) 2-year bioassays. The authors analyzed the tissues for *Kras* mutations in codons 12, 13, and 61. A high frequency of *Kras* mutations (23/23 examined, 100%) was observed in the lung neoplasms in EtO-exposed mice compared to spontaneous lung neoplasms (27/108, 25%). The lung neoplasms in EtO-exposed mice predominantly exhibited GGT-to-GTT mutations in codon 12 (21/23), a transversion that was rare in spontaneous lung tumors (1/108). A similar spectrum

of *Kras* mutations was detected in the lung neoplasms in EtO-exposed mice regardless of histological subtype (adenomas or carcinomas) or dose group. In the case of Harderian gland neoplasms, a high frequency (18/21, 86%) of *Kras* mutations was detected in neoplasms from EtO-exposed mice compared to spontaneous tumors (2/27, 7%). The predominant mutations in the Harderian gland neoplasms in EtO-exposed mice consisted of GGC-to-CGC transversions at codon 13 and GGT-to-TGT transversions at codon 12, neither of which was observed in the spontaneous tumors. When the six uterine neoplasms from EtO-exposed mice were examined (there were no uterine tumors in the controls), the predominant mutation was a GGC-to-GGT transition in codon 13 (5/6, 83%). Based on the above results, the authors propose that the prominent targeting of guanine bases in the lung and Harderian gland neoplasms suggests that the formation of N7-HEG adducts by EtO plays a role in the induction of these tumors. The authors further propose that EtO can specifically target the *Kras* gene in multiple types of tissues and that interaction with this gene is a critical component of EtO-induced tumorigenesis and is of potential relevance to humans.

In an earlier study by the same group of authors (Houle et al., 2006), mammary carcinoma tissues from the same NTP study of mice exposed to EtO (0, 50, or 100 ppm) mentioned above were examined for p53 protein expression and for *Trp53* (exons 5−8) and *Hras* (codon 61) mutations. The authors supplemented the number of spontaneous mammary carcinomas with tissues from female control mice in other NTP studies from the same time period. P53 protein expression was detected in 67% (8/12) of the mammary carcinomas in EtO-exposed mice and 42% (8/19) of the spontaneous tumors; however, expression levels were about sixfold higher in the tumors in the EtO-exposed mice than in the spontaneous tumors. *Trp53* mutations were observed in 67% (8/12) of the mammary carcinomas in EtO-exposed mice and 58% (7/12) of the spontaneous tumors. *Hras* mutations were detected in 33% (4/12) of the mammary carcinomas in EtO-exposed mice and 26% (5/19) of the spontaneous tumors. While the mutation levels for these two genes were not substantially elevated in the mammary carcinomas in EtO-exposed mice compared to the spontaneous tumors, a shift in the mutational spectrum was observed. *Hras* mutations in the tumors from EtO-exposed mice exhibited a preference for A-to-G and A-to-T transversions, while spontaneous *Hras* mutations exhibited a preference for C-to-A transversions. *Trp53* mutations in the tumors from EtO-exposed mice exhibited a base preference for guanine, while spontaneous *Trp53* mutations exhibited a preference for cytosine. In addition, concurrent *Hras* and *Trp53* mutations were more common in the tumors in EtO-exposed mice than in the spontaneous tumors. Based on the results of the above two studies, it is suggested that the purine bases serve as primary targets for mutations induced by EtO, while mutations of these genes involving cytosine appears to be a more common spontaneous event.

In vivo exposure to EtO also induced heritable mutations or effects in germ cells in rodents (IARC, 1994b). EtO induces dominant lethal effects in mice and rats and heritable translocations in mice (Generoso et al., 1990; Lewis et al., 1986). Generoso et al. (1986) and Generoso et al. (1988) have reported that short bursts of EtO at high concentrations, such as those that may occur in the workplace, lead to a possibly greater risk of germ cell damage than cumulative, long-term exposure to lower levels.

Dominant-lethal mutations were investigated by Generoso et al. (1986) in two studies (dose-response and dose-rate) in mice exposed to different doses of EtO. Dominant-lethal responses were assessed based on matings involving sperm exposed as late spermatids and early spermatozoa because these are the stages most sensitive to EtO exposure. In the dose-response study, male mice were exposed by inhalation to 300 ppm, 400 ppm, or 500 ppm EtO, 6 hours per day, for 4 consecutive days. A dose-related increase in dominant-lethal mutations was observed. In the dose-rate study, mice were given a total exposure of 1,800 ppm × hours per day, also for 4 consecutive days, delivered either as 300 ppm in 6 hours, 600 ppm in 3 hours, or 1,200 ppm in 1.5 hours. Dominant-lethal responses increased with increasing concentration level, indicating a dose-rate effect for the production of dominant-lethal mutations.

C.2.2.3. *In Vivo Studies—Humans*

Workers occupationally exposed to EtO have been studied using different physical and biological measures (Tates et al., 1991). Blood samples from 9 hospital workers and 15 factory workers engaged in sterilization of medical equipment with EtO and from matched controls were collected. Average exposure levels during 4 months (the lifespan of erythrocytes) prior to blood sampling were estimated from levels of HEVal adducts in hemoglobin. The adduct levels were significantly increased in hospital workers and factory workers exposed to a 40-hour time-weighted average of 0.025 ppm and 5 ppm, respectively. Exposures were usually received in bursts, with EtO concentrations in air ranging from 22 to 72 ppm in hospital workers and 14 to 400 ppm in factory workers. All blood samples were analyzed for *HPRT* mutant frequencies, chromosomal aberrations, micronuclei, and SCEs. Mutant frequencies were significantly increased in factory workers but not in hospital workers. The chromosomal aberration and SCE results are discussed below in Sections C.3 and C.5, respectively.

The same authors (Tates et al., 1995) conducted another study of workers in an EtO production facility. *HPRT* mutations were measured in three exposed groups (one with high acute exposures and two with low chronic exposures) and one unexposed group (seven workers per group). Contrary to the earlier study, no significant differences in mutant frequencies were observed among the groups; this discrepancy may be attributable to lower overall exposures in

these workers than in the factory workers in the previous study and to the small number of subjects per group.

Major et al. (2001) measured *HPRT* mutations in female nurses employed in hospitals in Eger and Budapest, Hungary. This study examined a possible causal relationship between EtO exposure and a cluster of cancers (mostly breast) in nurses exposed to EtO in the Eger hospital. Controls were female hospital workers in the respective cities. The 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.

C.2.3. Gene Mutations—Summary

In summary, there is sufficient evidence for mutagenicity of EtO in various organisms (prokaryotes, eukaryotes, in vitro and in vivo in rodents and in vitro in human cells) tested in a variety of mutational assays. In addition, increases in mutations in specific proto-oncogenes and tumor suppressor genes in EtO-induced mouse tumors have been reported. Dominant-lethal mutations have also been observed in several in vivo studies. Although data in humans are limited, there is some evidence of increased frequencies of mutations from occupational studies.

C.3. CHROMOSOMAL ABERRATIONS

The induction and persistence of EtO-induced chromosomal alterations have been studied both in in vitro and in vivo systems in rodent and monkey models (Lorenti Garcia et al., 2001; Farooqi et al., 1993; Lynch et al., 1984; Kligerman et al., 1983). In addition, several studies examined the association of chromosomal aberrations and EtO exposure in humans (WHO, 2003; Lerda and Rizzi, 1992; Galloway et al., 1986; Clare et al., 1985; Sarto et al., 1984a; Stolley et al., 1984; Pero et al., 1981; Thiess et al., 1981). Chromosomal aberrations have been linked to an increased risk of cancer in several large prospective studies [e.g., (Boffetta et al., 2007; Rossner et al., 2005; Hagmar et al., 2004; Liou et al., 1999)]. This section discusses key studies on EtO and chromosomal aberrations.

Lorenti Garcia et al. (2001) studied the effect of EtO on the formation of chromosomal aberrations in rat bone-marrow cells and splenocytes following in vivo exposure. Rats were exposed to EtO either chronically by inhalation (50–200 ppm, 4 weeks, 5 days/week, 6 hours/day) or acutely by i.p. injection at doses of 50 or 100 mg/kg. Frequencies of both spontaneous and EtO-induced chromosomal aberrations (and other endpoints, such as micronucleus formation and SCEs, which are discussed in Section 3.3.3.3) were determined in the splenocytes and bone-marrow cells following in vivo mitogen stimulation. No significant

increase in chromosomal aberrations was observed from the chronic or acute exposures. In another study, by Kligerman et al. (1983), no increase in chromosomal aberrations was observed in peripheral blood lymphocytes from rats exposed to EtO by inhalation at concentrations of either 50, 150, or 450 ppm, for 6 hours per day, for 1 and 3 days.

A study by Donner et al. (2010) in mice, however, showed clear, statistically significant increases in chromosomal aberrations with longer durations of exposure (≥12 weeks). Male B6C3F$_1$ mice were exposed by inhalation to 0, 25, 50, 100, or 200 ppm EtO, 5 days/week, 6 hours/day, for 6, 12, 24, or 48 weeks. The frequency of total chromosomal aberrations in peripheral blood lymphocytes was statistically significantly increased after 12 weeks exposure to 100 or 200 ppm EtO. By 48 weeks, statistically significant increases were observed for all the exposure groups. In addition, reciprocal translocation frequencies were statistically significantly increased in spermatocytes for all the exposure groups at 48 weeks. Ribeiro et al. (1987) similarly observed chromosomal aberrations in mouse bone marrow cells and spermatocytes following 1-day and 2-week inhalation exposures to higher levels of EtO. Male Swiss Webster mice were exposed to 0, 200, 400, or 600 ppm EtO for 6 hours in 1 day or to 0, 200, or 400 ppm EtO for 6 hours/day, 5 days/week, for 2 weeks. Statistically significant increases in chromosomal aberrations were observed in bone marrow cells and in spermatocytes following a 1-day exposure of 400 or 600 ppm EtO or a 2-week exposure of 200 or 400 ppm EtO. Chromosomal aberrations in bone marrow cells were also reported in a study of acute EtO exposure in mice (Farooqi et al., 1993). Female Swiss albino mice were administered single doses of EtO in the range of 30–150 mg/kg by i.p. injection. A dose-related increase in chromosomal aberrations in the bone marrow cells was observed.

Chromosomal aberrations induced by long-term exposures to inhaled EtO were also investigated in the peripheral lymphocytes of cynomolgus monkeys (Lynch et al., 1984). Groups of 12 adult male monkeys were exposed at 0, 50, or 100 ppm EtO (7 hours/day, 5 days/week) for 2 years. Exposure to EtO at 100 ppm resulted in statistically significant increases in chromosome-type aberrations in monkey lymphocytes, and exposure at both 50 and 100 ppm resulted in statistically significant increases in chromatid-type aberrations and in chromosome- and chromatid-type aberrations in combination. No differences in the number of gaps were found.

Increases in chromosomal aberrations in peripheral blood lymphocytes have been consistently reported in studies of workers exposed to high occupational concentrations of EtO (>5 ppm, TWA). Effects observed at lower concentrations have been mixed (WHO, 2003). Chromosomal aberrations that have been detected in the peripheral blood lymphocytes of workers include breaks, gaps, and exchanges and supernumerary chromosomes (Lerda and Rizzi,

1992; Galloway et al., 1986; Clare et al., 1985; Sarto et al., 1984a; Pero et al., 1981; Thiess et al., 1981).

Clare et al. (1985) conducted chromosomal analyses of lymphocytes from 33 workers employed in the manufacture of EtO. A slightly higher frequency of chromatid aberrations was observed in workers exposed to EtO than in controls. Further, a positive correlation between length of employment in the EtO-exposed group and the number of aberrations was observed. In another study, Galloway et al. (1986) analyzed chromosomal aberration frequencies in 61 employees potentially exposed to EtO. Three work sites (I, II, and III) with different historical ambient levels of EtO were chosen for the study. Blood samples were drawn over a 24-month period and aberrations were analyzed in 100 cells per sample after culture for 48−51 hours. At work sites I and II, no consistent differences in aberration frequencies were found. However, at work site III, aberration frequencies in potentially exposed individuals were significantly increased when compared with controls. A previous study by the same group (Stolley et al., 1984) showed an association between SCE frequency and EtO exposure. When the aberrations were compared with the levels of SCEs, the authors found a weak overall association. In addition, Lerda and Rizzi (1992) showed a significant increase in chromosomal aberration frequencies in EtO-exposed individuals when compared with controls. Major et al. (1996) studied hospital nurses exposed to low doses and high doses of EtO to identify changes in structural and numerical chromosomal aberrations. Chromosomal aberrations were found to be significantly elevated in both the low-dose and the high-dose exposure groups. Deletions and, to a lesser extent, chromatid exchanges and dicentrics were detected in the low-dose exposure group; however, in the high-dose group, in addition to the increased number of deletions, the frequencies of dicentrics and rings showed a significant excess when compared with controls. The authors suggest that a natural radioactivity from local tap water may have been a confounding factor.

A study by Sarto et al. (1984a) showed significant increases in chromosomal aberrations after exposure to EtO. Chromosomal aberrations were detected in the peripheral lymphocytes of 41 workers exposed to EtO in the sterilizing units of eight hospitals in the Venice region compared to 41 age- and smoking-matched controls. In another study of 28 EtO-exposed sterilizer workers and 20 unexposed controls, Högstedt et al. (1983) reported a statistically significant increase in total chromosomal aberrations and gaps, but not breaks, in the peripheral blood lymphocytes of the exposed workers, adjusted for age, smoking, drug intake, and exposure to ionizing radiation; no significant increases in chromosomal aberrations were observed in bone marrow cells. Tates et al. (1991) reported a significant increase in chromosomal aberrations in hospital workers and in factory workers (details of this study are provided in the section on gene mutations above). Tompa et al. (2006) reported statistically significant increases in

chromosomal aberrations and SCEs in 66 Hungarian hospital nurses exposed to sterilizing gases in uncontrolled environments compared to 94 nonexposed controls; however, it is difficult to sort out any effects of EtO exposure from possible effects from smoking or exposure to ionizing radiation, formaldehyde, or other possible sterilizing gases in this study.

In summary, the above data clearly indicate that EtO is genotoxic and can cause a variety of chromosomal aberrations, including breaks, gaps and exchanges [reviewed in detail in Preston (1999)]. Chromosomal aberrations have been observed in both in vitro and in vivo studies in rodent models and mammalian cells. Increases in chromosomal aberrations in peripheral blood lymphocytes have been consistently reported in studies of workers exposed to EtO.

C.4. MICRONUCLEUS FORMATION

Micronucleus formation also demonstrates the genotoxic effects of a chemical. When appropriate methods are used to identify the origin of the micronucleus (kinetochore-positive or kinetochore-negative), this assay can provide information about a chemical's mechanism of action (e.g., if a chemical causes direct DNA damage resulting from strand breaks [clastogen] or indirect numerical changes [aneugen] resulting from spindle disruption). An association between increased micronucleus frequency and cancer risk has been reported in at least one large prospective study (Bonassi et al., 2007). Several in vitro and in vivo studies in both laboratory animals (Lorenti Garcia et al., 2001; Jenssen and Ramel, 1980; Appelgren et al., 1978) and humans (Ribeiro et al., 1994; Schulte et al., 1992; Mayer et al., 1991; Tates et al., 1991; Sarto et al., 1990; Högstedt et al., 1983) have been conducted to explore the induction of micronuclei as a result of exposure to EtO.

Lorenti Garcia et al. (2001) studied the effect of EtO on the formation of micronuclei in rat bone marrow cells and splenocytes following in vivo exposure. Rats were exposed to EtO either subchronically by inhalation (50–200 ppm, 5 days/week, 6 hours/day, for 4 weeks) or acutely by i.p. injection at doses of 50 or 100 mg/kg. Spontaneous and induced frequencies of micronuclei were determined in the bone marrow cells (only for acute EtO exposure) and splenocytes following in vitro mitogen stimulation. Following chronic exposure, no significant increase in micronuclei was observed in rat splenocytes. Following acute exposure, micronuclei increased significantly in rat bone marrow cells as well as splenocytes.

In the Högstedt et al. (1983) study of 28 EtO-exposed sterilizer workers and 20 unexposed controls, a statistically significant increase in micronuclei was observed in bone marrow cells (erythroblasts and polychromatic erythrocytes), but not in lymphocytes, in the exposed workers, adjusted for age, smoking, drug intake, and exposure to ionizing radiation.

The frequency of micronuclei in peripheral blood cells was increased in workers exposed to relatively high (3.7–60.4 mg/m^3) levels of EtO (Ribeiro et al., 1994; Tates et al., 1991).

Schulte et al. (1992) did not observe increased micronuclei in the lymphocytes of hospital workers with low levels of EtO exposure (up to 2.5 mg/m^3 8-hour TWAs). Sarto et al. (1990) studied micronucleus formation in human exfoliated cells of buccal and nasal cavities to monitor the genotoxic risk in a group of workers (n = 9) chronically exposed to EtO (concentrations lower than 0.38 ppm as time-weighted average). The mean frequencies of micronucleated buccal cells were similar to control values. The frequency of nasal micronucleated cells was higher than in controls (0.77 vs. 0.44); however, the difference was not statistically significant. In another group of three subjects that were acutely exposed (concentration not provided) to EtO, buccal cavity and nasal mucosa samples were taken 3, 9, or 16 days after acute exposure. The frequencies of micronucleated buccal cells did not change, while the frequencies of micronucleated nasal cells significantly increased.

Peripheral blood cells of 34 EtO-exposed workers at a sterilization plant and 23 unexposed controls were assessed for different biological markers, such as EtO-hemoglobin adducts, SCEs, micronuclei, chromosomal aberrations, DNA single-strand breaks and an index of DNA repair (Mayer et al., 1991). Neither chromosomal aberrations nor micronuclei differed significantly by exposure status, whether or not adjusted for smoking status.

In summary, increases in the frequency of micronuclei have been observed in in vivo animal studies. The frequency of micronuclei in peripheral blood cells was also increased in workers exposed to relatively high (3.7–60.4 mg/m^3) levels of EtO (Ribeiro et al., 1994; Tates et al., 1991). However, in the majority of human studies involving exposures at lower levels, no effects on the frequency of micronuclei were observed. Apparent inconsistencies in the data could reflect the influence of peak exposures, differences in exposure measurement errors, duration of exposure, and/or smoking status.

C.5. SISTER CHROMATID EXCHANGES (SCES)

There is a significant body of evidence for the induction of SCEs as a result of exposure to EtO. Studies have been conducted both in laboratory animals (Lorenti Garcia et al., 2001; Ong et al., 1993; Kelsey et al., 1988; Lynch et al., 1984; Kligerman et al., 1983; Yager and Benz, 1982) and in humans (Agurell et al., 1991; Galloway et al., 1986; Laurent et al., 1984; Sarto et al., 1984a, b; Stolley et al., 1984; Yager et al., 1983; Garry et al., 1979). In particular, several occupational exposure studies have yielded positive results when EtO-exposed workers were studied. The following is a summary of both the animal and human studies.

Inhalation studies with rats have shown that exposures to EtO at 50 ppm or more for 3 days result in an increase in SCEs in peripheral blood lymphocytes (Kligerman et al., 1983). Increased incidences of SCEs in the peripheral blood lymphocytes of monkeys exposed to EtO at 500 or 100 ppm were also reported by Lynch et al. (1984). A follow-up study in these same

monkeys by Kelsey et al. (1988) indicated that the high SCE counts persisted for 6 years after exposure.

Lorenti Garcia et al. (2001) studied the effect of EtO on the persistence of SCEs in rat bone marrow cells and splenocytes following in vivo exposure. Rats were exposed to EtO either subchronically by inhalation (50–200 ppm, 5 days/week, 6 h/day, for 4 weeks) or acutely by i.p. injection at dose levels of 50 or 100 mg/kg. Frequencies of SCEs were determined in the bone marrow cells and splenocytes after in vitro mitogen stimulation. Following chronic exposure, cytogenetic analyses were carried out at Days 5 and 21 in the splenocytes. In these experiments, EtO was effective in inducing SCEs, and marked increases in cells with high frequency SCEs were observed which persisted until Day 21 postexposure. Following acute exposure, SCEs were increased significantly in rat bone marrow cells as well as splenocytes.

New Zealand white male rabbits (n = 4) were exposed in inhalation chambers to 0, 10, 50, and 250 ppm EtO for 6 hours a day, 5 days a week, for 12 weeks (Yager and Benz, 1982). Peripheral blood samples were drawn in three regimes (before the start of exposure, at intervals during exposure, and up to 15 weeks after the end of exposure) to measure SCE rates. No change in SCE rates was observed from exposure to 10 ppm; however, an increase was seen after exposure to 50 and 250 ppm. Above-baseline levels were observed even after 15 weeks postexposure, although the levels were not as high as during exposure. These results indicate that inhalation exposure to the EtO results in a dose-related increase in SCEs.

Lynch et al. (1984) investigated the effect of long-term exposures to inhaled EtO on SCE rates in peripheral lymphocytes of monkeys. Groups of 12 adult male cynomolgus monkeys were exposed at 0, 50, or 100 ppm EtO (7 hours/day, 5 days/week) for 2 years. Statistically significant increases in SCE rates were observed in monkey lymphocytes in both exposure groups. Both exposure groups had increased numbers of SCEs/metaphase as compared to controls, and these numbers increased in a dose-dependent manner.

In an in vitro study of human cells, peripheral lymphocyte cultures were exposed to methyl bromide, EtO, and propylene oxide, as well as diesel exhaust (Tucker et al., 1986). SCE frequency was measured, and the frequency more than doubled in the cultures treated with EtO. Agurell et al. (1991) also studied the effect of EtO on SCEs in human peripheral blood lymphocytes in vitro. An increase in SCE frequency was observed as a result of exposure (0−20 mMh) to EtO. Similarly, Hallier et al. (1993) observed that the frequency of SCEs in human peripheral blood lymphocytes exposed in vitro to EtO was higher in cells isolated from individuals expressing low levels of glutathione S-transferase T1 than in cells from subjects expressing higher levels of this enzyme.

Several studies of EtO-exposed workers have also reported an increased incidence of SCEs in peripheral lymphocytes [e.g., Garry et al. (1979), Yager et al. (1983), Sarto et al.

(1984a), Sarto et al. (1984b), Galloway et al. (1986), Schulte et al. (1992)], although the Högstedt et al. (1983) study discussed in Sections C.3 and C.4 did not report significant increases in SCEs in the lymphocytes of the exposed workers.

Garry et al. (1979) analyzed SCEs in lymphocytes cultured from EtO-exposed individuals as well as comparable controls. Significant increases in SCEs were observed at 3 weeks and at 8 weeks following exposure. Although this study does not describe the exact exposure estimates, EtO was recognized as a mutagenic or genotoxic agent. Laurent et al. (1984) studied SCE frequency in workers exposed to high levels of EtO in a hospital sterilization service. Blood samples were obtained retrospectively from a group of 25 subjects exposed to high levels of EtO for a period of 2 years. A significant increase in SCEs was observed in the exposed group when compared with the control group. The authors concluded that the effect of exposure to EtO was sufficient to produce a cumulative and, in some cases, a persistent genetic change.

Peripheral blood lymphocytes of nurses exposed to low and high concentrations of EtO were studied by Major et al. (1996). SCEs were slightly elevated in the low-exposure group but were significantly increased in the high-exposure group. Similarly, several studies (Sarto et al., 1991; Sarto et al., 1990; Sarto et al., 1987; Sarto et al., 1984a, b) showed significant increases in SCEs.

Tates et al. (1991) studied workers occupationally exposed to EtO using different physical and biological measures. Blood samples from 9 hospital workers and 15 factory workers engaged in sterilization of medical equipment with EtO and from matched controls were collected. Exposures were usually received in bursts, with EtO concentrations in air ranging from 22 to 72 ppm in hospital workers and 14 to 400 ppm in factory workers. The mean frequency of SCEs was significantly elevated by 20% in hospital workers and by almost 100% in factory workers. In contrast, no significant increase in SCEs was observed in lymphocytes of workers who were accidentally exposed to high concentrations of EtO or of workers with low exposure concentrations (Tates et al., 1995).

Schulte et al. (1992) observed a statistically significant increase in SCEs in 43 workers exposed to EtO in U.S. hospitals compared to 8 unexposed hospital workers. The frequency of SCEs was also significantly associated with cumulative EtO exposure in a regression analysis that controlled for various potential confounding factors, including smoking. A similar relationship was not observed in 22 Mexican hospital workers. Schulte et al. (1992) hypothesized that the difference may have been due to longer shipping times of the Mexican specimens for the cytogenetic assays.

In summary, significant increases in the frequency of SCEs were observed in rats and in monkeys both by inhalation and i.p. injection. In humans, multiple occupational studies have reported positive responses, with significant increases in frequency of SCEs in peripheral blood

lymphocytes having been observed among individuals exposed to higher levels of EtO. In some studies, increases in the frequency of SCEs have been observed to persist after exposure has ceased. The results of studies of individual workers exposed to very low levels (<0.9 mg/m^3) of EtO have been mixed.

C.6. OTHER ENDPOINTS (GENETIC POLYMORPHISM, SUSCEPTIBILITY)

Dose-dependent effects of polymorphisms in the genes for epoxide hydrolase (*EPHX1*), different subfamilies of glutathione-*S*-transferase (*GSTM1*, *GSTP1*, *GSTT1*), and various DNA repair enzymes (*hOGG1*, *XRCC1*, *XRCC3*) on EtO-induced genotoxicity were evaluated by Godderis et al. (2006). Peripheral blood mononuclear cells from 20 individuals were exposed to three doses of EtO (0.45, 0.67, 0.9 mM), and genotoxicity was evaluated by measuring comet tail length and micronucleus frequencies in binucleated cells (MNBC). A dose-dependent increase in tail length (indicating DNA strand breaks) was observed in exposed individuals compared to controls. No change in MNBC was observed. None of the epoxide hydrolase or glutathione-*S*-transferase polymorphisms had a significant influence on the tail length or MNBC results for any EtO dose. Further analysis revealed a significant contribution of the *hOGG1* (involved in base excision repair) and *XRCC3* (involved in repair of cross-links and chromosomal double-strand breaks) genotypes to the interindividual variability of EtO-induced increases in tail length. Homozygous *hOGG1*^{326} wild-type cells showed significantly lower effects of EtO on tail length compared to the heterozygous cells. Also, significantly higher tail lengths were found in EtO-exposed cells carrying at least one variant *XRCC3*^{241} Met allele. For the latter effect, there was a significant interaction between the *XRCC3*^{241} polymorphism and dose, signifying a greater impact of the polymorphism on DNA damage at higher doses.

In contrast to the findings of no significant effect of glutathione-*S*-transferase polymorphisms on DNA breaks and micronuclei production by Godderis et al. (2006), Hallier et al. (1993) observed that the frequency of SCEs in human peripheral blood lymphocytes exposed in vitro to EtO was higher in cells isolated from individuals expressing low levels of GSTT1 than in cells from subjects expressing higher levels of this enzyme. Similarly, Yong et al. (2001) measured approximately twofold greater EtO-hemoglobin adduct levels in occupationally exposed persons with a *GSTT1*-null genotype than in those with positive genotypes.

In a study involving small numbers (n = 4−12 per group) of nonsmoking males and females exposed to EtO through the sterilization of medical equipment, Fuchs et al. (1994) reported 1.5-, 2.2-, and 1.5-fold increases in DNA single-strand breaks in peripheral blood mononuclear cells obtained from individuals exposed to EtO concentrations of 0.1−0.49 mg/m^3, 0.5−2.0 mg/m^3, and >2 mg/m^3, respectively. Fuchs et al. (1994) further noted that these nonsmokers could be divided into two distinct susceptibility groups, with 67% of the subjects

exhibiting approximately fivefold higher levels of DNA single-strand breaks in response to EtO exposure than the remaining subjects, and that the bimodal nature of the differential susceptibility suggested that the susceptibility was attributable to an unidentified polymorphism.

Primary and secondary cultures of lymphoblasts, breast epithelial cells, peripheral blood lymphocytes, keratinocytes, and cervical epithelial cells were exposed to 0–100 mM EtO, and DNA damage was measured using the comet assay (Adám et al., 2005). A dose-dependent increase in DNA damage was observed in all cell types without notable cytotoxicity. Breast epithelial cells (26% increase in tail length) were more sensitive than keratinocytes (5% increase) and cervical epithelial cells (5% increase) but less sensitive than lymphoblasts (51% increase) and peripheral lymphocytes (71% increase) at the same dose of 20 mM.

C.7. ENDOGENOUS PRODUCTION OF ETHYLENE AND ETO

Ethylene, a biological precursor of EtO, is ubiquitous in the environment as an air pollutant and is produced in plants, animals, and humans (Abeles and Heggestad, 1973). Ethylene is generated in vivo endogenously during normal physiological processes such as (1) oxidation of methionine, (2) oxidation of hemoglobin, (3) lipid peroxidation, and (4) metabolism of intestinal bacteria [reviewed by (Bolt, 2000; IARC, 1994a)]. Marsden et al. (2009) proposed that oxidative stress can induce the endogenous formation of ethylene, which can in turn be metabolized to EtO. Endogenous production of ethylene has been documented in laboratory animals and in humans (Filser et al., 1992; Shen et al., 1989; Ehrenberg et al., 1977; Chandra and Spencer, 1963).

Shen et al. (1989) reported an endogenous production rate of 2.8 and 41 nmol/h ethylene in Sprague-Dawley rats and humans, respectively, with similar thermodynamic partition coefficients between the two species. Filser et al. (1992) reported a low degree of endogenous production of ethylene (32 ±12 nmol/h) in healthy volunteers based on exhalation data. The authors indicated that the endogenous levels of ethylene would account for ~66% of the background level of EtO-hemoglobin adducts (HEVal), while the remaining one-third (15 ppb) is contributed by exogenous environmental ethylene exposure. Although the percentage of endogenous ethylene converted to EtO is not known, Törnqvist et al. (1989) have shown that in fruit-store workers exposed to 0.3 ppm ethylene, only 3% is metabolized to EtO. Thus, the amount of endogenous ethylene converted to EtO should be minimal. Furthermore, with inadequate laboratory animal and human evidence available for ethylene as a carcinogen (IARC, 1994a), exogenous ethylene exposure may not produce enough EtO to contribute significantly to carcinogenicity under standard bioassay conditions (Walker et al., 2000).

Ethylene formed from endogenous sources is converted to EtO by cytochrome P450-mediated metabolism (Törnqvist, 1996; IARC, 1994a). EtO formed from the endogenous

conversion of ethylene leads to 2-hydroxyethylation of DNA and forms N7-HEG adducts, contributing to the background levels of this adduct in unexposed humans and rodents. As shown in Table C-1, improvements in analytical methodology have led to the detection and quantification of background N7-HEG adducts in DNA of unexposed laboratory animals and humans (Marsden et al., 2009; Swenberg et al., 2008; Tompkins et al., 2008; Marsden et al., 2007; Swenberg et al., 2000; van Sittert et al., 2000; Walker et al., 2000; Eide et al., 1999; Farmer and Shuker, 1999; Wu et al., 1999b; Wu et al., 1999a; Zhao et al., 1999; Bolt et al., 1997; Zhao et al., 1997; Kumar et al., 1995; van Delft et al., 1994; Farmer et al., 1993; van Delft et al., 1993; Leutbecher et al., 1992; Walker et al., 1992; Cushnir et al., 1991; Föst et al., 1989). However, the levels of adducts detected in rodents and humans vary widely and appear to depend on the type of the analytical method used. Even with the most advanced techniques (Tompkins et al., 2008), minor DNA adducts such as O^6-HEG and N3-HEA are below the level of detection. Also, some researchers consistently demonstrate higher background levels of DNA adducts (Wu et al., 1999a; Walker et al., 1992). However, the higher background levels in some of these studies are possibly due to the methodology used, which may have caused an artifactual increase in the adduct levels.

Using sensitive detection techniques and a dual-isotype labeling approach designed to separately quantify both endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO treatment in F344 rats, Marsden et al. (2009) reported detectable levels of exogenous adducts in DNA of spleen and liver tissues at the lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days). The authors also reported 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 at or below the limit of accurate quantitation (0.2−0.5 adducts/10^{10} nucleotides). EtO doses of ≥0.001 and ≥0.05 mg/kg induced elevated endogenous N7-HEG levels in the liver and spleen, respectively, with endogenous adduct levels increasing in an apparent dose-responsive manner above ≥0.05 mg/kg in both tissues; endogenous adduct levels in the stomach, however, remained unchanged at any dose. 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. For example, an approximate calculation indicates that the low exposure level of 10 ppm for 6 hours/day used in the Snellings et al. (1984) bioassay of F344 rats is equivalent to a daily dose of about 1.7 mg/kg, which is over 10 times higher than the largest daily dose of 0.1 mg/kg used by Marsden et al. (2009).[9]

[9]This calculation uses the mean alveolar ventilation rate for rats of 52.9 mL/minute/100 g reported by Brown et al. (1998). Changing the units, this rate is equivalent to approximately 0.032 m^3/hour/kg. For a 6-hour exposure, this results in an alveolar inhalation of 0.19 m^3/kg. 10 ppm EtO is equivalent to 18.3 mg/m^3, so a 6-hour exposure

In summary, endogenous ethylene and EtO production, which contribute to background N7-HEG DNA adducts indicative of DNA damage, have been observed in unexposed rodents and humans. Although a constant reduction in DNA damage in vivo is carried out by DNA repair and DNA replicative synthesis, a certain steady-state background level of adducts is measurable at all times. The quantitative relationships between the background DNA damage and the spontaneous rates of mutation and cancer are not well established. Experimental evidence is needed that can unequivocally measure artifact-free levels of background DNA damage, including effects other than adducts, clearly establish mutagenic potency of such background lesions, and demonstrate the organ- and cell-type-specific requirements for the primary DNA damage to be expressed as heritable genetic changes (Gupta and Lutz, 1999).

Some investigators have posited that the high and variable background levels of endogenous EtO-induced DNA damage in the body may overwhelm any contribution from 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 Chapter 3, 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 above, Marsden et al. (2009) reported increases in exogenous adducts in DNA of the spleen and liver consistent with a linear dose-response relationship ($p < 0.05$), down to 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). Furthermore, while the contributions to cancer risk from low exogenous EtO exposures may be relatively small compared to those from endogenous EtO exposure, low levels of exogenous EtO may nonetheless be responsible for levels of risk (above background risk) that exceed *de minimis* risk (e.g., $>10^{-6}$). 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, which have a background lifetime incidence risk on the order of 3%, and breast cancer, which has a background lifetime incidence risk on the order of 15%.

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equates to about 3.48 mg/kg. IARC (2008) reports that measurements from Johanson and Filser (1992) indicate that only 50% of alveolar ventilation is available to be absorbed into the bloodstream, so the 6-hour exposure to 10 ppm EtO would approximate an absorbed daily dose of 1.7 mg/kg.

Table C-1. Levels of endogenous (background) N7-HEG adducts in unexposed human and rodent tissues

Species	Tissue	Detection method	Adduct levels reported	Adducts/10^7 nucleotides[a]	Reference
Human	Lymphocytes	GC/MS	8.5 pmol/mg DNA	28	Föst et al. (1989)
Human	WBC	Immuno-slotblot	0.34 adducts/10^6 nucleotides	3.4	van Delft et al. (1994)
Human	Blood	HPLC-fluorescence	3.2 pmol/mg DNA	11	Bolt et al. (1997)
Human	Lymphocytes	GC/MS	2–19 adducts per 10^7 nucleotides	2–19	Wu et al. (1999b)
Human	WBC	^{32}P/TLC/HPLC	0.6 adducts/10^7 nucleotides	0.6	Zhao et al. (1999)
Human	WBC	^{32}P/TLC/HPLC	2.9 adducts/10^7 nucleotides	2.9	Zhao et al. (1999)
Human	Lung	^{32}P/TLC/HPLC	4.0 adducts/10^7 nucleotides	4.0	Zhao et al. (1999)
Human	Granulocytes	GC-EC-MS	3.8 adducts/10^7 nucleotides	3.8	Yong et al. (2007)
Rat	Lymphocytes	GC/MS	5.6 pmol/mg DNA	18.48	Föst et al. (1989)
Mice/Rats	6 Control tissues[b]	HPLC-fluorescence	2–6 pmol/mg DNA	6.6–19.8	Walker et al. (1992)
Rat	Liver, kidney, spleen	^{32}P/GC/MS	0.4 to 1.1 adducts/10^7 nucleotides	0.4–1.1	Eide et al. (1999)
Mice/Rats	Spleen	GC/EC/NCI-HRMS	0.2 to 0.3 pmol/mmol guanine	0.5–0.8[c]	Wu et al. (1999a)
Rat	Lymphocytes, liver, kidney	^{32}P/TLC/HPLC	0.6 to 0.9 adducts/10^7 nucleotides	0.6–0.9	Zhao et al. (1999)
Rat	Liver	GC/MS	2.6 adducts/10^8 nucleotides	0.26	van Sittert et al. (2000)
Rat Study1 Study 2	Heart, colon, liver 7 control tissues[d]	LC-MS/MS LC-MS/MS	0.27–2.38 adducts/10^8 nucleotides 1.06–3.52 adducts/10^8 nucleotides	0.027–0.238 0.106–0.352	Marsden et al. (2007)
Rat	Liver	HPLC/ESI TMS	8 adducts/10^8 normal nucleotides	0.8	Tompkins et al. (2008)
Rat	Liver, spleen, stomach	HPLC/LC-MS/MS	233–373 adducts/10^{10} nucleotides	0.233–0.373	Marsden et al. (2009)

Table C-1. Levels of endogenous (background) N7-HEG adducts in unexposed human and rodent tissues (continued)

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[a]Adduct levels are normalized using the formula: 1 pmol adducts/mg DNA = 3.3 adducts/10^7 normal nucleotides.
GC/MS, gas chromatography mass spectrometry; HPLC, high-performance liquid chromatography; ^{32}P, ^{32}P-postlabeling assay; TLC, thin-layer chromatography; LC-MS, liquid chromatography mass spectrometry; ESI TMS, electrospray ionization tandem mass spectrometry; GC/EC/NCI-HRMS, gas chromatography/electron capture/negative chemical ionization high-resolution mass spectrometry; WBC, white blood cells.
[b]Brain, lung, spleen, kidney,liver, and testes.
[c]Estimated by Marsden et al. (2007).
[d]Liver, heart, colon, lung, kidney, spleen, and stomach.

C.8. CONCLUSIONS

The overall available data from in vitro studies, laboratory animal studies, and human studies indicate that EtO is both a mutagen and a genotoxicant. In addition, increases in mutations in specific oncogenes and tumor suppressor genes in EtO-induced mouse tumors have been reported. Stable translocations seen in human leukemias may arise from similar DNA adducts that produce chromosome breaks, micronuclei, SCEs, and even gene mutations observed in peripheral lymphocytes. Dominant lethal mutations, heritable translocations, chromosomal aberrations, DNA damage, and adduct formation in rodent sperm cells have been observed in a number of studies involving the exposure of rats and mice to EtO. Based on the likely role of DNA alkylation in producing the genotoxic effects in germ cells in laboratory animals exposed to EtO, as well as the lack of qualitative differences in the metabolism of EtO between humans and laboratory animals, EtO can also be considered a likely human germ cell mutagen (WHO, 2003). There is consistent evidence that EtO interacts with the genome of cells within the circulatory system in occupationally exposed humans and overwhelming evidence of carcinogenicity and genotoxicity in laboratory animals. Based on these considerations, there is a strong weight of evidence suggesting that EtO would be carcinogenic to humans (see Chapter 3, Section 3.4).

APPENDIX D. REANALYSES OF ETHYLENE OXIDE EXPOSURE-RESPONSE DATA

[EDITORIAL NOTE: This Appendix contains a revised version of the report submitted by Dr. Kyle Steenland in 2010 summarizing the results of analyses he conducted under contract to the EPA. The terminology originally used by Steenland to designate the different exposure-response model forms has been changed to be consistent with that used in the rest of this assessment (see end of Section 4.1, pages 4–5 to 4–6). Models that are linear in log RR and which were previously referred to as "linear" models have been renamed "log-linear" or "Cox regression" models (except in some cases where it is stated that they are log RR models). Models of the form RR = 1 + β × exposure, which were previously referred to as "excess relative risk" (ERR) models, have been renamed "linear" models in most cases. In addition, section headings, figures, and tables were renumbered for the table of contents, and some supplemental analyses performed by Steenland after the main report was submitted have been added. Finally, after the SAB review of the 2014 draft assessment (U.S. EPA, 2014a, b), some additional analyses of the NIOSH data were conducted and the results of these analyses have been included here, and some of the text has been edited for consistency with the new analyses. The breast cancer incidence data are not publically available, and so, were no longer accessible to Steenland, as he is no longer at NIOSH. Therefore, the revised breast cancer incidence analyses, primarily categorical and linear RR analyses and the reexamination of lag periods, have been performed by Dr. James Deddens of NIOSH. Supplemental analyses of the lymphoid cancer mortality data were again conducted by Steenland under contract to the EPA. No further analyses were done of the all-lymphohematopoietic cancer or breast cancer mortality data because these analyses are not of primary interest in this assessment but rather are provided for comparison with the lymphoid cancer mortality and breast cancer incidence results. Some of the original pieces (e.g., figures and risk assessment sections) of the report submitted by Steenland in 2010 have been deleted because revised versions are presented in Chapter 4. Finally, some minimal technical editing was done.]

This report contains the results of reanalyses of the National Institute for Occupational Safety and Health cohort of workers exposed to ethylene oxide (EtO) conducted for the U.S. Environmental Protection Agency. The report begins with an overview of the modeling strategy used, followed by the results of reanalyses of the breast cancer incidence, breast cancer mortality, lymphoid cancer mortality, and finally, hematopoietic cancer mortality databases. Various models were used for these reanalyses, as discussed in this report. The report concludes

with the results of some sensitivity analyses and discussions of the possible influences of the healthy worker survivor effect and exposure mismeasurement.

Introduction. Modeling strategy for ethylene oxide (EtO) risk assessment

The modeling strategy adopted here for EtO risk assessment relies principally on the usual epidemiologic models in which the log of the rate ratio (RR) is some function of exposure, in this case cumulative exposure with a lag to reflect a length of time that is likely necessary before an exposure can result in (observable or fatal) cancer. We have relied primarily on Cox regression as a flexible method of modeling the log RR; however, we have also included some linear relative risk models. Cumulative exposure is typically the exposure metric of interest in predicting chronic disease. Transformations (natural log and sqaure root) of cumulative exposure have been used in some of the Cox regression models. 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.

We have also used two-piece spline models, in which log RR (in the log-linear model) or RR (in the linear model) is a function of two lines that join continuously at a single point of inflection, or knot. These two-piece spline models have been described as part of a general description of exposure-response modeling by Steenland and Deddens (2004) and have been used previously in risk assessment [e.g., see the risk assessment for dioxin by Steenland et al. (2001)]. The two-piece log-linear model has the form log $RR = \beta_0 + \beta_1 \times cumexp + \beta_2 \times (max(0,cumexp-knot))$, where cumexp is cumulative exposure, the last term equals either 0 or cumexp-knot, whichever is greater, and the knot is the point of inflection or point of change of slope for the two linear pieces. The slope of the last term is $\beta_1 + \beta_2$ for cumulative exposure values above the knot.

Log RR models are not linear when the log RR function is transformed via exponentiation back to a nonlogarithmic function, but they are nearly so in the low-dose region of interest. The splines are linear using the linear RR model.

"Plateau-like" exposure-response curves, in which the exposure-response curve begins steeply but is attenuated at higher exposure, have been seen for many occupational carcinogens. This may occur for a variety of reasons, including depletion of susceptible subpopulations, mismeasurement at high exposure resulting in attenuation, and the healthy worker survivor effect (Stayner et al., 1993). Attenuation of the exposure-response relationship occurs for the breast cancer and (lympho)hematopoietic endpoints of interest for EtO. For these endpoints, a simple linear model, where the log RR (for the log-linear model) or the RR increases linearly with cumulative exposure, does not fit the data well, based on simple visual inspection of the categorical data.

Frequently, such plateau-like curves may be modeled by using the log of cumulative exposure rather than cumulative exposure itself, but this approach has the disadvantage that the curve is usually highly supralinear at low doses. Two-piece spline models are particularly useful in modeling exposure-response relationships in which the log RR or RR increases initially with increasing exposure but then tends to increase less or plateau at high exposures. The two-piece spline models lessen this supralinearity in the low-dose region (Steenland and Deddens, 2004).

The shape of the two-piece spline model, in particular the slope of the curve in the low-dose region, depends on the choice of the point of inflection where the two splines are joined. Here, we have chosen the point of inflection based on the best model likelihood, trying a range of points of inflection (knots) across the range of exposure starting from 0 and incrementing by 100, 500, or 1,000 ppm-day intervals. The model likelihood often does not change much across these different points of inflection, but it does change some and we have chosen the point of inflection resulting in the best model likelihood. The model likelihood used to find the best fit in all models used in this analysis is the usual partial likelihood (Langholz and Richardson, 2010), as used with the Cox models, which maximizes the probability, across all the cases, that a case fails (the numerator) relative to its case-control risk set (which includes the case) (the denominator) and has the form

$$L(\beta) = \varphi_{case}(Z;\beta)/\Sigma_{j \text{ cases and controls}} \varphi_j(Z_j;\beta),$$

where $\varphi(Z;\beta)$ is some function of a vector of covariates Z and the parameters of interest β. For example, for the linear RR model with only cumulative exposure in the model, $\varphi(Z;\beta) = 1 + z\beta$, where z is cumulative exposure and β is the exposure-response coefficient of interest. For the log RR (i.e., log-linear) model, $\varphi(Z;\beta) = e^{(z\beta)}$.

In contrast to log-linear RR models, for which the Wald approach was used to estimate confidence intervals, linear RR models may not have symmetrical confidence intervals (Langholz and Richardson, 2010).[10] For linear RR models, a profile likelihood approach was used to derive confidence intervals. To obtain profile likelihood confidence intervals for the first linear piece of the two-piece linear spline model, the sample space for the beta coefficient for the first piece (beta1) of the two-piece spline model was searched manually for a beta value that was 1.92 lower in likelihood value from the optimal model, on either side of the MLE (< MLE, > MLE). The resulting profile likelihood confidence interval is a 90% interval. The upper bound of this interval corresponds to a 95% one-sided upper confidence limit. The profile

[10] This is because beta is constrained in nonlog-linear hazard functions because the hazard cannot be less than 0; beta in log-linear models is unconstrained because $e^{\beta x}$ will never be less than 0.

likelihood bounds are time-consuming to calculate, and lower bounds and the upper bounds on beta2 were not uniformly reported.

D.1. BREAST CANCER INCIDENCE BASED ON THE SUBCOHORT WITH INTERVIEWS

D.1.1. Exposure Distribution among EtO-Exposed Women in Breast Cancer Incidence Subcohort with Interviews (*n* = 5,139)

The estimated daily exposure to EtO across different jobs and time periods ranged from 0.05 ppm to 77 ppm. Exposure intensities from this broad range were multiplied by the length of time in different jobs to get estimates of cumulative exposure. The duration of exposure had a mean of 10.8 years (std. dev. 9.1), and a median of 7.4 years. The range was from 1.00 to 50.3 years. The 25th percentile was 2.8 years and the 75th percentile was 17.6 years. Multiplying exposure intensity and exposure duration results in a wide range of cumulative exposures.

Cumulative exposure at the end of follow-up, with no lag, had a mean of 13,524 ppm-days (37.0 ppm-years), with a standard deviation of 13,254 ppm-days. These data are highly skewed, with a range from 5 to 253,848 ppm-days. The 25th percentile is 926 ppm-days, while the 75th is 10,206 ppm-days. Log transformation of these data results in an approximately normal distribution of the data.

As a caveat, it should be remembered that cumulative exposure at the end of follow-up may be misleading, as it is not relevant to standard analyses, all of which treat cumulative exposure as a time-dependent variable which must be assessed at specific points in time. For example, standard life-table analyses calculate cumulative exposure at different times during follow-up for each person. Subsequently, both person-time and disease events are put into categories of cumulative exposure. A given person may pass through many such categories, contributing person-time to each. Poisson regression, analogous to life-table analyses (and often based directly on output from life table programs), similarly relies on person-time (and disease occurrence) categorized by cumulative exposure. Both of these types of analyses are inherently categorical.

In the analyses presented here, we have used Cox regression in which age is the time variable. The basic approach is to compare each case to a set of 100 randomly chosen controls, whose exposure is evaluated at the same age at which the case fails (gets disease or dies of disease). Using 100 controls generally would be expected to give the same result as the full risk set and shortens analysis time (Steenland and Deddens, 1997). Hence, cumulative exposure is again time dependent. For the case who fails at an early age, the cumulative exposure of the case and many of his or her controls at that same age may be low. For the case who fails late in life, the cumulative exposure of the case and his or her controls will be higher. When cumulative

exposure is lagged so that no exposure is counted until after a lag period (e.g., 15 years) is fulfilled, many cases and their respective controls will be "lagged out" (i.e., will have no cumulative exposure, if the case fails at an early age). Note that Cox regression uses individual data, and there is no inherent categorization typical of life-table analyses and Poisson regression, although categorical analyses can still be done in Cox regression and are often useful.

For these reasons, it is difficult to describe the cumulative exposure distribution of all subjects in the Cox regression. Controls may appear more than once matched to different cases, and their cumulative exposure will differ each time depending on the age of the case. However, cases only appear once in the data and their exposure distribution can be easily presented. In our situation, we have used Cox regression with a 15-year lag to analyze breast cancer incidence. The exposure distribution of the cases, by deciles above the lagged out category, is shown in Table D-1. Creating deciles such that cases are equally distributed is a good a priori way of creating categories in which rate ratios will have approximate equal variance, a desirable feature. The lagged out cases are women who got incident breast cancer within 15 years of first exposure.

Table D-1. Distribution of cases in Cox regression for breast cancer morbidity analysis after using a 15-year lag

Cumulative exposure, 15-year lag	Mean cumulative exposure (ppm-days)	Number of incident breast cancer cases
0 (lagged out)		62
>0–<364 ppm-days	178	17
364–<854 ppm-days	524	17
854–<1,379 ppm-days	1,107	17
1,379–<2,207 ppm-days	1,767	17
2,207–<3,895 ppm-days	2,918	17
3,895–<5,542 ppm-days	4,638	17
5,542–<8,012 ppm-days	6,442	17
8,012–<14,551 ppm-days	10,447	17
14,551–<25,458 ppm-days	19,506	17
≥25,458 ppm-days	44,778	17

D.1.2. Lag Selection for the Breast Cancer Incidence Data

After the SAB review of the 2014 draft assessment, the issue of lag selection was revisited. Table D-2 provides −2 log-likelihood results comparing different models with different lags. Table D-2 also presents the Akaike information criterion (AIC) values for the same models, to facilitate comparison with the two-piece spline models, which include an extra

parameter. [The knot is preselected and is not considered a parameter in these analyses, consistent with the SAB's concept of parsimony (SAB, 2015)].[11]

For four of the eight models, the lowest –2 log-likelihoods (and AICs) occur with a lag of 15 years, consistent with the lag used in the original published paper (Steenland et al., 2003). For both the log-linear and linear log cumulative exposure models, the lowest –2 log-likelihoods (and AICs) occur with no lag, which is not biologically likely. For both the log-linear and linear two-piece spline models, the lowest –2 log-likelihoods (and AICs) occur with a lag of 20 years, but the differences between the results for the 20-year lag and the 15-year lag is small, less than 1 AIC unit in each case. Thus, for consistency in comparisons and to optimize the best fitting lag overall, a lag of 15 years was selected for analyzing the breast cancer incidence data. Selecting the lag time based on the strongest associations is a common statistical approach (Checkoway et al., 2004). A lag of 15 years is also biologically plausible for a solid tumor like breast cancer. Sensitivity of the results to choice of lag time is examined in Sections D.1.5 and D.1.6 below.

D.1.3. Modeling of Breast Cancer Incidence Data Using a Variety of Models

D.1.3.1. *Cox Regression (Log RR) Models*

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2003). Age was the time variable in proportional hazards (Cox) regression. For breast cancer incidence, family history of breast cancer, date of birth (quartiles), and parity were included in models along with exposure variables. For our exposure variable, we used cumulative exposure lagged 15 years, which was found in prior analyses to provide the best fit to the data (Steenland et al., 2003).

Using log RR models, we used a categorical model, a (log-)linear model, a two-piece (log-)linear model, a log-transform model, a cubic spline model, and a square-root transform model. We also ran a number of analogous models using linear RR models.

The categorical analysis used deciles, as indicated in Table D-3. Deciles were used instead of the original quintiles from the publication (Steenland et al., 2003), because the relatively large sample size enabled more extensive categorization. Results of the categorical decile analysis are in Table D-3 below.

[11] "in some settings the principle of parsimony may suggest that the most informative analysis will rely upon fixing some parameters rather than estimating them from the data. The impact of the fixed parameter choices can be evaluated in sensitivity analyses. In the draft assessment, fixing the knot when estimating linear spline model fits from relative risk regressions is one such example" [page 12 of SAB (2015)].

Table D-2. Minus 2 × log-likelihood results and AICs for different models and different exposure lag times

Minus twice LL	Lag					To get AIC
	0.0	5.0	10.0	15.0	20.0	
LOG-LINEAR EXPOSURE MODELS						
CUMEXP	1,946.5	1,945.9	1,945.5	1,944.7	1,946.0	add 12
LCUMEXP	1,943.7	1,946.8	1,944.0	1,944.2	1,947.0	add 12
SQRT_CUMEXP	1,945.1	1,944.4	1,943.6	1,941.0	1,943.1	add 12
2-PIECE	1,943.8	1,943.1	1,943.5	1,940.5	1,939.6	add 14
knot[a]	500.0	6,250.0	500.0	5,500.0	5,500.0	
LINEAR EXPOSURE MODELS						
CUMEXP	1,946.1	1,945.2	1,944.5	1,942.5	1,944.7	add 12
LCUMEXP	1,943.3	1,947.3	1,944.4	1,944.8	1,947.3	add 12
SQRT_CUMEXP	1,944.9	1,944.3	1,943.4	1,940.5	1,942.6	add 12
2-PIECE	1,943.8	1,943.2	1,942.9	1,940.4	1,939.7	add 14
knot[a]	500.0	6,250.0	500.0	5,750.0	5,750.0	
NULL	1,967.8					
NONEXPOSURE COVARIATES	1,948.9					
AIC	**Lag**					
	0.0	5.0	10.0	15.0	20.0	
LOG-LINEAR EXPOSURE MODELS						
CUMEXP	1,958.5	1,957.9	1,957.5	1,956.7	1,958.0	
LCUMEXP	1,955.7	1,958.8	1,956.0	1,956.2	1,959.0	
SQRT_CUMEXP	1,957.1	1,956.4	1,955.6	1,953.0	1,955.1	
2-PIECE	1,957.8	1,957.1	1,958.1	1,954.5	1,953.6	
LINEAR EXPOSURE MODELS						
CUMEXP	1,958.1	1,957.2	1,956.5	1,954.5	1,956.7	
LCUMEXP	1,955.3	1,959.3	1,956.4	1,956.8	1,959.3	
SQRT_CUMEXP	1,956.9	1,956.3	1,955.4	1,952.5	1,954.6	
2-PIECE	1,957.8	1,957.2	1,956.9	1,954.4	1,953.7	

[a]knots for two-piece spline models were obtained by doing a grid search by increments of 500 ppm x days and then interpolating.
CUMEXP: cumulative exposure.
LCUMEXP: log (ln) cumulative exposure.
SQRT_CUMEXP: square root of cumulative exposure.
AIC: Akaike information criterion

Table D-3. Categorical analysis of breast cancer incidence by deciles (exposures lagged 15 years)

Parameter	Estimate	SE	RR	Lower RR	Upper RR
CAT 1	-0.1171	0.29340	0.88953	0.50051	1.58091
CAT 2	-0.02152	0.29716	0.97871	0.54665	1.75228
CAT 3	0.1925	0.29767	1.21226	0.67642	2.17257
CAT 4	0.1438	0.29972	1.15471	0.64172	2.07776
CAT 5	-0.00308	0.29966	0.99692	0.55410	1.79364
CAT 6	0.4381	0.30283	1.54977	0.85605	2.80568
CAT 7	0.3955	0.30573	1.48513	0.81568	2.70401
CAT 8	0.2980	0.30652	1.34711	0.73874	2.45649
CAT 9	0.5583	0.31129	1.74774	0.94950	3.21703
CAT 10	0.7732	0.31304	2.16675	1.17311	4.00199

SE = standard error.
−2 Log-likelihood = 1,937.0; degrees of freedom = 15 (10 exposure terms, 5 covariates)
AIC = 1,967.0

We then fit a cubic spline (restricted at the ends to be linear) which presents a description of the data similar to the categorical analyses but using a smooth curve. The exposure metric was cumulative exposure with a 15-year lag, which was found in earlier analyses to be the optimal lag (Steenland et al., 2003). Five knots for the cubic spline were chosen using every other midpoint from the categorical analysis (598, 1,774, 4,647, 11,187, and 37,668 ppm-days) (using Steenland's 2010 cutpoints, which were slightly different from those currently used).

We then ran a two-piece (log-)linear log RR model. The knot, or inflection point, was chosen to be the one where the model likelihood was highest, which was at 5,800 ppm-days. To choose this knot, we looked at possible inflection points over the range 100 to 15,000 ppm-days by 100 ppm-day increments. Figure D-1 shows the –2 log-likelihood graphed against the knots. In this figure, the lower peak corresponds to the highest likelihood.[12]

Figures D-2 and D-3 show the results of the two-piece (log-)linear, the categorical, the (log-)linear, and the cubic spline log RR models. In these figures, the categorical points are the midpoints of the decile categories, with the final category assigned the final cutpoint plus 50%, using Steenland's 2010 cutpoints, so the decile RR estimates differ somewhat from those reported in the current assessment.

[12]Editorial note: −2 × (natural) log likelihood is reported because the difference in this value for any two models is the value of the test statistic commonly used to compare model fit (likelihood ratio test). Under certain assumptions, the probability distribution for this statistic is approximately χ^2 with degrees of freedom equal to the difference in degrees of freedom between the two (nested) models.

It appears that the two-piece log-linear curve in Figure D-2 approximates the shape of the exposure-response seen in the decile and cubic spline log RR analyses, better than the log-linear curve in Figure D-3.

The log-linear curve appears to have a low slope versus the other models, suggesting possible influential observations in the upper tail of exposure. To further explore this, we excluded from the analysis increasing amounts of the upper tail of the data using the log-linear model (i.e., via excluding the upper 1, 2.5, 5, 10, 15, 20, and 27% of exposure) based on the exposure distribution of the cases (the last amount, 27%, corresponds to excluding subjects with cumulative exposure above 6,000 ppm-days, which was close to the knot in the two-piece log-linear model [5,800 ppm-days]). The ratios of the slope (coefficient) for the linear term (log RR model) with these exclusions versus the slope for the linear term (log RR model) with no exclusions were 1.5, 2.3, 3.2, 2.5, 3.1, 6.1, and 9.2, respectively. As expected, the slope increases markedly as the data are restricted to the lower range of exposure. For example, a modified log-linear curve after excluding the upper 5% of the data is seen in Figure D-4, along with the full log-linear curve from Figure D-3. Nonetheless, even the log-linear curve from these truncated data has a markedly lower slope in the low-exposure region than the two-piece log-linear (or spline) curves. For example, inspection shows that the RR for 6,000 ppm-days is about 1.2 for the log-linear curve from the truncated data and 1.6 from the two-piece log-linear model. Use of the log-linear curve based on truncated data has the disadvantage of having to choose rather arbitrarily where to truncate the data. This disadvantage is avoided by using the two-piece log-linear model.

A two-piece log-linear model, then, is preferred for estimating risk parsimoniously in the low-exposure region. For comparison purposes, we also show the model using the logarithm of exposure (see Figure D-5), which we have not used for risk assessment because it is supralinear in the low-dose region.

We also fit a square-root transformation (square root of cumulative exposure, 15-year lag) log RR model, which is shown in Figure D-6. This model also fits the breast cancer morbidity well, and can be used for risk assessment, but with the disadvantage that it is not linear or approximately linear in the low-dose region. For this reason, we prefer the two-piece log-linear curve, with is approximately linear in the low-dose region (and strictly linear in the linear RR models discussed below). Excess lifetime risk does not vary greatly among all these models (see below), with the exception of the log RR model with a linear term for cumulative exposure, which is below other excess risk estimates.

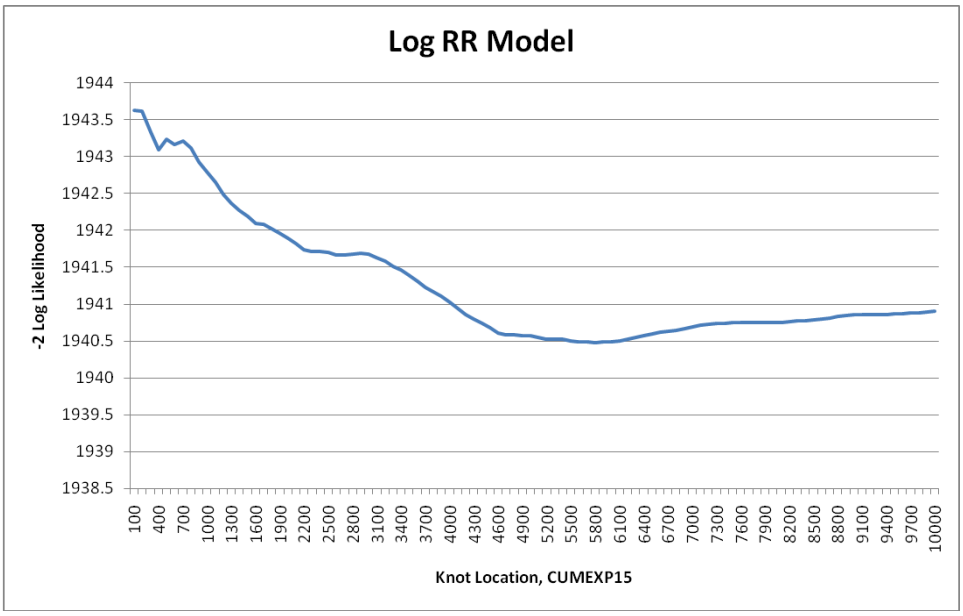


Figure D-1. Likelihoods vs. knots, two-piece log-linear spline model for breast cancer incidence.

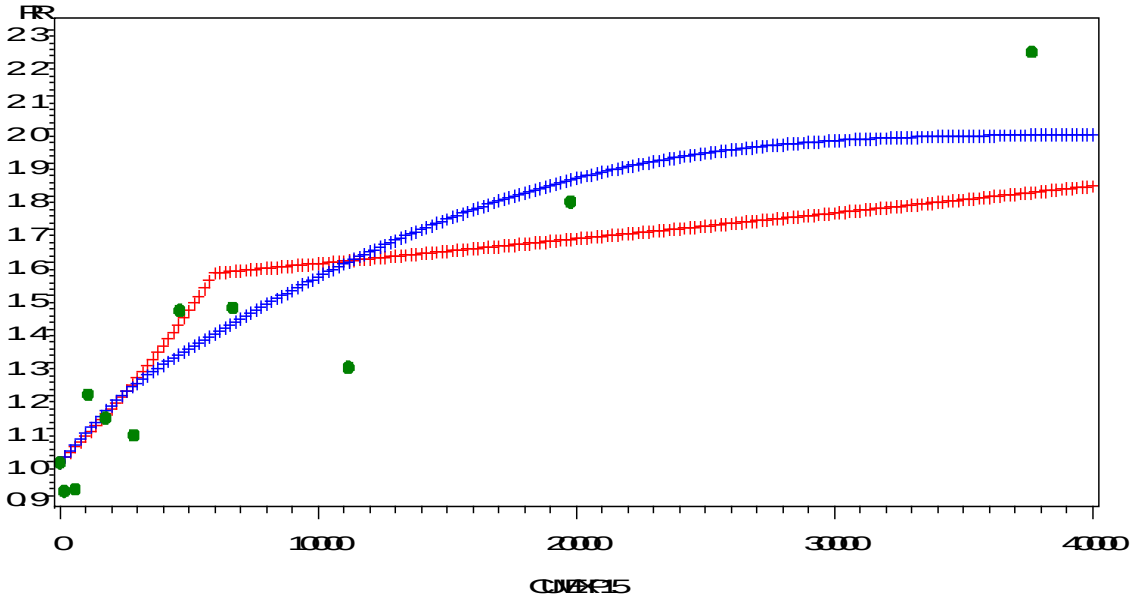


Figure D-2. Breast cancer incidence—two-piece log-linear spline model.

Plot of the two-piece log-linear spline dose-response relationship overlaid with a plot of restricted cubic (log RR) splines with continuous exposure. Dots represent the effect of exposure grouped in deciles. Deciles were formed by allocating cases approximately equally in ten groups, above lagged-out cases (using Steenland's 2010 cutpoints, so the decile RR estimates differ somewhat from those reported in the current assessment). The *y*-axis is rate ratio and the *x*-axis is cumulative exposure lagged 15 years, in ppm-days.

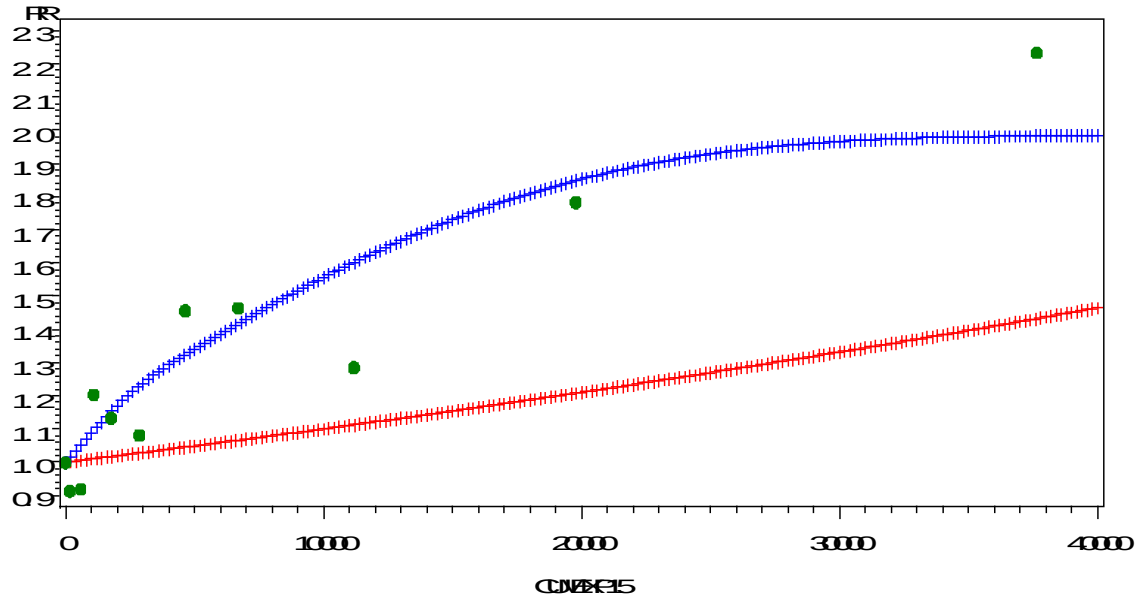


Figure D-3. Breast cancer incidence—log-linear (Cox regression) model.

Plot of the log-linear dose-response relationship overlaid with a dose-response relationship generated using restricted cubic (log RR) spline model with continuous exposure. Dots represent the effect of exposure grouped in deciles. Deciles were formed by allocating cases approximately equally in ten groups, above lagged-out cases (using Steenland's 2010 cutpoints, so the decile RR estimates differ somewhat from those reported in the current assessment).

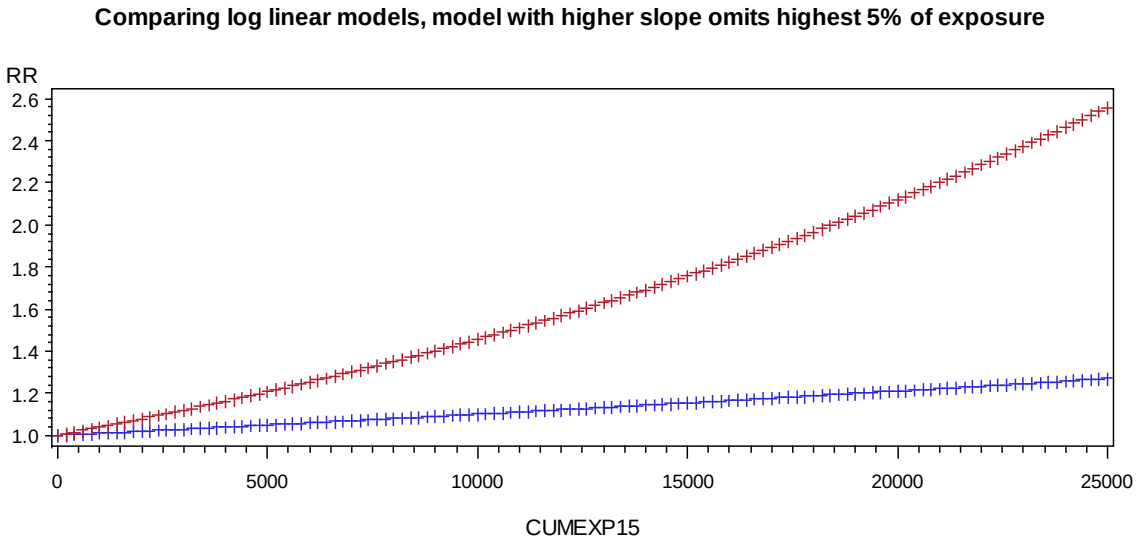


Figure D-4. Breast cancer incidence—effect on log-linear model of omitting highest exposures.

Comparison of log-linear curve (log RR = β × cumexp) with all the data (lower blue curve) and the log-linear curve (higher red curve) after excluding those in the top 5% of exposure (>27,500 ppm-days).

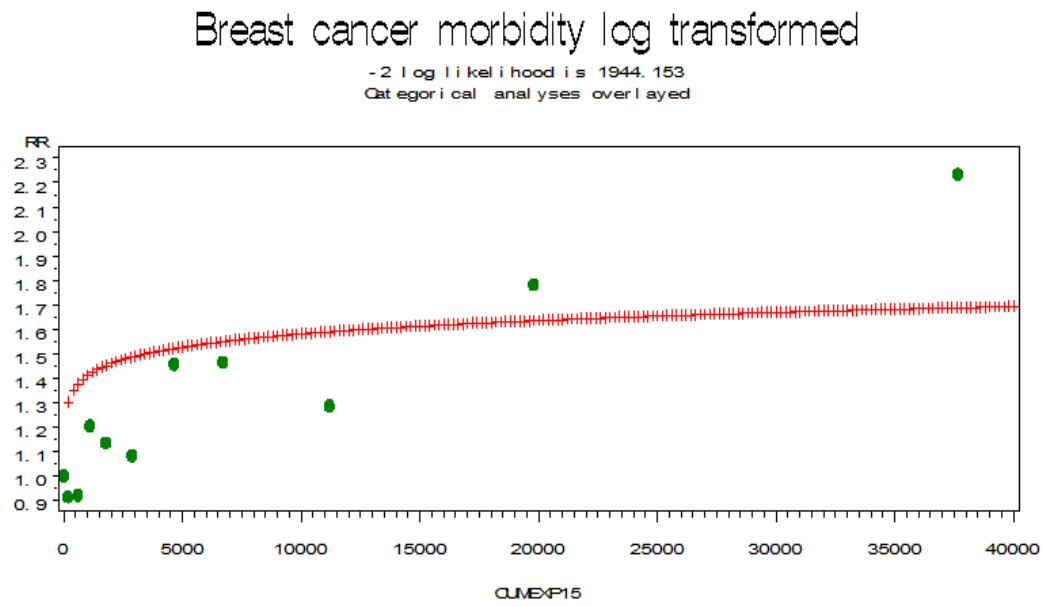


Figure D-5. Breast cancer incidence—log-linear model with log cumulative exposure.

Plot of a logarithmic transformation log RR dose-response model [log RR = β × log(cumexp)] overlaid with categorical RR results (deciles). Deciles were formed by allocating cases approximately equally in ten groups, above lagged-out cases (using Steenland's 2010 cutpoints, so the decile RR estimates differ somewhat from those reported in the current assessment).

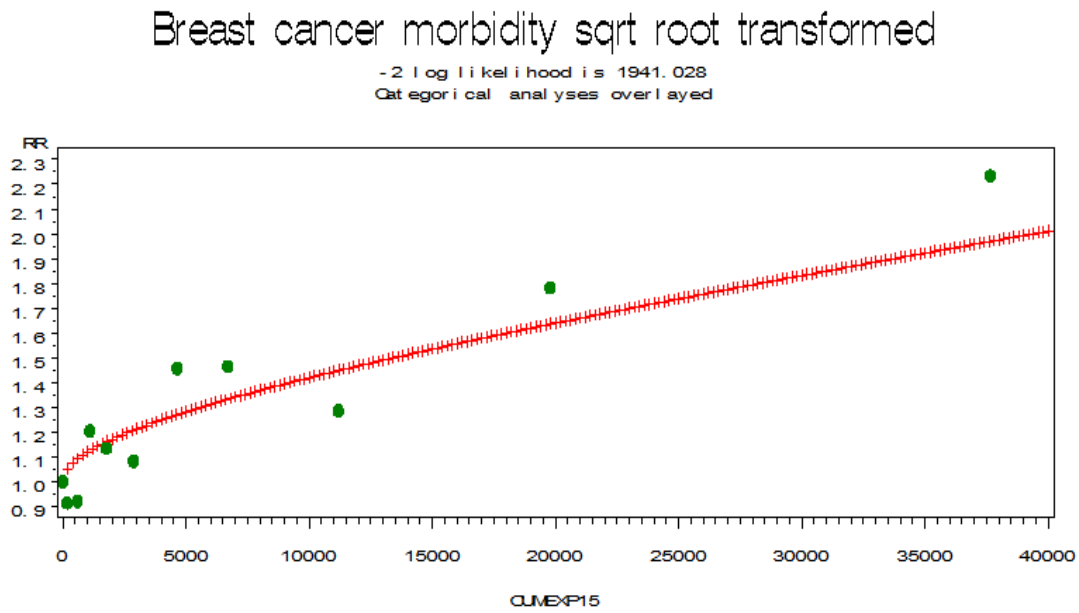


Figure D-6. Breast cancer incidence—log-linear model with square root of cumulative exposure.

Plot of a square-root transformation log RR dose–response model overlaid with categorical RR results (deciles). Deciles were formed by allocating cases approximately equally in ten groups, above lagged-out cases (using Steenland's 2010 cutpoints, so the decile RR estimates differ somewhat from those reported in the current assessment).

Tables D-4, D-5, D-6, and D-7 below present the model fit statistics for the two-piece log-linear, the log-linear, the square root log RR model, and the log-transform log RR model seen above. Table D-8 summarizes the goodness-of-fit data with regard to the exposure term. Table D-8 shows that the addition of exposure terms to the various models results in similar model fits. The exposure terms in the two-piece log-linear model improve model fit marginally better than those in the other models except the square root log RR model, with which the two-piece log-linear model is tied. If one adds a degree of freedom to the χ^2 test for the two-piece log-linear model, on the assumption that the choice of the knot is equivalent to estimating another parameter, the *p*-value increases to 0.04, in the same range as the log-linear and log-transform log RR models. Our argument here, however, is not that the two-piece log-linear model fits the data dramatically better than other models in purely statistical terms. Rather we believe that the fit conforms to the categorical and cubic spline models well in the low-exposure region of interest, and that the nearly linear exposure-response relationship in that region (strictly linear with the linear RR model) is a reason to prefer the two-piece log-linear model to the other models. In particular, among the parametric models, the log-transform and square root log RR models are supralinear in the low-exposure region.

The effects of these departures from linearity in the low-exposure region can be seen in the risk assessment results for the EC_{01} (estimate of effective concentration resulting in 1% extra risk) in Section 4.1.2.3 of the assessment (with the exception of the cubic spline results, which are not part of Section 4.1.2.3, Steenland's original risk assessment sections were deleted because they were based on older mortality and disease rates than were the analyses presented in the current Section 4.1.2.3). While we do not recommend the use of the cubic spline model for risk assessment due to its complexity, the EC_{01} based on the cubic spline model, presented in Section D.1.4 below, provides a good comparison to other parametric models.

Table D-4. Fit of two-piece log-linear model to breast cancer incidence data, Cox regression[13]

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
−2 LOG L	1,967.813	1,940.485			
AIC	1,967.813	1,954.485			
SBC	1,967.813	1,978.612			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	27.3281	7	0.0003		
Score	29.0949	7	0.0001		
Wald	28.4426	7	0.0002		
Analysis of maximum likelihood estimates					
Variable	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LIN_0 (β1)	0.0000770	0.0000317	5.4642	0.0194	1.000
LIN_1	-0.0000724	0.0000334	4.1816	0.0409	1.000
DOB1	0.08770	0.21805	0.1618	0.6875	1.092
DOB2	0.41958	0.24430	2.9496	0.0859	1.521
DOB3	0.55168	0.29096	3.5950	0.0580	1.736
PARITY1	-0.23398	0.18793	1.5502	0.2131	0.791
FREL_BR_CAN1	0.47341	0.17934	6.9686	0.0083	1.605

Covariance lin0 and lin1: -1×10^{-9}

[13]For environmental exposures, only exposures below the knot are of interest. Below the knot, RR = $e^{(\beta 1 * \text{exposure})}$.

Table D-5. Fit of log-linear model to breast cancer incidence data, Cox regression (RR = $e^{(\beta \times exposure)}$)

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
−2 LOG L	1,967.813	1,944.675			
AIC	1,967.813	1,956.675			
SBC	1,967.813	1,977.356			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	23.1374	6	0.0008		
Score	25.8389	6	0.0002		
Wald	25.3594	6	0.0003		
Analysis of maximum likelihood estimates					
Variable	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUMEXP15 (β)	9.5482E-6	4.09902E-6	5.4261	0.0198	1.000
DOB1	0.13558	0.21676	0.3912	0.5316	1.145
DOB2	0.53147	0.23741	5.0116	0.0252	1.701
DOB3	0.74477	0.27425	7.3748	0.0066	2.106
PARITY	−0.23394	0.18882	1.5351	0.2154	0.791
FREL_BR_CAN1	0.46449	0.17928	6.7126	0.0096	1.591

Table D-6. Fit of the square root transformation log RR model to breast cancer incidence data, Cox regression ($RR = e^{(\beta \times sqrt(exposure))}$)

Model fit statistics					
Crtierion	**Without covariates**	**With covariates**			
-2 LOG L	1,967.813	1,941.028			
AIC	1,967.813	1,953.028			
SBC	1,967.813	7,973.708			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	26.7851	6	0.0002		
Score	28.9446	6	< .0001		
Wald	28.5277	6	< .0001		
Analysis of maximum likelihood estimates					
Variable	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**
DOB1	1	0.09778	0.21756	0.2020	0.6531
DOB2	1	0.43872	0.24177	3.2929	0.0696
DOB3	1	0.58623	0.28404	4.2596	0.0390
sqrtcumexp15 (β)	1	0.00349	0.00118	8.7489	0.0031
PARITY1	1	−0.22539	0.18787	1.4393	0.2302
FREL_BR_CAN1	1	0.46937	0.17922	6.8589	0.0088

Table D-7. Fit of the log-transform model to breast cancer incidence data, Cox regression ($RR = e^{(\beta \times \ln(\text{exposure}))}$)

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	1,967.813	1,944.176				
AIC	1,967.813	1,956.176				
SBC	1,967.813	1,976.856				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	23.6371	6	0.0006			
Score	24.0044	6	0.0005			
Wald	23.5651	6	0.0006			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
DOB1	1	0.08605	0.21943	0.1538	0.6949	1.090
DOB2	1	0.38780	0.25363	2.3378	0.1263	1.474
DOB3	1	0.47303	0.31528	2.2509	0.1335	1.051
LCUMEXP15 (β)	1	0.04949	0.02288	4.6787	0.0305	1.051
PARITY1	1	−0.25908	0.18638	1.9322	0.1645	0.772
FREL_BR_CAN1	1	0.47620	0.17923	7.0595	0.0079	1.610

Table D-8. Change in –2 log-likelihood for log RR models for breast cancer incidence, with addition of exposure term(s)[a]

Log RR model	Change (χ^2)	DF	*p*-value
Log transform	4.8	1	0.03
Linear	4.2	1	0.04
Categorical	12.0	10	0.29
Cubic spline	8.8	4	0.07
Two-piece linear	8.4	2	0.01
Square root	7.7	1	0.006

[a]All models had 3 variables for date of birth, 1 for family history, and 1 for parity.

D.1.3.2. *Linear Relative Risk Models for Breast Cancer Incidence*

We also ran linear relative risk models for breast cancer incidence, using the techniques described by Langholz and Richardson (2010) to use SAS to fit these models, using the same data as used for the log RR models. The form of these linear RR models is $RR = 1 + \beta x$, where x

can be cumulative dose, the log of cumulative dose, a two-piece linear function of cumulative dose, and so on.

To choose the knot for the linear two-piece spline model, Deddens examined inflection points over the range 500 to 10,000 ppm-days by 500 ppm-day increments, and then interpolated where appropriate.

Figure D-7 shows the likelihood profile for different possible knots for the two-piece linear spline, with the search conducted by using increments of 500 ppm-days. The best knot was 5,750 ppm-days, similar to the knot of 5,800 ppm-days for the two-piece log-linear model.

Table D-9 shows the model fit statistics for the linear RR models. These models tend to fit slightly better than their log RR counterparts, although generally the improvement in the χ^2 does not attain significance at the 0.05 level. For the two-piece linear model, the model likelihood is 1940.36 versus a likelihood of 1940.49 for the two-piece log-linear model. Among the linear RR models, the two-piece spline model fits better than the other models, although not significantly so. Table D-10 gives the exposure parameter values for the linear RR models.

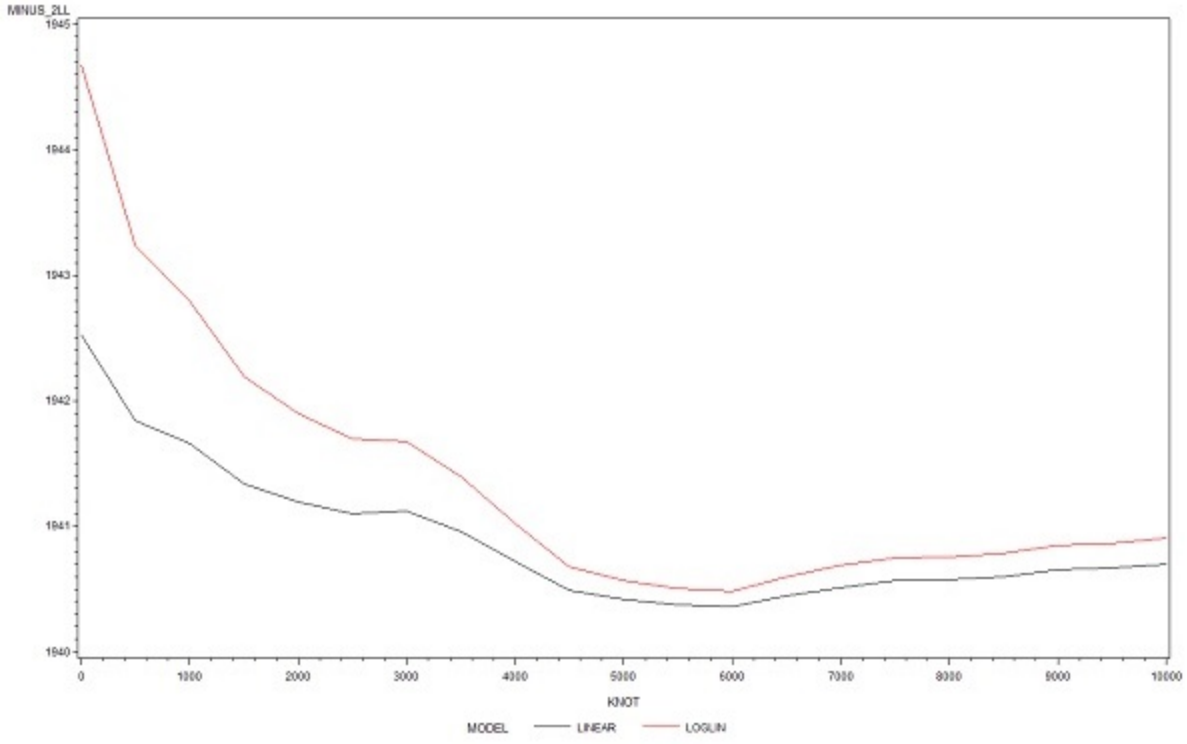


Figure D-7. Likelihoods vs. knots, two-piece linear spline model, breast cancer incidence (units are ppm-days, 15-year lag).

Table D-9. Model fit statistics for linear RR models, breast cancer incidence[a]

Linear RR model	DF (full model)[b]	–2 LL (full model)	–2 LL (model without exposure)	–2 LL (model without any covariates)	*p*-value (full model)	*p*-value (for addition of exposure terms)[c]
CUMEXP15	6	1,942.526	1,948.935	1,967.813	0.0030	0.0113
Sqrt(CUMEXP15)	6	1,940.501	1,948.935	1,967.813	0.0001	0.0037
Spline, knot = 5,750, CUMEXP15	7	1,940.360	1,948.935	1,967.813	0.0003	0.0137

[a]For the linear RR models, all nonexposure covariates were included multiplicatively.
[b]Degrees of freedom for full model.
[c]Based on change in likelihood for breast cancer incidence linear RR models with addition of exposure term(s) to model with date of birth, parity, and breast cancer in first degree relative. Degrees of freedom for addition of exposure terms is (degrees of freedom for the full model − 5)
LL = log likelihood

Table D-10. Model coefficients for linear RR models, breast cancer incidence

Linear RR model	Parameter(s)	SE	Profile likelihood 95% (one-sided) upper bounds[c]
CUMEXP15	$B = 2.2964 \times 10^{-5}$	$SE = 1.210 \times 10^{-5}$	$UB = 4.666 \times 10^{-5}$
Sqrt(CUMEXP15)	$B = 5.531 \times 10^{-3}$	$SE = 2.585 \times 10^{-3}$	UB = 0.01067
Spline, knot = 5,750, CUMEXP15[a,b]	$B1 = 8.9782 \times 10^{-5}$ $B2 = -7.7859 \times 10^{-5}$	$SE1 = 5.378 \times 10^{-5}$ $SE2 = 5.930 \times 10^{-5}$	$UB1 = 1.837 \times 10^{-4}$ $UB2 = 4.309 \times 10^{-6}$

SE = standard error.
[a]$Var1 = SE1^2 = 2.892 \times 10^{-9}$; $Var2 = SE2^2 = 3.516 \times 10^{-9}$; $Covariance = -3.11 \times 10^{-9}$.
[b]For estimating risks from occupational exposures (see Section 4.7), both pieces of the two-piece linear spline model are used. For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × [exp – knot]). For the (one-sided) 95% upper confidence limit, the Wald approach is used as an approximation because it was not possible to obtain a formula for the profile likelihood upper-bound estimates that could be used in the life-table analysis. Thus, for exposures below the knot, RRu = 1 + ([B1+ 1.645 × SE1] × exp); for exposures above the knot, RRu = 1 + (B1 × exp + B2 × [exp-knot] + 1.645 × sqrt[exp^2 × var1 + $(exp-knot)^2$ × var2 + 2 × exp × (exp-knot) × covar]), where exp = cumulative exposure, var = variance, covar = covariance. As shown in Figure D-8, the difference between the Wald upper-bound estimates and the profile likelihood upper-bound estimates is not large. In the range of occupational exposures of interest (i.e., up to 12,775 ppm × days), the Wald RRu estimate is at most ~5% less than the profile likelihood RRu estimate (at the extreme, i.e., at 12,775 ppm × days).
[c]Calculating the profile likelihood bounds is computationally difficult and estimating the lower bounds was not pursued here.

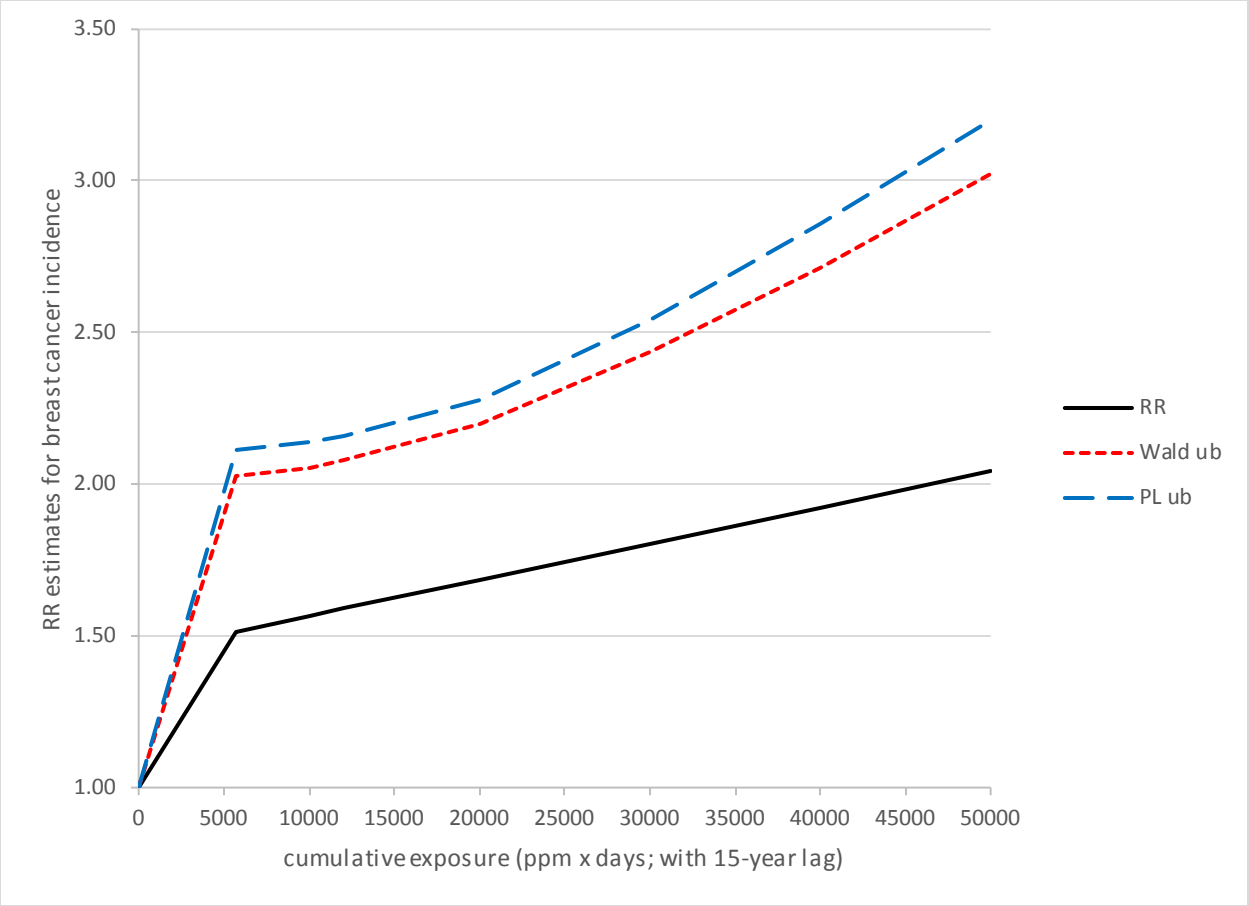


Figure D-8. Comparison of Wald and profile likelihood (one-sided) 95% upper-bound estimates for 2-piece linear spline model.

D.1.4. Risk Assessment for Breast Cancer Incidence Using the Cubic Spline Curve Log RR Model

Risk assessment using the spline curve is more difficult due to the semiparametric complicated nature of the restricted cubic spline function. The cubic spline function for the breast cancer incidence rate ratio is:

```
RR=exp((ns_0*cumexp15) + ns_1*(((cumexp15-598)**3)*(cumexp15>= 598) -
((37668-598) /(37668-11187)) *(((cumexp15-11187)**3) *(cumexp15>= 11187)) +
((11187 -598)/(37668 - 11187)) *(((cumexp15-37668 )**3) *(cumexp15>= 37668))
) +  ns_2*(((cumexp15-1774)**3)*(cumexp15>= 1774) - ((37668-1774) /(37668-
11187)) *(((cumexp15-11187)**3) *(cumexp15>= 11187)) + ((11187 -1774) /(37668
- 11187))*(((cumexp15-37668 )**3) *(cumexp15>= 37668)) ) + ns_3*(((cumexp15-
4647)**3)*(cumexp15>= 4647) - ((37668-4647) /(37668-11187)) *(((cumexp15-
11187)**3) *(cumexp15>= 11187)) + ((11187 -4647)/(37668 - 11187))
*(((cumexp15-37668 )**3) *(cumexp15>= 37668)) )).
```

The coefficients ns_0, ns_1, ns_2, and ns_3 used in this function are 0.00008294999811, −0.00000000000310, 0.00000000000425, and −0.00000000000114, respectively. The expression "cumexp15>=" is a logical statement whereby the term is 0 when "cumexp" is less than the specified value.

Here we calculate only the EC_{01} (without the LEC_{01}) for comparison with the corresponding EC_{01} from the two-piece log-linear model. The point is to show that the cubic spline log RR model and the two-piece log-linear spline give similar answers, not to propose the cubic spline for use in risk assessment, given its relatively complicated formula above. Calculation of the LEC_{01} is also particularly complicated because to do it correctly one must use not only the standard errors for four coefficients but also their covariances.

For breast cancer incidence, the EC_{01} using the cubic spline log RR model is 0.0138 ppm, similar to the value of 0.0152 ppm using the two-piece log-linear model. [Note that although these EC_{01} values are internally consistent for the comparison made here, they are not directly comparable to values reported in Chapter 4 because the calculations presented here were made using background mortality and incidence rates from 1997−2001 and were not updated for the current assessment. Nonetheless, the difference between the value of 0.0152 ppm presented here for the two-piece log-linear model and the value of 0.0155 ppm reported in Chapter 4 is negligible.]

D.1.5. Supplemental Results: Results for Cumulative Exposure and Log Cumulative Exposure Cox Regression Models with Different Lag Times

Sensitivity of the exposure parameter estimates to choice of exposure lag time (no lag, 5 years, 10 years, 15 years, and 20 years) for the log-linear cumulative exposure (standard Cox regression) and log cumulative exposure models is summarized in Table D-11.

Table D-11. Comparison of some log-linear model results with different lag periods; cumulative exposure in ppm × days

Model	Lag (years)	−2 log-likelihood	Likelihood ratio test p-value for exposure term	Exposure parameter estimate (per unit exposure)	Standard error (per unit exposure)
Log-linear cumulative exposure (standard Cox model)	0	1,946.492	0.09	5.93879×10^{-6}	3.52892×10^{-6}
	5	1,945.875	0.06	6.8565×10^{-6}	3.59626×10^{-6}
	10	1,945.521	0.04	7.75726×10^{-6}	3.80799×10^{-6}
	15	1,944.675	0.02	9.54826×10^{-6}	4.09902×10^{-6}
	20	1,946.040	0.055	1.01×10^{-5}	5.27041×10^{-6}
Log-linear log cumulative exposure	0	1,943.662	0.02	0.09294	0.04097
	5	1,946.843	0.16	0.04458	0.03135
	10	1,944.040	0.03	0.05654	0.02594
	15	1,944.176	0.03	0.04949	0.02288
	20	1,947.020	0.17	0.02970	0.02151

D.1.6. Sensitivity of Unit Risk Estimates to Change in Lag Period

Sensitivity of the unit risk estimates to changes in exposure lag time for the two-piece linear spline model with the knot at 5,750 ppm × days is summarized in Table D-12.

Table D-12. Comparison of unit risk estimates from two-piece linear spline models with different lag periods; cumulative exposure in ppm × days, knot at 5,750 ppm × days

Lag (years)	−2 log-likelihood	Parameter estimate for 1st spline segment (per ppm × day)	Profile likelihood 95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)
0	1,944.5	5.9472×10^{-5}	1.5063×10^{-4}	0.0160	6.29×10^{-3}	1.59
5	1,943.2	6.7229×10^{-5}	1.5589×10^{-4}	0.0153	6.59×10^{-3}	1.52
10	1,943.9	4.5903×10^{-5}	1.2655×10^{-4}	0.0245	8.88×10^{-3}	1.13
15	1,940.4	8.9782×10^{-5}	1.8372×10^{-4}	0.0138	6.75×10^{-3}	1.48
20	1,939.6	1.3725×10^{-4}	2.4727×10^{-4}	0.0101	5.59×10^{-3}	1.79

The sensitivity analysis for choice of lag reveals that the unit risk estimates for the selected two-piece linear spline model with the knot at 5,750 ppm × days 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 the best fitting 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).

The optimal knot for the two-piece linear spline model with a 20-year lag was the same as that for the model with a 15-year lag [i.e., 5,750 ppm × days (see Table D-2)]. For lags of 0 and 10 years, the optimal knot was in the vicinity of 500 ppm × days. Using this lower knot would have yielded higher regression coefficients for the 1st spline segment and correspondingly higher unit risk estimates. For the lag of 5 years, the optimal knot was slightly higher (6,250 ppm × days) than the knot for the selected model (5,750 ppm × days), which would have yielded a slightly lower unit risk estimate than that presented in Table D-12. However, even with the optimal knot, the models for lags of 0 and 5 years had *p*-values > 0.05 for the exposure terms (0.077 and 0.057, respectively), and the model with a lag of 10 years had $p = 0.049$.

D.1.7. Sensitivity of Unit Risk Estimates to Value of Knot

Sensitivity of the unit risk estimates to value of knot for the two-piece linear spline model is summarized in Table D-13, with knots of 5,750 ± 1,000 ppm × days.

Table D-13. Comparison of unit risk estimates from two-piece linear spline models with different knot; cumulative exposure in ppm × days, with lag of 15 years

Knot (ppm × days)	−2 log-likelihood	Parameter estimate for 1st spline segment (per ppm × day)	Profile likelihood 95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)
4,750	1,940.43	1.0008×10^{-4}	2.0898×10^{-4}	0.0124	5.93×10^{-3}	1.69
5,750	1,940.36	8.9782×10^{-5}	1.8372×10^{-4}	0.0138	6.75×10^{-3}	1.48
6,750	1,940.48	8.0280×10^{-5}	1.6357×10^{-4}	0.0154	7.58×10^{-3}	1.32

The sensitivity analysis for knot selection in the two-piece linear spline model shows very little difference in the unit risk estimates for knots 1,000 ppm × days below and above the selected knot of 5,750 ppm × days. The unit risk estimates for these alternate knot values are about 14% greater and 11% lower, respectively, than the unit risk estimate for the selected model (with the knot at 5,750 ppm × days).

D.1.8. Sensitivity of Unit Risk Estimates to Exclusion of Covariates

Sensitivity of the unit risk estimates to exclusion of (nonexposure) covariates (i.e., significant breast cancer risk factors) for the two-piece linear spline model is summarized in Table D-14.

Table D-14. Comparison of unit risk estimates from two-piece linear spline models with exclusion of nonexposure covariates; cumulative exposure in ppm × days with 15-year lag, knot at 5,750 ppm × days

Excluded covariates	−2 log-likelihood	Parameter estimate for 1st spline segment (per ppm × day)	Profile likelihood 95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)
None	1,940.4	8.9782×10^{-5}	1.8372×10^{-4}	0.0138	6.75×10^{-3}	1.48
Parity	1,941.8	9.0441×10^{-5}	1.8516×10^{-4}	0.0137	6.70×10^{-3}	1.49
Parity and breast cancer in 1st-degree relative	1,948.0	8.7427×10^{-5}	1.8109×10^{-4}	0.0142	6.84×10^{-3}	1.46

The sensitivity analysis for exclusion of covariates in the two-piece linear spline model shows very little difference in the unit risk estimates. Excluding parity and both parity and breast cancer in a first-degree relative would change the unit risk estimate by only about 1% from the unit risk estimate derived for the selected model (i.e., with inclusion of both covariates).

D.1.9. Analysis of Age Interaction for the Exposure Terms in the Two-piece Linear Spline Model

Table D-15 shows the *p*-values for the inclusion of age interaction terms for the spline exposure regression coefficients. The interaction terms have *p*-values well above 0.05, indicating that the exposure terms are independent of age (i.e., the proportional hazards assumption is validated).

Table D-15. Evaluation of age interaction for the exposure terms in the two-piece linear spline model with knot at 5,750 ppm × days; cumulative exposure in ppm × days, with lag of 15 years

Parameter	−2 log likelihood without age interaction term	−2 log likelihood with age interaction term	Difference in −2 log likelihoods	*p*-value for the inclusion of age interaction term
Beta1	1940.360	1940.167	0.193	0.66
Beta2	1940.360	1940.284	0.076	0.78

D.1.10. Sensitivity of Unit Risk Estimates to Upper-Bound Estimation Approach—Wald vs. Profile Likelihood

Sensitivity of the unit risk estimates to the approach used to estimate the upper bound on the first spline piece from the two-piece linear spline model is summarized in Table D-16. According to Langholz and Richardson (2010), the distribution of estimated parameters in nonlog-linear models (hazard functions) 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. The Wald-based result is 3% lower than the profile-likelihood-based estimate.

Table D-16. Comparison of unit risk estimates for breast cancer incidence from two-piece linear spline model using Wald-based and profile-likelihood-based upper-bound estimates on the 1st spline piece

Estimation approach	Beta1 estimate (per ppm × day)	Wald SE1 estimate (per ppm × day)	95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)
Wald	8.98×10^{-5}	5.38×10^{-5}	1.78×10^{-4}	0.0138	6.95×10^{-3}	1.44
Profile likelihood	8.98×10^{-5}	--	1.84×10^{-4}	0.0138	6.75×10^{-3}	1.48

D.1.11. Sensitivity of Occupational Extra Risk Estimates to Change in Lag Period

In Section 4.7, extra risk estimates from the selected model are presented for some occupational exposure scenarios of interest (35-year exposures to 8-hour TWAs ranging from 0.1 to 1 ppm between ages 20 and 55 years), because the scenarios include cumulative exposures above the level at which the unit risk estimate is valid. Here, the sensitivity of the selected model (i.e., the two-piece linear spline model with the knot at 5,750 ppm × days and a lag of

15 years) to changes in lag is explored. Parameter estimates for the two-piece linear spline model with the knot at 5,750 ppm × days and different lag periods (0, 5, 10, 15, and 20 years) are presented in Table D-17. The Wald approach was used as an approximation to derive the upper-bound estimates because it was not possible to obtain a formula for the profile likelihood upper-bound estimates that could be used in the life-table analysis. As shown in Figure D-8, the difference between the Wald upper-bound estimates and the profile likelihood upper-bound estimates is not large (the Wald upper-bound RR estimates are about 4% lower than the profile likelihood upper-bound RR estimates in the region of the second spline segment, and the difference is even less than that in the region of the first spline segment). The equations for deriving the MLE and upper-bound estimates across the range of exposures are presented in footnote 2 of Table D-10. Sensitivity of the extra risk estimates for the occupational exposure scenarios to changes in exposure lag time for the two-piece linear spline model with the knot at 5,750 ppm × days is summarized in Table D-18.

Table D-17. Parameter estimates for the two-piece linear spline model with the knot at 5,750 ppm × days for different lag periods; cumulative exposure in ppm × days

Lag (years)	Beta1 (per ppm × day)	Beta2 (per ppm × day)	SE1 (per ppm × day)	SE2 (per ppm × day)	Covariance [per (ppm × day)^2]
0	5.947×10^{-5}	-5.472×10^{-5}	4.606×10^{-5}	4.889×10^{-5}	-2.23×10^{-9}
5	6.723×10^{-5}	-6.134×10^{-5}	4.602×10^{-5}	4.913×10^{-5}	-2.23×10^{-9}
10	4.590×10^{-5}	-3.544×10^{-5}	4.402×10^{-5}	4.797×10^{-5}	-2.07×10^{-9}
15	8.978×10^{-5}	-7.786×10^{-5}	5.378×10^{-5}	5.930×10^{-5}	-3.11×10^{-9}
20	1.373×10^{-4}	-1.343×10^{-4}	6.387×10^{-5}	6.969×10^{-5}	-4.36×10^{-9}

Table D-18. Comparison of breast cancer incidence extra risk estimates from two-piece linear spline models with different lag periods; cumulative exposure in ppm × days, knot at 5,750 ppm × days

8-hour TWA	15-year lag	0-year lag	Ratio to 15-year-lagged estimates	5-year lag	Ratio to 15-year-lagged estimates	10-year lag	Ratio to 15-year-lagged estimates	20-year lag	Ratio to 15-year-lagged estimates
MLEs									
0.1	0.0128	0.0106	0.83	0.0114	0.89	0.00728	0.57	0.017	1.33
0.2	0.0255	0.0211	0.83	0.0227	0.89	0.0145	0.57	0.0336	1.32
0.3	0.0379	0.0315	0.83	0.0338	0.89	0.0217	0.57	0.0499	1.32
0.4	0.0502	0.0417	0.83	0.0448	0.89	0.0288	0.57	0.0659	1.31
0.5	0.0595	0.0481	0.81	0.052	0.87	0.034	0.57	0.0786	1.32
0.6	0.0643	0.0498	0.77	0.0545	0.85	0.0368	0.57	0.0854	1.33
0.7	0.068	0.0511	0.75	0.0565	0.83	0.0393	0.58	0.0901	1.33
0.8	0.0708	0.0521	0.74	0.0578	0.82	0.0413	0.58	0.0929	1.31
0.9	0.0736	0.053	0.72	0.0592	0.80	0.0433	0.59	0.0957	1.30
1	0.0757	0.0539	0.71	0.0602	0.80	0.045	0.59	0.0973	1.29
95% one-sided UCLs									
0.1	0.0253	0.024	0.95	0.0241	0.95	0.0186	0.74	0.0298	1.18
0.2	0.0498	0.0473	0.95	0.0476	0.96	0.0369	0.74	0.0585	1.17
0.3	0.0736	0.07	0.95	0.0704	0.96	0.0547	0.74	0.0862	1.17
0.4	0.0967	0.0921	0.95	0.0926	0.96	0.0722	0.75	0.113	1.17
0.5	0.114	0.105	0.92	0.106	0.93	0.0841	0.74	0.134	1.18
0.6	0.121	0.107	0.88	0.11	0.91	0.0886	0.73	0.144	1.19
0.7	0.126	0.109	0.87	0.113	0.90	0.092	0.73	0.151	1.20
0.8	0.13	0.109	0.84	0.114	0.88	0.0943	0.73	0.155	1.19
0.9	0.133	0.11	0.83	0.115	0.86	0.0967	0.73	0.16	1.20
1	0.136	0.111	0.82	0.116	0.85	0.0984	0.72	0.162	1.19

The sensitivity analysis for choice of lag reveals that the MLEs of extra risk for the selected two-piece linear spline model with the knot at 5,750 ppm × days for different lag periods (0, 5, 10, 15, and 20 years) ranged from about 40% less than (10-year lag) to about 30% greater than (20-year lag) the estimates for the selected model (15-year lag). The 95% (one-sided) upper bounds of extra risk ranged from about 25% less than (10-year lag) to about 20% greater than (20-year lag) the estimates for the selected model. Of these specific models, the model with the 20-year lag was the best fitting model, based on log likelihood (see Table D-12). 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).

For lags of 0 and 10 years, the optimal knot was in the vicinity of 500 ppm × days, and for the lag of 5 years, the optimal knot was slightly higher (6,250 ppm × days) than the knot for the selected model (5,750 ppm × days). Comparisons to extra risk results with these different optimal knots cannot be made without knowledge of the parameter estimates for the regression coefficients, which the EPA did not obtain from NIOSH because these additional analyses are outside of the scope of the intended lag sensitivity analyses, as changing the knot results in an entirely different (and inferior in terms of likelihood) model. With the optimal knot, the models for lags of 0 and 5 years had *p*-values > 0.05 for the exposure terms (0.077 and 0.057, respectively), and the model with a lag of 10 years had $p = 0.049$. The exception is the two-piece linear spline model with a 20-year lag, which had the optimal knot at the same value as two-piece linear spline model with a 15-year lag (i.e., 5,750 ppm × days [see Table D-2]). This two-piece linear spline model with a 20-year lag is the best fitting model of all the two-piece spline models with optimal knots and all the models with the knot at 5,750 ppm × days but with different lags. As noted above, for the occupational exposure scenarios of interest, the two-piece linear spline model with a 20-year lag yielded MLEs of extra risk about 30% greater than and 95% (one-sided) upper bounds about 20% greater than those for the selected model (15-year lag).

D.2. BREAST CANCER MORTALITY

D.2.1. Exposure Distribution among Women and Breast Cancer Deaths in the Cohort Mortality Study (*n* = 9,544)

In the Cox regression analyses of Steenland et al. (2004), the data on breast cancer mortality was found to be fit best using cumulative exposure with a 20-year lag. Table D-19 shows the distribution of the 102 breast cancer deaths by exposure quartile with a 20-year lag. The cutpoints are those used in the published data (Steenland et al., 2004). Regarding the women in the cohort as a whole, cumulative exposure at the end of follow-up, with no lag, had a

mean of 8.2 ppm-years, with a standard deviation of 38.2. This distribution was highly skewed; the median was 4.6 ppm-years.

Table D-19. Distribution of cases in Cox regression analysis of breast cancer mortality after using a 20-year lag

Cumulative exposure, 20-year lag[a]	Number of breast cancer deaths
0 (Lagged out)	42
>0–646 ppm-days	17
647–2,779 ppm-days	16
2,780–12,321 ppm-days	15
>12,321 ppm-days	12

[a]Mean exposures for females with a 20-year lag for the categorical exposure quartiles were 276, 1,453, 5,869, and 26,391 ppm × days. Median values were 250, 1,340, 5,300, and 26,676 ppm × days. These values are for the risk sets but should provide a good approximation to the full cohort values.

D.2.2. Modeling of Breast Cancer Mortality Data Using a Variety of Models

D.2.2.1. *Cox Regression (Log RR) Models*

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2004). Age was the time variable in proportional hazards (Cox) regression. For breast cancer mortality, only exposure variables were included in models. Cases and controls were matched on sex (all female), date of birth, and race.

Using log RR models, we used a categorical model, a (log-)linear model, a two-piece (log-)linear model, a log-transform model, and a cubic spline model. We also ran a number of analogous models using linear RR models (see Section D.2.2.2 below).

The categorical log RR model for breast cancer mortality was run using the originally published cutpoints to form four categories above the lagged-out group, as shown in Table D-19. To graph the categorical points, each category was assigned the midpoint of the category as its exposure level, except for the last one which was assigned 50% more than the last cutpoint 12,322 ppm-days.

For the two-piece log-linear model, the single knot was chosen at 700 ppm-days based on a comparison of likelihoods assessed every 100 ppm-days from 0 to 7,000 (see Figure D-9). We also explored knots beyond 7,000 ppm-days by looking at increments of 1,000 ppm-days from 0 to 25,000 (see Figure D-10 shows the results for knots up to 15,000 ppm-days). None of these outperformed the knot at 700 ppm-days, although Figure D-10 suggests a local maximum likelihood near 13,000 ppm-days.

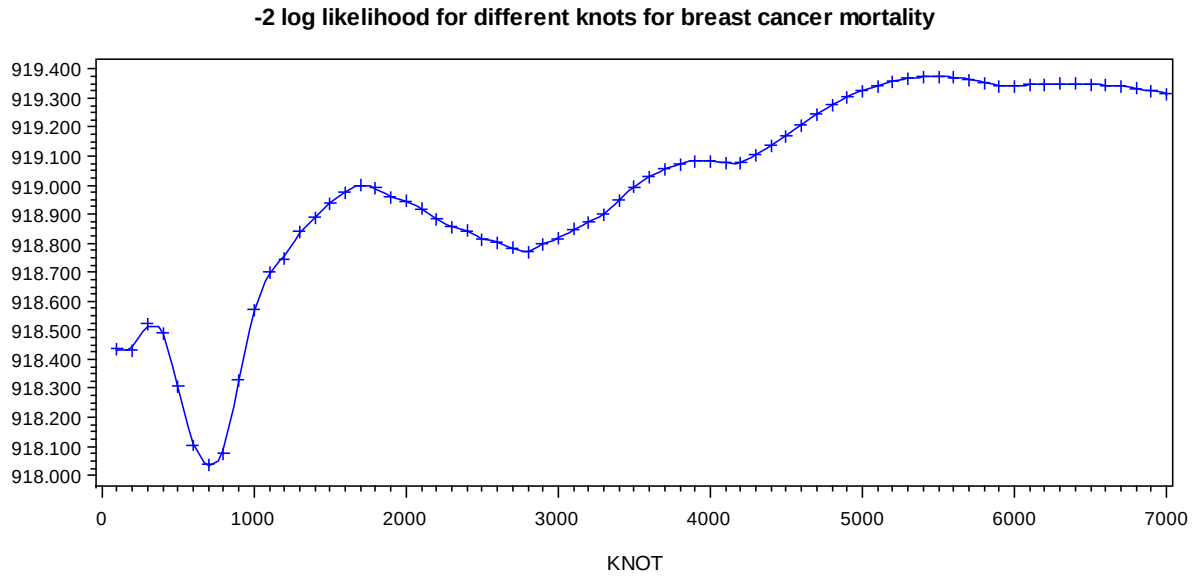


Figure D-9. Likelihoods vs. knots for the two-piece log-linear model, breast cancer mortality.

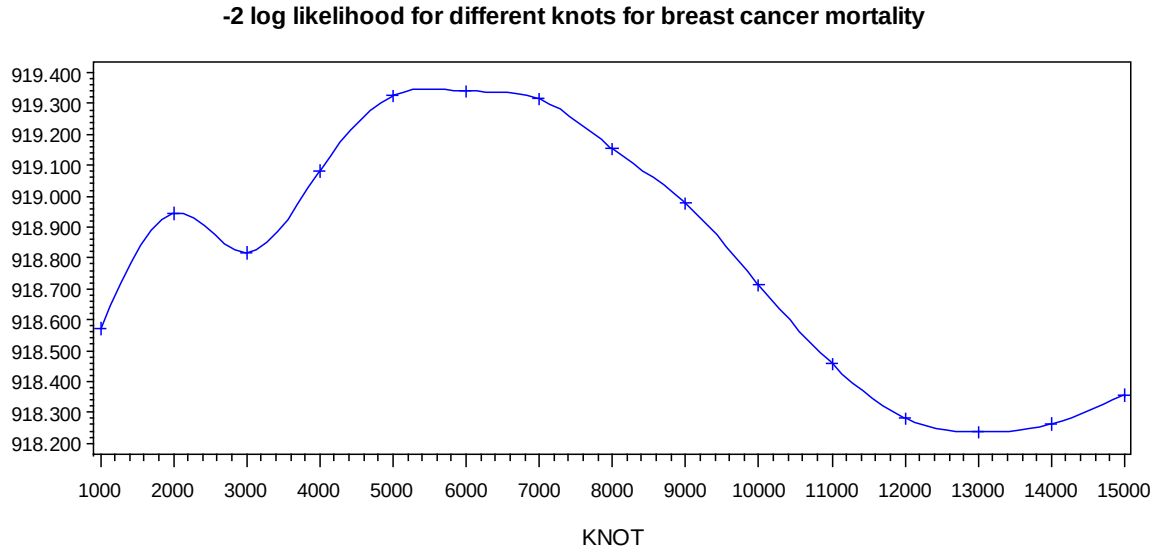


Figure D-10. Likelihoods vs. knots for the two-piece log-linear model, breast cancer mortality, up to 15,000 ppm-days.

In Figure D-11 below, we show the categorical and two-piece log-linear spline models, as well as the log-linear model and the log-linear model after cutting out the top 5% of exposed subjects.

The log-linear model was clearly highly sensitive to exclusion of the most highly exposed. As a sensitivity analysis, we excluded 1, 2.5, 5, and 10% of the upper tail of exposure. The 5% cutoff was at 15,000 ppm-days, while the 10% cutoff was at 13,000 ppm-days. The slope of the linear exposure-response relationship increased by 1.2, 1.6, 5.9, and 4.5 times, respectively, with the exclusion of progressively more data. It would appear that the upper 5% of the exposure range most affects the linear slope and is responsible for the attenuation seen in the exposure-response at high exposures.

The two-piece log-linear spline model in Figure D-11 fits reasonably well but appears to underestimate the categorical RRs at higher exposures. This may be due to the influence of the top 5% of the exposed, which appear to have a strong attenuating influence on the slope (see below).

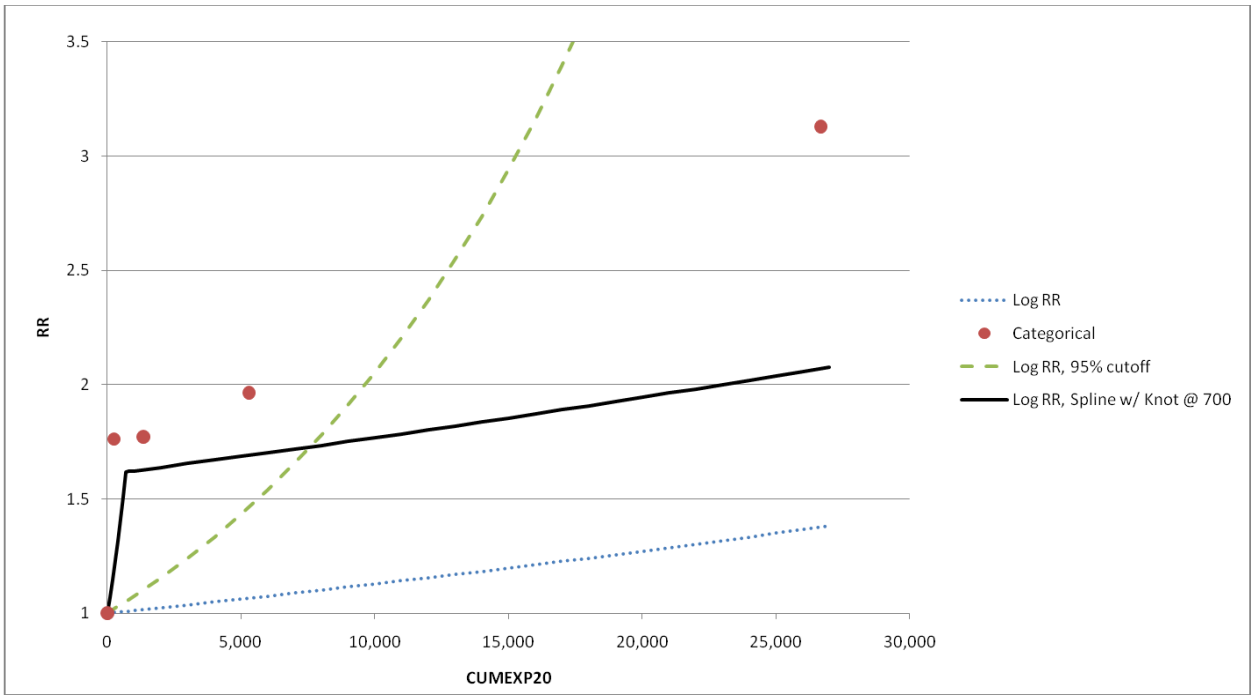


Figure D-11. Dose-response models for breast cancer mortality.

Plot of the dose-response relationship of continuous exposure (lagged 20 years) for breast cancer mortality, with the two-piece log-linear spline, the categorical, and the log-linear RR models (labeled "log RR"). Also shown is the log-linear curve (log RR = β × cumexp20) after cutting out the top 5% of exposure subjects (log RR 95% cutoff). Dots represent the categorical results (quartiles).

For comparison purposes, we also show the logarithmic transformation log RR model in Figure D-12 (which we have not used for risk assessment because it is supralinear in the low dose region).

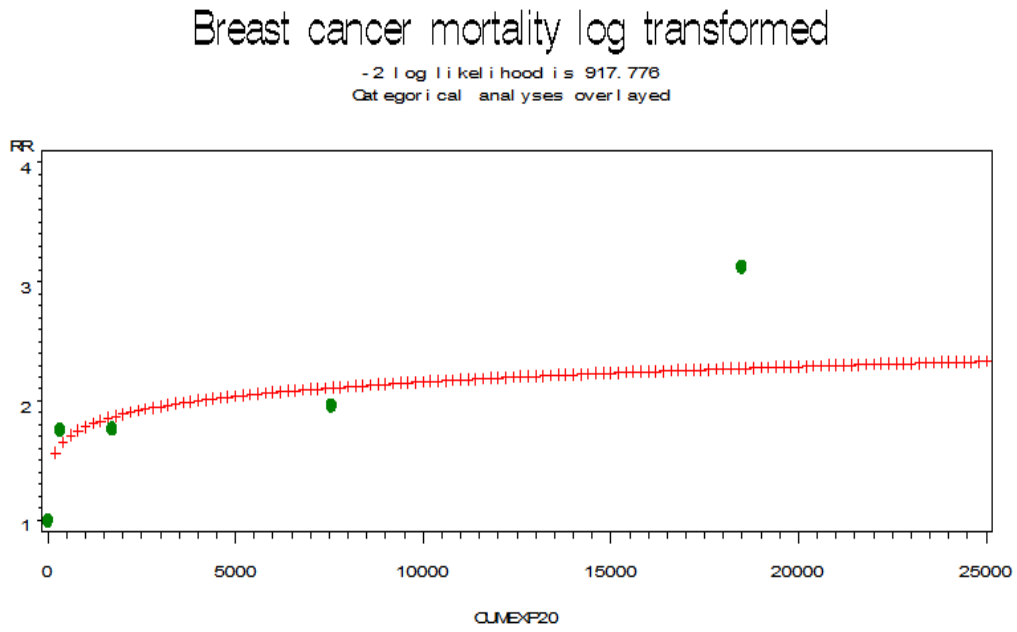


Figure D-12. Breast cancer mortality—log-linear model with log cumulative exposure.

Plot of the dose-response relationship of continuous exposure (lagged 20 years) for breast cancer mortality, using a logarithmic transformation log RR model. Dots represent the categorical results (quartiles).

Outputs from the categorical, two-piece log-linear spline, and log-linear RR models are given below in Tables D-20 to D-24. The two-piece log-linear model performed similarly to the log-linear model but appeared to fit the categorical log RR model points and the cubic spline log RR model much better. The log-linear spline model is at the border of statistical significance ($p = 0.07$). In any case, models with relatively sparse data may not achieve conventional statistical significance (at the 0.05 level) but still provide a good fit to the data, as judged by conformity with categorical and cubic spline analysis, and may still be useful for risk assessment.

Table D-20. Categorical output breast cancer mortality, 20-year lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
−2 LOG L	923.433	915.509				
AIC	923.433	923.509				
SBC	923.433	934.009				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	7.9244	4	0.0944			
Score	8.5160	4	0.0744			
Wald	8.3993	4	0.0780			
Analysis of maximum likelihood estimates						
Variable	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUM201	1	0.56653	0.33920	2.7894	0.0949	1.762
CUM202	1	0.57236	0.35505	2.5987	0.1070	1.772
CUM203	1	0.67537	0.37632	3.2207	0.0727	1.965
CUM204	1	1.14110	0.40446	7.9598	0.0048	3.130

Table D-21. Two-piece log-linear spline, breast cancer mortality, 20-year lag, knot at 700 ppm-days

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
−2 LOG L	923.433	918.037			
AIC	923.433	922.037			
SBC	923.433	927.287			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood	5.3967	2	0.0673		
Score	6.0153	2	0.0494		
Wald	5.8857	2	0.0527		
Anlysis of maximum likelihood estimates					
Parameter	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LIN_0	0.0006877	0.0004171	2.7178	0.0992	1.001
LIN_1	−0.0006782	0.0004188	2.6229	0.1053	0.999

[a]Covariance lin0 and lin1: -1.75 x 10^{-7}

Table D-22. Log-linear model, breast cancer mortality, 20-year lag

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
−2 LOG L	923.433	920.647			
AIC	923.433	922.647			
SBC	923.433	925.272			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	2.7865	1	0.0951		
Score	3.7383	1	0.0532		
Wald	3.6046	1	0.0576		
Analysis of maximum likelihood estimates					
Variable	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUMEXP20	0.0000122	6.40812E-6	3.6046	0.0576	1.000

Table D-23. Log-transform log RR model, breast cancer mortality, 20-year lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
−2 LOG L	923.433	917.743				
AIC	923.433	919.743				
SBC	923.433	922.368				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	5.6908	1	0.0171			
Score	5.7676	1	0.0163			
Wald	5.7688	1	0.0163			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LCUM20	1	0.08376	0.03487	5.7688	0.0163	1.087

Table D-24. Two-piece log-linear spline model, breast cancer mortality, 20-year lag, knot at 13,000 ppm-days

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
−2 LOG L	923.433	918.237			
AIC	923.433	922.237			
SBC	923.433	927.487			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	5.1963	2	0.0744		
Score	5.9044	2	0.0522		
Wald	5.7813	2	0.0555		
Analysis of maximum likelihood estimates					
Variable	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LIN_0	0.0000607	0.0000309	3.8539	0.0496	1.000
LIN_1	−0.0000583	0.0000371	2.4761	0.1156	1.000

D.2.2.2. *Linear Relative Risk Models for Breast Cancer Mortality*

Finally, we also ran linear RR models for these data, as shown in Figure D-13 below (denoted "ERR" models), which also includes the RRs from the log RR categorical model as shown in other graphs. Again, the linear curve, highly influenced by the upper 5% tail of exposure, underestimates the categorical points, while the log transform and two-piece spline capture better the initial increase in risk followed by an attenuation. Parameter estimates for these models can be found in Table D-25.

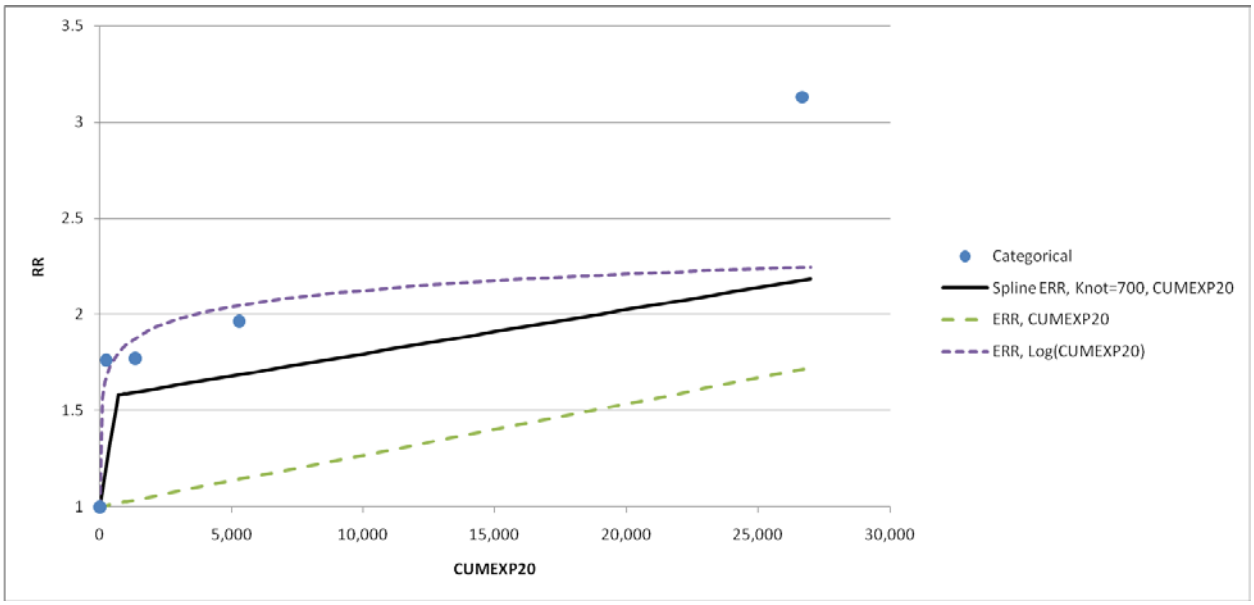


Figure D-13. Linear RR models for breast cancer mortality.

[Editorial note: "ERR" refers to linear RR models.]

Table D-25. Model results for breast cancer mortality, linear RR models[b]

Linear RR model	Parameter(s)	SE	–2 Log-likelihood
CUMEXP20	B = 2.6779×10^{-5}	SE = 2.1537×10^{-5}	920.122
Log(CUMEXP20)	B = 0.122090	SE = 0.061659	917.841
Spline, knot = 700, CUMEXP20[a]	B1 = 8.30×10^{-4}, B2 = -8.07×10^{-4}	SE1 = 6.14×10^{-4}, SE2 = 6.19×10^{-4}	918.058

SE = standard error.
[a]Covariance 2 pieces of spline: -3.80×10^{-7}.
[b]Editorial note: Confidence intervals were determined using the Wald approach. Confidence intervals for linear RR models, however, in contrast to those for the log-linear RR models, may not be symmetrical. For breast cancer incidence, the EPA used the profile likelihood approach for the linear RR models (Langholz and Richardson, 2010), which allows for asymmetric CIs. The unit risk estimate for breast cancer mortality presented in this assessment does not rely on any of the linear RR models, thus CIs calculated using the profile likelihood method are not shown here.

D.3. LYMPHOID CANCER MORTALITY (SUBSET OF ALL HEMATOPOIETIC CANCERS COMBINED) (*n* = 17,530).

D.3.1. Exposure Distribution in Cohort and among Lymphoid Cases in the Cohort Mortality Study

The estimated daily exposure to EtO across different jobs and time periods ranged from 0.05 to 77 ppm. Exposure intensities from this broad range were multiplied by the length of time in different jobs to get estimates of cumulative exposure. The duration of exposure for the full cohort at the end of follow-up had a mean of 8.7 years and a standard deviation of 9.3 years. Cumulative exposure at the end of follow-up, with no lag, had a mean of 27 ppm-years and a median of 6 ppm-years, indicating that these data are highly skewed. Log transformation of these data results in an approximately normal distribution of the data. For additional details about the exposure and other characteristics of the full cohort and the lymphoid cancer risk sets, see Section D.5 of Appendix D.

As noted in Section D.1.1, cumulative exposure at the end of follow-up may be misleading, as it is not relevant to standard analyses, all of which treat cumulative exposure as a time-dependent variable which must be assessed at specific points in time. See Section D.1.1 for more discussion.

In modeling lymphoid cancer, a subset of all (lympho)hematopoietic cancer, we used a 15-year lag for cumulative exposure as in the prior publication (Steenland et al., 2004), and we also used the same cutpoints as in the publication. Lymphoid cancer consists of non-Hodgkin lymphoma, lymphocytic leukemia, and myeloma (ICD-9 200, 202, 203, 204). The distribution of cases for lymphoid cancer mortality is presented in Table D-26.

Table D-26. Exposure categories and case distribution for lymphoid cancer mortality

Cumulative exposure, 15-year lag[a]	Male lymphoid cancer deaths	Female lymphoid cancer deaths	Total lymphoid cancer deaths
0 (lagged out)	6	3	9
>0–1,200 ppm-days	2	8	10
1,201–3,680 ppm-days	4	7	11
3,681–13,500 ppm-days	5	5	10
>13,500 ppm-days	10	3	13

[a]The means of the categories were 0, 446, 2,143, 7,335, and 39,927 ppm-days, respectively. The medians were 374, 1,985, 6,755, and 26,373 ppm-days, respectively. These values are for the full cohort.

D.3.2. Lag Selection for the Lymphoid Cancer Mortality Data

After the SAB review of the 2014 draft assessment, the issue of lag selection was revisited. Table D-27 provides −2 log-likelihood results comparing different models with different lags. Table D-27 also presents the AIC values for the same models to facilitate comparison with the two-piece spline models, which include an extra parameter. [The knot is preselected and is not considered a parameter in these analyses, consistent with the SAB's concept of parsimony (SAB, 2015)].[14]

Of the nonspline models (i.e., linear and log-linear cumulative and log cumulative exposure models), only the models with log cumulative exposure and a 15-year lag were statistically significant ($p = 0.02$ for both the linear and log-linear RR models). For the four spline model options—log-linear or linear, with the knots at the global maximum likelihood or the local maximum likelihood—the lowest –2 log likelihoods (and AICs) occur with a lag of 15 years in three of the cases. For the log-linear spline model with the knot at the global maximum likelihood, the lowest –2 log likelihood (and AIC) occurs with no lag, which is not biologically likely. The next lowest –2 log likelihood (and AIC) occurs with a lag of 15 years, and the AIC is within 2 AIC units of the lowest value, suggesting a negligible difference in fit. Thus, for consistency in comparisons and to optimize the best fitting lag overall, a lag of 15 years was selected for analyzing the lymphoid cancer mortality data. Selecting the lag time based on the strongest associations is a common statistical approach (Checkoway et al., 2004). A lag of 15 years is somewhat long for a lymphohematopoietic cancer, but within the range of plausible values, especially for mortality, as opposed to incidence. Sensitivity of the results to choice of lag time is examined in Section D.3.5 below.

[14] "in some settings the principle of parsimony may suggest that the most informative analysis will rely upon fixing some parameters rather than estimating them from the data. The impact of the fixed parameter choices can be evaluated in sensitivity analyses. In the draft assessment, fixing the knot when estimating linear spline model fits from relative risk regressions is one such example" [page 12 of SAB (2015)].

Table D-27. Minus 2 log-likelihood results and AICs for different models and different exposure lag times

Minus twice LL	LAG					To get AIC
	0.0	5.0	10.0	15.0	20.0	
LOG-LINEAR MODELS						
CUMEXP	460.8	460.8	461.6	462.4	463.6	add 2
LCUMEXP	462.0	463.5	463.0	458.4	461.6	add 2
2-PIECE	456.4	460.5	460.9	457.8	461.1	add 4
knot[a]	100	1,575	1,600	125	125	
2-PIECE	459.2			458.6	461.8	add 4
alt knot[b]	775			1,600	1,600	
LINEAR MODELS						
CUMEXP	460.8	460.8	460.9	461.2	463.1	add 2
LCUMEXP	461.4	463.2	462.8	458.2	461.2	add 2
2-PIECE	458.9	460.6	460.5	457.3	460.8	add 4
knot[a]	125	1,575	1,600	125	125	
2-PIECE	459.3			458.1	461.4	add 4
alt. knot[b]	775			1,600	1,600	
NULL LOG-LINEAR MODELS[c]		463.9				
NULL LINEAR MODELS[c]		463.5				

AIC	LAG				
	0.0	5.0	10.0	15.0	20.0
LOG-LINEAR EXPOSURE MODELS					
CUMEXP	462.8	462.8	463.6	464.4	465.6
LCUMEXP	464.0	465.5	465.0	460.4	463.6
2-PIECE	460.4	464.5	464.9	461.8	465.1
2-PIECE (alt. knot)	463.2			462.6	465.8
LINEAR EXPOSURE MODELS					
CUMEXP	462.8	462.8	462.9	463.2	465.1
LCUMEXP	463.4	465.2	464.8	460.2	463.2
2-PIECE	462.9	464.6	464.5	461.3	464.8
2-PIECE (alt. knot)	463.3			462.1	465.4

[a]knots were obtained by doing a grid search by increments of 100 ppm x days and then interpolating where appropriate.
[b]For models with very low knots, alternate knots were obtained from local maximum likelihoods because of the small number of cases informing the slope of the low-exposure spline for low knots (see Figure D-14).
[c]The log-linear and linear models were obtained using different SAS procedures which gave different -2LL results for the null model.

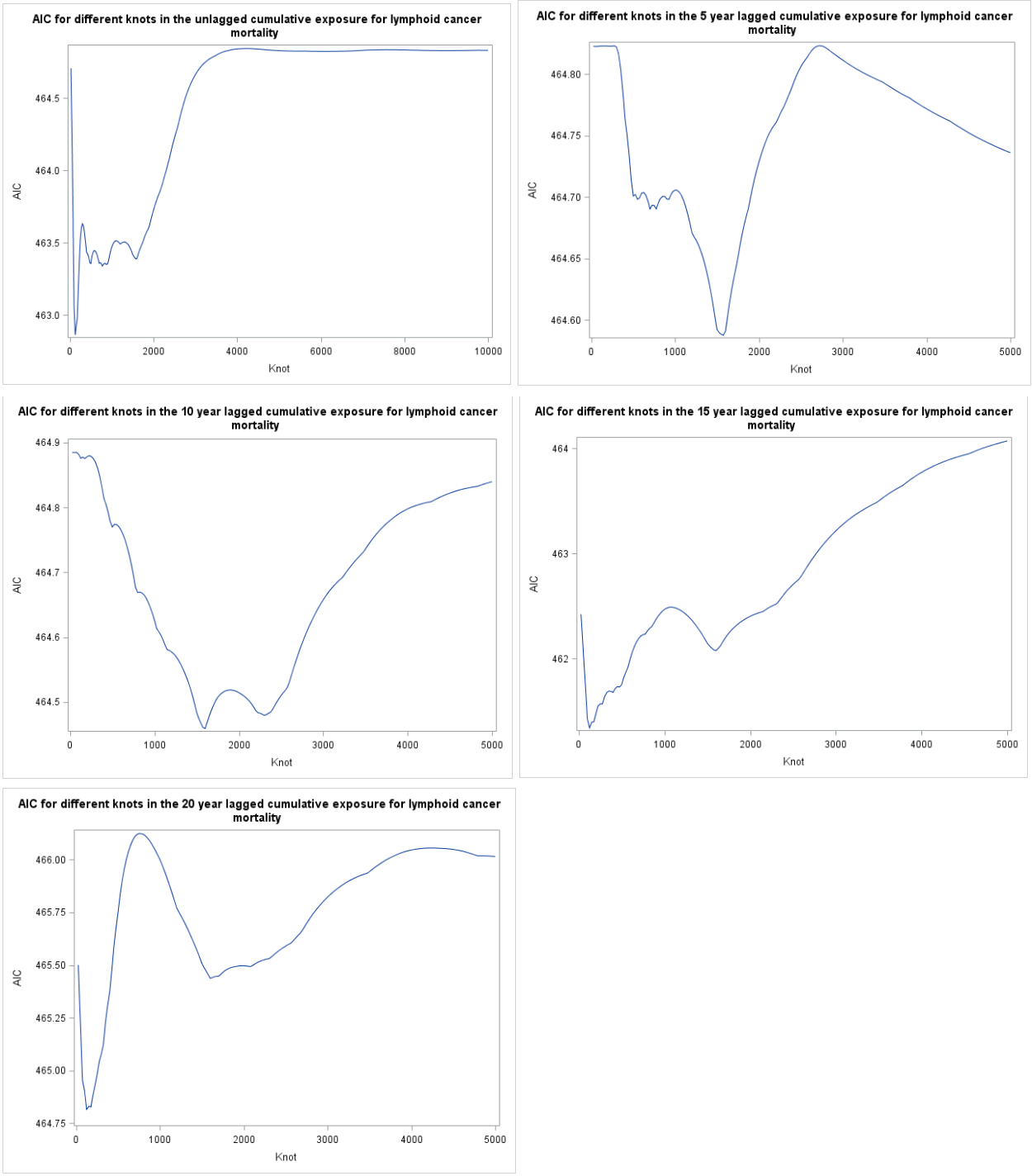


Figure D-14. AIC vs. knot for different lag periods for two-piece linear spline models.

(Graphs for the two-piece log-linear spline models were visually indistinguishable from these graphs for the linear spline models.)

D.3.3. Modeling of Lymphoid Cancer Mortality Data Using a Variety of Models

D.3.3.1. *Cox Regression (Log RR) Models*

While the published results in Steenland et al. (2004) focused on males [Table 7 in Steenland et al. (2004)], males and females in fact do not differ greatly in categorical results using a 15-year lag. A formal chunk test (Kleinbaum, 1994) for four interaction terms between exposure and sex is not close to significance ($p = 0.58$), although such tests are not very powerful in the face of sparse data such as these. Table D-28 below shows the categorical odds ratio results for men and women separately and combined. In the analyses presented here, males and females are combined.

Table D-28. Lymphoid cancer mortality results by sex

Cumulative exposure, 15-year lag	Odds ratios (95% CI) males	Odds ratios (95% CI) females	Odds ratios (95% CI) combined
0 (lagged out)	1.00	1.00	1.00
>0–1,200 ppm-days	0.91 (0.16–5.23)	2.25 (0.41–12.45)	1.75 (0.59–5.25)
1,201–3,680 ppm-days	2.89 (0.65–12.86)	3.26 (0.56–18.98)	3.15 (1.04–9.49)
3,681–13,500 ppm-days	2.71 (0.65–11.55)	2.16 (0.34–13.59)	2.44 (0.80–7.50)
>13,500 ppm-days	3.76 (1.03–13.64)	1.83 (0.25–13.40)	3.00 (1.02–8.45)

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2004). Age was the time variable in proportional hazards (Cox) regression. For lymphoid cancer mortality, only exposure variables were included in the model. Cases and controls were within risk sets matched on age, sex, and race.

Using log RR models, we used a categorical model, a (log-)linear model, a two-piece (log-)linear model, and a log-transform model. We also ran a number of analogous models using linear RR models (see Section D.3.3.2 below).

The categorical model for lymphoid cancer mortality was run using the originally published cutpoints to form four categories above the lagged-out group, as shown in Table D-28. To graph the categorical points, each category was assigned the midpoint of the category as its exposure level, except for the last one which was assigned 50% more than the last cutpoint.

For the two-piece log-linear model, the single knot was chosen at 100 ppm-days based on a comparison of likelihoods assessed every 100 ppm-day from 100 to 15,000. The best likelihood was at 100 ppm-days. Figure D-15 below shows the likelihood versus the knots. Figure D-15 also suggests a local maximum likelihood near 1,600 ppm-days.

Model results for the categorical and two-piece linear log RR models are shown in Tables D-29 and D-30. Tables D-31 and D-32 give the results for the log-transform model and linear log RR models; the latter does not fit the data well. Table D-33 shows the model results for the two-piece log-linear spine model with the knot at the local maximum likelihood of 1,600 ppm-days.

Figure D-16 shows the graphical results for the categorical, (log-)linear, two-piece (log-) linear, and log-transform log RR models. There is a very steep increase in risk at very low exposures. The knot for the two-piece log-linear curve is a low 100 ppm-days. The steep slope at low exposures may be unrealistic as a basis for risk assessment, dependent as it is on relatively sparse data in the low-exposure region. Table D-34 lists the cumulative exposures with a 15-year lag for all the lymphoid cancer cases (e.g., there are no cases below the knot of 100 ppm-days).

We further explored the sensitivity of the log-linear (standard Cox regression) model to high exposures, by excluding progressively more of the upper tail of exposure. We excluded 5, 10, 20, 30, 40, and 55% of the upper tail of exposure. The 55% cutoff was at 2,000 ppm-days. The slope of the log-linear exposure-response model increased by 0.4, 1.7, 7.9, 5.6, 26.7, and 113.7 times, respectively, with the exclusion of progressively more data. It is clear that the curve changes substantially once the top 20% of the exposure range is truncated.

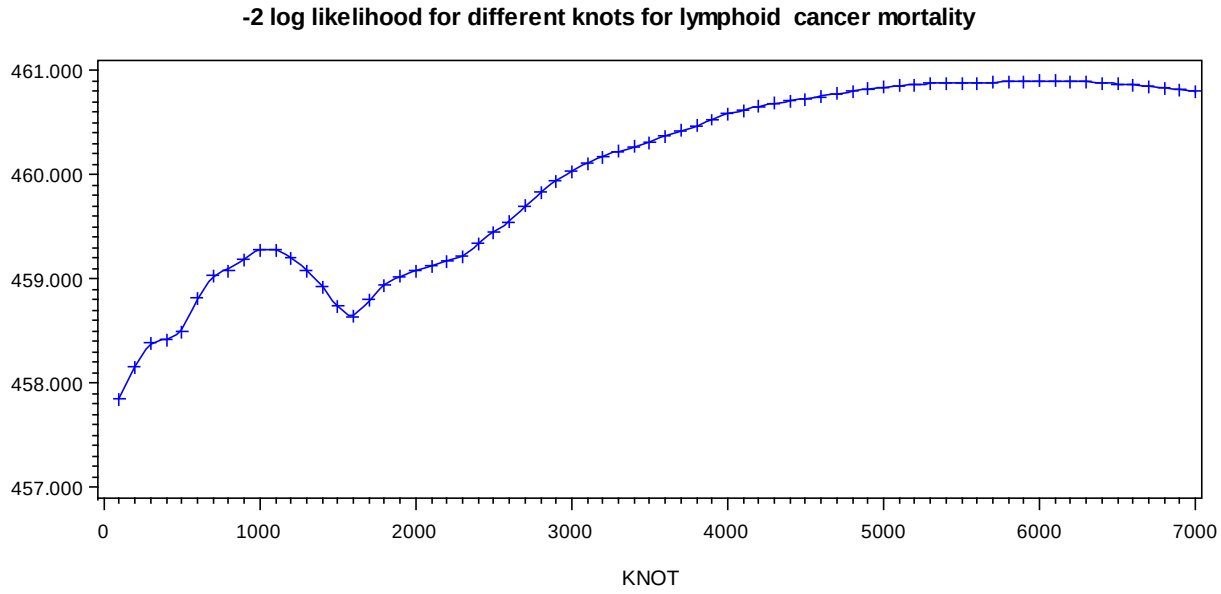


Figure D-15. Likelihoods vs. knots for two-piece log-linear model, lymphoid cancer mortality.

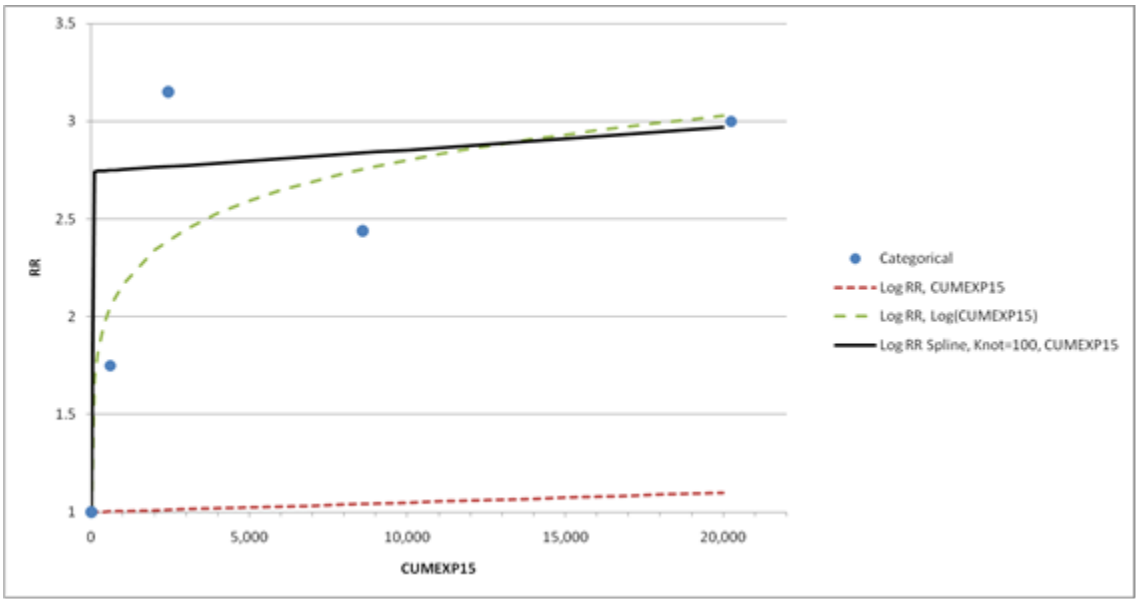


Figure D-16. Exposure-response models for lymphoid cancer mortality.

Plot of continuous exposure (with 15-year lag) and lymphoid cancer mortality rate ratios estimated using the two-piece log-linear spline model with the knot at 100 ppm-days overlaid with other log RR curves and categorical (quartile) points.

Table D-29. Categorical results for lymphoid cancer mortality, men and women combined

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	463.912	458.069				
AIC	463.912	458.069				
SBC	463.912	473.950				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	5.8435	4	0.2111			
Score	5.7397	4	0.2195			
Wald	5.6220	4	0.2292			
Analysis of maximum likelihood estimates						
Variable[a]	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUM151	1	0.56036	0.55981	1.0020	0.3168	1.75
CUM152	1	1.14581	0.56351	4.1344	0.0420	3.15
CUM153	1	0.89001	0.57391	2.4049	0.1210	2.44
CUM154	1	1.09998	0.55112	3.9837	0.0459	3.00

[a]Categorical exposure groups are quartiles of cumulative exposure with 15-year lag; from Table D-26 the exposure ranges are >0–1,200, 1,201–3,680, 3,681–13,500, and >13,500 ppm-days.

Table D-30. Results of two-piece log-linear spline model for lymphoid cancer mortality, men and women combined, knot at 100 ppm-days

Model fit statistics					
Criterion	**Without covariates**	**With covariates**			
-2 LOG L	463.912	457.847			
AIC	463.912	461.847			
SBC	463.912	465.787			
Testing global null hypothesis: BETA = 0					
Test	**χ^2**	**DF**	**Pr > ChiSq**		
Likelihood ratio	6.0658	2	0.0482		
Score	5.9648	2	0.0507		
Wald	5.8246	2	0.0544		
Analysis of maximum likelihood estimates					
Parameter	**Parameter estimates**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LIN_0	0.01010	0.00493	4.1997	0.0404	1.010
LIN_1	-0.01010	0.00493	4.1959	0.0405	0.990

Table D-31. Results of the log-transform log RR model for lymphoid cancer mortality, both sexes combined

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	463.912	458.426				
AIC	463.912	460.426				
SBC	463.912	462.396				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	5.4868	1	0.0192			
Score	5.3479	1	0.0207			
Wald	5.2936	1	0.0214			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LCUM15	1	0.11184	0.04861	5.2936	0.0214	1.118

Table D-32. Results of the log-linear model for lymphoid cancer mortality, both sexes combined

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	463.912	462.413				
AIC	463.912	464.413				
SBC	463.912	466.383				
Testing global null hypothesis: BETA = 0						
Teset	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	1.4998	1	0.2207			
Score	2.0403	1	0.1532			
Wald	1.9959	1	0.1577			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUMEXP15	1	4.7367E-6	3.35285E-6	1.9959	0.1577	1.000

Table D-33. Results of two-piece log-linear spline model for lymphoid cancer mortality, men and women combined, knot at 1,600 ppm-days

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
2- LOG L	463.912	458.640				
AIC	463.912	462.640				
SBC	463.912	466.581				
Testing global null hypothesis: BETA = 0						
Criterion	**Without covariates**	**With covariates**				
Likelihood ratio	5.2722	2	0.0716			
Score	5.2666	2	0.0718			
Wald	5.1436	2	0.0764			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LIN_0	1	0.0004893	0.0002554	3.6713	0.0554	1.000
LIN_1	1	0.0004864	0.0002563	3.6014	0.0577	1.000

Table D-34. Distribution of cumulative exposures with a 15-year lag for the lymphoid cancer deaths

CUMEXP15 (ppm-days)	Frequency	Percent	Cumulative frequency	Cumulative percent
0	9	16.98	9	16.98
100.063	1	1.89	10	18.87
130.644	1	1.89	11	20.75
181.819	1	1.89	12	22.64
272.09525	1	1.89	13	24.53
395.421	1	1.89	14	26.42
485.994	1	1.89	15	28.30
493.608	1	1.89	16	30.19
568.53575	1	1.89	17	32.08
777.045	1	1.89	18	33.96
860.77075	1	1.89	19	35.85
1,506.756	1	1.89	20	37.74
1,566.99	1	1.89	21	39.62
1,597.44	1	1.89	22	41.51
1,603.636	1	1.89	23	43.40
1,646.75225	1	1.89	24	45.28
2,147.01925	1	1.89	25	47.17
2,307.05	1	1.89	26	49.06
2,318.89425	1	1.89	27	50.94
2,567.721	1	1.89	28	52.83
2,592.742	1	1.89	29	54.72
3,478.642	1	1.89	30	56.60
3,776.718	1	1.89	31	58.49
4,556.3585	1	1.89	32	60.38
5,643.896	1	1.89	33	62.26
6,981.06375	1	1.89	34	64.15
7,127.132	1	1.89	35	66.04
7,549.875	1	1.89	36	67.92
10,485.87	1	1.89	37	69.81
11,127.772	1	1.89	38	71.70
12,279.195	1	1.89	39	73.58
13,498.377	1	1.89	40	75.47
15,696.735	1	1.89	41	77.36
17,507.77125	1	1.89	42	79.25
18,294.186	1	1.89	43	81.13
18,702.43425	1	1.89	44	83.025
23,611.25325	1	1.89	45	84.91

Table D-34. Distribution of cumulative exposures with a 15-year lag for the lymphoid cancer deaths (continued)

CUMEXP15 (ppm-days)	Frequency	Percent	Cumulative frequency	Cumulative percent
35,839.34525	1	1.89	46	86.79
43,955.86	1	1.89	47	88.68
49,101.02825	1	1.89	48	90.57
55,334.747	1	1.89	49	92.45
74,666.586	1	1.89	50	94.34
126,761.401	1	1.89	51	96.23
128,092.08075	1	1.89	52	98.11
146,460.07075	1	1.89	53	100.00

After the SAB review of the 2014 draft assessment, Steenland also provided modeling results for models with a square-root transformation of cumulative exposure. Results for the log-linear model with square root of exposure are presented in Table D-35.

Table D-35. Model fit statistics and coefficients for log-linear RR model with square-root of cumulative exposure, with a 15-year lag, lymphoid cancer mortality

Log-linear RR model	–2 Log-likelihood (full model)	AIC	*p*-value[a]	Parameter(s)	SE
sqrt(CUMEXP15)	460.8	462.8	0.08	$B = 2.83 \times 10^{-3}$	$SE = 1.5 \times 10^{-3}$

SE = standard error.
[a]From likelihood ratio test.

D.3.3.2. *Linear Relative Risk Models*

Table D-36 shows the model fit statistics and coefficients for the linear RR models. Results for linear RR models are seen in Figure D-18 (denoted as "ERR" models). They are quite similar to the log RR results in Figure D-16. Again there is a very steep rise in the exposure-response curve at very low exposures. The knot for the two-piece linear curve is again at 100 ppm-days.

Table D-36. Model fit statistics and coefficients for linear RR models, lymphoid cancer mortality

Linear RR model	–2 Log-likelihood (full model)	AIC	*p*-value[a]	Parameter(s)	Profile likelihood 95% one-sided confidence bounds
CUMEXP15	461.2	463.2	0.13	B = 1.227×10^{-5}	LB = -2.2×10^{-6} UB = 4.71×10^{-5}
Log(CUMEXP15)	458.2	460.2	0.02	B = 0.2083	LB = 0.0183 UB = 0.768
Sqrt(CUMEXP15)	459.8	461.8	0.053	B = 6.14×10^{-3}	NR[b]
Spline, knot = 100, CUMEXP15[c,d]	457.4	461.4	0.046	B1 = 0.015198 B2 = –0.015179	LB1 = 1.056×10^{-5} UB1 = 0.05901
Spline, knot = 1,600, CUMEXP15[c,d,e]	458.1	462.1	0.07	B1 = 7.58×10^{-4} B2 = -7.48×10^{-4}	LB1 = 4.52×10^{-6} UB1 = 2.983×10^{-3}

[a]From likelihood ratio test.

[b]Not reported: Confidence intervals for linear RR models, in contrast to those for the log-linear RR models, may not be symmetrical. The EPA did not apply the profile likelihood approach (Langholz and Richardson, 2010), which allows for asymmetric CIs, to develop CIs for this model because the model was not used further in the assessment.

[c]For estimating risks from occupational exposures (see Section 4.7 of the Carcinogenicity Assessment Document), both pieces of the two-piece linear spline model are used. For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × [exp – knot]). For the (one-sided) 95% upper confidence limit, the Wald approach is used as an approximation because it was not possible to obtain a formula for the profile likelihood upper-bound estimates that could be used in the life-table analysis. Thus, for exposures below the knot, RRu = 1 + ([B1 + 1.645 × SE1] × exp); for exposures above the knot, RRu = 1 + (B1 × exp + B2 × [exp-knot] + 1.645 × sqrt[$\text{exp}^2 \times \text{var1} + [\text{exp-knot}]^2 \times \text{var2} + 2 \times \text{exp} \times [\text{exp-knot}] \times \text{covar}$]), where exp = cumulative exposure, var = variance, covar = covariance. As shown in Figure D-17, the Wald upper-bound estimates are about half-way between the MLE RR estimates and the profile likelihood upper-bound estimates. In the range of occupational exposures of interest (i.e., up to 12, 775 ppm × days) the Wald-based RRu estimates are about 67% of the profile-likelihood-based RRu estimates.

[d]Calculating the profile likelihood bounds is computationally difficult and estimating the bounds for B2 was not pursued here.

Var1 = $SE1^2 = (6.32 \times 10^{-4})^2 = 3.99 \times 10^{-7}$; Var2 = $SE2^2 = (6.31 \times 10^{-4})^2 = 3.98 \times 10^{-7}$; Covariance = -3.99×10^{-7}.

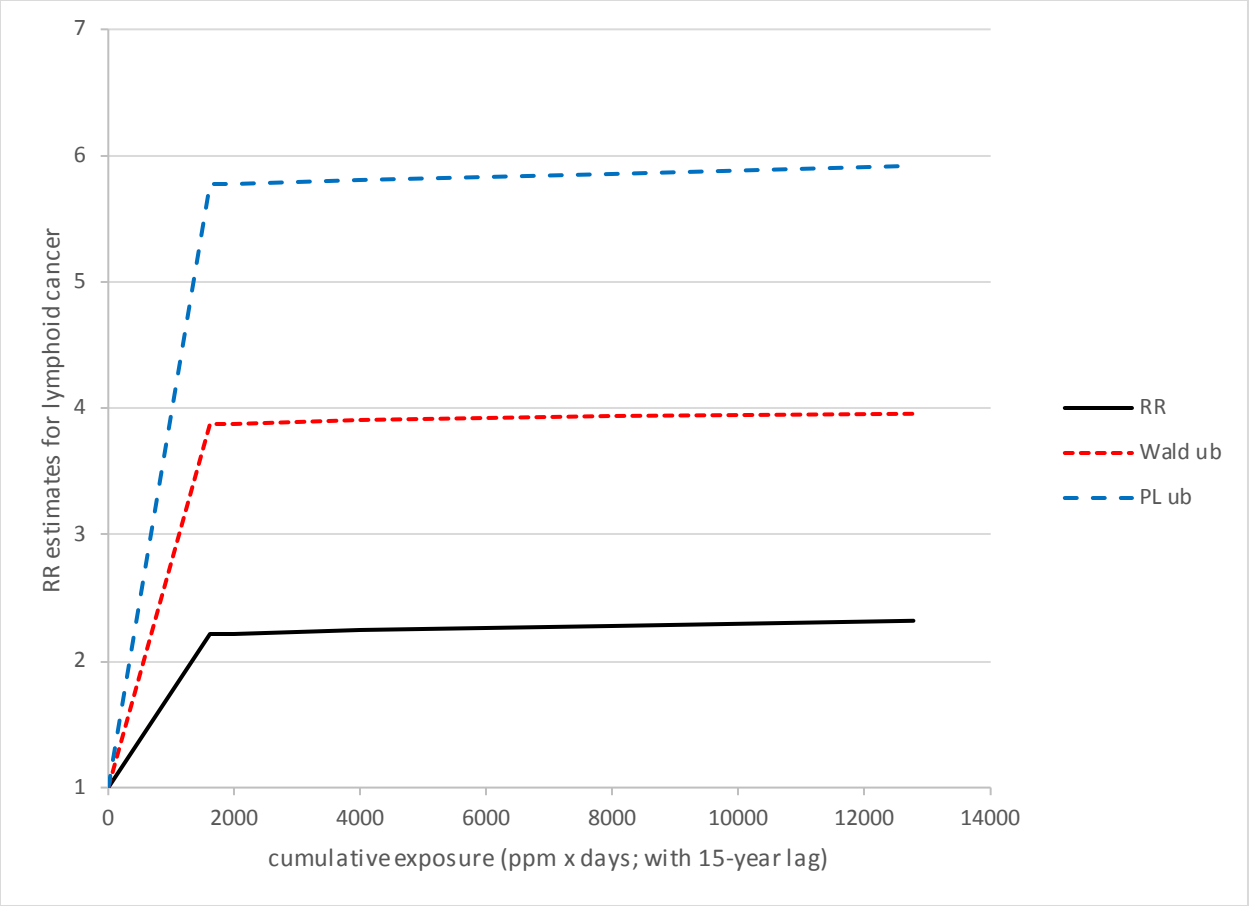


Figure D-17. Comparison of Wald and profile likelihood (one-sided) 95% upper-bound estimates for two-piece linear spline model.

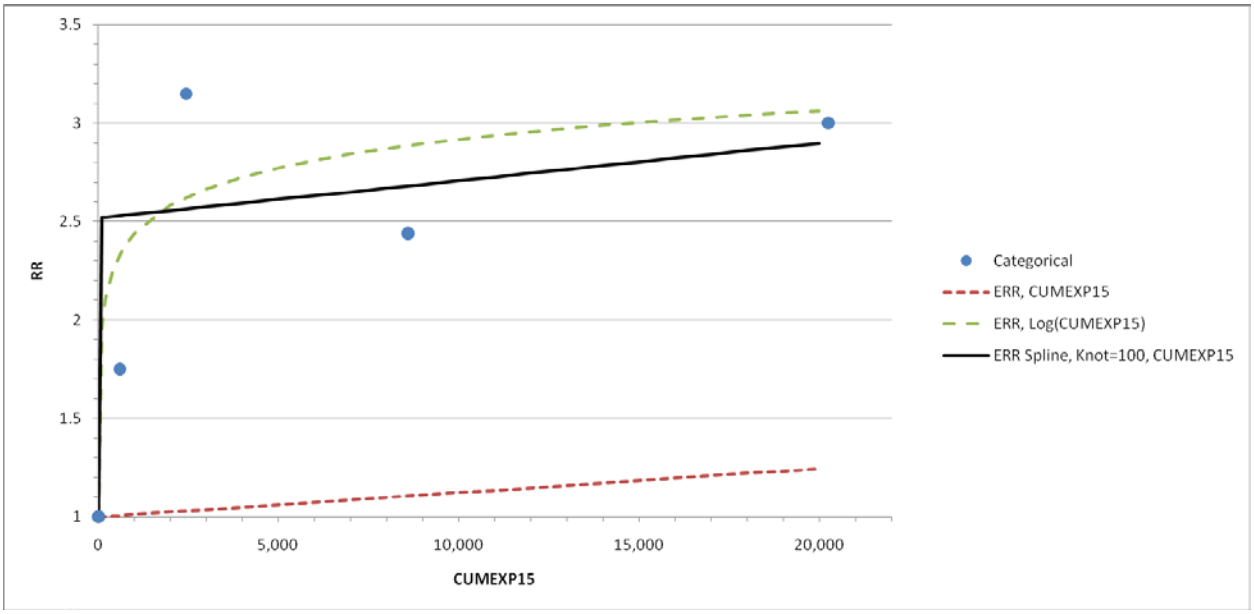


Figure D-18. Linear RR models for lymphoid cancer.

[Editorial note: "ERR" refers to linear RR models.]

D.3.4. Supplemental Results: Results for Log Cumulative Exposure Cox Regression Model with No Lag

Model fit statistics and parameter coefficients for the log cumulative exposure Cox regression model with no lag are presented in Table D-37.

Table D-37. Results for log cumulative exposure Cox regression model with no lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	463.912	462.014				
AIC	463.912	464.014				
SBC	463.912	465.984				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	1.8987	1	0.1682			
Score	1.8589	1	0.1728			
Wald	1.8530	1	0.1734			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LCUMEXP	1	0.10230	0.07515	1.8530	0.1734	1.108

D.3.5. Sensitivity of (Incidence) Unit Risk Estimates to Change in Lag Period

Sensitivity of the (incidence) unit risk estimates to choice of exposure lag time for the two-piece linear spline model is summarized in Table D-38.

Table D-38. Comparison of unit risk estimates for lymphoid cancer incidence from two-piece linear spline models with different lag periods; cumulative exposure in ppm × days, knot at 1,600 ppm × days

Lag (years)	−2 log-likelihood	Parameter estimate for 1st spline segment (per ppm × day)	Profile likelihood 95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)[a]
0	459.4	5.9×10^{-4}	6.7×10^{-3}	7.47×10^{-3}	6.57×10^{-4}	15.2
5	460.6	1.59×10^{-4}	1.549×10^{-3}	0.0300	3.07×10^{-3}	3.26
10	460.5	2.11×10^{-4}	1.427×10^{-3}	0.0245	3.63×10^{-3}	2.75
15	458.1	7.58×10^{-4}	2.983×10^{-3}	7.48×10^{-3}	1.90×10^{-3}	5.26
20	461.4	4.33×10^{-4}	1.745×10^{-3}	0.0145	3.59×10^{-3}	2.79

[a]Calculated for lymphoid cancer incidence; see Section 4.1.1.3.

The sensitivity analysis for choice of lag reveals that the unit risk estimates for the selected two-piece linear spline model with the knot at 1,600 ppm × days for different lag periods (0, 5, 10, 15, 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). The models for lags of 0, 5, 10, and 20 years all had *p*-values > 0.10 for inclusion of the exposure terms (0.12, 0.23, 0.21, and 0.35, respectively).

D.3.6. Sensitivity of (Incidence) Unit Risk Estimates to Value of Knot

Sensitivity of the (incidence) unit risk estimates to value of knot for the two-piece linear spline model is summarized in Table D-39, with knots of 1,600 ± 1,000 ppm × days.

Table D-39. Comparison of unit risk estimates for lymphoid cancer incidence from two-piece linear spline models with different knot; cumulative exposure in ppm × days, with lag of 15 years

Knot (ppm × days)	−2 log-likelihood	Parameter estimate for 1st spline segment (per ppm × day)	Profile likelihood 95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)[a]
600	458.0	2.26×10^{-3}	9.27×10^{-3}	2.51×10^{-3}	6.11×10^{-4}	16.37
1,600	458.1	7.58×10^{-4}	2.983×10^{-3}	7.48×10^{-3}	1.90×10^{-3}	5.26
2,600	458.8	4.03×10^{-4}	1.61×10^{-3}	0.0141	3.52×10^{-3}	2.84

[a]Calculated for lymphoid cancer incidence; see Section 4.1.1.3.

Unlike with the sensitivity analysis for knot selection for breast cancer incidence (see Section D.1.7), where the knots were at higher values of cumulative exposure, the sensitivity analysis for knot selection in the two-piece linear spline model for lymphoid cancer shows notable differences in the unit risk estimates for knots 1,000 ppm × days below and above the selected knot of 1,600 ppm × days. The unit risk estimates for these alternate knot values are about 3 times greater and 50% lower, respectively, than the unit risk estimate for the selected model (with the knot at 1,600 ppm × days).

D.3.7. Analysis of Age Interaction for the Exposure Terms in the Two-Piece Linear Spline Model

Table D-40 shows the *p*-values for the inclusion of age interaction terms for the spline exposure regression coefficients. The interaction terms have *p*-values well above 0.05, indicating that the exposure terms are independent of age (i.e., the proportional hazards assumption is validated).

Table D-40. Evaluation of age interaction for the exposure terms in the 2-piece linear spline model with knot at 1,600 ppm × days; cumulative exposure in ppm × days, with lag of 15 years

Parameter	*p*-value for the inclusion of age interaction term
Beta1	0.82
Beta2	0.82

D.3.8. Sensitivity of (Incidence) Unit Risk Estimates to Upper-Bound Estimation Approach—Wald vs. Profile Likelihood

Sensitivity of the (incidence) unit risk estimates to the approach used to estimate the upper bound on the first spline piece from the selected two-piece linear spline model is summarized in Table D-41. According to Langholz and Richardson (2010), the distribution of estimated parameters in nonlog-linear models (hazard functions) 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. The Wald-based result is 40% lower than the profile-likelihood-based estimate.

Table D-41. Comparison of unit risk estimates for lymphoid cancer incidence from two-piece linear spline model using Wald-based and profile-likelihood-based upper-bound estimates on the 1st spline piece

Estimation Approach	Beta1 estimate (per ppm × day)	Wald SE1 estimate (per ppm × day)	95% one-sided upper-bound estimate for 1st spline segment (per ppm × day)	EC_{01} (ppm)	LEC_{01} (ppm)	Unit risk estimate (per ppm)[a]
Wald	7.58×10^{-4}	6.32×10^{-4}	1.80×10^{-3}	7.48×10^{-3}	3.15×10^{-3}	3.17
Profile likelihood	7.58×10^{-4}	--	2.98×10^{-3}	7.48×10^{-3}	1.90×10^{-3}	5.26

[a]Calculated for lymphoid cancer incidence; see Section 4.1.1.3.

D.3.9. Sensitivity of Occupational Extra Risk Estimates to Change in Lag Period

In Section 4.7, extra risk estimates for lymphoid cancer mortality and incidence from the selected model are presented for some occupational exposure scenarios of interest (i.e., 35-year exposures to 8-hour TWAs ranging from 0.1 to 1 ppm between ages 20 and 55 years) because the scenarios include cumulative exposures above the level at which the unit risk estimate is valid. Here, the sensitivity of the selected model (i.e., the two-piece linear spline model with the knot at 1,600 ppm × days and a lag of 15 years) to changes in lag is explored. Parameter estimates for the two-piece linear spline model with the knot at 1,600 ppm × days and different lag periods (0, 5, 10, 15, and 20 years) are presented in Table D-42. The Wald approach was used as an approximation to derive the upper-bound estimates because it was not possible to obtain a formula for the profile likelihood upper-bound estimates that could be used in the life-table analysis. As shown in Figure D-17 above, the Wald upper-bound estimates are about halfway between the MLE RR estimates and the profile likelihood upper-bound estimates. 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. The equations for deriving the MLE and upper-bound estimates across the range of exposures are presented in footnote c of Table D-36. Sensitivity of the extra risk estimates for lymphoid cancer incidence for the occupational exposure scenarios to changes in exposure lag time for the two-piece linear spline model with the knot at 1,600 ppm × days is summarized in Table D-43.

Table D-42. Parameter estimates for the two-piece linear spline model with the knot at 1,600 ppm × days for different lag periods; cumulative exposure in ppm × days

Lag (years)	Beta1 (per ppm × day)	Beta2 (per ppm × day)	SE1 (per ppm × day)	SE2 (per ppm × day)	Covariance (per (ppm × day)^2)
0	5.9×10^{-4}	-5.8×10^{-4}	7.58×10^{-4}	7.53×10^{-4}	-5.71×10^{-7}
5	1.59×10^{-4}	-1.51×10^{-4}	3.65×10^{-4}	3.63×10^{-4}	-1.33×10^{-7}
10	2.11×10^{-4}	-2.01×10^{-4}	3.6×10^{-4}	3.59×10^{-4}	-1.29×10^{-7}
15	7.58×10^{-4}	-7.48×10^{-4}	6.32×10^{-4}	6.31×10^{-4}	-3.99×10^{-7}
20	4.33×10^{-4}	-4.32×10^{-4}	4.05×10^{-4}	4.09×10^{-4}	-1.65×10^{-7}

Table D-43. Comparison of extra risk estimates for lymphoid cancer incidence from two-piece linear spline models with different lag periods; cumulative exposure in ppm × days, knot at 1,600 ppm × days

8-hour TWA	15-year lag	0-year lag	Ratio to 15-year-lagged estimates	5-year lag	Ratio to 15-year-lagged estimates	10-year lag	Ratio to 15-year-lagged estimates	20-year lag	Ratio to 15-year-lagged estimates
MLEs									
0.1	0.0240	0.0213	0.89	0.00565	0.24	0.00720	0.30	0.0126	0.53
0.2	0.0331	0.0276	0.83	0.00757	0.23	0.00981	0.30	0.0182	0.55
0.3	0.0343	0.0282	0.82	0.00793	0.23	0.0103	0.30	0.0189	0.55
0.4	0.0349	0.0286	0.82	0.00824	0.24	0.0107	0.31	0.0193	0.55
0.5	0.0354	0.0290	0.82	0.00854	0.24	0.0111	0.31	0.0194	0.55
0.6	0.0359	0.0294	0.82	0.00884	0.25	0.0114	0.32	0.0196	0.55
0.7	0.0362	0.0298	0.82	0.00912	0.25	0.0118	0.33	0.0196	0.54
0.8	0.0365	0.0301	0.82	0.00941	0.26	0.0121	0.33	0.0197	0.54
0.9	0.0369	0.0305	0.83	0.00969	0.26	0.0125	0.34	0.0198	0.54
1	0.0372	0.0308	0.83	0.00998	0.27	0.0128	0.34	0.0198	0.53
95% one-sided UCLs									
0.1	0.0558	0.0646	1.16	0.0266	0.48	0.0270	0.48	0.0316	0.57
0.2	0.0762	0.0826	1.08	0.0348	0.46	0.0362	0.48	0.0453	0.59
0.3	0.0784	0.0838	1.07	0.0353	0.45	0.0373	0.48	0.0471	0.60
0.4	0.0794	0.0845	1.06	0.0355	0.45	0.0380	0.48	0.0480	0.60
0.5	0.0800	0.0852	1.07	0.0355	0.44	0.0387	0.48	0.0486	0.61
0.6	0.0806	0.0858	1.06	0.0354	0.44	0.0394	0.49	0.0492	0.61
0.7	0.0808	0.0862	1.07	0.0350	0.43	0.0401	0.50	0.0497	0.62
0.8	0.0811	0.0867	1.07	0.0346	0.43	0.0409	0.50	0.0502	0.62
0.9	0.0813	0.0871	1.07	0.0339	0.42	0.0417	0.51	0.0509	0.63
1	0.0815	0.0875	1.07	0.0331	0.41	0.0425	0.52	0.0516	0.63

The sensitivity analysis for choice of lag reveals that the MLEs of extra risk for the selected two-piece linear spline model with the knot at 1,600 ppm × days for different lag periods (0, 5, 10, 15, and 20 years) 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.

For lags of 5 and 10 years, the optimal knots for the two-piece linear spline model (1,575 ppm × days and 1,600 ppm × days, respectively) were in the vicinity of the selected knot (1,600 ppm × days), so these sensitivity analyses serve as comparisons for the optimal-knot models as well as for the selected model with alternative knots; however, as noted above, neither the 5- nor 10-year lagged models had a good statistical fit. For the lag of 20 years, the optimal knot was 125 ppm × days, and a local minimum AIC (maximum likelihood) was observed at 1,600 ppm × days, similar to the case with the 15-year lag (see Figure D-14). Even with the optimal knot, however, the 20-year lagged linear spline model had an inadequate statistical fit ($p = 0.26$). For the linear spline model with no lag, the optimal knot was also 125 ppm × days, and no clear alternative local minimum AIC was observed (see Figure D-14). Even with the optimal knot, the unlagged linear spline model had a poorer fit than the selected model (AIC of 462.9 vs. 462.1).

D.4. HEMATOPOIETIC CANCER MORTALITY (ALL HEMATOPOIETIC CANCERS COMBINED) (*n* = 17,530)

D.4.1. Exposure Distribution in Cohort and among All (Lympho)hematopoietic Cases in the Cohort Mortality Study

In modeling hematopoietic cancer, we used a 15-year lag for cumulative exposure, as in the prior publication (Steenland et al., 2004), and we also used the same cutpoints as in that publication. The distribution of cases for hematopoietic cancer mortality is presented in Table D-44.

Table D-44 Exposure categories and case distribution for hematopoietic cancer mortality

Cumulative exposure, 15-year lag[a]	Male hematopoietic cancer deaths	Female hematopoietic cancer deaths	Total hematopoietic cancer deaths
0 (lagged out)	9	4	13
>0–1,200 ppm-days	4	13	17
1,201–3,680 ppm-days	5	10	15
3,681–13,500 ppm-days	8	7	15
>13,500 ppm-days	11	3	14

[a]Mean exposures for both sexes combined with a 15-year lag for the categorical exposure quartiles were 446, 2,143, 7,335, and 39,927 ppm × days. Median values were 374, 1,985, 6,755, and 26,373 ppm × days. These values are for the full cohort.

D.4.2. Modeling of the Hematopoietic Cancer Mortality Data Using a Variety of Models

D.4.2.1. *Cox Regression (Log RR) Models*

While the published results of these data in Steenland et al. (2004) focused on males [Table 8 in Steenland et al. (2004)], in fact males and females do not differ greatly in categorical results using a 15-year lag. A formal chunk test for four interaction terms between exposure and sex is not close to significance (χ^2 4.5, 4 DF; $p = 0.34$), although such tests are not very powerful in the face of sparse data such as these. Table D-45 below shows the categorical odds ratio results for men and women separately and combined. Males and females were combined in all analyses for hematopoietic cancer here.

Table D-45. All hematopoietic cancer mortality categorical results by sex

Cumulative exposure, 15-year lag	Odds ratio (95% CI) males	Odds ratio (95% CI) females	Odds ratio (95% CI) combined
0 (lagged out)	1.00	1.00	1.00
>0–1,200 ppm-days	1.23 (0.32–4.74)	3.76 (1.01–17.23)	2.33 (0.93–5.86)
1,201–3,680 ppm-days	2.53 (0.69–9.27)	4.93 (1.01–23.99)	3.46 (1.33–8.95)
3,681–13,500 ppm-days	3.14 (0.95–10.37)	3.31 (0.64–17.16)	3.02 (1.16–7.89)
>13,500 ppm-days	3.42 (1.09–10.73)	2.11 (0.33–13.74)	2.96 (1.12–7.81)

CI = confidence interval.

Analyses used a case-control approach, with 100 controls per case, as in Steenland et al. (2004). Age was the time variable in proportional hazards (Cox) regression. For lymphoid

cancer mortality, only exposure variables were included in the model. Cases and controls were matched within risk sets on age, sex, and race.

Using log RR models, we used a categorical model, a (log-)linear model, a two-piece (log-)linear model, and a log-transform model. We also ran a number of analogous models using linear RR models (see Section D.4.2.2 below).

The categorical log RR model for hematopoietic cancer mortality was run using the originally published cutpoints to form four categories above the lagged-out group, as shown in Table D-45. To graph the categorical points, each category was assigned the midpoint of the category as its exposure level, except for the last one which was assigned 50% more than the last cutpoint.

For the two-piece log-linear model, the single knot was chosen based on a comparison of likelihoods assessed every 100 ppm-days from 0 to 7,000 ppm-days. The best likelihood was at 500 ppm-days (see Figure D-19). In Figure D-20 below we show the categorical, two-piece (log-)linear spline and log-transform log RR model results.

Model results for the categorical and two-piece (log-)linear log RR models are shown in Tables D-46 and D-47, and the results of the log-transform and (log-)linear log RR models in Table D-48 and Table D-49. Again the log-linear model appears to substantially underestimate the exposure-response relationship and does not provide a good model fit.

We further explored the sensitivity of the log-linear model to high exposures by excluding progressively more of the upper tail of exposure. We excluded 5, 10, 20, 30, 40, and 53% of the upper tail of exposure. The 53% cutoff was at 2,000 ppm-days. The slope of the log-linear exposure-response model increased by 0.8, 1.0, 9.3, 28.6, 58.2, and 191.4 times, respectively, with the exclusion of progressively more data. It appears the curve is flat in the top 20% of exposure.

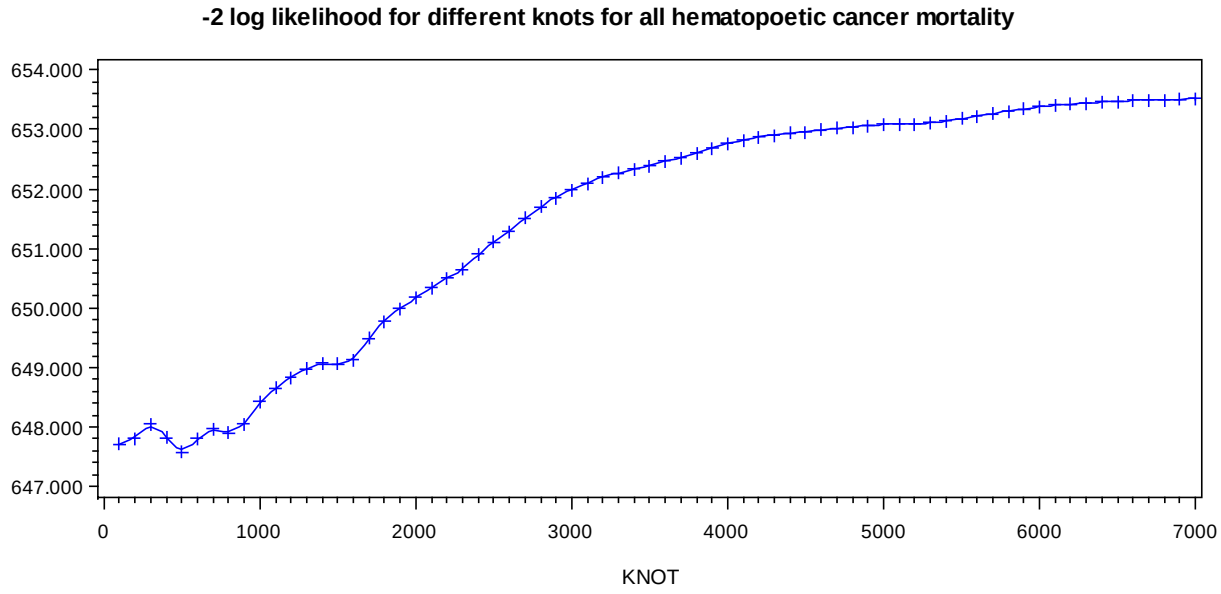


Figure D-19. Likelihood vs. knots for two-piece log-linear model, all hematopoietic cancer.

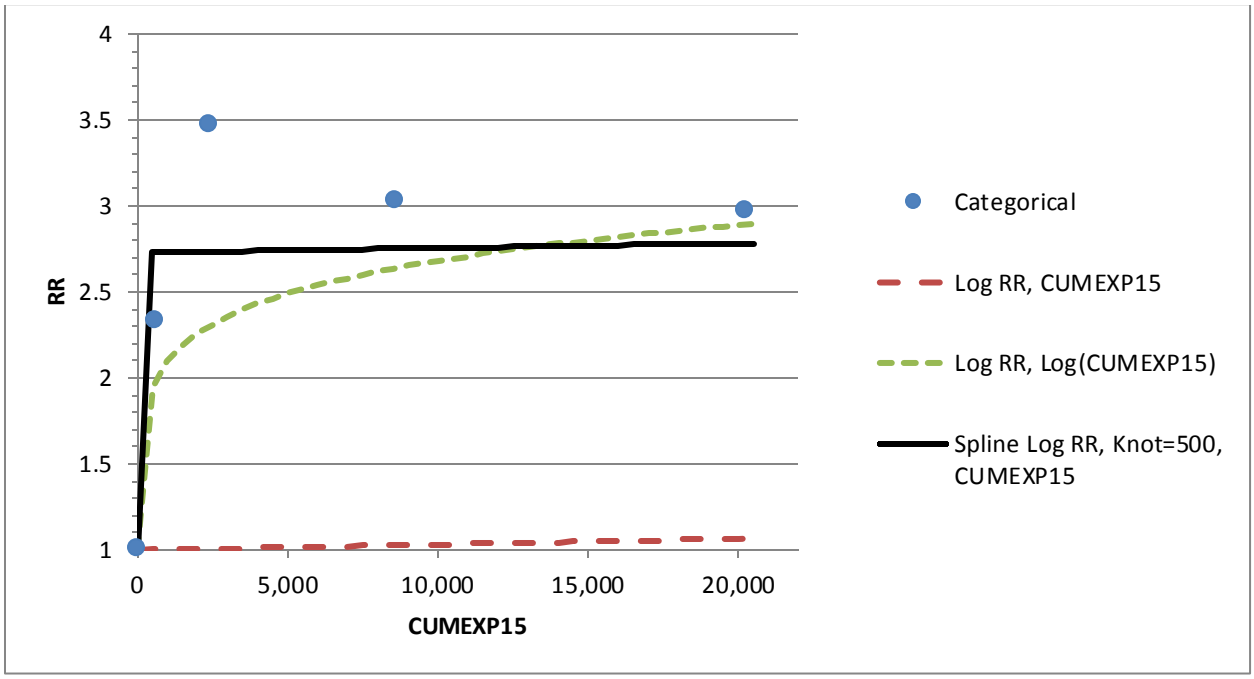


Figure D-20. Exposure-response models for hematopoietic cancer mortality.

Plot of continuous exposure (with 15-year lag) and all hematopoietic cancer mortality rate ratios estimated using the two-piece log-linear spline model with the knot at 500 ppm-days overlaid with other log RR curves and categorical (quartile) points.

Table D-46. Categorical results for all hematopoietic cancer mortality, men and women combined, cumulative exposure with a 15-year lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	655.643	647.806				
AIC	655.643	655.806				
SBC	655.643	665.022				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	7.8371	4	0.0977			
Score	7.3994	4	0.1162			
Wald	7.2354	4	0.1240			
Analysis of maximum likelihood estimates						
Variable	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiS**	**Hazard ratio**
CUM151	1	0.84746	0.46956	3.2573	0.0711	2.33
CUM152	1	1.23989	0.48571	6.5166	0.0107	3.46
CUM153	1	1.10664	0.48943	5.1126	0.0238	3.02
CUM154	1	1.08360	0.49603	4.7723	0.0289	2.96

Table D-47. Results of two-piece log-linear spline model for all hematopoietic cancer mortality, men and women combined, cumulative exposure with a 15-year lag; knot at 500 ppm-days

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	655.643	647.581				
AIC	655.643	651.581				
SBC	655.643	656.189				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	8.0615	2	0.0178			
Score	7.5092	2	0.0234			
Wald	7.3467	2	0.0254			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
SP11	1	0.00201	0.000731	6.7457	0.0094	1.002
SP12	1	-0.00201	0.0007738	6.7249	0.0095	0.998

Table D-48. Results of log-transform log RR model for all hematopoietic cancer mortality, men and women combined, cumulative exposure with a 15-year lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	655.643	648.825				
AIC	655.643	650.825				
SBC	655.643	653.129				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	6.8177	1	0.0090			
Score	6.6260	1	0.0100			
Ward	6.5593	1	0.0104			
Analysis of maximum likelihood estimates						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
LCUM15	1	0.10706	0.04180	6.5593	0.0104	1.113

Table D-49. Results of log-linear model for all hematopoietic cancer morality, men and women combined, cumulative exposure with a 15-year lag

Model fit statistics						
Criterion	**Without covariates**	**With covariates**				
-2 LOG L	655.643	645.922				
AIC	655.643	656.922				
SBC	655.643	659.226				
Testing global null hypothesis: BETA = 0						
Test	**χ^2**	**DF**	**Pr > ChiSq**			
Likelihood ratio	0.7213	1	0.3957			
Score	0.8783	1	0.3487			
Wald	0.8739	1	0.3499			
Analysis of maximum likelihood estimate						
Parameter	**DF**	**Parameter estimate**	**Standard error**	**χ^2**	**Pr > ChiSq**	**Hazard ratio**
CUMEXP15	1	3.26052E-6	3.48788E-6	0.8739	0.3499	1.000

D.4.2.2. *Linear Relative Risk Models for Hematopoietic Cancer Mortality*

For completeness, we also present the results of the linear RR models below (see Table D-50 and Figure D-21; linear RR models are denoted "ERR" models in the figure). They look much like their counterparts for the log RR models. Again, the high slope of the exposure-response relationship in the low-dose region for the two-piece linear and log-transform curves, and the low overall slope of the linear curve, call into question the use of these models for risk assessment.

Table D-50. Model fit statistics and coefficients for linear RR models, hematopoietic cancer mortality

Linear RR model	–2 Log likelihood (full model)	AIC	*p*-value[a]	Parameter(s)	SE[b]
CUMEXP15	654.64	656.64	0.32	B = 6.257×10^{-6}	SE = 8.187×10^{-6}
Log(CUMEXP15)	648.13	650.13	0.006	B = 0.2322	SE = 0.1437
Spline, knot = 500, CUMEXP15[c,d]	646.95	650.95	0.01	B1 = 3.673×10^{-3} B2 = -3.668×10^{-3}	SE1 = 2.345×10^{-3} SE2 = 2.345×10^{-3}

SE = standard error.
[a]From likelihood ratio test.
[b]Editorial note: Confidence intervals for linear RR models, in contrast to those for the log-linear RR models, may not be symmetrical. The EPA did not apply the profile likelihood approach (Langholz and Richardson, 2010), which allows for asymmetric CIs, to develop CIs for these models because the models were not used further in the assessment.
[c]Covariance of two pieces of linear spline: -5.70×10^{-6}.
[d]For Wald estimates, for the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × [exp – knot]). For the 95% upper confidence limit, for exposures below the knot, RR = 1 + ([B1 + 1.645 × SE1] × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × [exp-knot] + 1.645 × sqrt[exp^2 × var1 + $[exp\text{-}knot]^2$ × var2 + 2 × exp × [exp-knot] × covar]), where exp = cumulative exposure, var = variance, covar = covariance.

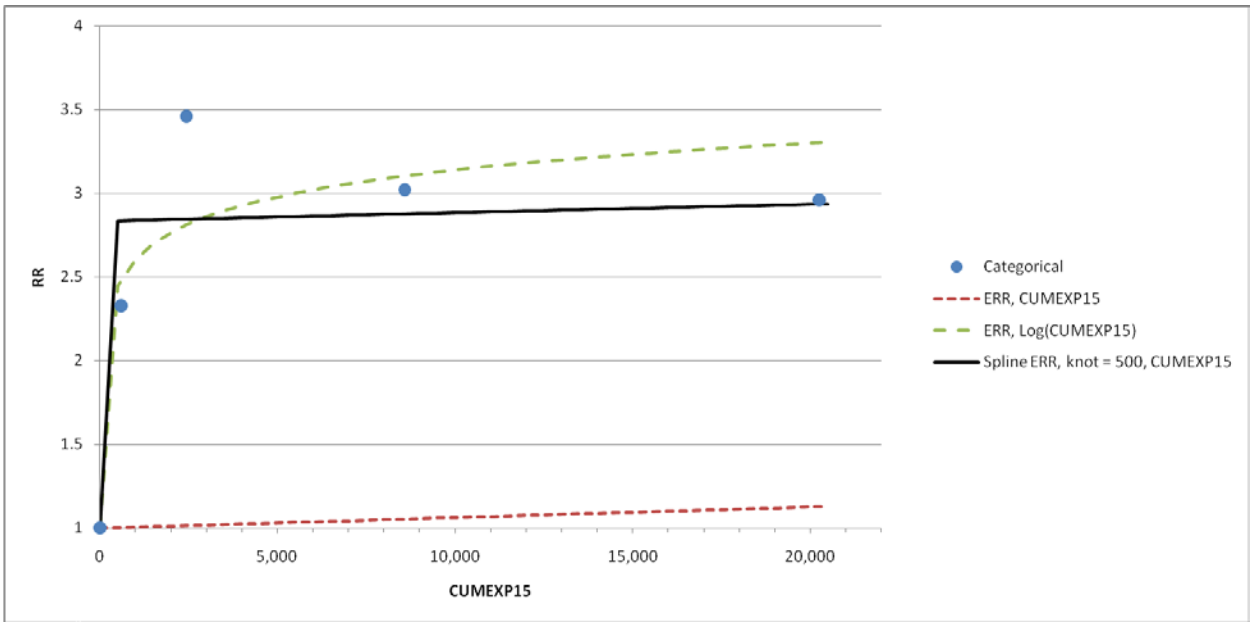


Figure D-21. Linear RR models for hematopoietic cancer mortality.

[Editorial note: "ERR" refers to linear RR models.]

D.5. FURTHER CHARACTERIZATION OF THE NIOSH COHORT

D.5.1. Further Characterization of the Exposure Distributions and Other Characteristics in the Full Cohort

Tables D-51–D-60 and Figures D-22–D-24 summarize characteristics of the full cohort, which comprises all persons enrolled in the cohort. Within this context, a case is someone who was ever diagnosed with a lymphoid cancer and a control is someone who was never diagnosed with any lymphohematopoietic cancer.

Table D-51. Marginal summaries of workers' exposures, and years of entry to employment and age at end of follow-up in full cohort

	N	Mean	Minimum	25th percentile	Median	75th percentile	Maximum
Year of birth	17,148	1940	1884	1931	1943	1950	1968
Year of entry	17,148	1970.6	1938	1967	1971	1975	1986
Exposures (ppm-yr)	17,148	26.7	0.01	1.46	5.60	23.25	135.2
Age at end of follow-up	17,148	56.3	17.5	47.3	54.6	65.2	100.1

Table D-52. Cumulative exposure to EtO by year of entry to employment in full cohort

Analysis variable: exposure (ppm-yr)					
Year of entry into employment	N	Mean	Minimum	Median	Maximum
< 1965	3,793	52.0	0.03	9.5	1,352
1965–1969	4,307	26.4	0.04	6.3	767
1969–1972	2,983	20.8	0.02	5.3	738
1972–1975	2,626	18.1	0.04	5.0	396
≥ 1975	3,415	11.0	0.01	3.9	257

Table D-53. Cumulative exposure to EtO by duration of employment in full cohort

Analysis variable: exposure (ppm-yr)					
Duration of employment	N	Mean	Minimum	Median	Maximum
< 0.9 years	3,441	3.2	0.02	1.7	65
0.9–2.6 years	3,386	7.4	0.02	3.3	173
2.6–7.0 years	3,441	18.8	0.01	8.1	367
7–17 years	3,442	41.9	0.01	18.0	638
≥ 17 years	3,414	62.4	0.01	21.3	1,352

Table D-54. Cumulative exposure to EtO in each of the risk categories in full cohort

Analysis variable: exposure (ppm-yr)					
Risk category[a]	N	Mean	Minimum	Median	Maximum
< 1,200 ppm-days	6,627	1.20	0.01	0.99	3.29
1,200–3,680 ppm-days	3,726	5.89	3.29	5.45	10.07
3,680–13,500 ppm-days	3,713	20.14	10.08	13.69	36.97
≥ 13,500 ppm-days	3,082	114.7	36.97	51.28	1,352

[a]Risk category cutpoints chosen based on exposure distributions for all lymphohematopoietic cancer; same as in Steenland et al. (2004).

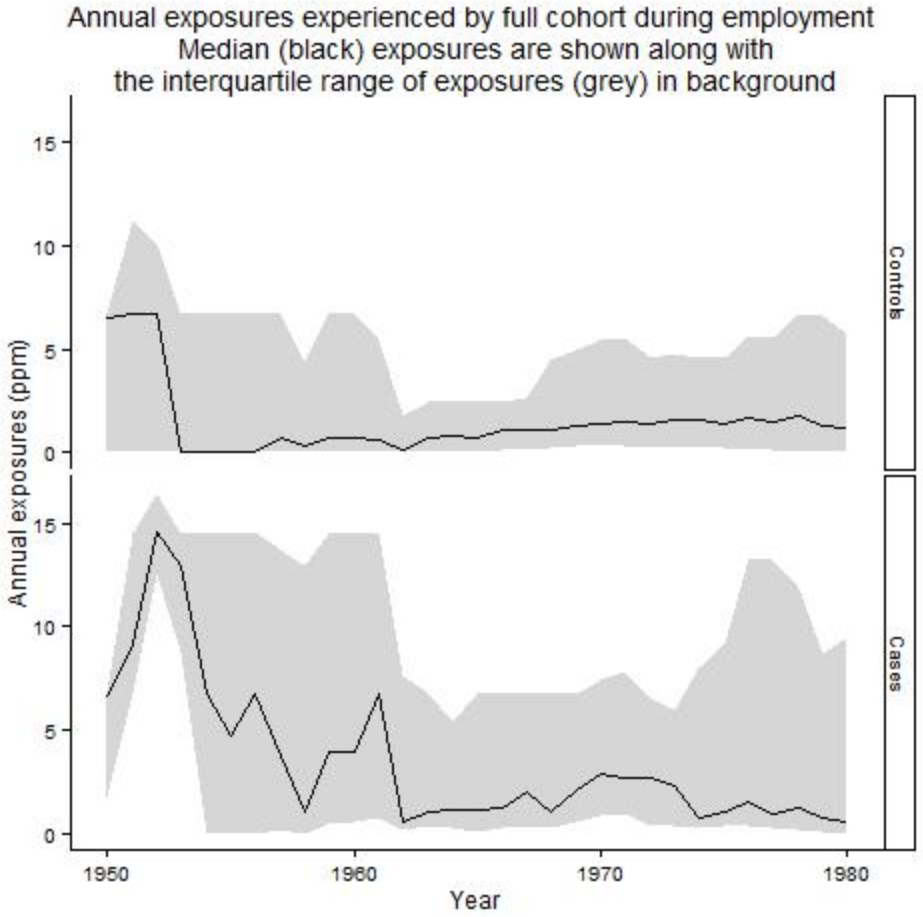


Figure D-22. Estimated annual exposures experienced by cases and controls in the full cohort while working[1]—medians and interquartile ranges[2]

[1]Annual exposure histories taken from NIOSH deidentified exposure records; include 382 workers ultimately removed from the analysis due to inconsistencies in the record.
[2]Prior to 1962, fewer than five cases were working in any given year. This resulted in a number of years where the 25th, 50th, and 75th percentiles of the exposure distribution were identical or nearly so.

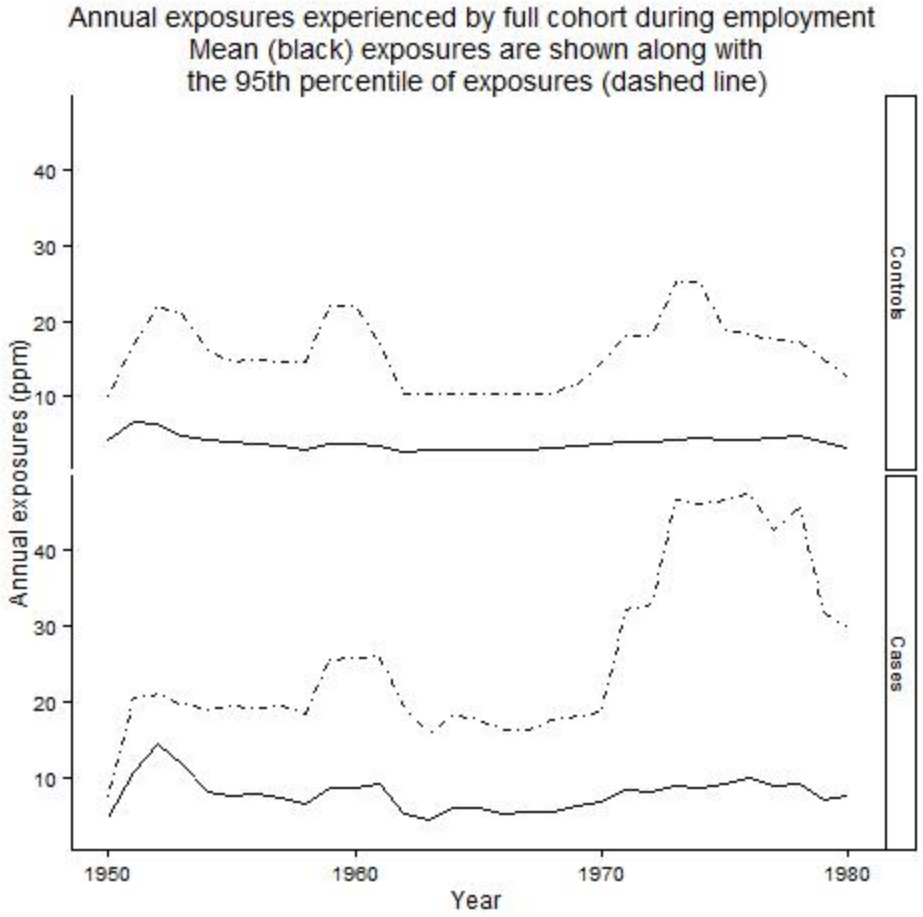


Figure D-23. Estimated annual exposures experienced by cases and controls in the full cohort while working[1]—means and 95th percentiles

[1]Annual exposure histories taken from NIOSH deidentified exposure records; include 382 workers ultimately removed from the analysis due to inconsistencies in the record.

Table D-55. Sex distribution over time—case and control sexes by the year they entered the workforce

		< 1950	1950–1960	1960–1970	1970–1980	1980–1990
Cases	Men	4	5	14	4	0
	Women	1	4	15	7	0
Controls	Men	172	501	3,171	3,176	547
	Women	144	873	4,095	4,026	365

Figure D-24. Sex ratios for currently working populations.

Sex ratios are calculated for each year with a working case, and include all persons of case or control status currently working at least part of that year.

Table D-56. Year of entry to the EtO workforce

	N	Mean	5th percentile	25th percentile	Median	75th percentile	95th percentile
Case	54	1963	1948	1960	1964	1969	1975
Control	17,070	1970	1956	1966	1970	1974	1981

Table D-57. Age of entry to the EtO workforce

	N	Mean	5th percentile	25th percentile	Median	75th percentile	95th percentile
Case	54	38.55	21.53	29.77	39.17	45.72	54.08
Control	17,070	29.46	18.30	20.97	26.30	36.29	49.65

Table D-58. Duration of employment in the EtO workforce

	N	Mean	5th percentile	25th percentile	Median	75th percentile	95th percentile
Case	54	11.14	0.95	2.53	8.99	18.18	31.88
Control	17,070	8.55	0.34	1.18	4.31	14.38	27.36

Table D-59. Year of departure/retirement from the EtO workforce

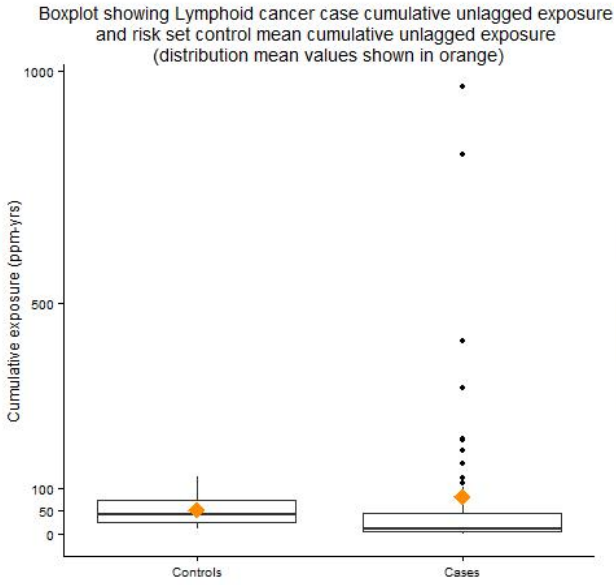
	N	Mean	5th percentile	25th percentile	Median	75th percentile	95th percentile
Case	54	1975	1961	1967	1974	1981	1986
Control	17,070	1978	1965	1971	1977	1985	1996

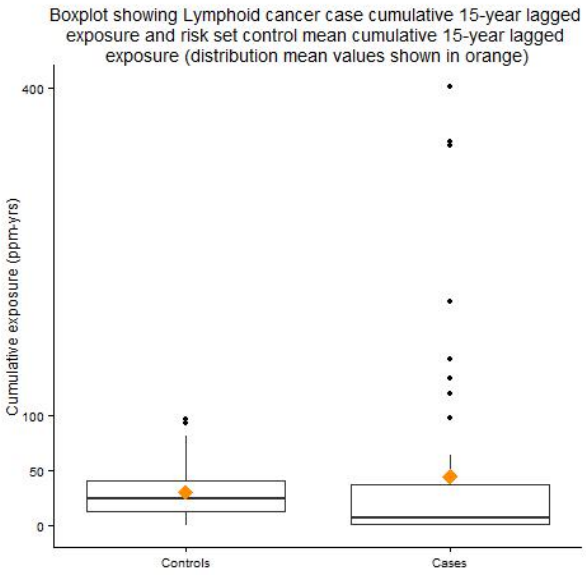
Table D-60. Age of departure/retirement from the EtO workforce

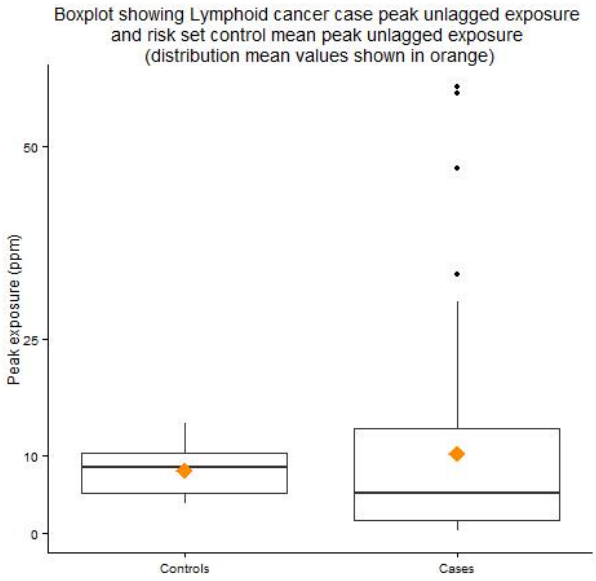
	N	Mean	5th percentile	25th percentile	Median	75th percentile	95th percentile
Case	54	49.69	29.68	39.90	49.88	61.47	65.83
Control	17,070	38.01	19.88	24.98	35.12	49.69	63.42

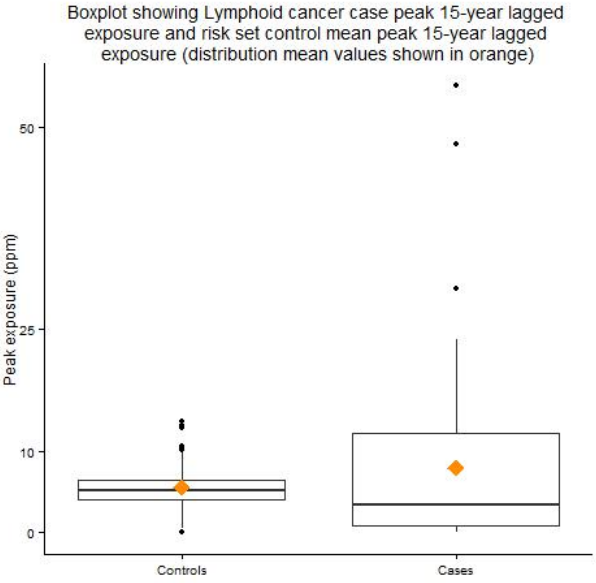
D.5.2. Further Characterization of the Exposure Distributions and Other Characteristics in the Risk Sets

Figures D-25 and D-26 and Table D-61 summarize characteristics of the risk sets, which each comprise a lymphoid cancer case and its matched set of ~100 controls. Controls were matched on age, plant, race, and sex and randomly selected from the pool of all those who had survived without lymphohematopoietic cancer to at least the age of the case in that set. Exposures were truncated at the case failure age within each set. In this context, statistics for controls are calculated via the average values for each set.


Boxplot showing Lymphoid cancer case cumulative unlagged exposure and risk set control mean cumulative unlagged exposure (distribution mean values shown in orange)
Cumulative exposure (ppm-yrs)
1000
500
100
50
0
Controls
Cases


Boxplot showing Lymphoid cancer case cumulative 15-year lagged exposure and risk set control mean cumulative 15-year lagged exposure (distribution mean values shown in orange)
Cumulative exposure (ppm-yrs)
400
100
50
0
Controls
Cases


Boxplot showing Lymphoid cancer case peak unlagged exposure and risk set control mean peak unlagged exposure (distribution mean values shown in orange)
Peak exposure (ppm)
50
25
10
0
Controls
Cases


Boxplot showing Lymphoid cancer case peak 15-year lagged exposure and risk set control mean peak 15-year lagged exposure (distribution mean values shown in orange)
Peak exposure (ppm)
50
25
10
0
Controls
Cases

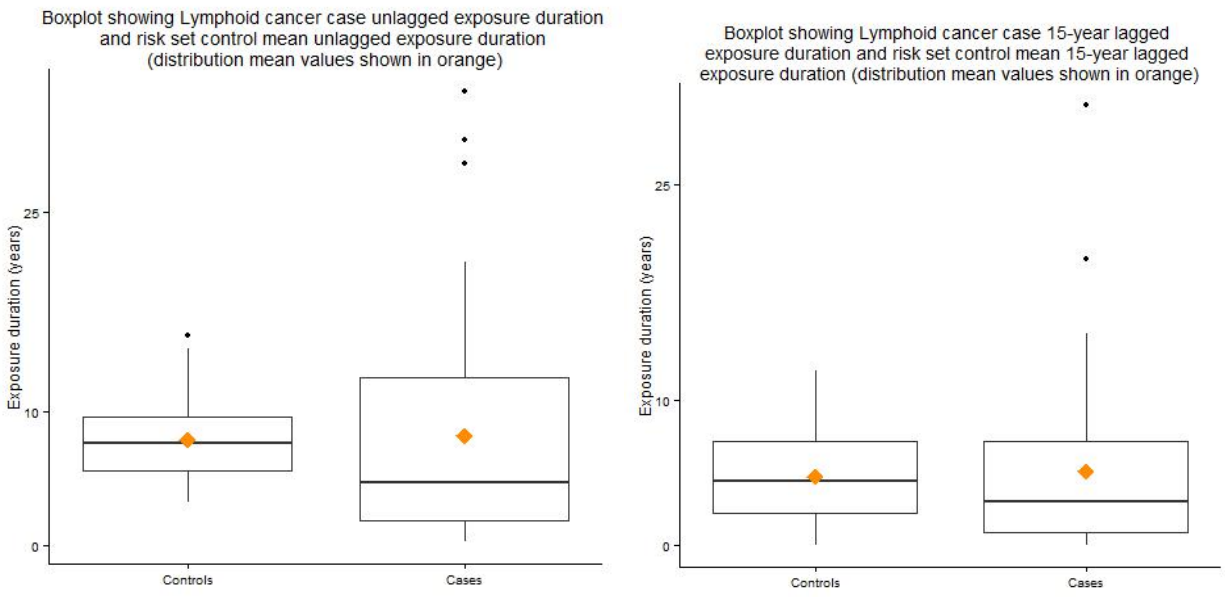
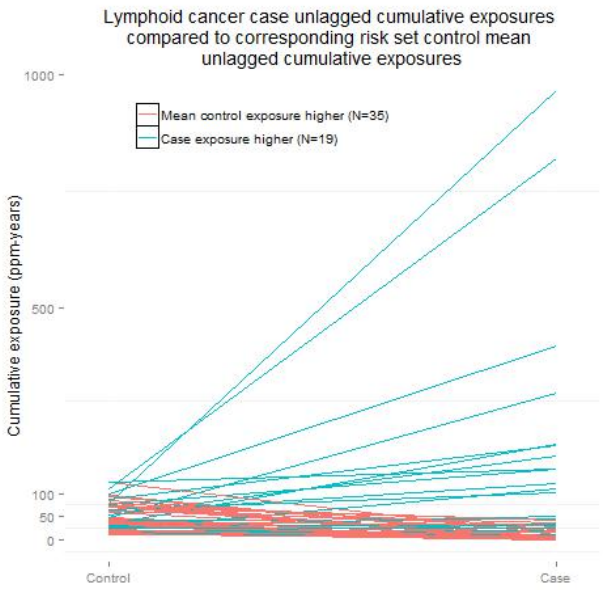
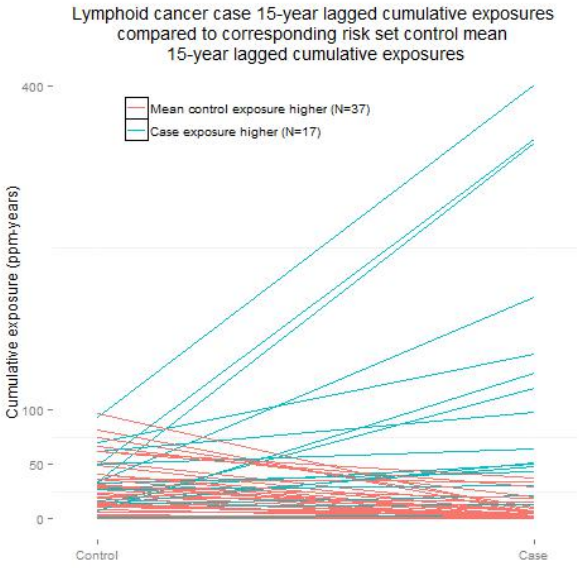
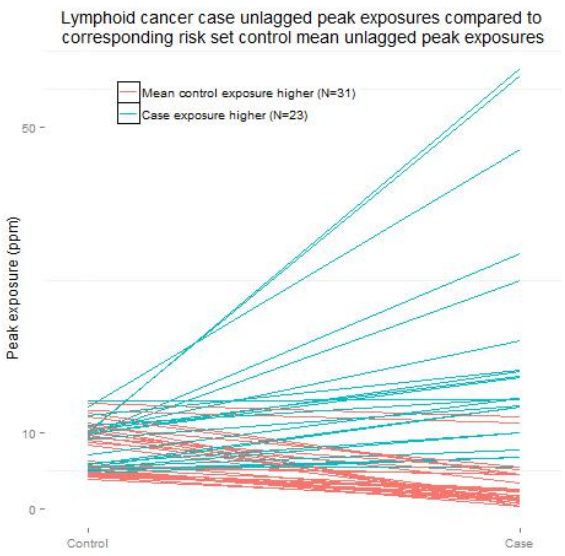


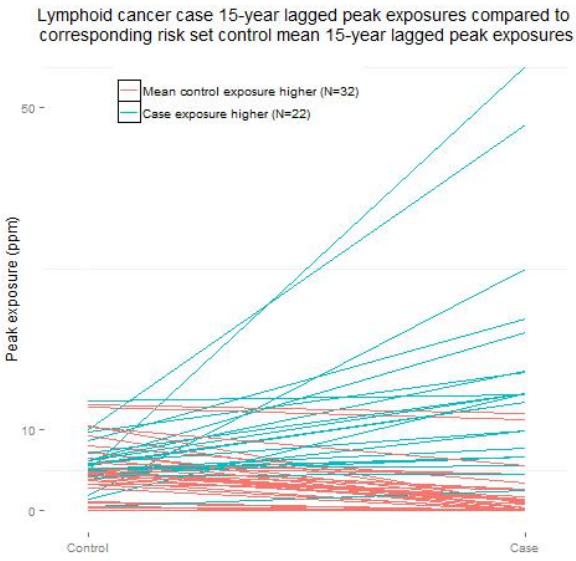
Figure D-25. Box plots[1] of both unlagged and 15-year lagged cumulative total exposures, peak exposures, and exposure durations for risk sets.

[1]Upper and lower sides of box correspond to 75th and 25th percentiles, spanning the interquartile range (IQR); the line in the middle of the box represents the median; the diamond depicts the mean; the upper/lower whisker extend from the top/bottom of the box to 1.5 × IQR from the top/bottom of the box; and the points beyond the whiskers are data outside that range.


Lymphoid cancer case unlagged cumulative exposures compared to corresponding risk set control mean unlagged cumulative exposures
Mean control exposure higher (N=35)
Case exposure higher (N=19)
Cumulative exposure (ppm-years)
1000
500
100
50
0
Control
Case


Lymphoid cancer case 15-year lagged cumulative exposures compared to corresponding risk set control mean 15-year lagged cumulative exposures
Mean control exposure higher (N=37)
Case exposure higher (N=17)
Cumulative exposure (ppm-years)
400
100
50
0
Control
Case


Lymphoid cancer case unlagged peak exposures compared to corresponding risk set control mean unlagged peak exposures
Mean control exposure higher (N=31)
Case exposure higher (N=23)
Peak exposure (ppm)
50
10
0
Control
Case


Lymphoid cancer case 15-year lagged peak exposures compared to corresponding risk set control mean 15-year lagged peak exposures
Mean control exposure higher (N=32)
Case exposure higher (N=22)
Peak exposure (ppm)
50
10
0
Control
Case

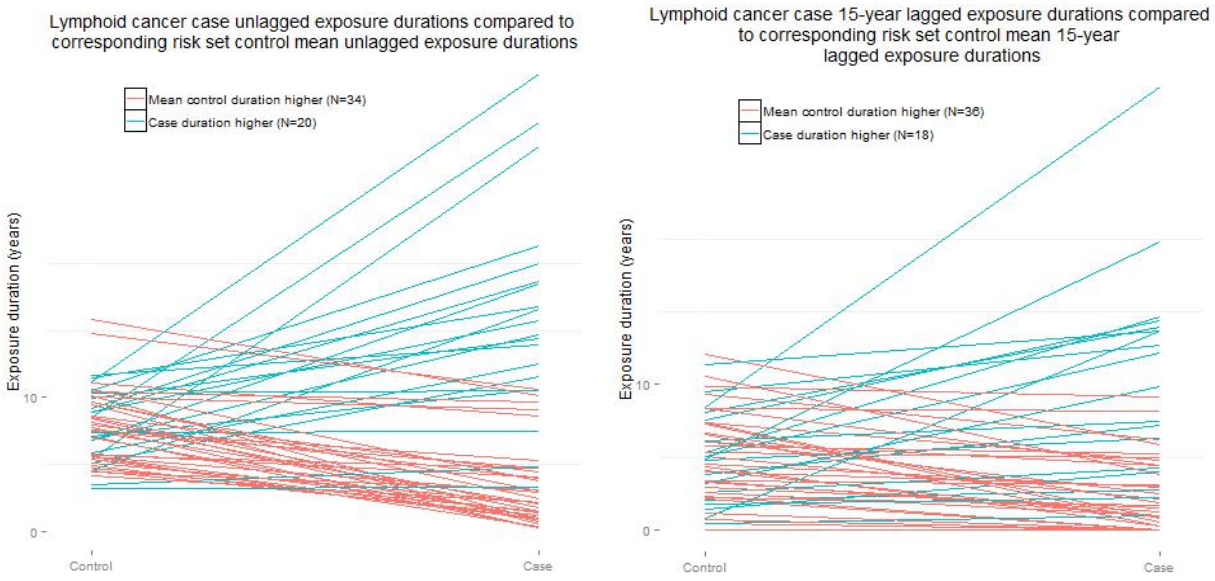


Figure D-26. Lymphoid cancer case exposures compared to corresponding risk set control mean exposures for cumulative total exposures, peak exposures, and exposure durations both unlagged and with a 15-year lag.

Table D-61. Summary of percentage of total case and control individual exposures in the risk set worker histories that are excluded when the lag of 15 years is imposed[a]

	Cases		Controls	
	Mean	**Median**	**Mean**	**Median**
Cumulative exposure	33.37%	1.23%	39.62%	12.48%
Peak exposure	23.36%	0%	30.05%	0%
Exposure duration	32.77%	1.94%	40.90%	22.43%

[a]Calculated using the equation:

$$\%Exposure\ excluded\ by\ lagging = \frac{Unlagged\ exposure - Lagged\ exposure}{Unlagged\ exposure} * 100$$

D.6. POSSIBLE INFLUENCE OF THE HEALTHY WORKER SURVIVOR EFFECT

The healthy worker survivor effect is the effect of healthy workers remaining in the workforce as sick workers leave, independently of any damaging effects of exposure. It is a selection bias via which healthier workers remain in the workforce. It tends to create a

downward bias in exposure-response coefficients when the exposure metric is cumulative exposure, which is by definition correlated with duration of exposure and almost always with duration of employment (Steenland et al., 1996). Given a true effect of exposure on disease incidence or mortality in the case of ethylene oxide, it is possible that the healthy worker survivor effect has caused some negative bias in observed exposure-response coefficients. However, there are no standard methods to correct for this bias because leaving work is both a confounder and an intermediate variable on a pathway between exposure and disease. Therefore, standard analyses would need to adjust for employment status as a confounder, but should not adjust for it because it is an intermediate variable. Robins et al. (1992) proposed some solutions using G-estimation to address this problem, but to date these solutions are not commonly used and can be difficult to implement. The degree to which the healthy worker survivor effect confounds measured exposure-response trends is not known, but it is likely that lagging exposure, as has been done here, diminishes such confounding (Arrighi and Hertz-Picciotto, 1994).

D.7. POSSIBLE INFLUENCE OF EXPOSURE MISMEASUREMENT

Exposure estimation in the EtO studies considered here is subject to errors in measurement. The method for exposure estimation used here involved assigned estimated average exposures in a given job, at a given time period in a given plant, to each worker in that job. Estimated average exposures were taken from observed measurements in a given job, or estimated likely average exposures in that job derived from a regression model based on observed measurements (Hornung et al., 1994). Errors in measurement in this type of situation are typically errors of the Berkson type, rather than classical errors (Armstrong, 1998, 1990). In Berkson errors, the model for errors is

$$\text{exposure}_{\text{true}} = \text{exposure}_{\text{observed}} + \text{error}$$

and the error is independent of the observed exposure. The classical error model is

$$\text{exposure}_{\text{observed}} = \text{exposure}_{\text{true}} + \text{error}$$

and the error is independent of the true exposure. Assuming the errors are unbiased (i.e., their expected value is 0) in the classical error model, it is well known that measurement error will bias exposure-response coefficients towards the null in regression analyses. However, in the Berkson error model, exposure-response coefficients will be unbiased in linear regression models, although their variance may be increased. In log-linear regression models, Berkson

error in some instances may result in biased exposure-response estimates (Deddens and Hornung, 1994; Prentice, 1982). This may occur when the variance of the errors increases with the true exposure level, which is often the case in occupational studies, when the disease is relatively rare (also typical), and when the true exposure is distributed log-normally (again typical of occupational exposures). In this situation, Steenland et al. (2000) have shown that exposure-response coefficients using cumulative exposure can be biased either upward or downward. The direction and degree of bias depends on the degree of increase in the variance of exposure error as exposure level increases and on the variance of duration of exposure. When the standard deviation of duration of exposure is less than or equal to its mean, as is the case in the EtO cohort studied here, simulations have shown that the exposure-response coefficients are approximately unbiased (Steenland et al., 2000). An added complication not considered in the simulations conducted by Steenland et al. (2000) is the possible correlation between measurement error and outcome. If this correlation is strong, which may occur when there is a strong exposure-response relationship, it is important to take it into account. Estimating the effect of exposure measurement in the presence of this correlation can be done using Bayesian models and special software (WINBUGS), but the calculations are complex and require a good deal of time.

Hornung et al. (1994) provide an estimate of the log-normal distribution of measured exposure based on personal samples, as well as the likely distribution of error in assigning the job-specific means to estimate individual exposures. Assignment of such job-specific means was shown to involve some bias as well as random error. This provides a rich source of information with which one could simulate the effect of measurement error on exposure-response coefficients. Based on the exposure estimates used in the study, and some assumptions about the error of such measurement in terms of bias and random error, as well as the assumption of a Berkson error model, one could simulate what the true job-specific exposure means were likely to have been, and then in turn simulate likely true personal exposure distributions. Using the latter in exposure-response analysis, one could estimate the true exposure-response coefficient. However, such analyses are rather involved and beyond the scope of the current task.

APPENDIX E. LIFE-TABLE ANALYSIS

A spreadsheet illustrating the extra risk calculation for the derivation of the LEC_{01} for lymphoid cancer incidence is presented in Table E-1.

Table E-1. Extra risk calculation[a] for lymphoid cancer incidence from environmental exposure to 0.00190 ppm (the LEC_{01})[b] using the two-piece linear spline model with knot at 1,600 ppm × days[c]

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	Age interval	All cause mortality ($\times 10^5$/yr)	lymphoid cancer incidence ($\times 10^5$/yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	lymphoid cancer hazard rate (h)	Cond prob of lymphoid cancer incidence in interval (Ro)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed lymphoid cancer hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of lymphoid cancer in interval (Rx)
1	<1	632.7	1.9	0.0063	0.9937	1.0000	0.0000	0.00002	0	0.00	0.00002	0.0063	0.9937	1.0000	0.00002
2	1–4	27.2	8.5	0.0011	0.9989	0.9937	0.0003	0.00034	0	0.00	0.00034	0.0011	0.9989	0.9937	0.00034
3	5–9	12.0	4.7	0.0006	0.9994	0.9926	0.0002	0.00023	0	0.00	0.00024	0.0006	0.9994	0.9926	0.00023
4	10–14	14.5	3.5	0.0007	0.9993	0.9920	0.0002	0.00017	0	0.00	0.00018	0.0007	0.9993	0.9920	0.00017
5	15–19	50.7	3.4	0.0025	0.9975	0.9913	0.0002	0.00017	2.5	5.27	0.00017	0.0025	0.9975	0.9913	0.00017
6	20–24	87.7	3.5	0.0044	0.9956	0.9888	0.0002	0.00017	7.5	15.82	0.00018	0.0044	0.9956	0.9888	0.00018
7	25–29	97.6	4.3	0.0049	0.9951	0.9845	0.0002	0.00021	12.5	26.37	0.00023	0.0049	0.9951	0.9845	0.00023
8	30–34	111.8	6.0	0.0056	0.9944	0.9797	0.0003	0.00029	17.5	36.91	0.00033	0.0056	0.9944	0.9796	0.00033
9	35–39	141.4	9.1	0.0071	0.9930	0.9742	0.0005	0.00044	22.5	47.46	0.00052	0.0071	0.9929	0.9741	0.00050
10	40–44	206.9	13.8	0.0103	0.9897	0.9673	0.0007	0.00066	27.5	58.01	0.00081	0.0105	0.9896	0.9672	0.00078
11	45–49	327.5	21.0	0.0164	0.9838	0.9574	0.0011	0.00100	32.5	68.56	0.00126	0.0166	0.9835	0.9572	0.00120
12	50–54	497.4	32.9	0.0249	0.9754	0.9418	0.0016	0.00153	37.5	79.10	0.00203	0.0253	0.9751	0.9414	0.00189
13	55–59	714.3	49.0	0.0357	0.9649	0.9187	0.0025	0.00221	42.5	89.65	0.00311	0.0364	0.9643	0.9179	0.00280
14	60–64	1,022.1	72.4	0.0511	0.9502	0.8865	0.0036	0.00313	47.5	100.20	0.00470	0.0522	0.9492	0.8851	0.00406
15	65–69	1,521.5	106.9	0.0761	0.9267	0.8423	0.0053	0.00434	52.5	110.74	0.00711	0.0778	0.9251	0.8401	0.00575
16	70–74	2,341.0	139.5	0.1171	0.8895	0.7806	0.0070	0.00514	57.5	121.29	0.00950	0.1196	0.8873	0.7772	0.00696
17	75–59	3,746.0	176.0	0.1873	0.8292	0.6944	0.0088	0.00557	62.5	131.84	0.01226	0.1908	0.8263	0.6896	0.00770
18	80–84	6,164.8	198.6	0.3082	0.7347	0.5758	0.0099	0.00492	67.5	142.38	0.01415	0.3125	0.7316	0.5699	0.00692
							Ro =	0.03055						**Rx =**	0.04022
extra risk = (Rx−Ro)/(1−Ro) = 0.00998															

Table E-1. Extra risk calculation[a] for lymphoid cancer incidence from environmental exposure to 0.00190 ppm (the LEC_{01})[b] using the two-piece linear spline model with knot at 1,600 ppm × days[c] (continued)

Column A: Interval index number (i).
Column B: 5-yr age interval (except <1 and 1−4) up to age 85.
Column C: All-cause mortality rate for interval i (× 10^5/yr) (2008−2012 data from CDC).
Column D: Lymphoid cancer incidence rate for interval i (× 10^5/yr) (2008−2012 SEER data).[d]
Column E: All-cause hazard rate for interval i (h^*_i) (= all-cause mortality rate × number of years in age interval).[e]
Column F: Probability of surviving interval i (without being diagnosed with lymphoid cancer) (q_i) [= $\exp(-h^*_i)$]. This column is intended to represent the probability of surviving the interval without a diagnosis of lymphoid cancer; however, because lymphoid cancer incidence rates are negligible compared to all-cause mortality rates, no adjustment was made to the population at risk to account for the probability of a lymphoid cancer diagnosis. For breast cancer incidence, on the other hand, the age-specific "mortality" rates (representing the rates at which the population at risk is decreased in each interval) were adjusted to include the age-specific breast cancer incidence rates and to exclude the age-specific breast cancer mortality rates, this latter adjustment so that the probability of death from lymphoid cancer is not counted twice, i.e., both as an incident case and as a component of the all-cause mortality.
Column G: Probability of surviving up to interval i (without having been diagnosed with lymphoid cancer) (S_i) ($S_1 = 1$; $S_i = S_{i-1} \times q_{i-1}$, for i > 1).
Column H: Lymphoid cancer incidence hazard rate for interval i (h_i) (= lymphoid cancer incidence rate × number of years in interval).
Column I: Conditional probability of being diagnosed with lymphoid cancer in interval i [= $(h_i/h^*_i) \times S_i \times (1-q_i)$], i.e., conditional upon surviving up to interval i (without having been diagnosed with lymphoid cancer) (Ro, the background lifetime probability of being diagnosed with lymphoid cancer = the sum of the conditional probabilities across the intervals).
Column J: Exposure duration at midinterval (taking into account 15-yr lag) (xtime).
Column K: Cumulative exposure midinterval (xdose) (= exposure level [i.e., 0.00190 ppm] × 365/240 × 20/10 × xtime × 365) [365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures; xtime × 365 converts exposure duration in years to exposure duration in days].
Column L: Lymphoid cancer incidence hazard rate in exposed people for interval i (hx_i) (= $h_i \times (1 + \beta \times \text{xdose})$, where $\beta = 0.002983$ per ppm × day is the profile likelihood 95% (one-sided) upper-bound estimate for the regression coefficient for the first spline of the two-piece linear spline model (see Section 4.1.1.2); note that the cumulative exposures are below the knot of 1,600 ppm × days for each interval, so only the first spline is used.
Column M: All-cause hazard rate in exposed people for interval i (h^*x_i) [= $h^*_i + (hx_i - h_i)$].
Column N: Probability of exposed people surviving interval i (without being diagnosed with lymphoid cancer) (qx_i) [= $\exp(-h^*x_i)$].
Column O: Probability of exposed people surviving up to interval i (without having been diagnosed with lymphoid cancer) (Sx_i) ($Sx_1 = 1$; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for i > 1).
Column P: Conditional probability of exposed people being diagnosed with lymphoid cancer in interval i [= $(hx_i/h^*x_i) \times Sx_i \times (1-qx_i)$] (Rx, the lifetime probability of being diagnosed with lymphoid cancer for exposed people = the sum of the conditional probabilities across the intervals).

[a]Using the methodology of BEIR (1988).
[b]The estimated 95% lower bound on the continuous exposure level that gives a 1% extra lifetime risk of lymphoid cancer incidence.
[c]Based on the results of Steenland et al. (2004), reanalyzed by Steenland for both sexes combined (see Appendix D), with a 15-year lag, as described in Section 4.1.1.
[d]Background cancer incidence rates are used to estimate extra risks for cancer incidence under the assumption that the exposure-response relationship for cancer incidence is the same as that for cancer mortality (see Section 4.1.1.3).
[e]For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval – the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. For the lymphoid cancer incidence

Table E-1. Extra risk calculation[a] for lymphoid cancer incidence from environmental exposure to 0.00190 ppm (the LEC_{01})[b] using the two-piece linear spline model with knot at 1,600 ppm × days[c] (continued)

calculations, this adjustment was ignored because the lymphoid cancer incidence rates are small when compared with the all-cause mortality rates. For the breast cancer incidence calculations, on the other hand, this adjustment was made in the all-cause hazard rate (see Section 4.1.2.3).

APPENDIX F. EQUATIONS USED FOR WEIGHTED LINEAR REGRESSION OF CATEGORICAL RESULTS

[Source: Rothman (1986), p. 343−344]

Linear model: $RR = 1 + bX$

where RR = rate ratio, X = exposure, and b = slope.

Slope (b) can be estimated from the following equation:

$$\hat{b} = \frac{\sum_{j=2}^{n} w_j x_j \hat{RR}_j - \sum_{j=2}^{n} w_j x_j}{\sum_{j=2}^{n} w_j x_j^{\,2}} \qquad \text{(F-1)}$$

where j specifies the exposure category level and the reference category ($j = 1$) is ignored.

The standard error of the slope can be estimated as follows:

$$SE(\hat{b}) \approx \sqrt{\frac{1}{\sum_{j=2}^{n} w_j x_j^{\,2}}} \qquad \text{(F-2)}$$

The weights, w_j, are estimated from the confidence intervals (as the inverse of the variance):

$$Var(\hat{RR}_j) \approx \hat{RR}_j^{\,2} Var[\ln(\hat{RR}_j)] \approx \hat{RR}_j^{\,2} \times \left[\frac{\ln(\overline{RR}_j) - \ln(\underline{RR}_j)}{2 \times 1.96}\right]^2 \qquad \text{(F-3)}$$

where $\overline{RR}_j$ is the 95% upper bound on the RR_j estimate (for the jth exposure category), and $\underline{RR}_j$ is the 95% lower bound on the RR_j estimate.

APPENDIX G. MODEL PARAMETERS IN THE ANALYSIS OF ANIMAL TUMOR INCIDENCE

Table G-1. Analysis of grouped data, NTP (1987) mouse study[a]; multistage model parameters

Tumor	Multistage[b] polynomial degree	q_0	q_1[c] $(mg/m^3)^{-1}$	q_2 $(mg/m^3)^{-2}$	q_3 $(mg/m^3)^{-2}$	*p* value (χ^2 goodness of fit)
Males						
Lung adenomas plus carcinomas	1	2.52×10^{-1}	1.52×10^{-2}			0.92
Females						
Lung adenomas plus carcinomas	2	3.87×10^{-2}	0.0	4.80×10^{-4}		0.39
Malignant lymphoma	3	1.74×10^{-1}	0.0	0.0	1.13×10^{-5}	0.18
Uterine carcinoma	2	0.0	0.0	9.80×10^{-5}		0.90
Mammary carcinoma	1[d]	2.27×10^{-2}	1.09×10^{-2}			–

[a]The exposure concentrations were 0, 50, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$, where d is inhaled ethylene oxide exposure concentration.
[c]Even though q_1 is zero in some cases, the upper bound of q_1 is nonzero.
[d]The 100-ppm dose was deleted; the fit was perfect with only two points to fit.

Table G-2. Analysis of grouped data from the Lynch et al. (1984a,c) study of male F344 rats[a]; multistage model parameters

Tumor	Multistage[b] polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	*p*-value (χ^2 goodness of fit)
Splenic mononuclear cell leukemia	1[c]	3.12×10^{-1}	1.48×10^{-2}	–
Testicular peritoneal mesothelioma	1	3.54×10^{-2}	6.30×10^{-3}	0.34
Brain mixed-cell glioma	1	0	1.72×10^{-4}	0.96

[a]The exposure concentrations were 0, 50, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$, where d is inhaled ethylene oxide exposure concentration.
[c]The 100-ppm dose was deleted; the fit was perfect with only two points to fit.

Table G-3. Analysis of grouped data from the Garman et al. (1985) and Snellings et al. (1984) reports on F344 rats[a]; multistage model parameters

Tumor	Multistage[b] polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	*p*-value (χ^2 goodness of fit)
Males				
Splenic mononuclear cell leukemia	1	1.63×10^{-1}	8.56×10^{-3}	0.34
Testicular peritoneal mesothelioma	1	2.38×10^{-2}	4.74×10^{-3}	0.68
Primary brain tumors	1	5.88×10^{-3}	2.92×10^{-3}	0.46
Females				
Splenic mononuclear cell leukemia	1	1.08×10^{-1}	2.37×10^{-2}	0.75
Primary brain tumors	1	5.94×10^{-3}	1.65×10^{-3}	0.80

[a]The exposure concentrations were 0, 10, 33, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k)]$, where d is inhaled ethylene oxide exposure concentration.

Table G-4. Time-to-tumor analysis of individual animal data from the NTP (1987) mouse study[a]; multistage-Weibull model[b] parameters

Tumor	Multistage polynomial degree	q_0	q_1 $(mg/m^3)^{-1}$	z
Males				
Lung adenomas plus carcinomas	1	3.44×10^{-1}	2.03×10^{-2}	5.39
Females				
Lung adenomas plus carcinomas	1	5.35×10^{-2}	1.76×10^{-2}	7.27
Malignant lymphoma	1	1.91×10^{-1}	8.80×10^{-3}	1.00
Uterine carcinoma	1	0.0	3.81×10^{-3}	3.93
Mammary carcinoma	1	3.78×10^{-2}	5.10×10^{-3}	1.00

[a]The exposure concentrations were 0, 50, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d, t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, where d is inhaled ethylene oxide exposure concentration. The length of the study was 104 weeks. The times t and t_0 as expressed in the above formula are scaled so that the length of the study is 1.0. Then, q_0 is dimensionless, and the coefficients q_k are expressed in units of $(mg/m^3)^{-k}$.

APPENDIX H. SUMMARY OF 2007 EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

A draft of this assessment document entitled *Evaluation of the Carcinogenicity of Ethylene Oxide* (dated August 2006) (U.S. EPA, 2006a) was available for public comment and underwent a formal external peer review in accordance with EPA guidance on peer review (U.S. EPA, 2006b). At the request of the EPA's Office of Research and Development, the EPA Science Advisory Board (SAB) convened a panel of 15 experts external to the Agency to review the ethylene oxide (EtO) assessment document. An external peer-review meeting was held in January 2007, and a final peer-review report was released in December 2007 (SAB, 2007).

The primary purpose of this draft assessment was to review and characterize the available data on the carcinogenicity of EtO and to estimate the lifetime unit cancer risk from inhalation exposure. The SAB panel was asked to comment primarily on three main issues including carcinogenic hazard, cancer risk estimation, and uncertainty associated with the hazard characterization and quantitative risk estimation. The SAB panel was charged with answering a number of specific questions that addressed key scientific issues relevant to the assessment. The comments made by the panel in the Executive Summary of the SAB report (SAB, 2007) in response to the charge questions are presented verbatim below followed by the EPA's responses; the comments and responses are arranged by charge question.

In addition, a number of comments from the public were received during the public comment period. An extract of the significant scientific public comments and the EPA's responses are also included in a separate section of this appendix (see Section H.2).

Following the 2007 SAB review, a revised draft was developed and released for public comment in July 2013. The comments on the 2013 draft are summarized in Appendix K along with the EPA's responses. The 2013 draft was further revised in response to the public comments and submitted for additional SAB review in August 2014. Comments on the 2014 SAB review draft and the EPA's responses are presented in Appendix I.

H.1. SAB PANEL COMMENTS

The statement of the issues as contained in the Agency's charge to the SAB panel are listed below in italics followed by (1) the panel's comments quoted directly from the Executive Summary of the panel's report (SAB, 2007) and (2) the Agency's response to the comments.

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

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

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

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

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

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

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

EPA RESPONSE: As supported by the majority of the panel, the EPA is retaining the conclusion that the combined weight of the epidemiological, laboratory animal, and mutagenicity evidence is sufficient to characterize EtO as carcinogenic to humans. Some panel members were of the opinion that the descriptor "Likely to be Carcinogenic to Humans" was more appropriate, as they found the epidemiological evidence to be weak and the data insufficient to conclude that key precursor events were observed in humans [SAB (2007), p.10]. The EPA and the majority of the SAB panel disagree that the epidemiological evidence is weak. The EPA has strengthened the summary review of these data in the human evidence section (see Section 3.1) and in the hazard characterization section (see Section 3.5.1). In addition, the revised assessment specifically addresses the precursor data for rodents and humans. While the databases for humans and rodents contain different types of studies, the EPA did not find any inconsistency and concluded that the data support a finding of a mutagenic mode of action (relevant to humans), a finding with which the SAB concurred. The EPA has expanded the discussion of these data, specifically in Sections 3.3.3.2, 3.3.3.3, and 3.4.1.

In response to the panel recommendations, the EPA has added an introduction at the beginning of Chapter 3 that provides a brief description of the information presented in the chapter and has provided a clearer explanation of the criteria used to evaluate the strengths and weaknesses of epidemiological studies (at the beginning of Section 3.1). With respect to the recommendation to put material from Appendix A into the main body of the document, the EPA determined that the in-depth level of detail in Appendix A was not appropriate for the main body of the document. Instead, the EPA has added two shorter summary tables of the lymphohematopoietic cancer (see Table 3–1) and breast cancer (see Table 3–2) findings in the various epidemiology studies to Section 3.1.1. The EPA has also added a cross-reference to summary Table A-5 in Appendix A at the beginning of Section 3.1. The main body of the document provides a summary of the findings of all the epidemiological studies, referencing Appendix A for further details.

The EPA notes that the panel agreed with the EPA's use of "internal" estimates rather than "external" comparisons.

The 2006 draft assessment did not refer to or characterize the magnitude of the unit risk associated with EtO exposure as "weak." Rather, it was with respect to the Hill considerations for causality (Hill, 1965) in the weight-of-evidence analysis for hazard characterization (see Section 3.5.1) that the draft assessment noted that there was little strength in the association, as reflected by the modest magnitude of the (relative) risk estimates from the epidemiology studies. The exposure-response models used to develop the unit risk estimates are derived from the NIOSH data and are thus consistent with the results of the NIOSH epidemiology study, as can be seen in the figures depicting RR versus exposure for the various exposure-response models. The unit risk estimates are derived from these exposure-response models and are similarly consistent with the results of the NIOSH study, as long as they are used in the low-exposure range, as intended. Because the exposure-response relationships for the cancers of interest in the NIOSH study are generally supralinear, the unit risk estimates will overpredict the NIOSH results if applied to exposure levels that correspond to the region of the exposure-response relationships where the responses plateau.

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

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

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

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

EPA RESPONSE: The discussion of endogenous metabolic production of EtO and its significance and contribution to the formation of background adducts in rodents and humans has been expanded (see Sections 3.3.2 and 3.3.3.1 and Section C.7 of Appendix C). A discussion of the endogenous production of ethylene during normal physiological processes and its metabolism to EtO under certain conditions has been added (see Section C.7 of Appendix C). It should be noted that the endogenous production of EtO due to the metabolism of endogenous

ethylene will be present in all test animals or subjects (including controls); hence, this factor is considered inherently in the analysis of effects of EtO exposure.

The discussion of DNA adduct formation resulting from EtO exposure has also been expanded to add breadth and depth (see Section 3.3.3.1 and Section C.1.1 of Appendix C). Section C.1.1 of Appendix C includes discussion of general DNA adduct formation, sensitivity of the methods used to detect DNA adducts, and DNA adduct studies, both in vitro and in vivo, that have been conducted in animals and humans.

The EPA agrees with the majority of the panel that data on (exogenous) ethylene should not be included in the assessment. One caveat provided on page 12 of the SAB report is that the ethylene bioassays administered ethylene concentrations with such low EtO equivalents that they would appear "to be below the limit of detection for a tumor response over the spontaneous background in the F344 rat." Thus, the ethylene data would not be very informative for the EtO assessment, for which there are already adequate EtO bioassays.

The EPA considered all 34 references listed by the SAB panel in its report (p. 13–15), and the revised draft cites all but 10 of them. The 10 references that were not cited were considered to be not particularly relevant or necessary to the assessment: one was on propylene oxide, one was on N-nitrosocompounds, two were on ethylene, two were related to OSHA's review of EtO, two were mutagenicity papers from the 1970s published in a Russian journal, one was a 1979 mutagenicity paper published in a French journal, and the last was a paper on the emission of EtO from the frying of foods.

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

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

EPA RESPONSE: The EPA has expanded the discussion of DNA adduct formation (see Section 3.3.3.1 and Section C.1.1 of Appendix C), mutagenicity (see Section 3.3.3 and Sections C.2–C.5 of Appendix C), and possible mechanisms (see Section 3.4) in the revised assessment document.

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

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

EPA RESPONSE: A more rigorous and balanced hazard characterization was incorporated into the revised assessment (see Section 3.5.1). To address the issue of precursor events, the genotoxicity (see Section 3.3.3 and Appendix C) and mode-of-action (see Section 3.4.1) sections have been revised to provide a more complete and balanced discussion of EtO-induced precursor events in laboratory animals and humans. As addressed in the EPA response under Charge Question 1.a above, while the databases for humans and rodents contain different types of genotoxicity studies, the EPA did not find an inconsistency in EtO-induced precursor events and concluded that the data support a finding of a mutagenic mode of action (relevant to humans) and that the key precursor events are anticipated to occur in humans (see Sections 3.3.3.2, 3.3.3.3, 3.4.1, and 3.5.1).

Issue 2: Risk Estimation (Section 4 and Appendices C and D of the EPA Draft Assessment) ***Do the available data and discussion in the draft document support the approaches taken by EPA in its derivation of cancer risk estimates for EtO? In your response, please include consideration of the following:***

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

SAB COMMENT: The Panel concurred that the NIOSH cohort is the best single epidemiological data set with which to study the relationship of cancer mortality to the full range

of occupational exposures to EtO. That said, the Panel encouraged the EPA to broadly consider all of the epidemiological data in developing its final Assessment. In particular, the Panel encourages the EPA to explore uses for the Greenberg et al. (1990) data including leukemia and pancreatic cancer mortality and EtO exposures for 2,174 Union Carbide workers from its two Kanawha Valley, West Virginia facilities. [Also described in (Teta et al., 1999; Teta et al., 1993)].

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

EPA RESPONSE: The EPA re-evaluated all of the epidemiological studies with quantitative exposure-response data and has revised the assessment to include an expanded discussion of study selection, including a summary table of important considerations, in Section 4.1, as well as expanded discussions of the exposure assessments for the Union Carbide (see Appendix A, Section A.2.20) and NIOSH (see Appendix A, Section A.2.8) studies.

In regard to the possible use of other epidemiologic data for exposure-response modeling, the assessment document includes a detailed discussion of the studies of workers at the Union Carbide facilities in West Virginia. Since the 2007 SAB review, analyses of the data from an extended follow-up (through 2003) of the Union Carbide cohort, focused on the 1,896 EtO production workers who did not work in the chlorohydrin unit, have been published by Swaen et al. (2009) and Valdez-Flores et al. (2010). This cohort is about one-tenth the size of the NIOSH cohort. At the end of the 2003 follow-up, only 27 lymphohematopoietic cancer deaths (including 12 leukemias and 11 NHLs) were observed in the cohort. Thus, even after extended follow-up, the number of cases is small compared to the NIOSH study, which had 74 lymphohematopoietic cancer deaths, 53 from lymphoid cancers.

Furthermore, the Union Carbide study has a less extensive exposure assessment than the NIOSH study. In part, the deficiency is inherent in a chemical production setting, where it is difficult to find workers with relatively uniform work histories that involve relatively constant exposure to EtO. The exposure assessment used by Swaen et al. (2009) for the Union Carbide study was relatively crude, based on just a small number of department-specific (high-, medium-, and low-exposure intensity) and time period-specific (1925–1939, 1940–1956, 1957–1973, and 1974–1988) categories, and with exposure estimates for only a few of the categories derived from actual measurements (see Section A.2.20 of Appendix A for the details). This is in contrast to the sterilization plants studied by NIOSH, where workers can be grouped into relatively common jobs/work zones, facilitating assignment of exposure. Furthermore, extensive sampling

data (2,350 measurements from 1975 to 1986, reduced to 205 annual job-specific means, representing 80% of the data; another 20% were not included but used as a validation sample) were used in the NIOSH study to estimate exposure in different jobs and years. Such sampling data were not used in estimating exposures in the Union Carbide cohort. Finally, the NIOSH regression model for estimating EtO exposure included data not only on job/work zone, but also on variables such as size of sterilizer, type of product, freshness of product, and exhaust systems for sterilizers. This regression model explained 85% of the variance in the EtO validation data set. As a result, the exposure estimates in the NIOSH study are expected to be more accurate.

In addition to its larger size, greater number of cases, and more thorough exposure assessment, the NIOSH study had other advantages over the Union Carbide cohort, such as the inclusion of female workers and the absence of occupational coexposures, as documented in Section 4.1. Furthermore, because of the lack of comparability in the exposure estimates across the two studies, it is not possible to group together the NIOSH cohort and the Union Carbide cohort for a rigorous combined quantitative exposure-response analysis. Thus, the EPA used the NIOSH study alone as the basis for quantitative risk estimates, consistent with the concurrence of the SAB panel that the NIOSH study is the best single study for that purpose.

The EPA has investigated the issue about potential instability that may result from interaction between the chosen time metric for the dose-response model and the treatment of time in the estimated exposure (e.g., log cumulative exposure with 15-year lag) in the NIOSH cohort and does not consider it to be a substantial problem. The concern is apparently that the 15-year lag in the exposure metric, which discounts the most recent exposures, may cause an over-reliance in the exposure-response analysis on exposures which were estimated prior to 1978, which may be less accurate because the NIOSH exposure model assumed that the effect of calendar year was constant before 1978. As discussed by Hornung et al. (1994), including the engineering controls in the NIOSH exposure model could not completely explain the decreases in EtO levels observed since the late 1970s. Thus, Hornung et al. (1994) also included calendar year in the model as a surrogate for improvements in work practices, above and beyond the engineering controls, resulting from increased awareness in the late 1970s of the potential carcinogenicity of EtO. Fitting the measurement data from 1976 to 1985 showed that the effect of calendar year on exposure estimates was maximal between 1976 and about 1978–1979 and reduced exposure estimates after that. Thus, the calendar year effect in the exposure model was fixed at 1978 for years prior to 1978. Assuming the effect of calendar year to be constant before 1978 was both consistent with the available data for exposure levels prior to 1978 and reasonable given that the increasing awareness of EtO carcinogenicity in the late 1970s could explain the calendar year effect decreasing exposures only after that time. Exposure estimates prior to 1978

were then determined entirely by the other variables in the model, for which data were available for the years before 1978.

There is inevitably more uncertainty about the estimation of exposures prior to 1976, when there were no sampling data. However, the use of a 15-year lag is unlikely to appreciably increase the uncertainty that exists in the cumulative exposure estimates due to potential measurement error in the pre-1976 exposure-level estimates, given that in the follow-up through 1998, exposures in the lagged out period for most workers would be substantially lower than exposure before the lag came into effect. See Section D.5.2 of Appendix D for more information on the impacts of the 15-year lag on exposure estimates.

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

SAB COMMENT: The Panel identified several important shortcomings in the linear regression modeling approach used to establish the point of departure for low dose extrapolation of cancer risk due to EtO [note added by the EPA: more detailed comments provided by the SAB panel about the linear regression approach and the EPA's responses are presented beginning on page H-25]. The Panel was unanimous in its recommendation that the EPA develop its risk models based on direct analysis of the individual exposure and cancer outcome data for the NIOSH cohort rather than the approach based on published grouped data that is presently used. The suggested analysis will require EPA to acquire or otherwise access individual data and develop appropriate methods of analysis. The Panel recommends that the Agency allocate the appropriate resources to conduct this analysis.

The Panel was divided on whether low dose extrapolation of risk due to environmental EtO exposure levels should be linear (following Cancer Guideline defaults for carcinogenic agents operating via a mutagenic mode of action) or whether plausible biological mechanisms argued for a nonlinear form for the low dose response relationship. With appropriate discussion

of the statistical and biological uncertainties, several Panel members strongly advocated that both linear and nonlinear calculations be considered in the final EtO Risk Assessment.

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

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

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

EPA RESPONSE: The above comment from the panel addresses a variety of issues and the EPA's responses to some of these issues are comparatively detailed; thus, the EPA has subdivided the response into separately titled subsections to make it easier to read.

EPA response on the modeling of the individual-level data: In response to the SAB comments, the EPA conducted extensive analyses using the individual-level (continuous) exposure and cancer outcome data for the NIOSH cohort. These analyses are described in

Section 4.1.1.2 for lymphoid cancer modeling and Section 4.1.2.3 for breast cancer incidence modeling (no further analyses were done with the all lymphohematopoietic cancer data because lymphoid cancer estimates are preferred or with the breast cancer mortality data because the incidence data set is preferred). These sections also include summary tables of the key models examined and the factors considered in model selection (see Tables 4–4 and 4–12 for lymphoid cancer and breast cancer incidence, respectively). More details on the various models and the model results are provided in Appendix D.

The underlying problem that makes the EtO data sets from the NIOSH cohort difficult to model (for the purposes of environmental risk assessment) is that the exposure-response relationships, particularly for lymphoid cancer and breast cancer mortality, are supralinear (i.e., the responses rise relatively steeply at low exposures and then attenuate or "plateau"). Supralinear exposure-response relationships are inherently difficult to model for the purposes of environmental risk assessment (i.e., to estimate risk at low exposures) because the standard single-parameter exposure-response models tend to exaggerate the low-exposure slope in order to simultaneously fit the plateauing at higher exposures. One approach attempted by the EPA, in consultation with Steenland, to address this difficulty was to use two-piece spline models, which provide more flexibility and allow for the lower-exposure and higher-exposure data to be fit with different spline segments.

For the breast cancer incidence data, the EPA was able to develop several continuous models that provided reasonable fits to the individual-level exposure data across the entire range of the data, consistent with the SAB recommendations. The best-fitting of these models, the two-piece linear spline model, now forms the basis for the EPA's unit risk estimate for breast cancer incidence (see Section 4.1.2.3).

For lymphoid cancer, however, despite the extensive modeling efforts, the various alternative continuous models investigated, including the two-piece spline models, proved problematic, as explained in detail in the text (see Section 4.1.1.2). In particular, the statistically significant models predicted extremely steep slopes in the low-dose region. Thus, the EPA has retained the approach used in the 2006 external review draft assessment and has based the preferred unit risk estimates for lymphoid cancer on a linear regression using the categorical data, excluding the highest exposure group. In consideration of the SAB recommendation, however, unit risk estimates from the most suitable alternative model based on the continuous exposure data were developed and added to the assessment for comparison purposes.

While the EPA understood and appreciated the SAB's recommendation and did much work to model the individual-level data for lymphoid cancer, it should be noted that modeling of grouped data is an important and well-recognized statistical methodology and its use is consistent with EPA guidance, policy, and past practice. For example, the EPA's 2005 *Guidelines for Carcinogen Risk*

Assessment (U.S. EPA, 2005a) specifically recognize the use of linear modeling of *grouped* epidemiological data ("For epidemiologic studies, including those with grouped data, analysis by linear models in the range of observation is generally appropriate unless the fit is poor," p. 3–11). In addition, the EPA's approach of using a weighted linear regression through the categorical relative risk estimates follows established statistical procedures (van Wijngaarden and Hertz-Picciotto, 2004; Rothman, 1986).

The breast cancer mortality data displayed similar extreme supralinearity, and the optimal two-piece spline model yielded an unrealistically steep low-dose slope estimate; thus, the EPA again used a linear regression of the categorical data, excluding the highest exposure group (see Section 4.1.2.2). In consideration of the SAB recommendation, however, a unit risk estimate for breast cancer mortality from the most suitable alternative model based on the continuous exposure data was developed and added to the assessment for comparison purposes. The breast cancer mortality data, however, are not critical to the assessment because the breast cancer incidence data set is preferred (see Section 4.1.2.3).

EPA response on the use of a nonlinear approach to low-exposure extrapolation: The EPA has given careful consideration to the range of perspectives provided in the SAB report on the issue of low-dose extrapolation, including the viewpoint expressed by several panel members who advocated that both linear and nonlinear calculations be considered in the EtO assessment. It is the EPA's judgment, as detailed below, that the inclusion of a nonlinear approach is not warranted.

As discussed in Chapter 3 of the assessment, EtO is a DNA-reactive, mutagenic, multisite carcinogen in humans and laboratory animal species; as such, it has the hallmarks of a compound for which low-dose linear extrapolation is strongly supported. The EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) specifically note the use of low-dose linear extrapolation for "agents that are DNA-reactive and have direct mutagenic activity." Appendix A of the SAB report also provides support for low-dose linearity for genetically acting agents, noting, for example, that additivity to background carcinogenic processes at low doses is expected to result in incremental risk that approaches linearity, as discussed by Crump et al. (1976). By comparison, the *Guidelines* recommend that, "A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses." The EPA's analysis indicates that EtO does not meet any of those conditions. For EtO, there is sufficient weight of evidence to support a mutagenic/genotoxic MOA, without compelling evidence of additional or alternative MOAs being operative (see Section 3.4.1).

The EPA specifically considered a two-hit MOA proposed by Kirman et al. (2004) to support a (nonlinear) quadratic model for leukemia. The Kirman et al. (2004) proposal was based on several assumptions, and the EPA concluded that the evidence was inadequate to substantiate the assumptions supporting use of the quadratic model, as discussed in detail in Section 3.4 of the assessment.

With regard to the particular comments of the SAB members advocating presentation of a nonlinear approach, the SAB report (p. 23) suggests that linear extrapolation "is a conservative assumption, given EtO's reactivity (which will diminish the amount reaching the nucleus), mutagenic mode of action, and that it is generated endogenously" and that "[s]ome repair seems likely and some threshold probably exists." The evidence is ample, however, that EtO from both endogenous and exogenous sources reaches the nucleus and forms adducts (see Section 3.3.3.1 and Section C.1.1 of Appendix C), and more recent data from Marsden et al. (2009) specifically demonstrate (nonsignificant) increases of DNA adducts for very low exposures to exogenous EtO (see Section 3.3.3.1). Any diminution of the amount of EtO reaching the nucleus is expected to affect the slope of the low-dose linear relationship but not linearity per se. Similarly, the fact that endogenous EtO is present and that some repair takes place is not considered evidence against low-dose linearity because the low doses of exogenous EtO are expected to contribute to background carcinogenic processes for the common cancers, such as lymphoid cancer and breast cancer, associated with EtO exposure. The SAB report itself, in that same paragraph presenting the argument for nonlinearity (p. 23), acknowledges that a "linear model per se at low doses is acceptable."

Additional reasons for using a nonlinear approach expanded upon in Appendix C of the SAB report were largely general suppositions 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, more recent data from Marsden et al. (2009) support a linear exposure-response relationship for EtO exposure and DNA adducts. 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, Marsden et al. (2009) reported statistically significant linear dose-response relationships ($p < 0.05$) for exogenous adducts in all three tissues examined (spleen, liver, and stomach) and measured increases of DNA adducts from exogenous EtO exposure above those from endogenous EtO for very low exposures to exogenous EtO, as discussed in detail in the assessment (see Section 3.3.3.1 and 4.5), providing evidence against the first reason proposed in support of a nonlinear approach in Appendix C of the SAB report. In support of the second reason, Appendix C of the SAB report presents two EtO-specific mutation data sets; however, the EPA's analysis of these data sets, summarized below, finds that they are in fact consistent

with low-dose linearity. In summary, the EPA's review of studies addressing dose-response patterns for adduct formation and mutagenesis by EtO finds these data to be supportive of the inferences made in the EtO assessment [and more broadly in the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)] regarding the plausibility of linear, nonthreshold, low-dose dose-response relationships for the biological effects of EtO, which is mutagenic and directly damages DNA.

The EPA further notes that the supralinear exposure-response relationships from the NIOSH data at low occupational exposures argue against the existence of a "threshold," practical or otherwise, at exposure levels anywhere near the POD. Also, the rodent bioassays do not suggest an absence of increased cancer risk at their lowest exposure levels.

Analysis of the EtO mutagenicity data sets presented in Appendix C of the SAB Report:

In Appendix C in the SAB report, one reviewer provided slides (numbers 25 and 26) showing dose-response data for *hprt* mutations in mice exposed to either EtO or to ethylene. For ethylene, a model estimate of an EtO-equivalent concentration was used to represent metabolism of ethylene to EtO. In both cases, mutations at the *hprt* locus of T-cells isolated from spleens of Big Blue mice were quantified. The EtO study results are from Walker et al. (1997), and it appears that the ethylene results are derived from experiments presented in Walker et al. (2000). In the latter case, there are some differences in the estimated EtO equivalents and the *hprt* mutation frequencies between the values given in the slide and those reported by Walker et al. (2000). The EPA performed statistical analyses using the data presented in slide 26 of Appendix C.

To examine these data, the EPA first analyzed the EtO data set (Walker et al., 1997) using maximum likelihood estimation (MLE). The EPA then looked at the consistency of the ethylene data set (Walker et al., 2000) with the EtO data set. The EtO data were fit with a linear model using a log-normal distribution of the individual animal response measurements due to the low mutant frequency that causes skewness of the data. As shown in Figure H-1, this model provided an adequate fit to the EtO data (open circles represent individual animal data for the EtO exposures; model goodness-of-fit $p = 0.09$; variance fit assuming homogeneous variance in log scale, $p = 0.64$). The MLE of the model is plotted (geometric mean [solid line] as an estimation of the median response along with the lower and upper 2.5 percentiles of the model [dashed lines]). The second, ethylene-derived, data set is plotted on the same graph (closed circles). The predicted EtO-equivalents from the ethylene data set fall well below the lowest dose level used in the EtO experiment, a range in which the EtO-based model would predict only a small response (i.e., no more than a 25% increase in mutation rate above background, a level

that cannot be expected to be detectable given the variability in the EtO experimental data; see Figure H-1). The fact that the ethylene exposures did not show measureable increases in *hprt* mutations is consistent with the modeled EtO results.

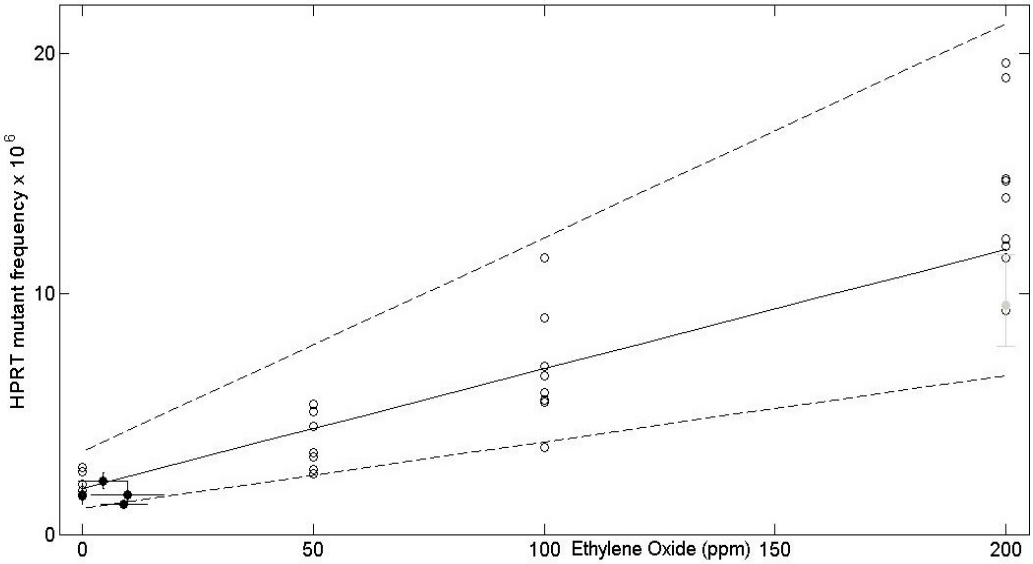


Figure H-1. Induction of *hprt* mutations by EtO (open circles and modeled fit) with data from ethylene (using estimated EtO equivalents) shown (solid circles).

Source: SAB (2007), Appendix C (slides 25 and 26); original experiments of Walker et al. (1997).

Note, however, that all medians of the ethylene-derived data are at or below the EtO-based model and one of the points is below the lower 2.5 percentile of the model, indicating that this point is unlikely to be consistent with the same model. To further investigate the compatibility of the data from the two experiments, the EPA analyzed the combined data set by including a term that represents the source of the data (the EtO vs. ethylene experiments) into the modeling (as above). This experimental variable was significant ($p < 0.05$), indicating that there is a systematic difference in response between the EtO and ethylene-derived data. As a further check, the EPA refit the data using an exponential model that provided an MLE fit with a degree of upward curvature (but still having low-dose linear behavior). Using a categorical experimental variable within this experiment also indicated a systematic dependence of results on data source (EtO vs. ethylene), indicating that this finding was not dependent on the choice of a straight-line dose-response model. As an additional sensitivity analysis, the EPA reran the

modeling using the values of EtO equivalents from ethylene exposure and *hprt* results directly from Walker et al. (2000) (rather than the values shown in the SAB Appendix C slide); the modeling results were essentially unchanged. Accordingly, the EPA concluded that combining the ethylene data with EtO data in evaluating dose-response relationships for the *hprt* mutations might not be appropriate.

Slide 27 of the SAB report presents data from Nivard et al. (2003) on the frequency of recessive lethal mutations in *Drosophila* exposed to EtO [full data set presented in Vogel and Nivard (1998)]. Plotting of mutation rate versus EtO concentration for wild-type *Drosophila* on nonlog-transformed axes shows a downward curving (supralinear) relationship indicating greater potency of EtO (per unit exposure) at low exposures as compared with high exposures (see Figure H-2). These data are adequately fit by a Michaelis-Menten-type relationship (downward curving, linear at low dose); the fit is somewhat improved with a fractional power Hill model, which would indicate even steeper low-dose response.

In conclusion, the EPA's review of the EtO mutagenicity data presented in Appendix C of the SAB report finds that these data do not show a disproportionate fall-off of mutagenic effects or an "inflection point" at low doses of EtO; that is, they do not indicate a low-dose nonlinear or threshold-type dose-response pattern. Thus, the EPA's review finds these data to be supportive of the inferences made in the assessment [and more broadly in the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)] regarding the plausibility of linear, nonthreshold, low-dose dose-response relationships for the carcinogenic effects of EtO, which is mutagenic and directly damages DNA.

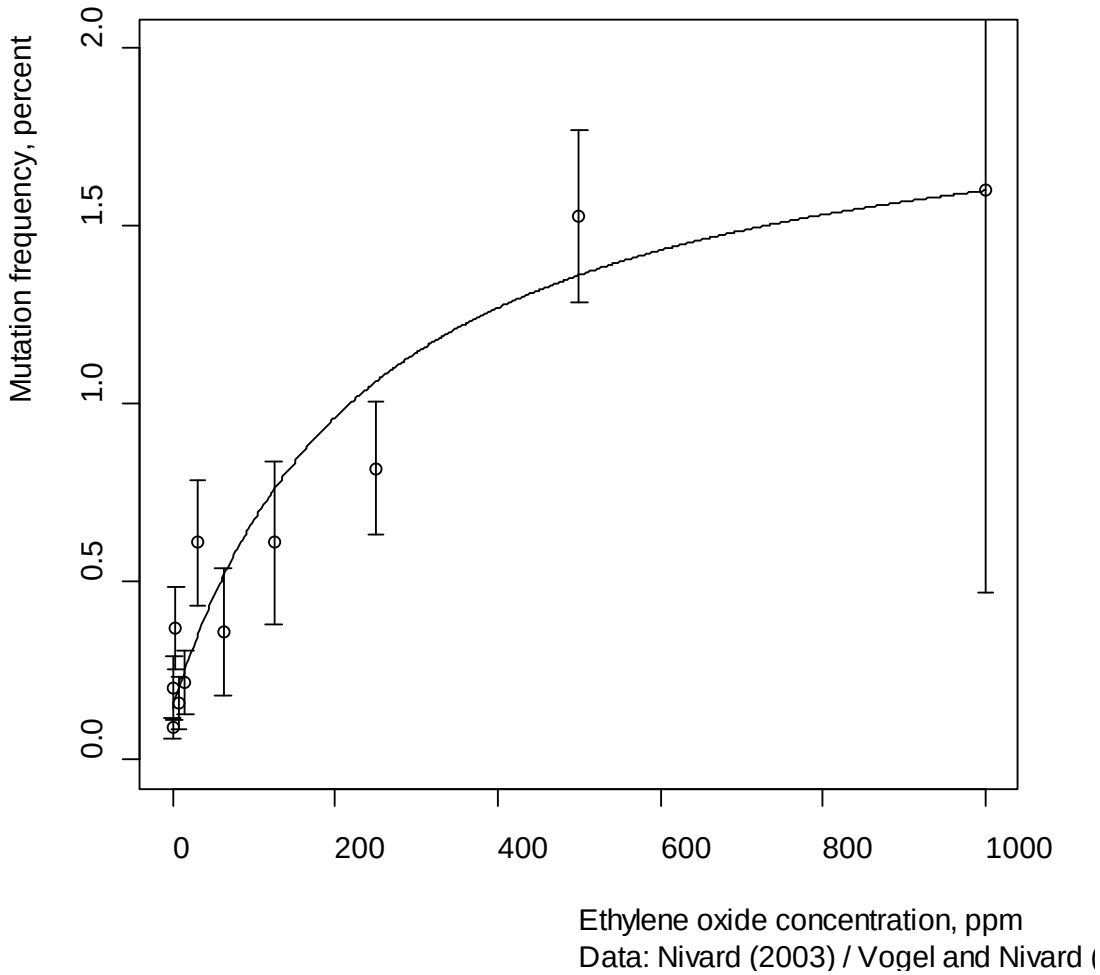


Figure H-2. Induction of recessive lethal mutations by EtO in *Drosophila* (wild-type).

Standard deviations are calculated as the square root of the number of mutations, assuming a Poisson distribution, and plotted as ± (SD × percent mutation frequency).

EPA response on using different data to estimate different dose-response curves: With respect to using different data to estimate different dose-response curves, the panel comment pertains only to the occupational exposure scenarios. This is addressed in the EPA's response to the SAB comment on Charge Question 2.d below.

EPA response on lymphohematopoietic cancer groupings: As recommended by the panel, the primary risk estimates in the revised assessment are based on the analysis of the lymphohematopoietic cancer subtype of lymphoid cancers (see Section 4.1.1.2), which was the

subtype with the strongest evidence of an EtO association in the NIOSH data set (Steenland et al., 2004). Analysis based on total lymphohematopoietic cancers is also included for completeness and comparison purposes.

EPA response on the use of only the male data for lymphohematopoietic cancers: Subsequent analyses by Steenland determined that there was not a statistically significant difference between the lymphohematopoietic cancer results for males and females (see Sections D.3.3.1 and D.4.2.1 of Appendix D). Thus, in the revised assessment, data on males and females were combined as appropriate, and unit risk estimates are now based on lymphoid cancers for males and females combined and breast cancer in females.

The following additional comments on page 31 of the SAB panel report under "2.b. Methods of Analysis: 7. Statistical issues," are quoted verbatim below followed by the EPA's responses:

SAB COMMENT:

7. Statistical issues

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

Conditional on the cancer slope factor results from the weighted least squares regression analysis, the life table (BEIR IV) approach to the determination of the LEC01 is programmed correctly.

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

Several Panel members commented on the use of the upper confidence limit for the estimated slope coefficient as the basis for estimating an LEC_{01}. The Panel encourages the EPA to present unit risk estimates based on the range of EC_{01} values corresponding to the lower 95%

confidence limit, the point estimate, and the upper 95% confidence limit for the estimated cancer slope coefficients from the final dose-response models.

EPA RESPONSE: The above comment from the panel addresses a variety of issues and the EPA has subdivided the response into separately titled subsections to make reading it easier.

EPA response on using the Committee on the Biological Effects of Ionizing Radiation [BEIR] approach to estimate incidence risks: In this assessment, the EPA's preferred unit risk estimates are those for cancer incidence, not mortality, as the cancers associated with EtO exposure (lymphohematopoietic, in particular lymphoid, and breast cancers) have substantial survival rates. Regarding the breast cancer incidence estimates, the assumption that a cancer mortality exposure-response model applies to cancer incidence was not needed because the model used for the breast cancer incidence estimates was based on incidence data. In addition, although the BEIR approach was designed for mortality estimates, the EPA believes it has made a suitable adjustment to the approach by redefining the population at risk as those alive and without a diagnosis of breast cancer at the beginning of the age interval (rather than those alive at the beginning of the interval). This adjustment was not made in the life-tables for the lymphoid cancer estimates because, unlike for breast cancer incidence rates, lymphoid cancer incidence rates (actually, the differential rates obtained by subtracting the mortality rates from the incidence rates) are negligible in comparison to the all-cause mortality rates.

Regarding the lymphoid cancers, the SAB provided the relevant comment that mathematically the BEIR formula would apply to the case where there is a proportional rate of incidence/mortality across the cancer types that are included in the grouped analysis. The EPA considered this in its application of the BEIR formula. The fact that the ratios of incidence to mortality are not strictly proportional contributes some uncertainty to the incidence estimates for the grouping of lymphoid cancers, but not a large amount. Uncertainties in using the life-table analysis approach to seek to develop reasonable estimates for incidence risk, including those noted by the SAB, are acknowledged in the assessment, and the impact of nonproportionality among cancer types is one of the uncertainties discussed (see Section 4.1.1.3). As illustrated in the assessment, these uncertainties do not have a major impact on the final risk estimates. The incidence unit risk estimate is about 120% higher than (i.e., 2.2 times) the mortality-based estimate, which is consistent with the relatively high survival rates for lymphoid cancers. Potential concern that the incidence unit risk values 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, in the cause-specific background rates in the life-table might inflate the incidence estimates). Multiple myelomas, however, constitute only 25% of the lymphoid cancer cases, and there is no evidence that multiple myeloma is driving the EtO-induced excess in lymphoid cancer mortality (25% is below the proportion of multiple myeloma deaths one would expect in the cohort based on age-adjusted background mortality rates of multiple myeloma, NHL, and chronic lymphocytic leukemia, and these 3 subtypes have the same pattern of mortality rates increasing as a function of age mostly above age 50, so the comparison with lifetime background rates is reasonable). 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 panel's suggestion to not use the BEIR approach for development of cancer incidence estimates for lymphoid cancer would not allow for the development of the desired cancer incidence risk estimates. Deriving incidence estimates from mortality data is consistent with EPA guidance, which suggests making adjustments to reflect the relationship between incidence and mortality [U.S. EPA (2005a), p. 3–12]. A possible alternative approach involving a crude survival adjustment to the mortality-based estimates would yield results with greater uncertainty than those from the life-table approach used. No alternative approaches were identified by the SAB. In the absence of an appropriate alternative approach to estimate risks of cancer incidence, the EPA has retained the application of the BEIR (life-table) approach, which it judges to provide a reasonable estimate of incidence risks. The EPA recognizes the uncertainties and assumptions outlined by the panel and has expanded the discussion of these in the carcinogenicity assessment (see Section 4.1.1.3). However, the EPA notes that deriving mortality estimates as the sole cancer risk estimates for lymphohematopoietic cancer would substantially underestimate cancer risk. In addition, the EPA presents the mortality-based estimates for comparison, and as discussed above, the lymphoid cancer incidence unit risk estimate is about 120% higher than (i.e., 2.2 times) the mortality-based estimate, which is considered reasonable, given the high survival rates for lymphoid cancers.

EPA response on the use of upper and lower confidence limits: In both the 2006 and revised drafts of the EtO assessment, the EPA presents 95% (one-sided) lower bounds and central estimates of the EC_{01}s as well as standard errors for the regression coefficients used in the modeling, which provide information about the variability in the modeled slope estimate. The EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) also recommend the calculation of a 95% upper bound on the central estimate (in this case the EC_{01}) related to the POD "to the extent practicable" [U.S. EPA (2005a), p. 1−14], and this value has been added to

the revised assessment for the selected breast cancer incidence model (see Section 4.1.2.3, Table 4–13, footnote j, based on the profile likelihood confidence limits for the regression coefficient). However, for the linear regression model used as the basis for the lymphoid cancer unit risk estimate, it was not practicable to calculate such a value, as it was undefined. Although there were models for lymphoid cancer from which upper bounds could have been calculated, the linear regression model was selected as the basis for the POD for the express purpose of obtaining a realistic slope estimate for the low-exposure region (see Section 4.1.1.2) and not for providing a realistic upper-bound estimate for the EC_{01}.

The EPA considered the SAB panel comment encouraging the EPA "to present unit risk estimates based on the range of EC_{01} values corresponding to the lower 95% confidence limit, the point estimate, and the upper 95% confidence limit." However, as a consequence of the two-step approach used by the EPA to generate cancer potency estimates from a POD rather than directly from the statistical model used to estimate the POD, potency estimates below the response level corresponding to the POD are no longer associated with the statistical model. Linear extrapolation from a POD that is the 95% (one-sided) lower bound on the central estimate of the exposure concentration associated with the selected (benchmark) response level (e.g., the LEC_{01}) might be generally expected to yield a reasonable upper bound on cancer risk for that data set (although not strictly a statistical "95%" upper bound). In contrast, estimates involving a linear extrapolation from the *upper* bound on that central estimate are not generally meaningful and could be misleading if they are mistaken for lower bounds on potency, as the actual exposure-response relationship may exhibit some sublinearity below the response level corresponding to the POD. Thus, it has not been EPA practice to develop potency estimates based on the upper 95% confidence limit on the EC_{01}, and the EPA did not undertake to develop any for this assessment. (The EPA does present the standard upper-bound unit risk estimates based on the LEC_{01}s [e.g., Table 4–22] as well as "$0.01/EC_{01}$" estimates [Table 4–23].)

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

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

EPA RESPONSE: The EPA has added a new subsection (see Section 4.4) detailing the application of the ADAFs.

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

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

EPA RESPONSE: For the breast cancer incidence risk estimates, a single model, the two-piece linear spline model, is now recommended for the occupational exposure scenarios. The two-piece linear spline model is a unitary model comprised of two linear pieces or segments with different slopes that are joined at a point referred to as a "knot." The two-piece linear model has the flexibility to represent situations, such as with EtO, where the relationship between exposure level and response changes over the range of exposure. For lymphoid cancer risk estimates, the preferred model for the occupational exposure scenarios of interest to the EPA, the log-cumulative exposure Cox regression model, is applicable over the entire range of occupational exposure scenarios of interest. A second model, the linear regression of the categorical results, is provided should exposure scenarios involving lower exposures be of interest at some future time or to other parties. Thus, two models are presented for the lower-exposure exposure scenarios, but just one of the models is recommended for the higher-exposure exposure scenarios; users have the option of using a single model across the range of exposure scenarios or of transitioning across models, depending on the exposure scenarios of interest, and some further guidance on choice of approach has been added in Section 4.7 of the revised assessment. As discussed in the assessment, the log-cumulative exposure model, which provides a good fit to the data in the plateau and is suitable for exposure scenarios with cumulative exposures in that region, is not appropriate for the low-exposure region (i.e., below the range of the occupational scenarios presented in this assessment) because such a steep increase in slope is considered to be biologically implausible and the good statistical global fit of the model should not be over-interpreted to infer that the model provides a meaningful fit to the low-exposure region. Likewise, the linear regression used to model the lower-dose exposure groups is not

intended to reflect the exposure-response relationship in the higher-exposure region. Hence, for lymphoid cancer, the use of both models may be required to cover a broader range of occupational exposure scenarios. Table 4–19 of the assessment shows how results from the two models compare over a range of exposure scenarios for which either model might be used.

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

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

EPA RESPONSE: The EPA notes the panel's support for the use of the ppm equivalence method. As the unit risk value is based on human data, the consideration of more sophisticated models was not warranted.

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

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

a. The assessment approach employed;

b. The use of assumptions and their impact on the assessment;

c. The use of extrapolations and their impact on the assessment;

d. Plausible alternatives and the choices made among those alternatives;

e. The impact of one choice versus another on the assessment;

f. Significant data gaps and their implications for the assessment;

g. The scientific conclusions identified separately from default assumptions and policy calls;

h. The major risk conclusions and the assessor's confidence and uncertainties in them; and

i. The relative strength of each risk assessment component and its impact on the overall assessment.

SAB COMMENT: The Panel has responded to Charge Questions 1 and 2 and has tried to incorporate their comments regarding Charge Question 3 within those responses. A separate

response for Charge Question 3 was not deemed necessary since issues of uncertainty were addressed in the responses to charge questions 1 and 2 [p. 9].

The following are detailed comments on the regression modeling used in the draft ethylene oxide assessment quoted from the SAB ethylene oxide panel report (related to Charge Question 2.b; p. 24–26) and the EPA response:

SAB COMMENT:

2. Linear regression model for categorical data

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

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

The approach described in the Draft Assessment uses a model based on categories defined by cumulative exposure ranges for male subjects in the NIOSH cohort. Steenland et al. identified several models that provide a significant ($p < 0.05$) fit to the exposure data; however, the EPA has elected to use model-based relative rate parameter estimates for categories of 15 year lagged, cumulative exposure. In Steenland et al. (2004) this model was not one that provided a significant fit to the NIOSH data ($p = 0.15$ for the likelihood ratio test of $\beta = [\beta 1, \beta 2, \beta 3, \beta 4] = 0$). The use of the weighted least squares regression fit of a linear regression line through the three data points defined by the estimated rate ratios and mean cumulative exposures for the first three exposure categories of the Steenland et al. 15 year lag, cumulative exposure category model is not a robust application of this technique. The Panel identified four weaknesses in the approach.

a) Model-based dependent variable: The dependent variables are model-based estimates of rate ratios for exposure categories. The rate ratio values used in the weighted least squares regression are derived from a cumulative exposure model (15 year lag) in which the estimated regression parameters in the proportional hazards regression model are not significantly different

from 0 at $\alpha = 0.05$ ($p = 0.15$). In Steenland et al. (2004), the only individually based (proportional hazards) model that fits the data for males in the NIOSH cohort is a model for log of individual exposure through t-15 years.

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

c) The model fitting does not conform exactly to the Rothman (1986) procedure: The 1998 (Second edition) of Rothman (Rothman and Greenland, 1998) describes the technique for estimating this risk from grouped data in Chapter 23. In that updated version of the original monograph the model that is fitted is:

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

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

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

The model estimated by the EPA method is:

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

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

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

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

Categorizing continuous variables results in a host of issues:

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

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

EPA RESPONSE: The EPA agrees that it may be generally preferable to develop risk models on the basis of direct analysis of individual exposure and cancer outcome data. The 2006 draft assessment included the presentation of models based on fitting Cox regression models to individual exposure-outcome data for EtO. The Cox regression models with log cumulative exposure provided reasonable fits to the data, as described by Steenland et al. (2004) and in the 2006 draft assessment. However, the EPA concluded that these models represented exposure-response relationships that were excessively sensitive to changes in exposure level in the low-dose region, and thus, were not biologically realistic. That is, in the low-dose region,

these models would yield extremely large changes in response for small changes in dose level. Accordingly, the judgment was that these models would not be suitable as the basis for low-dose unit risk values. This is what led the EPA to use the regression methodology with the published grouped data. The grouped data regression methodology is considered to be a valid procedure for analysis of such data, and, as mentioned above with respect to Charge Question 2.b, the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) specifically recognize the use of linear modeling of grouped epidemiological data (U.S. EPA, 2005a); therefore, the EPA has retained its use for some endpoints (lymphoid cancer and breast cancer mortality) in the revised assessment and implemented it as described by Rothman (1986) [also described in van Wijngaarden and Hertz-Picciotto (2004)].

The EPA followed the panel's recommendation and performed additional analyses of the individual data in collaboration with Steenland. The work performed by Steenland is described in Appendix D of the revised assessment. Working with Steenland, alternative models based on direct analysis of all individual data using (1) linear relative risk models (Langholz and Richardson, 2010) and (2) two-piece linear and log-linear spline models [e.g., Rothman et al. (2008)] were developed and evaluated. In the revised assessment, linear low-dose risk estimates based on the two-piece linear spline model (using the Langholz-Richardson linear relative risk approach) were used for breast cancer incidence risk estimates. Additional responses to specific comments follow:

a) Model-based dependent variable: The rate ratios for the exposure categories were not all statistically significant, likely due to loss of power from categorizing the data (in the draft that the SAB reviewed, which was based on the results in males only, it is true that none of the RR estimates for the lower three quartiles was statistically significantly elevated; in the revised draft, based on both sexes, the RR estimate for the 2nd quartile is statistically significant). The fact that the log cumulative Cox regression model is statistically significant for the continuous exposure data, however, establishes that there is an exposure-response trend for these data. Despite the lack of statistical significance for some of the categorical RR estimates, the EPA used the categorical results because they provide the best available estimates of the RRs for the limited exposure ranges reflected in each category, and these estimates were felt to be adequate, particularly for the three lowest quartiles (the highest exposure quartiles, which represent large, open-ended exposure ranges, were excluded from the linear regression models), for use in the linear regression model.

b) Grouped data regression: The panel comments identify assumptions inherent in the method. The EPA does not believe, however, that these assumptions preclude the use of the Rothman model in the context of the EtO cancer risk estimation. The EPA disagrees with the

suggestion that unmeasured confounders may have produced gross misclassification and somehow impaired the exposure model estimation for individuals. The estimation performed by NIOSH to estimate individual worker exposure (Hornung et al., 1994) was extensive and detailed. The resulting model used to estimate worker exposure accounted for 85% of the variation in average EtO exposure (see Section 4.1 and Section A.2.8 of Appendix A). Thus, unmeasured confounding, while possible, is unlikely to be substantial. The EPA agrees with the panel that the exposure analysis of Hornung et al. (1994) is an example of an "exemplary quantitative analysis of likely errors in exposure estimates." In response to the panel's suggestion that the Hornung analysis represents an "invaluable opportunity" for further analysis of the impact of possible errors in exposure estimation, the EPA investigated the possible use of the "errors in variables" approach (page 27 of the panel report). Steenland visited the NIOSH offices in Cincinnati in order to review the data and assess whether it would support an errors-in-variables analysis. Unfortunately, the electronic data files used in the exposure analysis were no longer available, so that analysis based on the errors-in-variables approach was not possible.

c) The EPA reviewed the statistical procedure for modeling categorical data using the methodology in Rothman (1986). This review confirmed that the Rothman procedure was followed closely. The equations used, which are the same as those in Rothman (1986) (pp. 341−344), are described in Appendix F. The equations are also provided in van Wijngaarden and Hertz-Picciotto (2004). The Rothman (1986) procedure, which is appropriate for case-control data such as the NIOSH data, is based on estimating the effect at each response level relative to the reference or baseline level. Thus, the effect estimates are relative rates (odds ratios), not absolute rates as used in the approach of Rothman and Greenland (1998) cited by the SAB. The rate ratio in the referent group (i.e., those with estimated cumulative exposure = 0) is 1.0, by definition and without an associated estimate of variability; hence, there is no intercept term in the model. As described by Rothman (1986) (p. 345), variability in the reference category is necessarily entrained in estimates of the slope. As Rothman (1986) points out, this can result in loss of estimation efficiency but nevertheless yields a valid estimate of trend. Thus, while it is true, as the comment states, that this procedure may not be optimal in a theoretical sense, it can provide a useful mechanism for estimating linear trend. The panel acknowledges that a linear regression may be the most appropriate approach when only grouped data are available. The EPA agrees but would add that when the objective is low-dose risk estimation, the approach may yield the most useful results from a pragmatic perspective. The availability of individual data does not preclude the use of the Rothman (1986) grouped data regression methodology. [See also the summary and review of the paper by Valdez-Flores and

Sielken (2013) in Section J.3.1 of Appendix J for a discussion of limitations in estimating the intercept when conducting a linear regression of the categorical results for the EtO data sets.]

In the case of the EtO data, it was possible to derive theoretically correct models via direct analysis of the individual data. In the case of the breast cancer incidence data, this approach yielded a model that provided a suitable basis for unit risk estimation. For the other data sets (breast cancer mortality, lymphoid cancer mortality), however, most of the models derived using all the individual data were not useful for unit risk estimation because of excessive sensitivity in the low-dose range. The large sensitivity of the models to small changes in low-dose values results in unstable low-dose risk estimates lacking in biological plausibility; thus, the Rothman procedure was used. In consideration of the SAB recommendation, however, unit risk estimates from the most suitable alternative models for lymphoid cancer and breast cancer mortality based on the continuous exposure data were developed and added to the revised assessment for comparison with the results of the linear regression of the categorical results, which was still the preferred model for reasons detailed in the revised assessment (see Sections 4.1.1.2 and 4.1.2.2).

Responses to SAB panel 'bullet' comments (contained within the SAB comment on page H-26 above):

• Assumption that the risk within the category boundaries is constant.

EPA RESPONSE: The EPA is not assuming that within-category risk is constant. Instead, the assumption is that observed risk within a category may be averaged over a category even though there may be a trend within the category. This is a conventional approach in epidemiological analyses in which categorical analysis is used.

• It is not known whether a given categorization is representative of the data since there are many ways of categorizing.

EPA RESPONSE: The data groupings used in the EPA analyses were based on sound statistical principles and standard epidemiological practice and were subject to peer review through the publications of Steenland et al. (2003) and Steenland et al. (2004). The categories were generally quartiles based on the distribution of cumulative exposures for the cases of the cancer of interest, resulting in essentially the same number of cancer cases per quartile, a typical approach in epidemiological studies.

• Loss of power and precision by spending degrees of freedom on each category.

EPA RESPONSE: There is some loss of power and precision in categorization. This can result in a failure to find a statistically significant effect when in fact there is a meaningful effect in the data.

• Misclassification at category boundaries (this can be minimized by choosing cut points where relatively few observations are present).

EPA RESPONSE: Misclassification can occur at category boundaries; however, this is expected to have a small impact on overall results. Moreover, the likely consequences of misclassification across boundaries are that if an RR is overestimated in one category, the RR in an adjacent category will be underestimated. Using a linear regression model across the categories may serve to smooth out some of this misclassification, if there is any.

• Categorizations can be manipulated to show the desired results.

EPA RESPONSE: This may be possible, but no manipulation of the EtO data was performed by the EPA to show "desired results." The data categories used in the EPA analyses were established a priori in the Steenland (2004; 2003) publications. The panel's recommendation to use "a large number of equally spaced exposure ranges" was not practical for lymphoid cancer because of the relatively small number of deaths.

H.2. PUBLIC COMMENTS

A number of public comments were received that addressed a range of technical issues related to the inhalation carcinogenicity of EtO. A number of comments were also received that are generally directed at what are referred to as "risk management" issues and, as such, are not addressed here. In the following, summaries of comments on technical risk assessment issues submitted by the public are provided followed by the EPA's responses (note that some duplicate comments were omitted).

PUBLIC COMMENT 1.0: The Draft Cancer Assessment Fails to Meet the Rigorous Standard of Quality Required Under the Information Quality Act and Cancer Guidelines. The Draft Cancer Assessment is "influential information" as set forth under the Information Quality Act (IQA) and therefore is subject to a rigorous standard of quality. EPA guidance and the Guidelines for Carcinogen Risk Assessment (Cancer Guidelines) (U.S. EPA, 2005a) require a

rigorous standard of quality, which necessitates ensuring that the Draft Cancer Assessment uses scientifically defensible analytical and statistical methods and has a higher degree of transparency than information considered noninfluential, particularly regarding the application of uncertainty factors in EPA's dose-response assessment and risk characterization. The Draft Cancer Assessment demonstrably fails to meet either the standard set forth under the IQA or the Cancer Guidelines. EPA must, therefore, substantially revise the assessment before the final EtO Integrated Risk Information System (IRIS) Risk Assessment (IRIS Assessment) is publicly disseminated or relied upon for any regulatory purposes.

EPA RESPONSE: Comments received from the SAB and from the public have been addressed and the EtO carcinogenicity assessment has been revised. It is the EPA's position that as a result of the extensive development, review, reanalysis, and revision, the revised assessment follows the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), uses scientifically defensible analytical and statistical methods, and meets a high standard of transparency. As such, the revised assessment is consistent with the EPA's Information Quality Guidelines.[15]

PUBLIC COMMENT 2.0: EPA failed to use all available epidemiologic data, including the Union Carbide Corporation (UCC) data and all the National Institute of Occupational Safety and Health (NIOSH) data that were available at the time EPA conducted its assessment.

EPA RESPONSE: The assessment describes and considers all relevant epidemiological data available at the time the assessment was conducted, including all the NIOSH and UCC data. The Union Carbide data and the publications that this public commentator referred to were evaluated and included in the assessment. The EPA also reviewed articles describing additional follow-up and analysis of the Union Carbide data that have been published after the panel's report was finalized. Ultimately, the EPA came to the conclusion that the shortcomings inherent in the Union Carbide data, particularly the crude assignment of exposure levels to subjects in the UCC cohort, are fundamental, and as a consequence, the data are not suitable for credible quantitative analysis of the carcinogenic risk due to exposure to EtO. In the NIOSH data, exposure estimates were based on a very large number of exposure measurements and a sophisticated modeling approach (Hornung et al., 1994), which took into account job category and other factors such as

[15]U.S. EPA (2002) Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by EPA. https://www.epa.gov/sites/production/files/2015-08/documents/epa-info-quality-guidelines.pdf

product type, exhaust controls, age of product, cubic feet of sterilizer, and degree of aeration. Hence, prediction and assignment of exposure levels for different workers in the NIOSH study would be expected to be much better than the more simplistic assignment methods used in the Union Carbide study. Although the recent follow-up of the UCC cohort has now been reported, there still remains a rather small number of cancers (27 lymphohematopoietic cancers vs. 79 in the NIOSH cohort and 12 vs. 31 NHLs). Consequently, for example, there was a 50% excess of NHL in the 9+ years of employment category in the Union Carbide study (Swaen et al., 2009), but it was based on only five cases and was thus not statistically significant. Also, the UCC cohort is restricted to men, making an analysis of breast cancer, which was seen to have a significant increase among female workers with high EtO exposures in the NIOSH cohort, impossible. In sum, the Union Carbide and NIOSH cohorts are not comparable on a number of levels, and the NIOSH cohort remains superior as a basis for exposure-response analyses. In the NIOSH cohort, exposure-response analyses are likely to involve much less misclassification of exposure and are based on greater numbers, and thus, would be expected to be more reliable. Analyses of the important breast cancer endpoint are only possible with the NIOSH cohort. See also the EPA's response to comments on Charge Question 2.a above.

PUBLIC COMMENT 3.0: EPA inappropriately based its evaluation on summaries of statistics available in various publications, rather than the primary source data, review of which and reliance upon are essential to conduct valid dose-response modeling. EPA should have based its calculations on readily available NIOSH data for individual subjects from the cohort mortality study.

EPA RESPONSE: The statistics used in the draft assessment were obtained from published journal articles describing the analysis of the NIOSH data. They are summary and categorical statistics that are commonly used in epidemiological research. The methodology for using such categorical data to perform dose-response analysis is well established in the epidemiological literature and is described in Rothman (1986), pp. 343−344, and van Wijngaarden and Hertz-Picciotto (2004). The categorical and summary statistics used by the EPA are constructed from the individual data in the NIOSH study. It is possible to perform analyses and construct models via direct analysis of the individual data and in some cases this is a preferable approach. In fact, the draft EPA assessment presented the results of such analyses in the form of the Cox regression models that were based on direct analysis of the individual data with exposure as a continuous variable. These models provided reasonable fits to the data. However, it was the judgment of the EPA that these models generated estimates of risk in the low-dose region that were excessively sensitive to changes in exposure level, and therefore, would not be suitable as the

basis for low-dose unit risk values. This is what led the EPA to use the regression methodology with the published grouped data. The EPA, in consultation with Steenland, performed analyses to fit additional models to the continuous exposure NIOSH data. The work by Steenland is described in Appendix D of the revised assessment. Working with Steenland, the EPA developed and evaluated sets of models using the individual data, including (1) linear relative risk models (Langholz and Richardson, 2010) and (2) two-piece linear and log-linear spline models [e.g., Rothman et al. (2008)]. In the revised assessment, linear low-dose estimates based on the two-piece spline model and using the Langholz-Richardson linear approach were used for breast cancer incidence risk estimates. See also the EPA's response to comments on Charge Question 2.b above.

PUBLIC COMMENT 4.0: EPA Statistical Analysis of the Data Is Flawed and Other Incorrect Procedures Grossly Overestimate Risk. Key flaws include:
PUBLIC COMMENT 4.1: EPA's risk assessments are invalid, based on linear regressions on odds ratios (ORs), rather than on individual subject data;

EPA RESPONSE: The odds ratios referrenced are summary statistics. Regression on categorical or summary statistics such as odds ratios is a valid statistical approach. See the response to Comment 1.2 and response to the SAB panel comment on this issue (Charge Question 2.b above).

PUBLIC COMMENT 4.2: EPA fails to include all available epidemiologic data;

EPA RESPONSE: This comment refers to the Union Carbide data. See response to Comment 2.0 and response to the SAB panel comment on this issue (Charge Question 2.b above).

PUBLIC COMMENT 4.3: EPA's rationale and methodology for exclusion of the highest exposure group is inappropriate;

EPA RESPONSE: The EPA did not use the data from the highest exposure group in estimating the unit risk because it was evident that the relationship between exposure and response changed over the range of exposure. The general pattern in the data indicated a steep increase in response in the low exposure range with a leveling or plateau in the high exposure range. Inclusion of the data from the highest exposure levels in either a Cox regression model or a linear regression yielded overall estimated relationships that were not suitable for risk assessment. Analyses conducted by Steenland excluding various percentages of the highest exposures confirmed that

the highest exposures are attenuating the slopes in such models (see Section D.3.3.1 of Appendix D). Although the Cox regression models with log cumulative exposure provided adequate fits to the different data sets, estimates of risk in the low-dose region were overly sensitive to changes in dose level, and thus, not biologically realistic. In order to obtain a suitable result for risk estimation at low exposures, the EPA used a linear regression model and excluded the highest exposure group in the draft assessment. An additional justification for not including the highest exposure category is that it represents a large, open-ended exposure range, which is less easily represented by a single exposure value, such as the mean exposure used for the narrower lower quartiles of exposure, for the purposes of the linear regression. The EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012) recognizes analyses omitting high-dose data points, when these data are not compatible with the development of suitable descriptive statistical analyses, as a viable analytical approach.

For the revised assessment, the EPA investigated the use of two-piece spline models that modeled the data as a combination of two splines or segments, one that increased steeply in the lower dose region joined with a second that increased at a lower rate in the higher dose region. This approach has the advantage of including all the (individual) data and incorporating into the overall model the change in the relationship over the observed range of exposure**.**

PUBLIC COMMENT 4.4: EPA's use of the heterogeneous broad category of distinct diseases of lymphohematopoietic (LH) cancers as the response increases sample size at the expense of validity and, thereby, reduces the ability to identify a valid positive dose-response relationship.

EPA RESPONSE: The EPA uses the narrower, less heterogeneous category of lymphoid cancer data for the primary risk estimates in the revised assessment.

PUBLIC COMMENT 5.0: Certain Policy Decisions EPA Implements in the Draft Cancer Assessment Are Scientifically Unsupported, Overly Conservative, Inappropriate and Have Not Been Reviewed by a Science Advisory Board. EPA made several policy decisions that compounded greatly the inherent conservatism in the risk estimates. These include, among others: (1) EPA's reliance on the lower bound of the point of departure, rather than the best estimate when using human data; (2) use of background incidence rates with mortality-based relative rates, thereby relying on unsupported assumptions that bias results; (3) EPA's assumption of an 85-year lifetime of continuous exposure and cumulative risk, rather than the more traditional 70-year lifetime; and (4) the application of adjustment factors for early-life exposures.

EPA RESPONSE: The EtO assessment has been reviewed by the SAB and the EPA has responded to their comments and revised the assessment. With regard to (1), use of the lower bound on the point of departure is consistent with the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a); (2), background incidence rates were used with mortality-based relative rates because the EPA's objective is to estimate incidence risk not mortality risk and making adjustments to the analysis when one has only mortality data is consistent with the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) (see also the EPA's response to this issue under the further statistical issues subsection at the end of Charge Question 2.b above); (3), the EPA did not assume an 85-year lifetime, rather exposures were considered up to age 85 (i.e., actual age-specific mortality and disease rates to age 85 were used in a life-table analysis; because most individuals die before age 85 years, the overall average lifespan from the analysis is about 75 years); (4), the EPA's application of adjustment factors for early life exposures in the EtO assessment was in accordance with the recommendations in the EPA's supplemental guidelines and the scientific data supporting the supplemental guidelines (U.S. EPA, 2005b). The application of these adjustment factors in this assessment was endorsed by the SAB. Moreover, the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and Supplemental Guidelines (U.S. EPA, 2005b) were both reviewed by the SAB.

PUBLIC COMMENT 6.0: EPA Improperly Relies Entirely on Males in Its Assessment of Lymphohematopoietic (LH) Cancer Mortality. To be scientifically defensible, EPA's LH cancer risk characterization must include both males and females, consistent with a "weight-of-evidence" approach that relies on *all* relevant information. In the NIOSH retrospective study, increased risks of LH cancer were observed in males but not females, even though the NIOSH cohort was large and diverse, and consisted of more women than men. EPA's exclusive reliance on male data is scientifically unsound without a mechanistic justification for treating males and females differently with respect to LH, which the analysis lacks.

EPA RESPONSE: In the revised assessment, the lymphohematopoietic cancer unit risk estimates are based on data for both sexes.

PUBLIC COMMENT 7.0: EPA's Draft Risk Estimates for Occupational Exposure Levels Rely on Invalid and/or Inappropriate Models. The models used to estimate risks from occupational exposure are flawed because they generate supralinear results, regardless of the observed data. These estimates also suffer from the same invalid methodology used in the environmental risk estimates. EPA must employ a dose-response model that would generate results consistent with the observed data.

EPA RESPONSE: It is the underlying data that indicate a supralinear exposure-response relationship, particularly for lymphoid cancer, all lymphohematopoietic cancer, and breast cancer mortality, as suggested by the categorical results as well as by the poorer fits of the Cox regression models with untransformed cumulative exposure data.

PUBLIC COMMENT 8.0: EtO is Considered by Many to be a Weak Mutagen and EPA Should Consider This in Proposing a Unit Risk Factor. A chemical's mutagenic potency is necessarily related to its carcinogenic potency. If genotoxicity is considered the means by which a chemical induces cancer, it follows that it will not induce cancer under conditions where it does not induce mutations, at either the chromosome or gene level, thus providing a mechanistic basis for estimating carcinogenicity. EtO has been shown only to be a weak mutagen; therefore, it should not be automatically considered a human carcinogen and certainly not a potent carcinogen. In addition, no treatment-related tumors were observed in rats exposed to EtO, even at the 100 ppm concentration level, at the 18 month sacrifice, and the most sensitive tumor type (i.e., splenic mononuclear cell leukemia) did not significantly increase in the exposed rats until 23 months, almost the end of their lifetime of exposures (Snellings et al., 1984). EPA's analysis should have reconciled these findings with its estimation of EtO's carcinogenic potency, but the analysis does not do so.

EPA RESPONSE: The EPA does not consider the mutagenicity and carcinogenicity findings to be in conflict with the potency estimates. EtO is a relatively weak mutagen when compared to strong mutagens such as cancer chemotherapeutic agents and diepoxides but not necessarily when compared to other environmental mutagens. Also, EtO is clearly carcinogenic in mice and rats. The inhalation unit risk estimate based on human data is notably larger than that based on rodent data (about 23 times larger), and the reasons for this discrepancy are unknown; however, such species differences are not unusual.

It would not be surprising if there was no statistically significant increase in tumors at 18 months in the Snellings et al. (1984) study. Because of the latency for cancer development, tumors generally occur later in life. Furthermore, only 20 animals per sex per dose group were killed at 18 months (and tissues from the animals in the low- and mid-dose group only got microscopically examined in the presence of a gross lesion), so there is low power to detect an effect. Nonetheless, Snellings et al. (1984) did report that incidences of brain tumors, which are a rather uncommon tumor type in F344 rats, were increased in the mid- and high-dose groups at the 18-month kill. In addition, for testicular peritoneal mesotheliomas, Snellings et al. (1984)

reported that when the rats with unscheduled deaths were included in the evaluation, EtO exposure appeared to be related to an earlier occurrence of mesotheliomas.

PUBLIC COMMENT 9.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.
PUBLIC COMMENT 9.1: The results of the Draft Cancer Assessment (resulting in negligible risk only at levels less than a part per trillion), are not reasonable when compared with the results generated for other substances that are considered potent mutagens and/or potent carcinogens, and do not comport with the results of other assessments EPA has undertaken.

EPA RESPONSE: The procedures used in this assessment comport with those used in other assessments the EPA has undertaken. Differences in relative potency across chemicals based on exposure levels may reflect differences in absorption, distribution, metabolism, excretion, or the pharmacodynamics of the chemicals.

PUBLIC COMMENT 9.2: The Draft Cancer Assessment grossly over predicts the observed number of cancer mortalities in the study upon which it is based by more than 60-fold.

EPA RESPONSE: The unit risk estimates are derived from, and are consistent with, the results of the NIOSH epidemiology study, as long as they are used in the low-exposure range, as intended. Because the exposure-response relationships for the cancers of interest in the NIOSH study are generally supralinear, the unit risk estimates will overpredict the NIOSH results if applied to the region of the exposure-response relationships where the responses plateau. The potency estimates derived in the assessment are constructed for use with low dose levels consistent with environmental exposure and are not appropriate for use with exposures in occupational settings, as stated explicitly in the document. Occupational exposure scenarios are addressed in Section 4.7 of the assessment document. Extra risks associated with occupational exposures are in the "plateau" region of the exposure-response relationships, and thus, increase proportionately less than risks in the low-dose region.

PUBLIC COMMENT 9.3: EPA's *de minimis* value from the Draft Cancer Assessment is 2 to 3 orders of magnitude below the endogenous level of EtO that is produced naturally in humans.

EPA RESPONSE: The EPA's risk estimates are for risk above background. The issue of endogenous levels is addressed in the revised assessment. See Section 4.5 for a discussion of the specific issue raised in this comment.

PUBLIC COMMENT 9.4: EPA's draft unit risk values for EtO are unreasonably large, given the evidence of carcinogenicity in a large body of epidemiology studies that is not conclusive, the weak mutagenicity data, and the lack of cancer response in rodents until very late in life. EPA must make the best use of all of the epidemiology, toxicology and genotoxicity data for EtO that provide valid information on the relationship between exposure and cancer response to improve the reasonableness of the unit risk values for EtO.

EPA RESPONSE: The EPA believes that it has made the best use of the available information in revising the assessment. The EPA's evaluation of the weight of evidence concludes that the epidemiological evidence is strong (see Section 3.5.1). In addition, the unequivocal evidence of rodent carcinogenicity and the supporting mechanistic evidence add sufficient weight for the characterization of "carcinogenic to humans" (see Section 3.5.1), which is beyond what is needed to support the derivation of quantitative risk estimates. This is thoroughly presented in the assessment and was supported by the SAB review. The unit risk estimates are derived from, and are consistent with, the results of the large, high-quality NIOSH epidemiology study. See also the response to Comment 8.0 above.

PUBLIC COMMENT 10.0: The Draft Cancer Assessment Does Not Use the Best Available Science as Required under the Information Quality Act and Cancer Guidelines.
PUBLIC COMMENT 10.1: EPA based its evaluation on summaries of statistics available in various publications. These data, however, are not sufficient to conduct valid dose-response modeling. EPA should have based its calculations on readily available National Institute of Occupational Safety and Health (NIOSH) data for individual subjects from the cohort mortality study.

EPA RESPONSE: See response to Comment 3.0.

PUBLIC COMMENT 10.2: EPA did not use all available epidemiologic data, including the Union Carbide Corporation (UCC) data and all NIOSH data that were available at the time EPA conducted its assessment. In particular, the Greenberg et al. (1990) UCC study reported the consistency of the death certificate diagnosis with a pathology review of medical records for leukemia cases, a validation not conducted for cases in the NIOSH study.

EPA RESPONSE: The EPA considered all the available epidemiological data, including NIOSH and UCC data, and the publications that the American Chemistry Council referred to in

its comments. See response to Comment 2.0 for more details on why the UCC data were not used for the derivation of quantitative risk estimates.

PUBLIC COMMENT 11.0: EPA Should Recognize That EtO Is Both a Weak Mutagen and Weak Animal Carcinogen.

EPA RESPONSE: The full text of this comment was essentially the same as Comment 8.0 and is addressed in the EPA's response to that comment above.

PUBLIC COMMENT 11.1: Among 26 alkylating agents studies by Vogel and Nivard (1998), EtO showed the second lowest carcinogenic potency.

EPA RESPONSE: The Vogel and Nivard (1998) study is not relevant to the EPA's assessment of the carcinogenicity of EtO. Most of the substances considered by Vogel and Nivard (1998) are chemotherapeutic chemicals that are, by design, intended to be strong alkylating agents.

PUBLIC COMMENT 11.2: Previous assessments of EtO inhalation time to tumor in rats showed that the increased risks observed at higher experimental doses did not extend to the lowest experimental dose. To comply with the Cancer Guidelines, EPA should include these and other relevant animal data in a weight-of-evidence characterization of EtO.

EPA RESPONSE: The carcinogenicity data reviewed in Section 3.2 reveal that, of 13 exposure-response relationships for the tumor types associated with EtO exposure from the three rodent bioassays, all but one show an increased incidence at the lowest exposure level, although not all the increases are statistically significant at that level.

PUBLIC COMMENT 12.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.
PUBLIC COMMENT 12.1: [This was the same as Comment 9.1 above.]
PUBLIC COMMENT 12.2: The results of the Draft Cancer Assessment are at odds with EPA's conclusion that EtO is a potent (*de minimis* level < 1 ppt) human carcinogen and EtO's potency seen in animal studies.

EPA RESPONSE: The risk estimates based on the rodent data are over an order of magnitude lower than (~1/23) the estimate based on the human data, for unknown reasons, but species differences are not unusual and human data are generally preferred over rodent data for

quantitative risk estimates because the uncertainties due to interspecies extrapolation are avoided.

PUBLIC COMMENT 12.3: EPA's draft unit risk values for EtO are not applicable to the general public. The Draft Cancer Assessment grossly over predicts the observed number of LH cancer mortalities in the study upon which it is based by more than 60-fold. Further, EPA's *de minimis* value is about 50 times lower than the lowest ambient concentration found at remote coastal locations. Based upon PBPK simulations, endogenous concentrations of EtO in humans are approximately 400-1700 times greater than EPA's proposed *de minimis* value of 0.00036 parts per billion.

EPA RESPONSE: The unit risk estimates are derived from, and are consistent with, the results of the NIOSH epidemiology study, as long as they are used in the low-exposure range, as intended; see response to Comment 9.2 above. Endogenous and ambient concentrations of EtO could be contributing to background rates of lymphohematopoietic cancer and breast cancer incidences, which are appreciable. The EPA values are not implausible upper-bound estimates.

APPENDIX I. EPA RESPONSES TO SAB COMMENTS ON 2014 EXTERNAL REVIEW DRAFT

This Appendix provides responses to the comments received from the Science Advisory Board (SAB) in their August 7, 2015 report (SAB, 2015) following their review of the EPA's 2014 SAB review draft (U.S. EPA, 2014a, b). A similar draft was reviewed by the public in 2013 (U.S. EPA, 2013a, b), and responses to the public comments are presented in Appendix K. Responses to SAB (SAB, 2007) and public comments on the EPA's 2006 external review draft (U.S. EPA, 2006a) are compiled in Appendix H. In response to charge questions, Appendices K (then L) and H were specifically reviewed by the SAB during their review of the 2014 draft. Public comments to the SAB on the 2014 draft are not addressed directly in this appendix; however, the SAB had all of the public comments for consideration and some of the public comments were explicitly reflected by the SAB in their comments to the EPA.

I. SAB RECOMMENDATIONS IN SAB LETTER TO THE ADMINISTRATOR WITH EPA RESPONSES

1. **COMMENT:** Overall the SAB finds the agency has been highly responsive to the 2007 SAB recommendations. The SAB finds that the National Institute of Occupational Safety and Health (NIOSH) dataset is still the most appropriate dataset to use and concurs with the agency's decision to not use the Union Carbide Corporation cohort data. The statistical and epidemiological issues in this assessment are complex and the agency is to be commended for conducting the additional exposure-response modeling in response to the 2007 SAB recommendations. The SAB believes that the advice and recommendations in this report can be addressed relatively quickly and that the draft assessment should move forward to be finalized.

   EPA RESPONSE: Consistent with the SAB's concurrence, the EPA has continued to use the NIOSH data set as the basis for the quantitative risk estimates and has not derived any estimates from the Union Carbide Corporation cohort data.

2. **COMMENT:** The draft assessment employed lagged exposure estimates in the derivation of cancer risk estimates. Although there is a scientific rationale for a period of latency between biologically important exposures and subsequent cancer incidence or mortality, the SAB did not find a strong biological or statistical argument supporting the particular selected latency periods applied for breast and lymphoid cancers. The EPA is

encouraged to perform a sensitivity analysis of various latency periods to determine what effect this selection had on risk estimates.

EPA RESPONSE: The lag period defines an interval before death, incidence, or end of follow-up during which any exposure is excluded from the calculation of the exposure metric. The EPA re-examined lag selection for both the lymphoid cancer mortality (see Section D.3.2 of Appendix D) and breast cancer incidence (see Section D.1.2 of Appendix D) data sets with a larger group of models than was considered in the 2014 draft and has again selected 15 years as the lag for each data set (endpoint). Sensitivity analyses were conducted with lags of 0, 5, 10, and 20 years to determine the effect of lag selection on the unit risk estimates (see Sections D.1.6 and D.3.5 of Appendix D) and on the extra risk estimates for the occupational exposure scenarios in Section 4.7 (see Sections D.1.11 and D.3.9 of Appendix D). For breast cancer, unit risk estimates from the selected model with the alternate lag periods varied by at most 35% from the primary estimate derived with the selected lag period of 15 years. For the occupational exposure scenarios, the upper-bound extra risk estimates varied by at most about 25% from the estimates derived with the selected lag. For lymphoid cancer, the unit risk estimates from the selected model with the alternate lag periods ranged from about 48% less than to about 190% greater than the estimate derived with the selected lag period of 15 years. For the occupational exposure scenarios, the upper-bound extra risk estimates varied by at most about 55% from the estimates derived with the selected lag.

3. **COMMENT:** A number of different statistical models were examined for estimating breast cancer incidence risk from low exposure to EtO. The draft assessment presents a number of considerations used in the selection of the preferred model. The SAB generally concurs with the selection of the two-piece spline model for estimating breast cancer incidence. However, the SAB has recommendations on improving the considerations used for model selection, including less reliance on the Akaike information criterion (AIC). However, if AIC is used for model selection, it should be used appropriately. There should be *a priori* considerations regarding the nature of the functional form being applied. Specifically, the SAB recommends prioritizing functional forms of the exposure that allow regression models with more local fits in the low exposure range (e.g., spline models). The draft assessment also presents risk estimates from other "reasonable models." Although much of this approach is scientifically appropriate, the SAB finds that a clear definition of "reasonable models" is lacking and

encourages some modifications and more transparency in the presentation. The SAB also provides recommendations on prioritizing statistical considerations in the selection of models. Any model that is to be considered reasonable for risk assessment must have a dose-response form that is both biologically plausible and consistent with the observed data.

EPA RESPONSE: The EPA has followed the SAB's recommendations for model selection. Model selection for both the breast cancer incidence (see Section 4.1.2.3) and lymphoid cancer (see Section 4.1.1.2) data prioritizes functional forms that allow more local fits in the low-exposure range (e.g., spline models), relies less on AIC, and includes consideration of biological plausibility. In addition, the EPA has confirmed that the AIC is being used appropriately—the different models being compared were fit using the same measures, the models had the same outcome variable, and the models were estimated with software routines that calculate AIC in the same way. [The EPA has determined that SAS proc NLP, which was used for the linear RR models for lymphoid cancer, consistently yielded −2 log likelihoods and AICs almost 0.4 units lower than those from proc PHREG, which was used for the log-linear models, for the same models (when the log-linear models were also run in proc NLP), including the null model. This small discrepancy is assumed to be related to computational processing differences. For breast cancer incidence, proc NLMIXED was used for the linear RR models, and this proc generated the same −2 log likelihoods and AICs as did proc PHREG.] The EPA has improved the clarity and transparency of the discussion of model selection, and the EPA no longer distinguishes a subset of "reasonable models." The EPA continues to use the two-piece spline model for the breast cancer incidence data, consistent with SAB concurrence (see Section 4.1.2.3).

4. **COMMENT:** For lymphoid cancer, the draft assessment presents a linear regression of categorical results using dose categories as the preferred model for the derivation of the unit risk estimate for low exposure to EtO. The SAB prefers the use of continuous individual-level exposure data over the use of categorical results. The linear regression of categorical results should not be selected unless the individual exposure model results are biologically implausible. The SAB recommends presentation of multiple estimates of the unit risk in sensitivity analyses and an updated justification of model selection.

EPA RESPONSE: In response to SAB comments, the EPA has changed its model selection for lymphoid cancer from the linear regression of categorical results to a model based on individual-level exposure data. The EPA presents unit risk estimates from multiple models for comparison (see Table 4−7) and has updated the justification for model selection (see Section 4.1.1.2). Consistent with SAB recommendations, the model selection now emphasizes use of the individual-level data, prioritization of functional forms that allow more local fits in the low-exposure range (e.g., spline models), the principle of parsimony, less reliance on AIC, a weighing of biological and statistical considerations, and prioritization of models that can be used for both environmental exposures and the occupational exposure scenarios. As a result of these model selection emphases, the EPA has selected the two-piece linear spline model with the knot at 1,600 ppm × days for the lymphoid cancer data (see Section 4.1.1.2).

5. **COMMENT:** The SAB suggests that the agency consider using the same model for both environmental and occupational exposures. The use of different models for environmental and occupational exposures should only be done with sufficient justification.

   EPA RESPONSE: In response to the SAB comments, the EPA now uses two-piece spline models, which can be applied to both environmental (see Section 4.1) and occupational (see Section 4.7) exposures, for both lymphoid cancer and breast cancer incidence.

6. **COMMENT:** The uncertainty discussions are generally clear, objective, and scientifically appropriate, but they can be improved and extended. Considerations about uncertainty directly pertaining to the analyses reported can be separated into uncertainty due to the data themselves (particularly from reliance on a single data set), and uncertainty of the results given the data. The SAB recommends adding descriptive summaries of the characteristics of the NIOSH cohort, better quantification of the results from the various models (such as reporting unit risk estimates and comparisons in sensitivity analyses), and down-weighting epidemiologic results based on external standards that may be subject to bias due to the healthy worker effect.

   EPA RESPONSE: In response to the SAB comments, the uncertainty discussion has been restructured to address uncertainty due to the data themselves

(see Section 4.1.4.1) separately from uncertainty of the results given the data (see Section 4.1.4.2). In addition, more descriptive summaries of the characteristics of the NIOSH cohort have been incorporated into the assessment, including sex distribution over time, age and year of entry to the EtO workforce, duration of employment in the EtO cohort, age and year of departure/retirement from the EtO cohort, cumulative total and peak exposures for individual cases and controls, percentage of total case and control individual exposures in the worker histories that are excluded when various lags are imposed, and mean, median, and 25^{th}, 75^{th}, and 95^{th} percentile values for annual exposures among cases and controls (see Section D.5 of Appendix D). Additional sensitivity analyses have also been included in the assessment, and all the results are reported as unit risk estimates. For both breast cancer incidence and lymphoid cancer, a variety of models are compared, and the sensitivity of the selected models to different lag periods and knots is examined. Also, mortality and incidence estimates as well as upper-bound estimation approaches are compared. For breast cancer incidence, further analyses include comparisons of the subcohort with interviews and the full cohort, of total breast cancer and invasive breast cancer only, and of the full (selected) model and the model with the exclusion of the nonexposure covariates (breast cancer risk factors). For lymphoid cancer, results are compared to the results for all lymphohematopoietic cancers. All of the EPA's quantitative estimates are based on internal comparisons.

7. **COMMENT:** The draft assessment presents an accurate, objective, and transparent summary of published studies on EtO genotoxicity. The SAB agrees that the weight of the scientific evidence from epidemiological studies, laboratory animal studies and *in vitro* studies supports the general conclusion that the carcinogenicity of EtO in laboratory animals and humans is mediated through a mutagenic mode of action. The SAB finds that several areas of the draft assessment can be improved to enhance the clarity of presentation and to provide a more detailed interpretation of findings within the context of more recent advances in the understanding of the biology of cancer and has specific recommendations and suggestions for revision detailed in the report.

   EPA RESPONSE: The EPA has retained the conclusion that there is sufficient weight of evidence that a mutagenic mode of action is operative in EtO carcinogenicity, but in response to SAB comments, the EPA has strengthened the presentation of the evidence. Section 3.3 (genotoxicity) has been revised to synthesize the information used to support a mutagenic MOA in a more systematic

and complete manner, including the addition of a substantially expanded summary that integrates study information in terms of dose-response and temporal relationships (see Section 3.3.3.4). Section 3.4 has been revised and reorganized to more clearly discuss the mechanisms by which the genotoxic effects might be instrumental in EtO carcinogenesis (see Section 3.4.1.1), particularly in the target organs (see Sections 3.4.1.2 and 3.4.1.3). In addition, the EPA has revised and expanded Table 3−6 (now 3−8) and provided additional summary tables (see Tables 3−6, 3−7, 3−9, and 3−10). (For more details on specific revisions, see responses to detailed comments in Part II.5, below.)

8. **COMMENT:** Appendix H of the draft assessment provides a summary of the 2007 SAB comments and the EPA's response to the comments. The responses are transparent, objective, and for the most part, accurate (exceptions are noted in the current report). In particular, the SAB supports the expanded discussion of endogenous EtO provided in the draft assessment and has suggestions for further improvement; agrees with the decision not to include a toxicity value for EtO based upon nonlinear extrapolation and recognizes and agrees with revisions to strengthen support for a classification of EtO as "carcinogenic to humans."

   EPA RESPONSE: Appendix H is largely unchanged. The EPA's response to any exceptions noted in the 2015 SAB report are addressed where they arise (see Section II.6.a and II.6.b below; pages I-35 to I-44).

9. **COMMENT:** In general, the literature review of new studies presented in Appendix J appears complete. The logic and progression of the review is clearly supported. The clarity can be improved by distinguishing between statements made by study authors and statements made by the EPA. The SAB concurs that inclusion of the new studies would not substantially alter the findings of the assessment, with the exception of the Mikoczy et al. (2011) study of Swedish sterilization workers, which can strengthen support for the hazard characterization of EtO and provide support for the modeling of the NIOSH data.

   EPA RESPONSE: The EPA has clarified what are Agency interpretations and what are study author statements. In addition, the EPA has incorporated discussion of the Mikoczy et al. (2011) study into the main body of the report, supporting the hazard characterization of the epidemiological evidence on breast cancer (see Sections 3.1 and 3.5) and the supralinear exposure-response relationship

observed with the NIOSH data (see Section 4.1.4). Also, a comparison was done of the Mikoczy et al. (2011) RR estimates with predicted RR values from the selected model derived from the NIOSH data (see Section 4.1.4 and Section J.2.2 of Appendix J). (see also response to Comment II.7.a.i below.)

10. **COMMENT:** Appendix L [now K] presents public comments on the July 2013 draft of the assessment and EPA responses to the public comments. The SAB finds that overall, the EPA has been very responsive to the public comments. The responses are thorough, clear, and appropriate.

    EPA RESPONSE: In response to specific comments detailed in Section II.8.a below (pages I-47 to I-54), the EPA has strengthened a few of the responses.

II. COMMENTS FROM THE SAB REPORT

1. More detailed comments regarding lag (p. 7–8 of SAB report)

a. COMMENT: [T]he SAB recommends the methods used to determine minimum latency estimates in the CDC 9/11 Working Group Guidelines (Howard, 2013) as a good framework for assessing latency in cancer onset. However, the disease-specific latency selections in the guidelines are specific to the World Trade Center Health Program and 9/11 agents, and are not relevant to the EtO draft assessment.

EPA RESPONSE: The EPA is interested in an optimal lag and not the minimum lag and has used standard epidemiological methods to determine an optimal lag (see Sections D.1.2 and D.3.2 of Appendix D). Nonetheless, the EPA has reviewed the CDC guidelines (Howard, 2013) and found that the method that the EPA used is also one of the methods discussed in the CDC guidelines—"Method 4A: Statistical Modeling—Estimates of cancer latency obtained by statistical modeling in epidemiologic studies of the association between exposure to an agent and a type of cancer."

b. COMMENT: The SAB encourages the EPA to refine the discussion of this uncertainty with a paragraph in the body of the assessment and a summary of an analysis (detailed in an appendix) that examines the sensitivity of estimates of unit risks over the plausible range of latency periods (i.e., 0–20 years). [...] The SAB

encourages the EPA to formalize the presentation and discussion of the quantitative results for the sensitivity analysis of exposure lags that is currently included in Appendix D, focusing on the sensitivity of the EPA's recommended models and a strongest competitor(s) to the length of the assumed latency period. The body of the draft assessment should include a short summary of the quantitative results of the sensitivity analysis described in detail in the appendix, accompanied by a qualitative discussion of how the results of the sensitivity analysis should factor into an overall assessment of the biological and statistical uncertainty of the unit risk estimates derived under the alternative models of exposure risk.

EPA RESPONSE: The EPA has conducted the sensitivity analyses recommended by the SAB. These are summarized in Sections 4.1.1.3 (lymphoid cancer) and 4.1.2.3 (breast cancer), discussed qualitatively in the context of overall uncertainty in Sections 4.1.4 (sources of uncertainty) and 4.5 (conclusions regarding the unit risk estimates), and detailed in Sections D.1.6 and D.3.5 of Appendix D. For breast cancer, unit risk estimates from the selected model with the alternate lag periods varied by at most 35% from the primary estimate derived with the selected lag period of 15 years, and a comparison is made with the strongest competitor, a 20-year lag. For lymphoid cancer, the unit risk estimates from the selected model with the alternate lag periods ranged from about 48% less than to about 190% greater than the estimate derived with the selected lag period of 15 years, and there is no good competitor. See also response to Comment I.2 above.

c. COMMENT: In summary, the SAB agrees that it is scientifically plausible, and even likely, for there to be a period of latency between biologically important exposures and subsequent cancer incidence or mortality.

EPA RESPONSE: The EPA agrees and has selected lag periods of 15 years of lymphoid cancer mortality (see Section D.3.2 of Appendix D) and 15 years for breast cancer incidence (see Section D.1.2 of Appendix D). See also response to Comment I.2 above.

2. More detailed comments regarding breast cancer incidence model selection

a. Bulleted summary recommendations regarding model selection (p. 11 of SAB report)

i. **COMMENT:** The SAB requests that the EPA provide better documentation of the NIOSH study data, particularly with respect to exposure.

EPA RESPONSE: The EPA has provided the additional details requested by the SAB, as detailed in SAB comments on Charge Question 4 (see Comment II.4.b.iii below); these are summarized in tables and figures in Section D.5 of Appendix D. Some of the new cohort details summarized in Section D.5 include mean, median, minimum, maximum, and 25^{th} and 75^{th} percentiles of cumulative exposure in the full cohort; cumulative exposures by year of entry and by duration of employment; sex distribution over time; distributions of year of entry, age of entry, duration of employment, and age and year of departure/retirement; distributions of cumulative and peak exposures for individual cases and controls; percentages of total case and control individual exposures in the worker histories that are excluded when the 15-year lag is imposed; and mean, median, and 25^{th}, 75^{th}, and 95^{th} percentile values for annual exposures among cases and noncases in the cohort.

ii. **COMMENT:** In selecting models for use in risk assessment, the SAB recommends less reliance on the AIC for model selection. If AIC is used for model selection, it should be used appropriately.

EPA RESPONSE: The EPA has followed the SAB's recommendations for model selection, including less reliance on AIC (see Section 4.1.2.3). In addition, the EPA has confirmed that the AIC is being used appropriately. See also response to Comment I.3 above and Comment II.2.d.iii and its response below.

iii. **COMMENT:** The SAB recommends prioritizing functional forms of the exposure that allow regression models with more local fits in the low-exposure range (e.g., spline models).

EPA RESPONSE: The EPA has followed the SAB's recommendations for model selection, including prioritizing functional forms that allow more local fits in the low-exposure range, and the EPA's selected model is a two-piece spline model (see Section 4.1.2.3).

iv. **COMMENT:** Any model that is to be considered reasonable for risk assessment must have a dose-response form that is both biologically plausible and consistent with the observed data.

EPA RESPONSE: Consistent with SAB concurrence (SAB letter to the Administrator), the EPA has selected a two-piece linear spline model. This general model shape is biologically plausible and the selected model is consistent with the data (see Section 4.1.2.3).

v. **COMMENT:** Sensitivity analyses should be reported for a range of results and should include the target quantity of interest (unit risk, excess risk). Although not all models are equally reasonable from a risk assessment perspective, full and transparent reporting of the target parameters of interest provides valuable context.

EPA RESPONSE: As detailed in Comment II.2.b.iv and its response below, the EPA reports ranges of results for various sensitivity analyses and, in response to SAB recommendations, these are reported as unit risk estimates (or, for the occupational exposure scenarios, as estimates of extra risk [see Section 4.7]).

b. More detailed comments from the text regarding model selection (p. 8–11 of SAB report)

i. **COMMENT:** There is not enough detail provided for the NIOSH exposure data for the SAB to determine the appropriateness of the data. Therefore the SAB response is conditional on the assumption that the NIOSH exposure data are appropriate.

EPA RESPONSE: The EPA has provided the additional information requested by the SAB in the detailed SAB comments on Charge Question 4 (see Comment II.4.b.iii below); these are summarized in tables and figures in Section D.5 of Appendix D. In particular, the SAB had concerns about some exposure data presented by public commenters, also detailed in SAB comments on Charge Question 4, and these concerns are addressed in the

response to those comments (see Comment II.4.b.iv below). In brief, contrary to public comments made at the SAB meeting, the NIOSH EtO exposure patterns were not anomalous, but rather reflected the underlying changes in variables predicting exposure over time. One of the principal drivers of the NIOSH exposure levels was the cubic feet of the sterilizers used, and sterilizer volumes increased over time in some plants.

ii. **COMMENT:** Although generally the EPA's model selection for breast cancer incidence is scientifically appropriate, it could be described more clearly and transparently. The EPA is encouraged to revise the discussion of the Cox model, or more generally, relative risk models, to use terminology that can be directly linked with the published literature. Prentice (1985) provides examples of this terminology and a discussion of relative risk models. Terminology describing the behavior of the models at the low-exposure range should be clearly defined, particularly terms that are used to make judgments, such as "unstable."

EPA RESPONSE: The EPA has improved the clarity and transparency of the discussion of model selection (see Section 4.1.2.3). In addition, the EPA has summarized the terminology it applies to the relative risk models at the beginning of Chapter 4, using terminology in the published literature (Langholz and Richardson, 2010). The EPA has clearly defined any terms used to describe low-exposure model behavior.

iii. **COMMENT:** The SAB supports the prioritization of incidence data and the choice of data to use for the breast cancer incidence analyses. The SAB also concurs with the reliance on analyses based on the individual estimates of cumulative exposure for risk assessment (in contrast to categorized exposure or other exposure metrics such as duration). Exposure duration is not as informative for risk assessment because the magnitude of exposure is not part of duration. Using an exposure lag is more biologically plausible than using no lag. The SAB commends the EPA for considering and documenting the results for a variety of different model specifications in terms relevant for the ultimate risk assessment. In particular, a good choice is the linear spline structure used to parameterize the exposure covariate in the relative risk function under an exponential (exp(f(x))) or linear (1+f(x)) relative risk model. A spline parameterization of f(x) has the advantage of allowing the shape of the relative risk function to vary over the range of exposure while ensuring that the behavior of the function in the low-

exposure range is not unduly influenced by the highest exposures. The linear spline parameterization has the disadvantage that it has a "corner" and a smooth dose-response function would be preferred. The draft assessment uses a cubic spline model to address this, but ultimately the simpler linear spline model was selected as the preferred model. The EC_{01} from the cubic spline model is similar to the one from the linear spline model and the SAB concurs with the EPA's preference for the much simpler linear spline model parameterization, recognizing the virtue of simplicity and transparency of reporting. Alternatives to using cumulative exposure in the model as a single untransformed term are log-transformation and square root transformation. These alternatives are less desirable because they produce more global fits to the entire exposure range, which would give the higher exposures more influence (compared to the more local spline models) on the fitted dose-response in the low-exposure range of the data. [...S]pline models have the advantage of being sensitive to local behavior in the data. They can also be chosen to be parsimonious (an example is a 2-piece linear spline). Models fit to exposure categories are similarly sensitive to local behavior in the data, but they require more parameters to be estimated and are thus less parsimonious than the spline models considered in the assessment.

EPA RESPONSE: The EPA agrees and has retained the prioritization of the incidence data and use of the subcohort with interviews from the NIOSH incidence study (Steenland et al., 2003). The EPA has also retained use of the cumulative exposure metric and the lag period. In addition, the EPA has retained its preference for the two-piece linear spline model.

iv. **COMMENT:** The SAB has some concern about the number of models that were fit to the data because over-reliance on the best-fitting results can lead to statistical artifacts [such as "random high bias" which has been defined in the context of hypothesis testing; e.g., see Fleming (2010)]. At this stage of the EtO risk assessment, the SAB's concern with the large number of models that have been explored can best be addressed by striving for comprehensive reporting of model results; i.e., sensitivity analyses should be reported for a range of results. These should include sensitivity to the functional form of the model (both the choice of relative risk function and the functional form of exposure within). Other aspects of the analysis should also be considered such as inclusion of confounding variables, choice of lag, and cohort (full cohort vs. those with

interviews). The SAB recommends inclusion of tables documenting the various estimates of the target parameter of interest (which is predominantly the unit risk estimate) from the many models that were considered for the risk assessment. Although not all models are equally reasonable from a risk assessment perspective, full and transparent reporting of the target parameters of interest provides valuable context. Appropriate use of appendices and thoughtfully designed tables in the main report can minimize the potential for confusion that may result from reporting so many estimates.

EPA RESPONSE: Additional sensitivity analyses have been included in the assessment, and all the results are reported as unit risk estimates. A variety of models are compared, both with different relative risk functions and different functional forms of exposure within, for the full incidence cohort and the subcohort with interviews (see Table 4−15). In addition, the sensitivity of the selected model to different lag periods, different knots, and the exclusion of covariates (breast cancer risk factors) is examined (see Tables D-12, D-13, and D-14 of Appendix D). All the models that were investigated are presented in the assessment.

v. **COMMENT:** [T]he draft assessment states that low-dose extrapolation was performed for risk assessment, but the document does not state whether or not the doses considered for the unit risk estimates were outside the range of the NIOSH exposure data. For instance, as given by the conversion shown in footnote "e" of Table 4−13, 5,800 ppm-days corresponds to 0.075 ppm (with the correction to the formula that one divides by 365). The tenth percentile of the breast cancer incidence data corresponds to 157 ppm-days of exposure and 17 incident cases have nonzero exposure at or below this level (using a 15-year lag; see Table D-1). Using the same formula, this corresponds to 0.00202 ppm. The LEC01 from the preferred model is 0.00576 ppm, more than twice 0.00202 ppm, suggesting there is no low-dose extrapolation in these data. Because there is no low-dose extrapolation in these data, there is less uncertainty of the unit risk estimate than would be otherwise present.

EPA RESPONSE: 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 the EPA's 2005 National Air Toxics Assessment data, the average exposure concentration of EtO from all sources [including background] in the United States is 0.0062 $\mu g/m^3$ [3.4×10^{-6} ppm]; the average background concentration is 0.0044 $\mu g/m^3$ [2.4×10^{-6} ppm]), and thus, there is uncertainty about the low-exposure extrapolation from the point of departure (6.75×10^{-3} ppm, or 12 $\mu g/m^3$, for breast 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 (see Table D-1 of Appendix D), but, for example, 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. The EPA has added a footnote to the uncertainty discussion (see Section 4.1.4) to clarify that there is still considered to be low-exposure extrapolation from the point of departure.

vi. **COMMENT:** In conclusion, the SAB concurs with the EPA's selected model for the breast cancer incidence data. However, it could be described more clearly and transparently and the SAB prefers a somewhat different set of criteria for selecting the most appropriate model. There are clear advantages to relying on parsimonious regression models directly fit to the individual-level cumulative exposure data using spline models to parameterize exposure. In addition, biologic plausibility and other external information (such as corroborating information from other studies) should help inform the model selection. For example, the incidence rate ratio (IRR) results reported for the Swedish sterilization workers study by Mikoczy et al. (2011) could be used to support the selected model. The

task of selecting a final model is more challenging when a set of plausible models gives widely disparate unit risk estimates. [Comments in Part II.2.d below] provide[] further advice on how to prioritize potentially plausible models. Ultimately though, the SAB expects that this preferred approach will result in selecting the same or a very similar model to the one selected by the EPA.

EPA RESPONSE: As noted in response to Comment I.3 above, the EPA has improved the clarity and transparency of the discussion of model selection and has followed the SAB's recommendations for model selection (see Section 4.1.2.3). The EPA has also cited the Mikoczy et al. (2011) study as supporting the selected model (see Section 4.1.4). Ultimately, the EPA selected a model virtually the same as that in the 2014 draft assessment—a two-piece linear spline model with the knot at 5,750 ppm × days.

c. Bulleted summary recommendations regarding discussion of "reasonable models" (p. 13 of SAB report)

i. **COMMENT:** Revise the discussion to provide more clarity and transparency as well as making the disposition easier to follow.

EPA RESPONSE: The EPA no longer distinguishes a group of "reasonable models" and so this particular discussion has been eliminated. The EPA has improved the clarity and transparency of the discussion comparing models.

ii. **COMMENT:** Discarding a model because the fitted curve is "too steep" is only acceptable when there is scientific justification.

EPA RESPONSE: The EPA has reworded the discussion and no longer discounts models as "too steep."

iii. **COMMENT:** Clearly articulate the criteria for determining that models are reasonable as well as providing transparent definitions for frequently used terms such as "too steep," "unstable," "problematic," and "credible."

EPA RESPONSE: The EPA has generally omitted these terms from the revised discussion. If used, they are clearly defined or not used in discussions of model selection.

iv. **COMMENT:** Assign weight to various models based on a modified combination of biological plausibility and statistical considerations; use somewhat different considerations for comparing AICs than those currently employed in the draft assessment.

EPA RESPONSE: See detailed Comment II.2.d.i and response below.

v. **COMMENT:** Use a different set of emphases in the priorities for the most reasonable models; detailed suggestions are provided by the SAB in this response.

EPA RESPONSE: The EPA no longer distinguishes a group of "reasonable models"; however, the EPA has adopted the SAB's recommended emphases in the overall model selection. See detailed Comment II.2.d.i and response below.

d. More detailed comments from the text regarding discussion of "reasonable models" (p. 11−13 of SAB report)

i. **COMMENT:** The SAB recommends assigning weight to certain types of models based on a modified combination of biologic plausibility and statistical considerations, and using somewhat different considerations for comparing AICs than those currently employed in the draft assessment. Regarding statistical considerations about various models, the SAB recommends a different set of priorities for establishing the most reasonable models and gives guidance on the preference for their ordering. First, prioritization should be given to regression models that directly use individual-level exposure data. [...] Second, among models fit to individual-level exposure data, models that are more tuned to local behavior in the data should be relied on more heavily. Thus, spline models should be given higher priority over transformations of the exposure. Third, the principle of parsimony should be considered. [...I]n some settings the principle of parsimony may suggest that the most informative analysis will rely upon fixing some parameters rather than estimating them from the data. The impact of the

fixed parameter choices can be evaluated in sensitivity analyses. In the draft assessment, fixing the knot when estimating linear spline model fits from relative risk regressions is one such example. Use of AIC can assist with adhering to this principle of parsimony, but its application cannot be used naïvely and without also including scientific considerations.

EPA RESPONSE: Consistent with SAB recommendations, the model selection now emphasizes use of the individual-level data, prioritization of functional forms that allow more local fits in the low-exposure range (e.g., spline models), the principle of parsimony (e.g., fixing the knot), less reliance on AIC, a weighing of biological and statistical considerations, and prioritization of models that can be used for both environmental exposures and the occupational exposure scenarios. In addition to the statistical considerations specified by the SAB, the EPA considered the biological plausibility of the exposure-response shapes for the cancer endpoints and overall consistency with the observed data in selecting the final models.

ii. **COMMENT:** [O]ne advantage of fitting and examining a wide range of models is to get a better understanding of the behavior of the data in the exposure regions of interest. For instance, the models shown in Table 4−13 and Figures 4−5 and 4−6 can be compared, ideally with one or more of these presentations augmented with a few more model fits, including the square root transformation of cumulative exposure, linear regression of categorical results given more categories, and several additional 2-piece linear spline models with different knots. From the comparisons, it is clear that these data suggest a general pattern of the risk rising very rapidly for low-dose exposures and then continuing to rise much more slowly for higher exposures. It is reassuring to observe that many of the fitted models reflect this pattern even though they have different sensitivity to local data.

EPA RESPONSE: The EPA has added square-root transformation models to Tables 4−13 and 4−14 and Figure 4−6. Additional linear regressions of categorical results were not conducted because the EPA has prioritized the individual-level data, consistent with SAB advice. Additional two-piece linear spline models with different knots are considered in the sensitivity analyses in Section D.1.7 of Appendix D. The EPA agrees that it is reassuring

that many of the fitted models reflect a general pattern of the risk rising rapidly for low-dose exposures and then continuing to rise more slowly for higher exposures. Ultimately, the two-piece spline model was selected, consistent with SAB concurrence.

iii. **COMMENT:** The application of AIC for selecting models is acceptable within some constraints as outlined in the following discussion. [...] (The following discussion is intended to be fairly comprehensive and thus covers points that the SAB did not identify as problematic in the draft assessment.) AIC is an appropriate tool to use for model selection for both nested and non-nested models, provided these models use the same likelihood formulation and the same data. AIC is not the preferred way to characterize model fit. For model selection, (1) AIC is not an appropriate tool for comparing across different models that are fit using different measures, such as comparing a Poisson vs. least squares fit to count data; (2) one should not use AICs to compare models using different transformations of the outcome variable; and (3) comparing AICs from models estimated using different software tools, including different implementations within the same statistical package can be challenging because many calculations of AIC remove constants in the likelihood from the estimated AIC. These AIC features require that users interested in comparing AICs across different software routines (even those within one statistical package) understand exactly what likelihood is being maximized and how the AIC is calculated. AIC can be used to compare the same regression model with the same outcome variable and different predictors whether or not these models are nested. This gives a consistent estimate of the mean-squared prediction error (MSPE), which is one criterion for choosing a model. Finally, the theory behind this MSPE criterion can break down with a large number of models. Thus, naïve applications of AIC for model selection can be problematic (but are not necessarily so in any particular application). In particular, differences in AICs could be an artifact of how the calculation was done. This is a possible difference between the linear and exponential relative risk models applied to the breast cancer incidence data.

EPA RESPONSE: The EPA notes that the SAB comment identifies general situations in which comparing AICs might be inappropriate, but it does not state that any of those situations arose in the EtO analyses. Nevertheless, the EPA has confirmed that the AIC is being used appropriately—the different

models being compared were fit using the same measures, the models had the same outcome variable, and the models (both linear and exponential relative risk models) were estimated with software routines that calculate AIC in the same way. See also response to Comment I.3 above.

e. More detailed comments from the text regarding knot selection (p. 13 of SAB report)

i. **COMMENT**: The method used to identify the knots involves a sequential search over a range of plausible knots to identify the value at which the likelihood is maximized. This is scientifically appropriate and a practical solution that is transparently described.

EPA RESPONSE: The EPA applies the same approach to knot selection in the revised assessment.

3. More detailed comments regarding lymphoid cancer model selection

a. Bulleted summary recommendations regarding rationale for selecting linear regression of categorical results (p. 15 of SAB report)

i. **COMMENT:** The SAB recommends that the linear regression of categorical estimates of lymphoid cancer mortality risk not be selected as the preferred model unless the individual exposure model results are biologically implausible.

EPA RESPONSE: The EPA no longer relies on the linear regression of categorical results as the preferred model but, rather, has selected a two-piece linear spline model based on the individual-level data. See also response to Comment I.4 above.

ii. **COMMENT:** In deriving unit risk estimates under a linear regression model for risk by exposure category the use of category median exposure rather than the mean exposure is recommended.

EPA RESPONSE: The EPA considers the mean exposure to be most suitable in this context (i.e., for RR as a linear function of cumulative

exposure, with bounded categories); however, because the EPA no longer relies on the linear regression of categorical results as the preferred model, it does not impact the conclusions of the assessment.

iii. **COMMENT:** The SAB recommends presentation of multiple estimates of the unit risk derived under the alternative models for individual and categorized exposures.

EPA RESPONSE: The EPA has expanded Table 4−7 to include more alternative models (e.g., linear two-piece spline models and other linear models of individual-level data as well as square-root-transformation models), and to present estimates of the unit risk for each model. Alternative linear regressions of categorical results were not conducted because the EPA has prioritized the individual-level data, consistent with the SAB's advice.

b. **More detailed comments from the text regarding model selection (p. 14−15 of SAB report)** mostly contain recommendations regarding the linear regression of categorical results in the event that the EPA retained that approach for the preferred model, but as the EPA has selected a two-piece linear spline model based on the individual-level data for the revised assessment, these comments are no longer relevant.

c. Bulleted summary recommendations regarding model selection for estimating low-exposure cancer risks and cancer risks from occupational exposure scenarios (p. 15 of SAB report)

i. **COMMENT:** As noted in the response to Charge Question 3a, the SAB recommends that the linear regression of categorical estimates of lymphoid cancer mortality risk not be selected as the preferred model unless the individual exposure model results are biologically implausible.

EPA RESPONSE: The EPA no longer relies on the linear regression of categorical results as the preferred model but, rather, has selected a two-piece linear spline model based on the individual-level data.

ii. **COMMENT:** The SAB finds the rationale for the selection of the preferred exposure-response model for lymphoid cancer to be lacking and not transparently communicated. The SAB refers to the response to Charge Questions 2a and 2b for general recommendations to strengthen the model selection rationale and transparency in the discussion of model inputs and model fitting for the lymphoid cancer data.

EPA RESPONSE: Consistent with SAB recommendations, the model selection now emphasizes use of the individual-level data, prioritization of functional forms that allow more local fits in the low-exposure range (e.g., spline models), the principle of parsimony, less reliance on AIC, a weighing of biological and statistical considerations, and prioritization of models that can be used for both environmental exposures and the occupational exposure scenarios.

d. More detailed comments regarding model selection for estimating low-exposure cancer risks and cancer risks from occupational exposure scenarios (p. 15 of SAB report)

i. **COMMENT:** The SAB suggests that the EPA consider using the same model for both environmental and occupational exposures. The use of different models needs sufficient justification.

EPA RESPONSE: Consistent with SAB recommendations, the EPA now uses the same model for both environmental and occupational exposures. See also response to Comment I.5 above.

e. More detailed comments from the text regarding the derivation of lymphoid cancer incidence estimates from mortality data (p. 15–16 of SAB report)

i. **COMMENT:** The approach used for deriving risk estimates for lymphoid cancer incidence and the rationale for using this approach are explained transparently and are scientifically appropriate. However, if the draft assessment were also intended for a broad audience, the approach could be more transparently described. The SAB suggests the EPA go through some more crudely estimated approaches so general readers can understand clearly all the different aspects of

obtaining the unit risk and excess risk estimates without having to rely on the more complex life table analyses. If the EPA judges it to be informative, the SAB suggests that extra lifetime risk be presented in terms of the number of lymphoid cancers that are due to the exposure to EtO in the cohort.

EPA RESPONSE: The EPA has added a more crude approach to illustrate the derivation of the estimates (see Sections 4.1.1.2 and 4.1.2.3). In addition, the estimated numbers of lymphoid and breast cancers in the cohort that are due to EtO exposure, assuming the selected exposure-response models, are shown in Table I-1.

Table I-1. Number of cancer cases in the cohort attributable to EtO exposure, assuming the selected models

Cancer type	Mean exposure (ppm × days, with 15-year lag)[a]	Selected model	RR estimate from selected model[b]	Attributable fraction[c]	Total cases in cohort	Number of cases in cohort attributable to EtO exposure[d]
Lymphoid cancer mortality	8,704	Two-piece linear spline model with knot at 1,600 ppm × days[e]	2.28	0.56	53	30
Breast cancer incidence (subcohort with interviews)	9,230	Two-piece linear spline model with knot at 5,750 ppm × days[f]	1.56	0.36	233	83

[a]From the risk sets.
[b]Calculated from selected model at mean exposure.
[c]Attributable fraction = (RR−1)/RR.
[d]Number of attributable cases = attributable fraction × total cases.
[e]$\beta_1 = 7.58 \times 10^{-4}$; $\beta_2 = -7.48 \times 10^{-4}$
[f]$\beta_1 = 8.978 \times 10^{-5}$; $\beta_2 = -7.786 \times 10^{-5}$

ii. **COMMENT:** [T]he risk estimates (Table 4-5, for example) would benefit by expressing these in scientific notation, rather than a list of leading zeros.

EPA RESPONSE: In cases in which there is more than one leading zero, most results are now expressed in scientific notation, consistent with SAB recommendations.

4. More detailed comments regarding the qualitative discussions of uncertainty in the cancer risk estimates

a. Bulleted summary recommendations regarding the qualitative discussions of uncertainty in the cancer risk estimates (p. 19 of SAB report)

i. **COMMENT:** The SAB recommends that the EPA consolidate the current discussion of exposure uncertainty that appears in various sections of Appendices D and H and also to include in the body of the draft assessment a qualitative discussion of the statistical uncertainty that is associated with the model-based predictions of annual exposures.

EPA RESPONSE: Instead of consolidating the discussions in Appendices D and H, the EPA has expanded the discussion of exposure uncertainty in Section 4.1.4.2.1, which is the main section of the document in which exposure uncertainty is addressed. Information on the statistical uncertainty that is associated with the model-based predictions of annual exposures, however, is lacking. The EPA had considered an errors-in-variables analysis, as discussed in Section D.7 of Appendix D (see also "grouped data regression" response on page H-28 of Appendix H); however, it was determined that such an analysis would be very time consuming and involve a lot of assumptions, and the analysis was deemed to be beyond the scope of this assessment.

ii. **COMMENT:** To better characterize the NIOSH worker samples and their exposure profiles, the SAB recommends that key demographic, work history and exposure characteristics of the NIOSH cases and controls be summarized in descriptive tables or figures in the body of the EtO risk assessment report.

EPA RESPONSE: The EPA has included the recommended results in Section D.5 of Appendix D.

iii. **COMMENT:** The EPA should ensure that they obtain a copy of the NIOSH individual data including all relevant data released from NIOSH to members of the public.

EPA RESPONSE: In response to SAB recommendations, the EPA has obtained the relevant publicly available data, which include the cohort mortality data and some exposure data pertaining to modeled exposure levels for the 13 plants.

iv. **COMMENT:** The SAB repeats its recommendation from previous charge questions that there be improvements in the quantification of the results from the models that were fit as a way of improving the qualitative discussion of uncertainty. Specifically, unit risks should be reported and compared in sensitivity analyses for a rich set of models.

EPA RESPONSE: Consistent with SAB recommendations, the EPA now reports unit risks for all the comparisons. See also response to Comment I.6 above.

v. **COMMENT:** The SAB recommends down-weighting all epidemiological results that are based on external standards (e.g., standardized mortality ratio, standardized incidence ratio).

EPA RESPONSE: All of the EPA's quantitative estimates are based on internal comparisons.

b. More detailed comments from the text regarding the qualitative discussions of uncertainty in the cancer risk estimates (p. 16–19 of SAB report)

i. **COMMENT:** The uncertainty discussions are generally clear, objective, and scientifically appropriate but they can be improved and extended. Considerations about uncertainty directly pertaining to the analyses reported can be separated into 1) uncertainty due to the data (particularly from reliance on a single dataset), and 2) uncertainty of the results.

EPA RESPONSE: The uncertainty discussion has been restructured to address uncertainty due to the data themselves (see Section 4.1.4.1) separately from uncertainty of the results given the data (see Section 4.1.4.2).

ii. **COMMENT:** The SAB supports the use of the NIOSH EtO worker cohort described in Steenland et al. (2004) and Steenland et al. (2003) as the primary data source for the modeling of cancer risk from EtO exposures. This is consistent with the support for the data source in the previous SAB (2007) review. The support of the NIOSH data is founded on study documentation of the original exposure measurements, procedures for exposure estimation (Hornung et al., 1994) and historical modeling (prediction) of exposures that occurred before the time period in which actual exposure measurements were systematically collected. All such model-based reconstructions of exposure data are subject to variable and potentially systematic sources of error (i.e., bias). [...] Appendices D and H of the current draft assessment provide a comprehensive response to most of the key questions of data or model uncertainty that were raised in the SAB (2007) review (see the response to Charge Question 5b [Section II.6 below]). [...T]he SAB recommends that the EPA consolidate the current discussion of exposure uncertainty that appears in various sections of Appendices D and H and also to include in the body of the draft assessment a qualitative discussion of the statistical uncertainty that is associated with the model-based predictions of annual exposures.

EPA RESPONSE: Instead of consolidating the discussions in Appendices D and H, the EPA has expanded the discussion of exposure uncertainty in Section 4.1.4.2.1, which is the main section of the document in which exposure uncertainty is addressed. Information on the statistical uncertainty that is associated with the model-based predictions of annual exposures, however, is lacking. The EPA had considered an errors-in-variables analysis, as discussed in Section D.7 of Appendix D (see also "grouped data regression" response on page H-28 of Appendix H); however, it was determined that such an analysis would be very time-consuming and involve a lot of assumptions, and the analysis was deemed to be beyond the scope of this assessment.

iii. **COMMENT:** On page 17 of the SAB report, the SAB recommends a list of characteristics of the NIOSH cases and controls to be summarized in tables or figures – gender distribution over time, year of entry and age of entry to the EtO workforce, duration of employment in the EtO cohort, and age and year of departure/retirement from the EtO cohort – as well as a list of exposure characteristics to summarize – box plot of cumulative total and peak exposures for individual cases and controls, time plot of the distribution of computed mean, median, and 25th, 75th, and 95th percentile values for annual exposures among cases and controls, and summary of percent of total case and control individual exposures in the worker histories that are excluded when various lags are imposed (e.g., 5, 10,15 and 20 years).

EPA RESPONSE: Each of these characteristics has been summarized in tables and figures in Section D.5 of Appendix D.

iv. **COMMENT:** The SAB is also concerned that public commenters had exposure data from the NIOSH cohort that the EPA did not have. For instance, a few selected graphs were presented in public comments to the Augmented CAAC that indicated exposure predictions for four jobs in two of the fourteen plants showed lower exposures in some or all years prior to 1975. The SAB was provided only a few carefully selected examples, and thus was unable to assess the extent of these surprising data. This is an uncertainty that can easily be ruled out. Upon reviewing the model equation in Hornung et al. (1994), the SAB finds the surprising historical behavior to be unlikely and could be explained by changes in processes in specific plants, rather than some failure of the model to capture historically larger exposures.

EPA RESPONSE: The EPA has obtained some exposure data from NIOSH and has ascertained that, contrary to public comments made at the SAB meeting, the NIOSH EtO exposure patterns are not anomalous, but rather reflect the underlying changes in variables predicting exposure over time. One of the principal drivers of the NIOSH exposure levels was the cubic feet of the sterilizers used [see Table III, Hornung et al. (1994)]. It was not uncommon in these plants for sterilizer volume to have increased over time as the demand for EtO-sterilized products increased. Increased sterilizer volume generally resulted in higher predicted average exposures until the late 1970s,

when increased controls were used after it became known that EtO might be dangerous. Table I-2 shows the sterilizer volume, as well as the model-predicted EtO levels, from the first example for Plant 1 presented at the SAB meeting (Dept 0I/Oper MP). The sterilizer volume in this plant increased until the mid-1970s and then decreased, and predicted exposure levels followed the same pattern. The other example presented at the SAB meeting for this plant, using Dept OQ/Oper AF, exhibits the same concordance.

Table I-2. Plant 1, sterilizer volume and predicted EtO exposure levels by year

Plant 1, Dept OI, Oper MP		
Year	**Sterilizer volume (cubic ft)**	**Predicted EtO level (ppm)**
1966	650	2
1967−1968	1,300	4.3
1969−1975	2,250	9.1
1976−1977	1,600	5.9
1978−1979	650	2

Plant 5 follows a similar pattern. Table I-3 shows the sterilizer volume, as well as the model-predicted EtO levels, from the first example for Plant 5 presented at the SAB meeting (Dept 1, Oper ZZ). The predicted exposure levels across time again follow closely the sterilizer volume. The same concordance is seen for the 2nd example in Plant 5 (Dept 01/Oper 82).

Table I-3. Plant 5, sterilizer volume and predicted ETO exposure levels by year

Plant 5, Dept 1, Oper ZZ		
Year	**Sterilizer volume (cubic ft)**	**Predicted ETO level (ppm)**
1943−50	887	6
1951−61	1,679	15
1962−70	1,304	10
1971−72	1,964	18
1973−76	2,624	26
1977−78	3,284	32

v. **COMMENT:** Although the SAB concurs with the EPA's decision to rely solely on the NIOSH dataset for the risk assessment, the use of only one dataset is a source of uncertainty. This uncertainty can be reduced by highlighting how the Swedish sterilization workers data (Mikoczy et al., 2011) help support the conclusions reached from the NIOSH data.

EPA RESPONSE: In response to SAB recommendations, the EPA has cited the Mikoczy et al. (2011) study as supporting the conclusions reached from the NIOSH data; this is discussed in the context of reducing the uncertainty associated with using a single study in Section 4.1.4.1 (see also response to Comment II.7.a.i below).

vi. **COMMENT:** The SAB recommends better quantification of the results from the models that were fit as a way of improving the qualitative discussion of uncertainty. In particular, as has been noted in responses to previous charge questions, the unit risks should be reported and compared in sensitivity analyses for a rich set of models. This could include analyses that e.g., differ according to the various outcomes, subcohorts, link functions, functional forms of the exposure (i.e., exposure parameterizations), exposure metrics, exposure lags (see response to Charge Question 1), confounder adjustments, and standard error estimation approaches (Wald vs. profile likelihood). Such information would provide context for the unit risk behavior across the range of plausible models. The SAB also encourages consideration of focusing the reporting of sensitivity analyses on the target parameters of interest (unit risk, excess risk).

EPA RESPONSE: Additional sensitivity analyses have been added to the assessment, and all the results are reported as unit risk estimates. For both breast cancer incidence and lymphoid cancer, various models are compared, including different relative risk functions and functional forms of the exposure, and the sensitivity of the selected models to different lag periods, knots, and upper-bound estimation approaches is examined. For breast cancer incidence, additional analyses include comparisons of the subcohort and the full cohort, of total breast cancer and invasive breast cancer only, and of the full model and the selected model with the nonexposure covariates (breast cancer risk factors) excluded. For lymphoid cancer, results are compared to the results for all lymphohematopoietic cancers. Sensitivity analyses were not

conducted for alternative exposure metrics because it is unclear how to derive unit risk estimates for metrics such as duration and peak exposure, and as noted in Comment II.2.b.iii, the SAB concurred with the reliance on analyses based on the individual estimates of cumulative exposure for risk assessment.

vii. **COMMENT:** If feasible, consideration of additional analyses using alternative exposure metrics is suggested. The December 4, 2014 EPA memo (U.S. EPA, 2014) notes that four exposure metrics were already considered by the agency. If additional metrics are available, it would be valuable to consider these as well.

EPA RESPONSE: No additional exposure metrics are available, and as noted in the response to Comment II.4.b.vi above, it was not considered feasible to derive unit risk estimates for alternative exposure metrics.

viii. **COMMENT:** The SAB encourages consideration of the following points in the document, either directly in the uncertainty discussion, or in other places, as appropriate. The first two points are observations, the third is a recommendation.

a) The dose-response model indicated by the NIOSH cohort that suggests risk increases sharply for low exposures and then increases further but less steeply for higher exposures. The biologic plausibility of this functional form is uncertain, and evidence that there are mechanistic explanations that support this form will inform the risk assessment.

EPA RESPONSE: The EPA is not aware of a mechanistic explanation for the shape of the exposure-response relationship in the NIOSH cohort data but notes that the SAB found it "reassuring to observe that many of the fitted models reflect this pattern" for breast cancer incidence data (p. 12 of the SAB report), and the same is true for the lymphoid cancer data. Similarly, the SAB noted that the results of the Mikoczy et al. (2011) study could be used to support the selected model for breast cancer incidence (p. 10 of SAB report). The EPA now cites the Mikoczy et al. (2011) study as supporting the selected model (see Section 4.1.4).

b) The analysis of NIOSH data relies on cumulative exposure as the dose metric. Given the status of the exposure data, it is unlikely that other more refined

exposure information can be used to better understand the mechanisms of EtO exposure in cancer initiation. Furthermore, it is often difficult to determine mechanisms from epidemiological data, particularly when these data are limited.

EPA RESPONSE: The EPA has considered the issue and agrees that it is unlikely that other more refined exposure information can be used to better understand the mechanisms of EtO exposure in cancer initiation and that it is often difficult to determine mechanisms from epidemiological data, particularly when these data are limited, as is the caes with EtO.

c) The SAB recommends down-weighting all epidemiological results that are based on external standards (e.g., standardized mortality ratio, standardized incidence ratio). The presence of the healthy worker effect cannot be denied in these occupational data and the use of an external standard for comparison does not avoid healthy worker types of biases.

EPA RESPONSE: The EPA agrees that internal comparisons are superior to external comparisons, and all of the EPA's quantitative estimates are based on internal comparisons.

5. More detailed comments regarding genotoxicity discussions

a. Bulleted summary recommendations regarding genotoxicity discussions (p. 19−21 of SAB report)

i. **COMMENT:** Table 3.6 should be revised to specify the sites involved and the relative importance (weight) assigned to each of the individual studies presented. In addition, a new table should be added to show the dose-response relationships for the formation of DNA adducts and the *in vivo* genotoxic effects in humans and comparative model systems.

EPA RESPONSE: Table 3.6 has been revised and is now Table 3−8. A similar table (see Table 3−7) was created showing the cytogenetic effects in laboratory animals. It has been made clearer that most of the studies are of peripheral blood lymphocytes. The relative importance of the studies is

considered primarily as a function of the genotoxic endpoint investigated and the estimated level of exposure. A discussion regarding the relative importance (qualitative weight) of various genotoxicity endpoints has been included (see Section 3.3.3.3), and the studies in Table 3−8 have been arranged roughly in order of increasing estimated exposure concentration. The studies of chromosomal aberrations and sister chromatid exchanges are generally positive at the higher exposure levels, while the data on micronuclei at the higher exposure levels are more limited. A "Comments" column has also been added to the table providing more study details. In addition, a similar table presenting the results from studies reporting DNA adducts and/or mutations following in vivo exposures in humans or laboratory animals has been added (see Table 3−6), along with two new summary tables showing the temporal and dose-response relationships for the in vivo formation of DNA adducts and mutations (see Table 3−9) or cytogenetic effects (see Table 3−10) in humans and laboratory animals.

ii. **COMMENT:** The rationale for decisions made regarding model selection for calculations of unit risk should be presented in this section, and elsewhere, within the context of MOA considerations and the initial key biological events involved in mutagenesis and carcinogenesis.

EPA RESPONSE: The models used for the epidemiologic data are essentially empirical curve-fitting models, and it is unclear how the available biological data can be used to guide general model selection. In one specific case, considerations of the biological data did inform the decision not to use a two-hit quadratic model for lymphohematopoietic cancers (see Section 3.4.1.2). In addition, the conclusion of a mutagenic mode of action resulting from direct EtO-DNA interactions occurring in the absence of any evidence for concurrent cytotoxicity or alternative modes of action (see Sections 3.4.1.1, 3.4.1.4, 3.4.2, and 3.4.3) provides support for the use of linear, low-exposure extrapolation for the derivation of the unit risk estimate (e.g., Section 4.1.1.2).

iii. **COMMENT:** Although the description of the database was found to be adequate, the synthesis of the information used to support a mutagenic MOA should be presented in a more systematic and complete manner. Section 3.4

should be reorganized around a broader evidence base for a mutagenic MOA to more clearly establish the framework for defining mutagenic MOA. Key elements of this framework, as informed by a recent review by Eastmond (2012) should include [details of the sub-bullets are presented in the SAB report]:

- Characterization of Molecular Alterations
- Characterization of mutagenic or clastogenic effects

EPA RESPONSE: Section 3.3.3 (genotoxicity) has been revised to synthesize the information used to support a mutagenic MOA in a more systematic and complete manner, including more detailed characterization of the molecular alterations (e.g., Section 3.3.3.1) and mutagenic (see Section 3.3.3.2) and other genotoxic (see Section 3.3.3.3) effects. In addition, a table (see Table 3−7) summarizing the cytogenetic effects in laboratory animals (comparable to the previous Table 3−6 [now Table 3−8] for humans) and a table (see Table 3−6) summarizing the dose-response information on DNA adducts and mutations in humans and laboratory animals have been added. Furthermore, a substantially expanded summary (see Section 3.3.3.4) of the genotoxicity section summarizes and integrates the mutagenicity and genotoxicity information and includes two new tables summarizing the temporal and dose-response findings for DNA adducts and mutations (see Table 3−9) and for cytogenetic effects (see Table 3−10) in humans and laboratory animals. Section 3.4 has been revised and reorganized to more clearly discuss the mechanisms by which the genotoxic effects might be instrumental in a mutagenic mode of action (see Section 3.4.1.1), particularly in the target organs (see Sections 3.4.1.2 and 3.4.1.3).

iv. **COMMENT:** In the absence of further mechanistic information, evidence for DNA interactions coupled with consistency in the occurrence of mutagenic/clastogenic effects provides a sound basis for applying a mutagenic MOA to risk assessment. Additional data that may be informative in revising the draft to support a mutagenic MOA includes [details of the sub-bullets are presented in the SAB report]:

- Genotoxic Effects in Cancer Target Organs
- Non-linearities
- Temporal Relationships
- Alternative Mechanisms

- Summary of Cancer MOA

EPA RESPONSE: Building on the information in Section 3.3, Section 3.4 has been revised and reorganized to more clearly discuss the mechanisms by which the genotoxic and mutagenic effects might be instrumental in EtO-induced carcinogenesis (see Section 3.4.1.1), particularly in the target organs (see Sections 3.4.1.2 and 3.4.1.3), i.e., how a mutagenic mode of action might be operating. The support for low-exposure linearity from the DNA adduct data at very low EtO doses (Marsden et al., 2009) is discussed in more detail in the derivation of the unit risk estimate (see Section 4.5); however, cross-referencing to that discussion has been added to Section 3.3.3.1. Temporal relationships are addressed in the expanded summary in Section 3.3.3.4 and in the new Tables 3−9 and 3−10. A short section (see Section 3.4.2) has been added on alternative mechanisms. A revised summary of the evidence for a mutagenic mode of action is provided in Section 3.4.1.4.

b. Bulleted suggestions regarding genotoxicity discussions (p. 21 of SAB report)

i. **COMMENT:** Inclusion of additional experimental details about the separation of endogenous from exogenous adducts as reported by Marsden et al. (2009) would help provide biological perspective for issues related to risk assessment considerations, especially linearity versus non-linearity of dose-response relationships.

EPA RESPONSE: The discussions of the Marsden et al. (2009) study in Section 3.3.3.1 and in Section C.7 of Appendix C were expanded. This study reported linear dose-response relationships for N7-HEG adducts in the three tissues evaluated from exogenous EtO dosing down to very low doses.

ii. **COMMENT:** The genotoxicity section would be improved by consideration of the role that differences in DNA repair capacity between different target cells in different tissues plays in relative vulnerability to mutagenesis. For example, genes known to regulate vulnerability of breast cancer in women, such as BRAC1, BRAC2 and XRCC1, are known to regulate DNA repair pathways in breast tissue (Shi et al., 2004; Hu et al., 2002). This line of thinking can help to

inform the biological bases to better understand the shape of the dose response in the low-dose region of the NIOSH dataset.

EPA RESPONSE: This material is covered in Section C.6 of Appendix C. Mention has also been added to Sections 3.4.1.1, 3.4.1.2, and 3.4.1.3. There was insufficient information to elucidate a basis for the supralinear exposure-response relationships observed for lymphoid and breast cancers in the NIOSH study.

iii. **COMMENT:** In light of the above discussion, the organization of the text can also be revised to include information about known differences in mutagenic and carcinogenic pathways for EtO at different tumor sites, as well as the degree to which biochemical differences at the cellular or tissue level differentially impact MOA. Furthermore, references made in page 3−29 to the levels of different adducts are presented without making a clear and necessary distinction between the putative or assigned biological impact for N-7 versus O-6 DNA adducts.

EPA RESPONSE: Not much is known about different pathways operating at different tumor sites, but the text has been more clearly organized to discuss possible mechanisms specific to lymphohematopoietic cancers (see Section 3.4.1.2) and breast cancer (see Section 3.4.1.3). Also, to the extent that there is information, the sensitivities of different tissues to EtO-induced mutagenicity and genotoxicity are discussed in these sections. In addition, the discussion of the different adducts and their biological implications in Section 3.3.3.1 has been expanded.

c. More detailed comments from the text regarding the genotoxicity discussions (p. 19 of SAB report)

i. **COMMENT:** Section 3.3.3 and Appendix C of the draft assessment present an accurate, objective and transparent summary of the results of research studies published up to July 2013 on EtO genotoxicity. The SAB agrees that the weight of the scientific evidence from epidemiological studies, laboratory animal studies and *in vitro* studies supports the general conclusion that the carcinogenicity of EtO in laboratory animals and humans is mediated through a mutagenic mode of action (MOA). Indeed, the genotoxicity database has firmly established that EtO

is a direct-acting agent, as evidenced by the formation of DNA adducts and highly reproducible, positive effects in a variety of *in vitro* and *in vivo* mutation and clastogenesis assays. The genotoxic studies examined showed adducts, mutagenesis, or clastogenesis at the bioassay doses and in some cases lower (Donner et al., 2010; Marsden et al., 2009; Recio et al., 2004; Walker et al., 1997), providing evidence of dose-response concordance for a mutagenic mode of action.

EPA RESPONSE: The EPA has retained the conclusion that there is sufficient weight of evidence that a mutagenic mode of action is operative in EtO carcinogenicity, but in response to SAB comments, the EPA has strengthened the presentation of the evidence (e.g., with the expanded discussion of temporal and dose-response relationships in Section 3.3.3.4).

6. More detailed comments regarding Section H.1 of Appendix H—responses to SAB comments regarding the 2006 draft

a. Bulleted summary recommendations regarding Section H.1 (p. 28 of SAB report)

i. **COMMENT:** Consider adding a brief introductory summary of purpose and highlights to each chapter 2, 3 and 4 to improve the readability of the assessment document.

EPA RESPONSE: Text boxes containing a brief summary of the purpose and the major conclusions of the chapter have been added to the beginning of Chapters 2, 3, and 4.

ii. **COMMENT:** Expand the description of endogenous sources of EtO to include formation from external exposure to ethylene.

EPA RESPONSE: Discussion of the conversion of exogenous ethylene to EtO has been added to Section 3.3.3.1.

iii. **COMMENT:** Summarize the key highlights of Dr. Steenland's further analysis as they reflect on the reliability of the cumulative exposure with 15-year lag metric used in the dose-response assessment.

EPA RESPONSE: In response to SAB comments, the EPA has summarized the key highlights of the response that Steenland provided to the 2007 SAB comments on Charge Question 2.a (see revised pages H-8 to H-9 of Appendix H).

b. More detailed comments from the text regarding Section H.1 (p. 21–28 of SAB report)

i. **COMMENT:** Appendix H provides a summary of the SAB (2007) peer review comments, followed by the agency's response. Overall, the EPA was highly responsive to the comments and recommendations presented in the SAB (2007) report. Responses are transparent, objective, and for the most part, accurate (exceptions are noted in the current review). The agency should be commended for this effort because this was a particularly challenging undertaking given the lack of consensus in the SAB (2007) report on several issues critical to key outcomes of the draft assessments. The EPA not only addressed all major consensus recommendations but also responded specifically to both the majority and minority opinions whenever divergent views were expressed.

EPA RESPONSE: The EPA thanks the committee.

ii. **COMMENT:** There are some recommendations or suggestions of the SAB (2007) peer review that are not implemented in the current draft assessment [...]. Feedback regarding these agency decisions is provided in the detailed response to this charge question and in responses to other charge questions. This feedback can be summarized as follows:

1. The SAB finds that EtO likely acts by a mutagenic MOA and therefore its potency should be modeled according to a linear low-dose model. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) note the following: "A nonlinear extrapolation method can be used for cases with sufficient data to ascertain the mode of action and to conclude that it is not linear at low doses ….." (p. 3-23). The SAB finds that the empirical data for EtO and its MOA are consistent with a linear low-dose extrapolation and the

database does not provide the type of evidence that the Cancer Guidelines would find sufficient to support a nonlinear MOA, which precludes the need for the presentation of nonlinear modeling approaches.

2. The SAB concurs with the decision not to use the Union Carbide Cohort data for unit risk derivation, but suggests that the agency discuss the weight of the evidence of the UCC, NIOSH, and Swedish sterilization workers studies. More suggestions regarding the Swedish sterilization workers study can be found in the response to charge question 6.
3. The SAB suggests that the EPA consider using the same model for both environmental and occupational exposures.
4. The SAB agrees with the decision to not move the contents of Appendix A to the main body of the draft assessment.

EPA RESPONSE: Consistent with SAB concurrence, the EPA has retained the use of linear low-exposure extrapolation, as well as the decisions not to use the UCC data for unit risk derivation and not to move the contents of Appendix A into the body of the assessment. In addition, in response to SAB comments, the EPA has considered the implications of the UCC and Mikoczy et al. exposure-response relationships in the uncertainty discussions of the unit risk estimates derived from the NIOSH data (see Section 4.1.4.1), and the EPA now uses the same exposure-response model for both environmental and occupational exposures for both cancer endpoints (see Sections 4.1.1, 4.1.2, and 4.7).

iii. **COMMENT:** This charge question asks specifically about responses to comments on endogenous EtO (p. H-4), a nonlinear approach (P. H-13 to H-17), and the cancer hazard characterization. Each of these topics is addressed in the detailed response to the charge question, but can be summarized as follows: (1) The SAB supports the expanded discussion of endogenous EtO provided in the draft assessment and has suggestions for further improvement; (2) as noted above, the SAB agrees with the decision not to include a toxicity value for EtO based upon nonlinear extrapolation, but recommends a more balanced and objective discussion of the subject; and (3) the SAB recognizes and agrees with revisions to strengthen support for a classification of EtO as "carcinogenic to humans."

EPA RESPONSE: In response to specific comments detailed further below (and above), the EPA has revised the assessment. For example, discussion of the conversion of exogenous ethylene to EtO has been added to Section 3.3.3.1 (see response to Comment II.6.a.ii above) and cross-referencing has

been added to the discussion of endogenous EtO exposure in Section 3.3.3.1 to link to the discussion of the relevance of endogenous EtO to the unit risk estimate in Section 4.5 (see response to Comment II.6.b.vi below). In addition, the discussion of not using a nonlinear approach has been made more balanced. For example, cautions about the Marsden et al. (2009) study have been added (e.g., page H-13 of Appendix H). Furtheremore, the related discussions of genotoxicity (see Section 3.3.3) and mode of action (see Section 3.4) have been made more comprehensive and balanced. Also, the EPA has included discussion of two more recent studies (Zhang et al., 2015b; Zhang et al., 2015a), which provide further support for a mutagenic mode of action and for oxidative stress not being an additional mode of action of concern (see Section J.4.1 of Appendix J).

iv. **COMMENT**: The SAB agrees with the decision not to transfer *in toto* materials from Appendix A – Critical Review of the Epidemiological Evidence to the main body of the assessment. The addition of the two brief summary tables on the hematopoietic and breast cancer studies is a good alternative for strengthening the chapter. This choice is consistent with the NRC (2011) recommendations that the main body of the assessment focus on the critical data, rationales, and analyses used to support the unit risk derivation and that, as much as possible, detailed description of key and other studies or analyses be summarized in appendices with appropriate cross-referencing in the main body of the assessment. If anything, the current document could benefit from transferring more materials to appendices, although it is acknowledged that the current draft assessment is not intended to conform completely to the new format for IRIS assessments.

EPA RESPONSE: In the interest of minimizing further delays in the finalization of this assessment, the EPA has not further condensed the main assessment text.

v. **COMMENT:** The EPA also clarified its designation of the unit risk estimate as "weak" in the prior draft assessment, and section 3.5.1 of the current draft assessment provides a good evaluation of the strength of the weight of the evidence in term of Hill's criteria for causality**.**

EPA RESPONSE: No response needed.

vi. **COMMENT:** Based on the discussion presented in the assessment and considering the weight of the evidence from human and animal studies, the SAB finds EPA's conclusion on endogenous exposure to EtO to be supported. Nonetheless (and also in light of the analyses presented on pages H-15 to H-17 and further insights derived from the SAB recommendations in the response to Charge Question 5a – Section 3.5 of this report), it appears that recognizing this source of metabolic EtO and briefly expanding on its relevance to the assessment would complete the description of sources of endogenous EtO and their relative importance for adduct formation. This could be readily done in detail in Appendix C with the expanded, but succinct description added to Chapter 3.0 and cross-referenced to the appendix.

EPA RESPONSE: The relevance of endogenous EtO exposure to the assessment is discussed in Section 4.5 in the context of the use of low-dose linear extrapolation in deriving the unit risk estimate. Cross-referencing to this section has been added to the discussion of endogenous EtO exposure in Section 3.3.3.1.

vii. **COMMENT:** The EPA added 24 of the 34 additional references recommended by the panel. There was no explanation for the reasons for not including 10 of the references suggested for inclusion.

EPA RESPONSE: All 34 of the references were considered; however, some of them were not particularly relevant to the assessment (e.g., one was on N-nitrosocompounds). The text in Appendix H has been expanded to provide reasons for the exclusions.

viii. **COMMENT:** The SAB finds that the EPA has been responsive in providing an expanded and more balanced discussion of human and animal studies of precursor events that support a mutagenic MOA. However, the supportive evidence for a mutagenic MOA needs further enhancement and discussion as indicated in the SAB response to Charge Question 5a (Section 3.5 of this report).

EPA RESPONSE: In response to SAB comments, the EPA has strengthened the presentation of the evidence supporting a mutagenic MOA. Section 3.3

(genotoxicity) has been revised to synthesize the information in a more systematic and complete manner, including the addition of a substantially expanded summary that integrates study information in terms of dose-response and temporal relationships (see Section 3.3.3.4). Section 3.4 has been revised and reorganized to more clearly discuss the mechanisms by which the genotoxic effects might be instrumental in EtO carcinogenesis (see Section 3.4.1.1), particularly in the target organs (see Sections 3.4.1.2 and 3.4.1.3). In addition, the EPA has revised and expanded Table 3–6 (now 3–8) and provided additional summary tables (see Tables 3–6, 3–7, 3–9, and 3–10). (For more details on specific revisions, see responses to detailed comments in Part II.5 above.)

ix. **COMMENT:** The selection of the NIOSH cohort and the decision not to combine these data with the Union Carbide cohort is better and more transparently justified in the revised draft assessment. [...] The SAB concurs with this assessment of the UCC data and concurs with the decision not to include the UCC data. However, the SAB considers that a more detailed description of the NIOSH cohort is needed as it relates to the derivation of exposure metrics, as indicated in the SAB response to Charge Question 2 (Section 3.2 of this report) for the current draft assessment.

EPA RESPONSE: The EPA has provided the additional details requested by the SAB, as indicated in the SAB comments on Charge Question 2 and detailed in SAB comments on Charge Question 4 (see Comment II.4.b.iii above); these are summarized in tables and figures in Section D.5 of Appendix D. Some of the new cohort details summarized in Section D.5 include mean, median, minimum, maximum, and 25^{th} and 75^{th} percentiles of cumulative exposure in the full cohort; cumulative exposures by year of entry and by duration of employment; sex distribution over time; distributions of year of entry, age of entry, duration of employment, and age and year of departure/retirement; distributions of cumulative and peak exposures for individual cases and controls; percentages of total case and control individual exposures in the worker histories that are excluded when the 15-year lag is imposed; and mean, median, and 25^{th}, 75^{th}, and 95^{th} percentile values for annual exposures among cases and noncases in the cohort.

x. **COMMENT:** It is not known if Dr. Steenland received only the comment as presented in the Executive Summary of the SAB (2007) report, or the more detailed discussion in pages 20–22 of the SAB (2007) report. The SAB considers that, although consultation with Dr. Steenland on the technical aspects of this recommendation is appropriate because of his intimate knowledge of the exposure model developed for the NIOSH EtO cohort studies, the EPA should have provided its own response to the SAB (2007) recommendation. Dr. Steenland indicates that he was not completely sure about the meaning of the recommendation and proceeded to present a set of reasonable arguments as to why the bias introduced by using this metric would not alter the analysis appreciably. It is also important to note that the exposure estimates likely to be of lower reliability (because there were no exposure measurement data that could be included in the exposure model prior to 1979) are also likely to be higher than the more recent exposures and, therefore, would play a less important role in the current derivation of the point of departure (POD). The response, however, has not completely clarified the issue of potential estimate instabilities introduced by interactions between time-varying predictor variables and the log cumulative exposure with a 15-year lag exposure estimate. This issue is addressed in the SAB response to Charge Question 2 (Section 3.2 of this report) for the current draft assessment.

EPA RESPONSE: The EPA has addressed the SAB comments raised regarding the NIOSH exposure estimates in response to other charge questions (e.g., see response to Comment II.4.b.iii above). For example, Section D.5 of Appendix D now presents time plots of the distribution of computed mean, median, and 25th, 75th, and 95th percentile values for annual exposures among cases and controls (see Figures D-22 and D-23), as well as a summary of the distribution of cumulative exposures as a function of year of entry into employment (see Table D-52). The EPA has added sensitivity analyses in which unit risk estimates (see Sections D.1.6 and D.3.5) and extra risk estimates for occupational exposure scenarios (see Sections D.1.11 and D.3.9) are derived from the selected models using cumulative exposure with different lag periods. Furthermore, in response to SAB comments, the EPA deleted Steenland's response on the issue of potential instability resulting from the interaction of the lag and the treatment of time in the exposure model and now provides its own response (pages H-8 to H-9 of Appendix H).

xi. **COMMENT:** The EPA was highly responsive in addressing concerns about the use of categorical data for POD derivation and contracted with Dr. Steenland, the principal investigator of the NIOSH studies, to perform multiple analyses of the NIOSH cohort data (including use of individual and categorical exposure estimates) using alternative modeling approaches. In addition, there was also an attempt to expand on the error analysis of the NIOSH cohort exposure estimation, although this could not be accomplished because the data files used in that assessment were no longer available. Results from the extensive additional analysis are detailed and well described in the current draft assessment, both in Chapter 4 and in Appendix D, together with the rationale for supporting the decisions by EPA in model selection. Problems with the implementation of the recommendations are described clearly. Outcomes from alternative models are summarized both in tables and graphical form, with justification for the preferred models. It is important to emphasize that Dr. Steenland's involvement in the additional analyses is a strength of the revised draft not only because of his intimate familiarity with the NIOSH cohort studies but his expertise in exposure modeling and occupational epidemiology. The revised assessment for breast cancer risk incidence is based on continuous exposure data. The analysis for LH cancer subtype is based on the NIOSH cohort lymphoid cancer results (results for all LH cancers are also presented) for both genders (no statistically significant gender differences were found). Results for individual and categorical data models are presented; EPA preferred the non-categorical model.

EPA RESPONSE: No response required.

xii. **COMMENT:** Although there are still significant concerns with the final selection of modeling approaches for derivation of unit risk in the current draft assessment (see the responses to Charge Questions 1–4, Sections 3.1–3.4 of this report), the EPA should be commended for the effort and the commitment of resources to address the comments and recommendations in the SAB (2007) report. Likewise, the EPA considered the SAB's extensive comments on the rationale for non-linear low-dose extrapolation including additional analysis of experimental animal data on mutations by EtO (pages H-15 to H-19 of Appendix H), concluding that the evidence did not indicate low-dose, non-linear extrapolation or threshold dose-response patterns. Thus, the rationale (including

more expansion on EPA guidance) for using low-dose, linear extrapolation is improved and stronger in the current assessment, but some concerns remain (see responses to Charge Questions 1–3 and 6, Sections 3.1–3.3 and Section 3.6 of this report).

EPA RESPONSE: No response required here; the EPA has addressed the SAB concerns related to the exposure-response modeling in the context of the other SAB comments (e.g., the EPA has revised its model selection for the lymphoid cancer data and now uses a continuous exposure model; see response to Comment I.4 above).

xiii. **COMMENT:** Concerns about the suitability of life table methodology for determination of LEC01 have been addressed. The EPA provides a convincing rationale (especially since alternative approaches are not available) for using the BEIR IV algorithm. The response to the request to present the range unit risk estimates for the upper and lower 95% confidence limits of the EC01 is also reasonable.

EPA RESPONSE: No response required.

xiv. **COMMENT:** The EPA also responded in detail to the comments provided in Appendix A of the SAB (2007) report. Many of the comments referred to the use of categorical exposure metrics and regression on group data that are also the subject of the current SAB review and are reflected in the responses to Charge Questions 1–3 (Sections 3.1–3.3 of this report).

EPA RESPONSE: No response required.

xv. **COMMENT:** The SAB finds this [response regarding expanded discussion of application of ADAFs (Section4.4)] to be responsive to the SAB (2007) comment.

EPA RESPONSE: No response required.

xvi. **COMMENT:** The SAB suggests that the EPA consider using the same model for both environmental and occupational exposures. (Please refer to the response to Charge Question 3 – Section 3.3 of this report).

EPA RESPONSE: The EPA is now using the same model for both environmental and occupational exposures for both cancer endpoints.

xvii. **COMMENT:** The SAB agrees with EPA's response [on the use of ppm equivalency for interspecies scaling of EtO exposure].

EPA RESPONSE: No response required.

xviii. **COMMENT:** SAB comments on uncertainty in the current draft assessment are reflected in the response to Charge Question 4 (Section 3.4 of this report).

EPA RESPONSE: No response required.

7. More detailed comments regarding Appendix J—new studies

a. Bulleted summary recommendations regarding Appendix J (p. 29 of SAB report)

i. **COMMENT:** Specific suggestions for expanded inclusion of the Swedish sterilization workers study results (Mikoczy et al., 2011) for breast cancer include:

• Discussion of the study should be moved to a more central position in the draft assessment.
• The Swedish sterilization worker study should be incorporated into an overall weight of evidence assessment of EtO effects at low doses.
• Consideration of using the word "strong" in its Bradford-Hill strength of association analysis.
• Consideration of characterizing the exposure assessment as high quality in light of the results of the exposure matrix for the early period of the study being validated by hemoglobin adduct levels (Hagmar et al., 1991).
• Consideration of a quantitative risk assessment based on the breast cancer data in the study.
• Alternately, consideration of applying NIOSH estimates to the Swedish sterilization workers study to assess the consistency of findings with:
  o Low dose exposure

o Attenuation of risk with higher exposures
o The observation of increased breast cancer risk with 16 more years of follow-up (latency)

EPA RESPONSE: The EPA has incorporated discussion of the Mikoczy et al. (2011) study into the main body of the report. For example, the study is now considered in the weight-of-evidence analysis and supports the characterization of the epidemiological evidence on breast cancer as "strong" (see Sections 3.1 and 3.5). In addition, the study is cited as supporting the supralinear exposure-response relationship observed with the NIOSH breast cancer incidence data (see Section 4.1.4). Also, a comparison was done of the Mikoczy et al. (2011) RR estimates with predicted RR values from the selected model derived from the NIOSH data; the selected model underestimated the Mikoczy et al. (2011) results (see Section 4.1.4 and Section J.2.2 of Appendix J).

ii. **COMMENT:** Consideration of separating agency interpretation of study findings from those of the studies' authors.

EPA RESPONSE: The EPA has clarified what are Agency interpretations and what are study author findings.

iii. **COMMENT:** Consideration of an expanded review of recent studies, including summary reviews, with specific focus on issues related to mode of action.

EPA RESPONSE: The EPA has added to Appendix J some more recent studies with significant new information pertaining to mode of action (Zhang et al., 2015b; Zhang et al., 2015a); however, at this stage of development of the assessment, the Agency did not further consider new studies unless they provided important new information.

iv. **COMMENT:** Consideration of emphasizing the importance of internal comparisons in occupational studies.

EPA RESPONSE: The preference for internal comparisons is listed among the considerations in evaluating epidemiological studies at the beginning of

Section 3.1. The statement that "[i]nternal 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" occurs later in Section 3.1, but was also brought forward as a footnote to the considerations at the beginning of the section.

b. More detailed comments from the text regarding Appendix J (p. 28–29 of SAB report)

i. **COMMENT:** In general, the logic and progression of the literature review are clearly supported. However, in the descriptions and assessments of the new studies, it is not entirely clear which statements are made by the study authors and which are made by the EPA. The discussion of the Kiran et al. (2010) case-control study is thorough. The conclusion that the Kiran et al. (2010) study overall supports the draft assessment is reasonable. The conclusion that small numbers of participants in the Morgan et al. (1981) and Ambroise et al. (2005) studies preclude more detailed analysis, but warrant inclusion in the review is reasonable. The summary of the Valdez-Flores and Sielken (2013) study discussion in Appendix J-3 is thorough, but parts of the discussion should be moved to the main body of the draft assessment. The SAB generally agrees that the new studies in Appendix J do not substantially alter the findings of the assessment with the exception of the Swedish sterilization workers study (Mikoczy et al., 2011; Hagmar et al., 1991). This study of EtO sterilization workers, with detailed exposure data at low doses with documented substantial effects on breast cancer has stronger implications than suggested in the draft assessment. The strong breast cancer results at low-dose exposures in this study greatly add to the overall findings. The observation of a 2.5–3.5-fold significantly elevated risk of breast cancer associated with low cumulative exposure in this study demonstrates strong evidence of carcinogenicity.

EPA RESPONSE: The EPA has clarified what are Agency interpretations and what are study author findings. The Morgan et al. (1981) and Ambroise et al. (2005) studies were ultimately omitted because the Morgan et al. (1981) study was included in the EPA's 1985 EtO health assessment (U.S. EPA,

1985) and the Ambroise et al. (2005) study, in addition to being too small to be informative, does not report EtO-specific results. The EPA has incorporated discussion of the Mikoczy et al. (2011) study into the main body of the report (see response to Comment II.7.a.i above for details). The discussion of the Valdez-Flores and Sielken (2013) study has been substantially shortened and has not been incorporated into the main report because the issue of modeling the categorical data is no longer of paramount importance, as the assessment now relies on models of the continuous exposure data for both cancer endpoints in the NIOSH study.

8. More detailed comments regarding Appendix K (then L) and Section H.2 of Appendix H—responses to public comments on the 2013 and 2006 drafts, respectively

a. **Detailed comments from the text regarding Appendix K (p. 29–32 of SAB report) (no bulleted summary recommendations were provided in response to this charge question)**

i. **COMMENT:** Appendix K presents a summary of the EPA responses to public comments on the July 2013 draft assessment. The section begins with a brief and clear summary of the comments received.

EPA RESPONSE: No response required.

ii. **COMMENT:** Before assessing the responses of the EPA to each of the specific comments, a general assessment of the nature of the comments received by the EPA, which primarily came from industry or industry organizations, is presented. In addressing this charge question, the primary focus is to evaluate the quality and thoroughness with which the EPA responded to the public comments rather than to evaluate the issues raised as these are covered in the responses to the other charge questions in the current report.

EPA RESPONSE: No response required.

iii. **COMMENT:** *Comment 1:* This comment claims that the EPA failed to follow NRC (2011) guidelines and failed to apply a systematic and weight-of-evidence

approach. The EPA response is clear but could be even stronger. There are several places in the draft assessment where the weight-of-evidence approach is discussed and justified. To strengthen the response to this question, some more detail listing places in the draft assessment where NRC (2011) and EPA guidelines as well as the systematic and weight-of-evidence approach are explained and justified would be helpful. There was additional comment on the use of NIOSH breast cancer incidence data that were not publically available. The EPA response clearly described their adherence to the EPA Information Quality Act Guidelines, which do not require all raw epidemiology data be publically available. Constraints due to confidentiality were also noted.

EPA RESPONSE: In response to SAB comments, the EPA has strengthened the response to the public comment by including more details of where the considerations used in the evaluation of the epidemiological studies (see Section 3.1), weight-of-evidence analysis (see Section 3.5), characterization of the cancer hazard (see Section 3.5), selection of the epidemiology study(ies) for quantitative risk estimation (see Section 4.1), and selection of exposure-response models (see Section 4.1) can be found.

iv. **COMMENT:** *Comment 2:* The comment states that the EPA did not properly explain the criteria used to evaluate studies and deem them to be of high quality for inclusion in their analysis. A summary of the characteristics used by EPA in the EtO assessment was revised in order to more clearly respond to this public comment. Criteria used to evaluate data quality are now discussed in much more detail than in the previous document.

EPA RESPONSE: No response required.

v. **COMMENT:** *Comment 3:* The comment states that lymphohematopoietic and lymphoid cancers should not be grouped because they are derived from different cells of origin. The response clearly states the rationale for grouping these together and notes that the SAB (2007) report agreed with the logic of that grouping for comparison purposes. This response is clear and appropriate.

EPA RESPONSE: No response required.

vi. **COMMENT:** *Comment 4:* The comment states that the evidence for breast cancer is too weak. The response notes that the document acknowledges that the breast cancer database is more limited than that for other cancers. Further, the response notes that the SAB (2007) report accepted the derivation of a unit risk factor based on that database. This response is clear and appropriate. Additionally, the EPA could also discuss the animal model data (Parsons et al., 2013; NTP, 1987) and Swedish sterilization workers study data (Mikoczy et al., 2011) to provide further support for breast cancer as a potential hazard from EtO exposure.

EPA RESPONSE: As suggested by the SAB, the EPA has expanded the response to include additional supporting information.

vii. **COMMENT:** *Comment 5:* The comment notes that EtO is a weak mutagen. Both the response and the draft assessment never claim that EtO is a strong mutagen. The "weakness" of EtO as a mutagen as compared to many anticancer compounds and other reactive epoxides is clearly stated. In their response, the EPA provides further justification by noting that there is seldom a good correlation between mutagenic and carcinogenic potencies. This response is clear and appropriate.

EPA RESPONSE: No response required.

viii. **COMMENT:** *Comment 6:* The comment states that a mutagenic MOA is not supported by the most recent scientific evidence; other MOAs, specifically oxidative stress and cell proliferation, should be considered. There are two major issues here with regard to the MOA. First, the database concerning the MOA is rather complex, which the draft assessment and the EPA response acknowledge. Second, and most significantly, the Parsons et al. (2013) study cited in the comment is considered to be flawed and does not adequately argue that other MOAs besides direct mutagenesis are involved. The response clearly states that there is no support for the conclusions in Parsons et al. (2013). In the response, the EPA cites another recent study (Nagy et al., 2013) that does not support oxidative stress. The response also provides a detailed discussion of the problems of inferring too much from K-ras mutation data. Even fewer data exist to support

a proliferative MOA. The EPA response methodically presents the reasoning behind this conclusion.

EPA RESPONSE: No response required. However, the EPA notes that more recent studies (Zhang et al., 2015b; Zhang et al., 2015a) similarly do not support the oxidative stress hypothesis; these studies have been added to Appendix J (see Section J.4.1).

ix. **COMMENT:** *Comment 7:* The comment criticizes the EPA for failing to incorporate the Union Carbide Corporation (UCC) data into the dose-response assessment. It goes on to state that the NIOSH exposure assessment also suffered from limitations. The EPA response is concise and clear. This issue is discussed in detail in the draft assessment and was supported by the SAB (2007) report. The NIOSH study meets the criteria of being a high-quality study much more strongly than the UCC data. This response is well-supported and appropriate. The SAB concurs with the EPA decision to not combine UCC EtO exposure data with those from the NIOSH study.

EPA RESPONSE: No response required.

x. **COMMENT:** *Comment 8:* This comment criticizes the EPA for using summary data rather than the individual data in the modeling of breast cancer mortality and lymphoid cancer despite the SAB (2007) recommendations. Two key points are made in the response. First, the rationale for the modeling procedures used and their consistency with the previous recommendations in the SAB (2007) report are noted. Second, the response notes that the current document adds additional models based on continuous exposure data and has added them to the assessment for comparison purposes. This response is appropriate. However, the SAB suggests that the model should only apply to low-dose exposures and that a range of doses should be specified over which the model applies.

EPA RESPONSE: The assessment has been revised so that models based on the continuous exposure data are used for both cancer endpoints. Both selected models are linear two-piece spline models, and the assessment notes that the linear extrapolations from these models are valid for exposures below the knots.

xi. **COMMENT:** *Comment 9:* A comment from two sources criticized the EPA for using a non-peer-reviewed supralinear spline model. The response notes that the model was published in 2011. Further, the response notes that use of the model will receive additional review by the SAB. This response is clear and appropriate.

EPA RESPONSE: No response required.

xii. **COMMENT:** *Comment 10:* A comment was made regarding other concerns about the modeling procedures used and how they lead to over-prediction of cancer deaths in the NIOSH study. In response to concerns raised by the two publications cited in the comment, the EPA provided additional discussion in Appendix J to specifically address concerns raised with respect to the Valdez-Flores and Sielken (2013) study. The response further suggested that the referenced citations did not provide convincing evidence of flaws in the modeling. Further, the EPA notes that the potential degree of over-prediction is far less than that claimed in the comment and the two papers. This response is appropriate.

EPA RESPONSE: No response required.

xiii. **COMMENT:** *Comment 11:* A comment was made from three sources that the EPA should present both linear and nonlinear extrapolation approaches. This subject is discussed at great length in the draft assessment and in Appendix H. The response further notes that the SAB (2007) report agreed that there was presently insufficient evidence to support use of a nonlinear extrapolation approach. This response is appropriate.

EPA RESPONSE: No response required.

xiv. **COMMENT:** *Comment 12:* A comment was made from two sources that combining breast cancer and lymphoid cancer unit risk estimates is not justified, and that the EPA did not discuss competing risks, different background populations, incidence vs. mortality, and the use of different exposure-response models. In their response, the EPA first notes that breast cancer and lymphoid cancers were first modeled separately and then combined. The rationale for combining these unit risk estimates is explained in detail in the draft assessment.

Further, the subject of competing and background risks is also discussed in detail in the draft assessment. Finally, the response concludes by noting the distinction between cancer incidence and cancer status. Standard practice in IRIS assessments is to estimate total cancer risk and not just the risk from individual cancer types; this practice is consistent with EPA guidelines and NRC recommendations. This response is appropriate.

EPA RESPONSE: No response required.

xv. **COMMENT:** *Comment 13:* A comment was made from three sources that the EPA should reexamine its risk determination given background and endogenous levels of EtO and that the EPA's risk estimates are unrealistically high. The EPA response explains how background rates for the cancers of interest have been taken into account in the risk determination. They also note that in one of the comments an unrealistic exposure concentration was used in arguing their point. This response is appropriate.

EPA RESPONSE: No response required.

xvi. **COMMENT:** *Comment 14:* Two sources commented that the EPA should not be deriving occupational exposure limits for EtO. The EPA response makes two clarifications. First, the EPA's Office of Pesticide Programs (OPP) is indeed responsible for deriving occupational exposure limits. Second, and more importantly, the response notes that such a derivation was not conducted in the present risk determination. Rather, the response notes that with the models used for the EtO cancer data, the unit risk estimate is not appropriate for the full range of occupational exposure scenarios of interest to OPP. For the purposes of OPP, the assessment provides sample risk estimates for exposure scenarios of interest to OPP for its own risk assessment of sterilization uses of EtO.

EPA RESPONSE: The EPA has clarified in its response that the Agency does not set "occupational exposure limits" for EtO but has the authority to consider occupational risks in labeling and regulation decisions.

xvii. **COMMENT:** *Overall Analysis of EPA Response to Public Comments in Appendix K (then L):* The responses provided by the EPA are focused, generally complete, and appear to be delivered in good faith.

EPA RESPONSE: The EPA confirms that the responses were delivered in good faith.

xviii. **COMMENT:** In addition to evaluating the EPA response (Charge Question 7) to public comments received on the July 2013 draft assessment, the EPA also presented their responses to public comments received on the 2006 draft assessment (U.S. EPA, 2006a) in Appendix H. Some of the comments were addressed by changes made in the current assessment. For example, one criticism was that the 2006 draft assessment (U.S. EPA, 2006a) had an improper reliance on data from only one sex. The current draft assessment uses data from both sexes. Another example was the EPA response to Comment 7 regarding the modeling procedures. Although the EPA response to the comment on the 2006 draft assessment (U.S. EPA, 2006a) was very brief and lacked sufficient detail, these issues are extensively addressed in the current draft assessment and the accompanying appendices. Several other comments were redundant with public comments made on the 2013 draft assessment. Examples include comments on EtO mutagenicity, lack of use of the UCC database, and the use of summary data versus individual data. In summary, the previous EPA responses in Appendix H as well as the changes that were instituted in the current draft assessment adequately and appropriately respond to the public comments on the 2006 draft assessment (U.S. EPA, 2006a).

EPA RESPONSE: No response required.

APPENDIX J. SUMMARY OF MAJOR NEW STUDIES SINCE THE LITERATURE CUTOFF DATE

The cutoff date for literature inclusion into the main body of this assessment was June 30, 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. An updated literature search was done in 2013, involving a systematic literature search 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. No studies were identified that would impact the assessment's major conclusions. Nonetheless, two new studies of high pertinence to the assessment had been published since the cutoff date, and these studies are reviewed briefly in this Appendix for transparency and completeness. Two additional highly pertinent studies published after the May 2013 literature search were identified from public comments received in October 2013 on the July 2013 public review draft of the EtO carcinogenicity assessment. These additional new studies similarly would not affect the assessment's major conclusions but are reviewed briefly here for transparency and completeness and to be responsive to the public comments. A final updated literature search, using the same approach as for the 2013 search, was conducted for the time period from May 2013 through August 2016. Once more, no studies were identified that would impact the assessment's major conclusions; however, two new studies of high pertinence to the assessment were published in that time frame, and these studies are also reviewed briefly in this appendix.

The Appendix first provides a description of the systematic literature search that was conducted to identify relevant new studies (see Section J.1) and then provides the reviews of the two major new studies identified in the May 2013 literature search (see Section J.2), the two additional major studies identified from the 2013 public comment period (see Section J.3), and the two major new studies identified in the 2016 literature search (see Section J.4). Sections J.2 and J.3 were part of the external review draft (U.S. EPA, 2014a, b) that was reviewed by the SAB in late 2014 (SAB, 2015); Section J.4 discusses studies published after completion of that review draft.

J.1. SYSTEMATIC LITERATURE SEARCH

Systematic literature searches were conducted in May 2013, covering the time frame from January 2006 to May 2013, and September 2016, for the time period of May 2013 through August 2016. The searches were conducted using the LitSearch tool in the EPA's HERO database, and the following three literature databases were searched: PubMed, Web of Science,

and ToxNet. The search terms involved Ethylene Oxide AND (carcinogenicity OR cancer OR mutagenicity OR mutation OR genotoxicity).

The May 2013 search identified 372 references, of which 56 were determined to be potentially relevant.[16] The disposition of the 56 potentially relevant references is summarized in Table J-1. In brief, for the purposes of this carcinogenicity assessment, 26 references that were primarily discussions of methods studies or exposure studies[17] or were reviews or other secondary source material were not generally considered further. The remaining 30 references were given further consideration to see if they represented major new studies. No new studies were identified that would impact the assessment's major conclusions. Two references were identified as highly pertinent studies, and these are reviewed briefly in Section J.2 of this appendix.

Table J-1. Disposition of 56 new references identified as potentially relevant in 2013

Category	References	Disposition
Exposure studies	Davis et al. (2006) Lin et al. (2007) Tateo and Bononi (2006)	Not considered further.
Methods studies	Ahn and Shin (2006) Tretyakova et al. (2012) Wu et al. (2011)	Not considered further.
Reviews or other secondary source material	Brown and Rushton (2012) Butterworth and Chapman (2007) Chan et al. (2006) Farmer and Singh (2008) Grosse et al. (2007) Hoenerhoff et al. (2009) Jarabek et al. (2009) Keshava et al. (2006a) Keshava et al. (2006b) Manservigi et al. (2010) McCarthy et al. (2009) Mosavi-Jarrahi et al. (2009) Okada et al. (2012) Smith-Bindman (2012)]	Not considered further.

[16]In this first part of the screening, any references of potential relevance to the carcinogenicity assessment of ethylene oxide were identified. References that pertained to other things and that were inadvertently captured in the literature search were excluded. For example, in an alphabetical listing of the 372 references by first author, the first reference is: Agarwal, A., Unfer, R. and Mallapragada, S. K. (2007), Investigation of in vitro biocompatibility of novel pentablock copolymers for gene delivery. J. Biomed. Mater. Res., 81A: 24–39. This reference discusses some copolymers of various chemicals, including poly(ethylene oxide), synthesized as vectors for gene delivery and tested in some cancer cell lines; this reference was not relevant to the assessment and was excluded from further consideration.

[17]This refers to general exposure studies; exposure studies related to any of the epidemiological studies of EtO would be considered further.

Table J-1. Disposition of 56 new references identified as potentially relevant (continued)

Category	References	Disposition
Reviews or other secondary source material (continued)	Snedeker (2006) Steinhausen et al. (2012) Weiderpass et al. (2011) Won (2010) WHO, 2008 [same as IARC (2008)]	Not considered further.
	IARC (2008)	Already cited in the assessment.
Cancer studies	Kiran et al. (2010) Mikoczy et al. (2011)	Reviewed in Section J.2.
	Swaen et al. (2009)	Already cited in the assessment.
	van Balen et al. (2006)	Not considered further. Primarily a study of risks to farmers. EtO left out of analysis because too few study subjects were exposed to it. Subjects were part of the EPILYMPH study analyzed by Kiran et al. (2010) (see Section J.2.1).
	Fondelli et al. (2007)	Not considered further. No EtO-specific results.
	Kim et al. (2011)	Not considered further. Case report study of 7 cases of malignant lymphohematopoietic disorders found in 2 semiconductor plants. Various carcinogens suspected of causing lymphohematopoietic cancers were investigated; EtO not found in processes of cases.
Genotoxicity/ mutagenicity studies	Donner et al. (2010) Godderis et al. (2006) Hong et al. (2007) Houle et al. (2006) Marsden et al. (2007) Marsden et al. (2009) Tompkins et al. (2008) Yong et al. (2007)	Already cited in the assessment.
	Mazon et al. (2009) Tompa et al. (2006) Tompkins et al. (2009)	Citations added to the assessment.
	Huang et al. (2011)	Not considered a major new study. Largely an exposure study; examined use of urinary N7-HEG as a biomarker of EtO exposure in EtO-exposed workers and smokers in Taiwan.
	Lindberg et al. (2010)	Not considered further. This study examined use of a micronucleus assay for detecting genotoxic damage in mouse alveolar Type II and Clara cells—EtO was used as a test agent but at a high concentration (>3 times higher than the highest exposure concentration used in the mouse cancer bioassay).
	Mazon et al. (2010)	Not considered further. Focused on a specific repair gene product in *E. coli*.
	Parsons et al. (2012) Tompkins et al. (2006)	Not considered further. Published abstracts, not full papers.

Table J-1. Disposition of 56 new references identified as potentially relevant (continued)

Category	References	Disposition
Other	Sielken and Valdez-Flores (2009a) Sielken and Valdez-Flores (2009b) Swenberg et al. (2008) Valdez-Flores et al. (2010)	Already cited in the assessment.
	Haufroid et al. (2007)	Citation added.
	Kensler et al. (2012)	Not relevant; focused on chemoprevention.
	Steenland et al. (2011)	Not considered further. Peer-reviewed publication of analyses already in the assessment.
	Valdez-Flores et al. (2011)	Not considered further. Quantitative risk assessment for occupational exposures—issues pertaining to the Valdez-Flores et al. risk assessment approach are already addressed in the assessment in discussions of the 2010 paper by the same authors (Valdez-Flores et al., 2010).
	Swenberg et al. (2011)	Not considered further. Largely a review; focused on implications of endogenous adducts for risk assessment—this issue is already addressed in the assessment (e.g., at the end of Section 4.5 and in the responses to SAB comments in Appendix H).

EPILYMPH = population-based case-control study of lymphoma in six European countries.

The September 2016 search identified 180 references, of which 17 were determined to be potentially relevant. The disposition of the 17 potentially relevant references is summarized in Table J-2. Eight references that were primarily discussions of methods studies or exposure studies or were reviews or other secondary source material were not considered further. The remaining 9 references were given further consideration to see if they represented major new studies. No new studies were identified that would impact the assessment's major conclusions. Two references were considered highly pertinent studies, and these are reviewed briefly in Section J.4 of this Appendix.

Table J-2. Disposition of 17 new references identified as potentially relevant in 2016

Category	References	Disposition
Exposure studies	Gabriel et al. (2013) Jacob et al. (2013) Kloth et al. (2014) St Helen et al. (2014)	Not considered further.
Methods studies	Breheny et al. (2014)	Not considered further.
Reviews or other secondary source material	Bukowska (2015) Eastmond et al. (2014) Konduracka et al. (2014)	Not considered further.
Cancer studies	Yuan et al. (2014)	Not considered further. Case-control study of lung cancer and urinary metabolites of a variety of pollutants – the urinary metabolite of EtO was not associated with increased risk of lung cancer.
Genotoxicity/ mutagenicity studies	Nagy et al. (2013) Parsons et al. (2013)	Already cited in the assessment (see Section J.3).
	Zhang et al. (2015b)	Reviewed in Section J.4.
	Philippin et al. (2014)	Not considered further. Focused on the capacity for *N7*-alkylguanine adducts to induce mutagenicity in *E. coli*.
	Zhang et al. (2016)	Not considered further. Study of *in silico* modeling using Pearson's hard and soft acids and bases theory to estimate the activation energies and other chemical characteristics of 36 epoxides and correlate these calculated activation energies against previously published mutagenicity results in *S. typhimurium* strain TA100.
Other	Valdez-Flores and Sielken (2013)	Already cited in the assessment (see Section J.3).
	Zhang et al. (2015a)	Reviewed in Section J.4.
	Filser et al. (2013)	Not considered further. Study of EtO levels in blood from ethylene exposure.

J.2. REVIEWS OF MAJOR NEW STUDIES IDENTIFIED IN THE 2013 LITERATURE SEARCH

As discussed in Section J.1, a systematic literature search was conducted in 2013 to determine whether any significant new or missed studies had been published since January 2006. No new studies were identified that would impact the assessment's major conclusions. Nonetheless, two studies of high pertinence to the assessment had been published since the June 2010 cutoff date for literature inclusion. The two studies are epidemiology studies of occupational exposure to EtO. These studies are reviewed briefly here for transparency and completeness, and key features of the studies are summarized in Table J-3.

Table J-3. New epidemiological studies of ethylene oxide and human cancer

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Population-based case-control study involving 22 centers in 6 European countries (Czech Republic, France, Germany, Italy, Ireland, Spain) [EPILYMPH study] Kiran et al. (2010)	2,347 cases (1,314 male, 1,033 female); 2,463 controls (1,321 male, 1,142 female), matched on sex, age group, and residence area	1.2% of study population defined as ever-exposed (31 cases, 27 controls)	All lymphoma (no. cases/no. controls) OR (95% CI) Unexposed (2,316/2,436) 1.0 [referent category] Ever exposed (31/27) 1.3 (0.7, 2.1) Confidence in exposure classification low (8/12) 0.8 (0.3, 1.9) med or high (23/15) 1.6 (0.8, 3.1) *p*-trend = 0.242 Exposure frequency (no. working hr) 1–5% (16/23) 0.8 (0.4, 1.4) >5% (15/4) 4.3 (1.4, 13.0) *p*-trend = 0.107 Exposure intensity (ppm) ≤0.5 (15/19) 0.9 (0.4, 1.7) >0.5 (16/8) 2.2 (0.9, 5.1) *p*-trend = 0.197 Duration (years) ≤10 (18/16) 1.2 (0.6, 2.4) >10 (13/11) 1.3 (0.6, 3.0) *p*-trend = 0.441 Cumulative exposure score ≤median (13/16) 0.9 (0.4, 1.8) >median (18/11) 1.8 (0.8, 3.9) *p*-trend = 0.246	Would vary by individual participant because it is not an industry-based study; however, inclusion of farm work and occupational exposure to solvents in the regression model did not affect the risk estimates	Low exposure prevalence in study population, so small numbers of exposed cases and controls Lymphoma subtype analyses, in particular, limited by small numbers Participation rate only 52% in population controls, but the positive association was observed across centers with different control types

EPILYMPH = population-based case-control study of lymphoma in six European countries.

Table J-3. New epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
Two plants that produced disposable medical equipment, Sweden Mikoczy et al. (2011) Same cohort as (Hagmar et al., 1995; Hagmar et al., 1991), followed an additional 16 years	2,171 (862 men, 1,309 women)	Exposure levels were up to 75 ppm in 1964 in Plant B and up to 40 ppm in 1970 in Plant A. By 1985, levels had dropped to below 1 ppm. For the 2,020 cohort members for whom job titles were available, the median was 0.13 ppm × years; the 75th percentile was 0.22 ppm × years; and the 90th percentile was 1.29 ppm × years.	*Lymphohematopoietic cancers:* *Mortality* (results not shown): Nonsignificant increases of deaths from leukemia and lymphoma were reported; with a 15-yr induction period, these increases were lowered; with a 15-yr induction period and restriction to workers with cumulative exposure estimates above the median, nonsignificant increases in leukemia deaths were reported *Incidence:* Cancer (ICD-7) [cases] SIR (95% CI) All lymphohematopoietic (200–209) [18] 1.25 (0.74, 1.98) NHL (200+202) [9] 1.44 (0.66, 2.73) Leukemia (204–205) [5] 1.40 (0.45, 3.26) Internal analysis of lymphohematopoietic cancers: Cum exp gp ppm × years [cases] IIR (95% CI) 0–0.13 [7] 1.00 0.14–0.21 [5] 1.17 (0.36, 3.78) ≥0.22 [5] 0.92 (0.28, 3.05)	Fluorochlorocarbons, methyl formate (1:1 mixture with EtO)	Still a youthful cohort (mean age 56 years), with small numbers of events for the study of the incidence and mortality of specific cancer types—203 total cancer cases (9.4%) and 171 total cancer deaths (7.9%) Estimated cumulative exposures were generally low. There was no unexposed referent group; internal analyses involved comparison of responses in the top quartiles of cumulative exposure to those in the lower 50% of cumulative exposures.

Table J-3. New epidemiological studies of ethylene oxide and human cancer (continued)

Population/ industry	Number of subjects	Extent of exposure to ethylene oxide	Health outcomes	Other chemicals to which subjects were potentially exposed	Limitations
			(continued from previous page) *Female breast cancer:* *mortality* (results not shown): Slight but nonsignificant decrease in the SMR was reported. With a 15-yr induction period included, the SMR for breast cancer was "somewhat increased." For workers with cumulative exposures above the median, with a 15-yr induction period, a "higher than expected" SMR, which was not statistically significant, was reported. *Incidence:* 41 female breast cancer cases vs. 50.9 expected (ICD−7 170); SIR = 0.81 (95% CI = 0.58, 1.09) Internal analysis: Cum exp gp ppm × yr [cases] — IIR (95% CI) 0−0.13 [10] — 1.00 0.14−0.21 [14] — 2.76 (1.20, 6.33) ≥0.22 [17] — 3.55 (1.58, 7.93)		

J.2.1. Kiran et al. (2010)

Kiran et al. (2010) investigated occupational exposure to EtO in a population-based case-control study of lymphoma in six European countries (the "EPILYMPH study"). Cases (n = 2,347) were consecutive adult patients with a first diagnosis of lymphoma, classified under the 2001 World Health Organization lymphoma classification system, in 1998−2004 at 22 centers in the six countries. Controls from Germany and Italy were randomly selected from the general population, matched on sex, 5-year age group, and residence area. Controls from the Czech Republic, France, Ireland, and Spain were matched hospital controls with diagnoses other than cancer, infectious diseases, and immunodeficient diseases (total controls = 2,463). Participation rates were 88% in cases, 81% in hospital controls, and 52% in population controls. All study subjects were interviewed in person using the same structured questionnaire, which included questions on sociodemographic factors, lifestyle, health history, and complete work history (including all full-time jobs held for ≥1 year). For each job, information was collected on type of industry, tasks performed, machines used, and exposure to 35 specific agents (or groups of agents) of interest, including EtO. Supplemental questionnaire modules for specific occupations were used to get additional details on jobs and exposures of interest.

Exposure was evaluated in each center by specially trained industrial hygienists who reviewed all the questionnaires and assessed frequency and intensity of exposure to each agent on a 4-point scale (unexposed and low, medium, and high exposures) as well as confidence in the assessment (low, medium, or high). Most of the exposed cases and controls were classified with medium or high confidence, although a greater proportion of cases than controls were thus classified (23/31 vs. 15/27). Because of the low prevalence (1.2%) of EtO exposure in the study, the medium and high categories of exposure frequency and intensity were combined in the statistical analyses. A cumulative exposure score for EtO was also developed for each study subject, integrating duration, frequency, and intensity of exposure; these scores were then categorized as above or below the median score among exposed subjects.

Risk was assessed for all lymphoma, B-cell lymphoma (which represented 80% of all the lymphoma cases), and the most common subtypes of B-cell lymphoma. The OR was calculated using unconditional logistic regression, adjusting for age, sex, and center. Including education, farm work, and exposure to solvents in the model, reportedly did not change the risk estimates (results not shown). Linear trends for the exposure metrics were calculated using the Wald test for trend.

Because of the low prevalence of EtO exposure in the study (1.2%), the number of exposed cases and controls was limited (31 and 27, respectively), especially for analyses of lymphoma subtypes. Results for all lymphoma for ever exposed and for the highest exposure

category for each of the different exposure metrics are presented in Table J-3. Increased risks were observed for ever exposed and for the highest exposure category for each of the exposure metrics, and the OR for medium or high frequency of exposure was statistically significant (4.3; 95% CI 1.4, 13.0). However, none of the trend tests was statistically significant. The overall association appeared to be stronger using hospital controls; however, when considering only subjects whose EtO exposures were assessed with medium or high confidence, the increased ORs were similar using either hospital or population controls. Results were similar when only B-cell lymphoma, which represented the majority of all lymphomas, was evaluated. The strongest associations were generally observed for chronic lymphocytic leukemia, and *p*-values for trend were ≤0.051 for all the exposure metrics for that lymphoma subtype. The investigators note that while random variation related to the low prevalence might account for some positive results, their combined probability test (Fisher method) indicated that the chance probability of an upward trend in chronic lymphocytic leukemia across the four metrics assumed to be independent (confidence, frequency, intensity, and duration) was 0.003.

In conclusion, this study adds further support to the weight-of-evidence finding obtained in Chapter 3 of strong, but less than conclusive, evidence of a causal association between EtO exposure and lymphohematopoietic cancers in humans. Because only categorical exposures were assessed, no quantitative risk estimates can be derived from this study.

J.2.2. Mikoczy et al. (2011)

This study is an update of the Hagmar et al. (1991) and Hagmar et al. (1995) studies discussed in Section 3.1 of the assessment and in Section A.2.11 of Appendix A. The first update (Hagmar et al., 1995) had a median follow-up time of only 11.8 years; this update extends the follow-up period through 2006, providing an additional 16 years of follow-up. The cohort consists of 2,171 (1,309 females and 862 males[18]), employed for at least 1 year prior to 1986, at two Swedish facilities that sterilized medical equipment using EtO (Plant A sterilization operations ran from 1970 to 1994; Plant B sterilization operations ran from 1964 to 2002). Vital status and emigration data at the end of follow-up were obtained from the Swedish population registry, cause of death for 1972−2006 was obtained from Statistics Sweden, and malignant tumor data for 1972−2006 were obtained from the Swedish Cancer Registry. At the end of follow-up, the mean age of the cohort was 56 years and the cohort had contributed 58,305 person-years of risk; 171 cohort members had died (7.9%) and 126 (5.8%) had emigrated and were of unknown vital status. Mean duration of employment in the cohort was 6.3 years.

[18]Without explanation, there is one additional male in the update; the 1991 and 1995 papers both reported 2,170 workers, including 861 males, in the cohort (Hagmar et al., 1995; Hagmar et al., 1991).

In the original study (Hagmar et al., 1991), individual cumulative exposure estimates were derived based on job-exposure matrices for each plant and exposure level estimates determined up to 1986. While exposure levels were high in the early years of the operations (e.g., peak levels of 75 ppm in 1964 in Plant B and exposure levels up to 40 ppm in 1970 in Plant A), 8-hour TWA levels had decreased to below 1 ppm by 1985 [see Hagmar et al. (1991) and Section A.2.11 of Appendix A for more details on the original exposure assessment]. For this update, worker histories for the 1,303 workers who were still employed at the two plants at the end of the original study (1986) were extended up until the cessation of sterilization operations in the plants, and exposure estimates for the follow-up period were determined from yearly statutory industrial hygiene measurements of EtO from 1986 on. Because of the low exposure levels after 1985, the impact of updating the cumulative exposure estimates was low (the largest impact was reportedly on the 90th percentile, which changed from 1.17 to 1.29 ppm × years). The mean and median cumulative exposures for the 2,020 cohort members for whom job titles were available were 2.92 ppm × years and 0.13 ppm × years, respectively.

Standardized mortality and incidence ratios (SMRs and SIRs) were obtained by comparing the number of deaths or incident cases observed to the number expected based on 5-year age group-, cause-, calendar year-, and sex-specific rates in the county (external referents). For cancer incidence (but not mortality), internal analyses were also conducted using Poisson regression analyses, adjusted for age group, sex, and calendar period, with no induction (latency) period. In the internal analyses, incidence rate ratios (IRRs) 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 of 0.13 ppm × years (internal referents). [Internal analyses are generally preferred by the EPA over external analyses because the referents are from the same cohort as the exposed subjects, potentially reducing confounding as well as the healthy worker effect, which can mask an increase in risk; however, in this study, some of the advantages of internal analyses may be mitigated by the absence of an unexposed referent group, which could itself dampen relative risk estimates.]

Results for cancer mortality and incidence for the cancer types of interest (i.e., lymphohematopoietic cancers and female breast cancer) are summarized in Table J-3. For lymphohematopoietic cancers, nonsignificant increases in SMRs and SIRs were reported. For the incidence data, the internal analysis shows no exposure-related association for lymphohematopoietic cancers, although the EPA found this analysis to be 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. The EPA also noted that data were not reported or analyzed for

the subgrouping of "lymphoid" cancers. In a crude comparison, ignoring the exposures in the referent group, the lack of a lag period, and the fact that the results are for all lymphohematopoietic cancers rather than lymphoid cancers, the EPA compared the Mikoczy et al. (2011) results with RR estimates obtained from the selected model for lymphoid cancer based on the NIOSH data (two-piece linear spline model with knot at 1,600 ppm × days; with a 15-year lag; see Section 4.1.1.2 and Figure 4–7) using the mean cumulative exposures for the highest two quartiles in the Mikoczy et al. (2011) study. The results obtained from the selected model for the NIOSH data were within the 95% confidence interval for the RR estimates reported by Mikoczy et al. (2011) (see Table J-4).

Table J-4. Comparison of Mikoczy et al. (2011) RR estimates with those obtained using the selected models based on the NIOSH study

Exposure group (ppm-years)	Mean cumulative exposure (ppm-yr)[a]	Mean cumulative exposure (ppm-days)	Reported RR estimate (n; 95% CI)		RR estimate from model based on NIOSH data[b]	
			Lymphohemato-poietic cancer	Breast cancer	Lymphoid cancer	Breast cancer
0 – 0.13	0.0745		1.00 (7)	1.00 (10)		
0.14 – 0.21	0.1737	63.40	1.17 (5; 0.36–3.78)	2.76 (14; 1.20–6.33)	1.05	1.01
≥ 0.22	14.1846	5,177	0.92 (5; 0.28–3.05)	3.55 (17; 1.58–7.93)	2.25	1.46

[a]Personal communication from Zoli Mikoczy to Jennifer Jinot, U. S. EPA, 15 September 2015.
[b]Ignoring the 15-year lag in model and the nonzero exposure in the referent group.

For breast cancer mortality (results not shown), a "slight but nonsignificant decrease" in the SMR was reported. With a 15-year induction period included, the SMR for breast cancer was reportedly "somewhat increased." For workers with cumulative exposures above the median, with a 15-year induction period, a "higher than expected" SMR, which was not statistically significant, was reported.

For breast cancer incidence (41 incident cases), SIRs were nonsignificantly decreased, both with and without a 15-year induction period. Internal analyses resulted in statistically significant increases in the IRRs for the two highest cumulative exposure quartiles as compared to the 50% of workers with cumulative exposures below the median (see Table J-3), despite having a low-exposed rather than an unexposed referent group.

The EPA noted that the cumulative exposure estimates for this study were very low compared to those in other studies. For example, in the Swaen et al. (2009) study of the UCC

cohort of male EtO production workers, the estimated average cumulative exposure was 67.16 ppm × years. In the more comparable NIOSH cohort of sterilization workers, cumulative exposure estimates at the end of follow-up for the full cohort, which included workers with <1 year of employment, had a mean of 27 ppm × years and median of 6 ppm × years (see Appendix D, Section D.1), and in particular, the mean cumulative exposure at the end of follow-up in the breast cancer incidence study cohort, which only included workers with ≥1 year of employment, was 37.0 ppm × years. Yet, the breast cancer incidence RRs for the categorical exposure groups reported in Steenland et al. (2003) for the NIOSH breast cancer incidence study were lower than those observed in the Mikoczy et al. (2011) study.

Thus, if unit risk estimates for breast cancer incidence were derived based on the Mikoczy et al. (2011) study, they would be higher than the estimates calculated from the NIOSH study. The EPA did not derive such estimates, however, because the reported grouped results from the Mikoczy et al. (2011) study are not well suited for derivation of a unit risk estimate, the EPA does not have the individual data to model, and the NIOSH study is preferred as the basis for the unit risk estimate in any event (see Section 4.1). Instead, as a crude comparison, ignoring the exposures in the referent group and the lack of a lag period, the EPA compared the Mikoczy et al. (2011) results with RR estimates obtained from the selected model for breast cancer incidence based on the NIOSH data (two-piece linear spline model with knot at 5,750 ppm × days; with a 15-year lag; see Section 4.1.1.2 and Figure 4–9) using the mean cumulative exposures for the highest two quartiles in the Mikoczy et al. (2011) study. The results obtained from the selected model for the NIOSH data were below the lower bound of the 95% confidence interval for the RR estimates reported by Mikoczy et al. (2011) (see Table J-4); i.e., the selected model used to derive the unit risk estimate for breast cancer incidence in this assessment underestimates the IRRs observed in the Mikoczy et al. (2011) study.

The EPA could not determine the reasons for the discrepancy between the observed IRRs and the predictions from the model based on the NIOSH data. As noted above, the cumulative exposure estimates for the Mikoczy et al. (2011) study are lower than those for the NIOSH study. 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 (Hagmar et al., 1991), but most of the NIOSH plants had lower estimated exposure levels (see Table J-5). However, exposure durations are shorter in the Mikoczy et al. (2011) study and more person-years would have accrued in more recent time periods, when exposure levels in the Swedish plants were lower. 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. Measurement data were available from 1973 for one plant ("A") and 1975 for the other ("B"); for earlier exposures, estimates were constructed taking into account information on changes in

production methods and environmental controls, subjective memories, and time trends (Hagmar et al., 1991). However, Plant A started operations in 1970 and Plant B in 1964, so the historical reconstructions did not have to go very far back in time and are thus probably subject to less uncertainty than most such retrospective reconstructions. Another major difference between the two studies is 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). Additionally, there was no information on potential breast cancer risk factors in the Mikoczy et al. (2011) study, as was available for the NIOSH subcohort, although accounting for these factors made little difference in the unit risk estimate derivation from the NIOSH data (see Section D.1.8 of Appendix D).

Table J-5. Comparison of highest exposure levels estimated for the NIOSH cohort plants with those in the Mikoczy et al. (2011) study plants[a]

Plant	Highest exposure level (ppm)	~ Years
Mikoczy et al. (2011) cohort plant		
A	40	1970-1972
B	75	1964-1966
NIOSH cohort plant[b]		
1	14	1969-1975
2	19	1976-1977
4	4	1971-1978
5	77	1977-1978
6	77	1977-1978
7	17	1969-1978
8	25	1967-1978
9	3	1969-1979
10	24	1974-1978
11	20	1970-1978
12	17	1972-1978
13	25	1970-1977
14	5	1976-1979

[a]8-hour TWAs for jobs/operations with the highest exposure levels per plant from NIOSH exposure data and Hagmar et al. (1991), compared for the earliest time periods of the two Mikoczy et al. (2011) study plants.
[b]Plant 3 did not have exposure data.

In conclusion, the EPA finds that the nonsignificant increases in SMRs and SIRs for lymphohematopoietic cancers reported in this study are consistent with an increase in lymphohematopoietic cancer risk but, overall, the study is underpowered for the analysis of lymphohematopoietic cancers and contributes little to the weight of evidence for these cancers. For breast cancer incidence, however, the statistically significant exposure-related increases in internal analyses add support to the weight-of-evidence finding obtained in Chapter 3 of strong, but less than conclusive, evidence of a causal association between EtO exposure and female breast cancer in humans. Although the Mikoczy et al. (2011) results are consistent with a higher unit risk estimate for breast cancer incidence than that obtained from the NIOSH study results, the Mikoczy et al. (2011) results support the general supralinear exposure-response relationship (i.e., steeper rise at lower exposure levels and then a plateauing of response at higher exposure levels) observed in the NIOSH study.

J.3. REVIEWS OF MAJOR STUDIES IDENTIFIED BETWEEN THE 2013 LITERATURE SEARCH AND THE 2014 SAB REVIEW DRAFT

Two additional major studies were identified from public comments on the July 2013 public review draft of the EtO carcinogenicity assessment, and a third study related to one of those studies was also discovered after the May 2013 literature search. These three studies are reviewed briefly here. These new studies would not affect the assessment's major conclusions but are reviewed here for transparency and completeness and to be responsive to the public comments.

J.3.1. Valdez-Flores and Sielken (2013)

Valdez-Flores and Sielken (2013) criticized the approach employed by the EPA in earlier drafts of the EtO carcinogenicity assessment of using a weighted linear regression of the RR estimates based on categorical exposure groups to derive exposure-response relationships for lymphoid cancer mortality and breast cancer mortality, stating that exposure-response modeling is best based on individual data. While the EPA does not agree with aspects of the Valdez-Flores and Sielken (2013) paper [see Section J.3.1 of Appendix J in (U.S. EPA, 2014a)], the EPA is no longer using the weighted linear regression of the categorical results as a selected model, and thus, the issues raised by Valdez-Flores and Sielken (2013) are not relevant to the current assessment.

J.3.2. Parsons et al. (2013) [and Nagy et al. (2013)]

As part of a larger study to examine potential key events in EtO-induced mouse lung carcinogenesis, Parsons et al. (2013) exposed Big Blue B6C3F$_1$ mice to various concentrations of EtO by inhalation for 4, 8, or 12 weeks (0, 10, 50, 100, or 200 ppm for 4 weeks or 0, 100, or 200 ppm for 8 or 12 weeks) and analyzed the levels of three specific K-*ras* codon 12 mutations (GGT→GAT, GGT→GTT, and GGT→TGT) in lung DNA samples using ACB-PCR (allele-specific competitive blocker PCR). Parsons et al. (2013) presented the first results to be published from this larger study. K-*ras* mutations were investigated because K-*ras* mutations, and more specifically codon 12 mutations, were identified in all of the lung tumors evaluated from EtO-exposed mice in the NTP cancer bioassay (Hong et al., 2007). Of the codon 12 mutations in the 23 mouse lung cancers evaluated, 21 were GGT→GTT mutations. Parsons et al. (2013) suggest that because 8-oxo-dG adducts[19] preferentially cause G:C→T:A mutations, an early increase of the GGT→GTT (and/or GGT→TGT) mutation relative to the GGT→GAT

[19]Same as 8-hydroxy-2′-deoxyguanosine (8-OHdG) adducts.

mutation would support the hypothesis that EtO causes oxidative stress in the mouse lung, resulting in the formation of 8-oxo-dG adducts.

Because many of the K-*ras* mutant fraction (MF) measurements were below the limit of accurate ACB-PCR quantification (10^{-5}), differences among treatment groups were assessed by analyzing the numbers of MFs greater than and less than 10^{-5} using a Fisher's exact test. Parsons et al. (2013) reported that for the GTT mutation at 4 weeks of EtO exposure, a significant increase in MF compared to concurrent controls occurred only in the 100-ppm group. "Surprisingly," as Parsons et al. (2013) noted, the MFs at 8 weeks in both the 100- and 200-ppm groups were statistically significantly *decreased* relative to concurrent controls, and at 12 weeks, the 200-ppm group had statistically significant decreases. A similar pattern was observed for the GAT mutation, with statistically significant increases in the 50-, 100-, and 200-ppm groups at 4 weeks and statistically significant decreases in the 100- and 200-ppm groups at 8 weeks compared to concurrent controls. The EPA noted that MFs were decreased in the 100- and 200-ppm groups at 12 weeks as well, but the results were almost all greater than 10^{-5}, and thus, the trend would not be apparent using the Fisher's exact test. For the TGT mutation, all of the measurements were less than 10^{-5}, and the investigators performed no further analyses. Parsons et al. (2013) also reported a "surprising amount of variability" in the GTT and GAT MF results among the 4-, 8-, and 12-week control groups, with the 8-week control GTT results and the 8- and 12-week control GAT results being statistically significantly increased compared to their respective 4-week control results.

Instead of observing an early preferential increase in GTT mutations, as anticipated, Parsons et al. (2013) reported an early induction of both GAT and GTT mutations, with a greater induction of the GAT mutation, which they note is the main K-*ras* codon 12 mutation observed by Hong et al. (2007) in "spontaneous" mouse lung tumors (11 of 17 K-*ras* codon 12 mutations in 108 lung tumors from control B6C3F$_1$ mice in the NTP 2-year cancer studies were GAT mutations). To explain these findings and the irregular pattern of results in which GTT and GAT MFs "did not accumulate straightforwardly with cumulative [EtO] dose or duration of treatment" and because "no induction of cytotoxicity or apoptosis was detectable" in another part of the larger study [results not presented by Parsons et al. (2013)], Parsons et al. (2013) proposed the following biphasic response. Parsons et al. (2013) hypothesized that "[EtO] may have caused a low level of oxidative stress and produced negatively charged molecules that modify Ras and Ras signaling...leading to an early expansion of K-*ras* mutant clones" but at higher EtO concentrations or longer exposure durations, or both, the amplification of existing K-*ras* mutations switches to the selective senescence or death of K-*ras* mutant cells. No explanation is proposed for the erratic control results.

The EPA notes several limitations of the Parsons et al. (2013) study and its reported findings. First, the study is looking at only three specific base-substitution mutations in one specific codon of one specific gene. Given that carcinogenesis is a multifaceted process, involving numerous genes, and that EtO can induce a variety of different types of mutation and other genotoxic effects, one should not infer too much about the mode of action for EtO-induced mouse lung carcinogenesis from this one study. In addition, the high degree of variability in most of the dose group MF results and the instability of the control results across different exposure durations suggest that the assay results might be unreliable. Nonetheless, Parsons et al. (2013) proposed some elaborate pathways to explain the "surprising" time- and dose-response patterns they observed. A more straightforward explanation for the highly variable dose group results, the erratic control group results, and the irregular time- and dose-response patterns is measurement error associated with the assay.

However, even if EtO caused a low level of oxidative stress and modified Ras signaling, resulting first in amplification and then in the death of K-*ras* mutant cells, as Parsons et al. (2013) proposed, which might explain some of their irregular time- and dose-response patterns (the erratic control results are still unexplained), their hypothesized explanation does not constitute a complete mode of action for the EtO-induced lung carcinogenicity observed in the NTP mouse cancer bioassay. Moreover, the Parsons et al. (2013) study, which found decreased levels of GAT and GTT mutations at 8 and 12 weeks compared to concurrent controls, does not elucidate the observations by Hong et al. (2007) of later-occurring K-*ras* codon 12 GTT or GAT mutations in all of the lung tumors evaluated from EtO-exposed mice in the NTP 2-year cancer bioassay.

Furthermore, these hypotheses have no independent support to date. In fact, this proposal disagrees with another 2013 study (Nagy et al., 2013) indicating that lung epithelial cells are relatively sensitive to the DNA alkylating effects of EtO and relatively resistant to oxidative DNA damage and that EtO does not induce oxidative damage. To investigate the relative susceptibility of different cell types to different types of DNA damage, Nagy et al. (2013) exposed human lung epithelial cells, peripheral blood lymphocytes, and keratinocytes for 1 hour in vitro to different concentrations (previously determined to be subcytotoxic) of EtO (TWA concentrations of 0, 16.4, 32.1, 55.5, or 237.5 µM) to assess alkylating damage or hydrogen peroxide (0, 1, 2, 5, or 10 µM) to assess oxidative damage. DNA damage was determined using the comet assay, and oxidative damage was detected by incorporating a step involving incubation with formamidopyrimidin DNA-glycosylase (Fpg)—a lesion-specific restriction endonuclease that can recognize oxidized purines and pyrimidines—into the assay. Nagy et al. (2013) reported that linear regression analyses showed a statistically significant positive correlation between EtO exposure and DNA damage as measured by both tail length and tail DNA for all three cell types.

The shallowest slope was for keratinocytes for both DNA damage parameters. The slope for the tail length parameter was higher for lung epithelial cells than for lymphocytes across the applied concentration range, and the slope for the tail DNA parameter was higher for lung epithelial cells than for lymphocytes across the concentration range for all but the highest concentration. A statistically significant positive correlation also was found between hydrogen peroxide exposure and oxidative DNA damage measured by both tail length and tail DNA for all three cell types. For oxidative DNA damage, however, the shallowest slope was for lung epithelial cells for both DNA damage parameters. Nagy et al. (2013) also reported that the oxidative potential of EtO was similarly evaluated, and no evidence of Fpg-dependent oxidative DNA damage was found in the examined cells at the applied concentrations (data not presented). Likewise, the more recent mouse lung studies by Zhang et al. (Zhang et al., 2015b; Zhang et al., 2015a) provided little support for the oxidative stress hypothesis (see Section J.4.1 below).

In addition, the EPA notes that none of the results presented by Parsons et al. (2013) preclude direct genotoxic effects of EtO. For example, Parsons et al. (2013) also reported that increased *cII* MFs were observed in lung tissues from the same EtO-exposed mice and that MFs increased significantly with EtO concentration at 8 and 12 weeks (results to be published separately), indicating that direct genotoxicity from EtO can occur elsewhere in the DNA. Furthermore, even the K-*ras* codon 12 mutations that Parsons et al. (2013) investigated can result directly from EtO—Parsons et al. (2013) themselves noted that the GAT mutation can result from EtO-induced O^6-HEG adducts, and even if 8-oxo-dG adducts from oxidative stress preferentially cause G:C→T:A mutations as indicated by Parsons et al. (2013), a variety of mutagens are known to cause G:C→T:A mutations as well (DeMarini, 2000).

J.4. REVIEW OF MAJOR STUDIES IDENTIFIED IN THE 2016 LITERATURE SEARCH

Two additional major studies were identified after the 2014 SAB review draft (U.S. EPA, 2014a, b), in the September 2016 literature search. These new studies would not affect the assessment's major conclusions but are reviewed briefly here for completeness.

J.4.1. Zhang et al. (2015a) and Zhang et al. (2015b)

In two studies published separately, Zhang et al. (2015a) and Zhang et al. (2015b) exposed male B6C3F$_1$ mice to ≤ 200 ppm EtO via whole-body inhalation for either 4 or 12 weeks and measured the resulting impact on lung levels of glutathione conjugates (Zhang et al., 2015a) or purine nucleotides and adducts (Zhang et al., 2015b), using liquid chromatography coupled with mass spectrometry to improve the simultaneous detection of various endpoints. Specifically, positive ions generated by electrospray ionization following separation by reverse phase chromatography were quantified using selective reaction monitoring in a Q-trap and

tandem mass spectrometry. To evaluate the effects of EtO exposure on lung levels of reduced and oxidized glutathione (GSH and GSSG, respectively), as well as 2-hydroxyethylated glutathione (HESG) resulting from EtO-alkylation of reduced glutathione, Zhang et al. (2015a) exposed male mice to 0, 10, 50, 100, or 200 ppm EtO for 6 hours/day, 5 days/week, for 4 weeks. The intra- and inter-day relative variation and accuracy were acceptable (≤ 13% and 87–113%, respectively), and the lower limit of quantification (LLOQ) was reported to be 0.002 µg/mL (~ 2 ppb) for all three analytes, which seems to the EPA to be sufficiently sensitive considering that GSH is present at µmol/g levels in rodent tissues (Pilon et al., 1988). To evaluate the effects of EtO exposure on purine nucleotide adduction, Zhang et al. (2015b) exposed male B6C3F$_1$ mice to 0, 100, or 200 ppm EtO on a similar schedule for a longer duration of 12 weeks. As with the glutathione conjugates, the intra- and inter-day relative variation and accuracy (≤ 19% and 87–120%, respectively) for a variety of guanine and adenine nucleotide adducts were acceptable, and the LLOQs ranged from low ppt for DNA adducts to ppm for the unmodified purine nucleotides.[20] The authors did not evaluate N3-HEA, which was previously reported in the spleens of F344 rats after 4 weeks of exposure to 300 ppm EtO (Walker et al., 1992), but did evaluate two other products of adenine n-alkylation (N1-HedA and N^6-HedA). While the authors did not evaluate the formation of the predominant EtO-guanine alkylation product, N7-HEG, they did measure the levels of guanine adducts likely to result from reactive oxygen species activity directly (8-OHdG), or indirectly following lipid peroxidation (CrotondG and N2,3-EthenodG) (see Footnote 20 for abbreviations).

In both studies, the lungs were not perfused, but were excised and snap-frozen immediately following the final exposure period. While Zhang et al. (2015a) reported exposing groups of 20 mice to each concentration evaluated and then combining 50 mg of lung tissue from subgroups of 4 mice to create five analytical samples for each concentration, this process was not clearly described in Zhang et al. (2015b), although they also reported five analytical samples per exposure group, and may have pooled tissue from multiple mice in a similar manner. The study authors presented tables of the biological sample measurements but did not provide any extensive analysis or qualitative discussion of the results, or perform any statistical analysis, in either report (Zhang et al., 2015b; Zhang et al., 2015a); thus, the EPA conducted its own statistical analyses. In mice exposed to ≤ 200 ppm for 4 weeks, lung levels of both GSH and GSSG decreased with increasing exposure concentrations, exhibiting a dose-response

[20] 0.025, 0.00125, 0.025, 0.00125, 0.025, 0.01, 2,342, and 2,500 ng/mL for 8-hydroxy-2′-deoxyguanosine (8-OHdG), α-methyl-γ-hydroxy-1,N2-propano-2′-deoxyguanosine (CrotondG), N2,3-etheno-2′-deoxyguanosine (N2,3-EthenodG), O^6-(2-hydroxyethyl)-2′-deoxyguanosine (O^6-HEdG), 1-(2-hydroxyethyl)-2′-deoxyadenosine (N1-HEdA), N^6-(2-hydroxyethyl)-2′-deoxyadenosine (N^6-HEdA), 2′-deoxyguanosine (dG), and 2′-deoxyadenosine (dA), respectively.

relationship consistent with a linear trend (see Table J-6), although by pairwise comparisons only concentrations ≥ 100 ppm induced statistically significant decrements in both endpoints [Table J-6; from Table 4 in Zhang et al. (2015a)]. The EPA notes that this observation is consistent with previous measurements of blood EtO-hemoglobin adducts in mice and rats indicating the potential for significant tissue glutathione depletion following exposures ≥ 100 ppm (see Section 3.3.2). While both GSH and GSSG decreased in the lungs of mice with increasing EtO exposure, the ratio of GSH:GSSG (a redox couple commonly evaluated as a measure of cellular oxidative stress) did not change. Furthermore, while below the limit of quantification in control samples, levels of the EtO-GSH alkylation product HESG increased in what also appeared to be a linear relationship with increasing exposure concentrations ≥ 10 ppm.

The EPA found it interesting that Zhang et al. (2015a) could not quantify HESG levels from the lungs of control mice, considering that N7-HEG DNA adducts resulting from endogenous EtO alkylation have been reported in various tissues including lungs from unexposed rats and mice [e.g., Wu et al. (1999a); Walker et al. (1992); see Section 3.3.3.4]. While presumably endogenous EtO would also form HESG adducts at some level in the mouse lung, these background levels must have been at least 10 times lower than the 14.3 µg/g average levels resulting from 10 ppm EtO exposure, given the stated LLOQ of 0.002 µg/mL (Zhang et al., 2015a). Levels of both nonoxidized glutathione (e.g., GSH + HESG) and total glutathione (GSH + GSSG + HESG) remained similar or may have increased marginally with treatment. The decrease in lung GSH and GSSG levels concomitant with increased HESG levels, together with the constant ratio of GSH:GSSG, following exposure to EtO concentrations from 10 to 200 ppm indicated to the authors that the lung GSH depletion resulted from EtO alkylation to HESG and not oxidation to GSSG. While the ratio of GSH:GSSG was unchanged, the ratio of free reduced:total other glutathione (i.e., GSH:[GSSG + HESG]) decreased dramatically from 13.1 in controls to 0.8 in the lungs of mice exposed to 200 ppm (see Table J-6), suggesting to the EPA that the capacity of the lungs to withstand oxidative stress induced by some other exogenous source may be severely compromised at higher EtO concentrations, consistent with previous reports evaluating EtO-hemoglobin adduction (see Section 3.2.2).

Table J-6. Evaluation of reported measurements of GSH, GSSG, and HESG; averages (SD) of pooled samples [µg/g tissue; Zhang et al. (2015a)][a]

Pooled exposure group (ppm)	GSH (from Table 4)	GSSG (from Table 4)	HESG (from Table 4)	GSH + HESG (from Table 4)	GSH/GSSG (from Table 4)	GSSG + HESG (calc)	GSH/[GSSG + HESG] (calc)	GSH + GSSG + HESG (calc)
0	702 (59.0)[b]	53.4 (4.78)[b]	<LOQ	702	13.2	53.4	13.1	755.4[c]
10	717 (29.5)	41.8 (3.16)**	14.3 (3.75)[b]	728	17.1	56.1	12.8	773.1
50	687 (38.1)	49.0 (6.23)	84.6 (23.8)**	746	14.0	133.6	5.1	820.6
100	590 (40.6)**	38.8 (6.15)***	206 (69.8)****	724	15.2	244.8	2.4	834.8*
200	336 (59)****	26.6 (4.71)****	407 (18.5)****	733	12.6	433.6	0.8	769.6

[a]Averages (standard deviation: SD) of five pooled samples, with tissue from groups of four male mice pooled together to create the five analytical samples per exposure group, except for the 200-ppm group, which had only four pooled samples. Authors presented no statistical analysis of results. Average (SD) data reported in Table 4 of Zhang et al. (2015a) were evaluated within each column (i.e., GSH, GSSH, and HESG independently) by the EPA using one-way ANOVA. Other values were calculated (calc) from data provided. Means significantly differed amongst the treatment groups for each endpoint ($p < 0.0001$), and significant pairwise changes compared with the 0-ppm group by Dunnett's multiple comparison posttest are indicated by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and **** ($p < 0.0001$).
[b]Linear trend between means and row number, $p < 0.0001$, using test for linear contrast (posttest for trend) in Graphpad (http://www.graphpad.com/guides/prism/6/statistics/index.htm?stat_posttesttrend.htm).
[c]Marginal difference among means ($p = 0.05$ by one-way ANOVA).

In the lungs of male B6C3F$_1$ mice exposed to 0, 100, or 200 ppm EtO via inhalation for 12 weeks, CrotondG and 8-OHdG were detected in all samples, and O^6-HEdG in all but 2/5 samples from the control group, while N1-HEdA and N^6-HEdA were quantifiable only in the samples from exposed mice, and N2,3-ethenodG was not detected in any sample (Zhang et al., 2015b). The only other analysis of rodent tissue O^6-HEdG formation resulting from EtO exposures only evaluated F344 rats exposed to 300 ppm for 4 weeks and concurrent controls, reporting measurable levels of O6-HEdG adducts in lung, brain, spleen, and kidney in the exposed rats, but not in the control animals (Walker et al., 1992). The more recent observations of O^6-HEdG adduct formation in mouse lungs are not inconsistent with that study, as Walker et al. (1992) used a less sensitive measurement technique, relying on fluorescence detection of analytes following HPLC separation, and did not evaluate O^6-HEdG levels in tissues from

EtO-exposed rats at any lower concentrations (see Section 3.3.3.4). From the Zhang et al. (2015b) mouse lung adduct data, the EPA's statistical analyses indicated that levels of O6-HEdG adducts increased in an apparently linear manner, with statistically significant increases observed in the 200-ppm samples by pair-wise comparisons (see Table J-7). Levels of both adenine adducts (i.e., N1-HEdA and N^6-HEdA) were unquantifiable in the control samples but were present in samples from mouse lungs in both exposure groups, with significant increases observed in samples from 200 versus 100 ppm. The EPA notes that along with the potentially mutagenic O^6-HEdG adduct, both N1-HEdA and N^6-HEdA could also be promutagenic, as adducts in these positions may interfere with base-pairing interactions. 8-OHdG can be formed by direct reaction of reactive oxygen species such as superoxide or hydroxyl radicals, but levels of this adduct were unaffected by EtO exposures $\leq$200 ppm (see Table J-7). While Zhang et al. (2015b) did not remark on changes in CrotondG adducts, which can form following lipid peroxidation, CrotondG levels appeared to have increased with exposure, and the levels in lung samples following 200-ppm exposures were significantly increased compared with control samples, although to a lesser extent than O6-HEdG or either adenine adduct.

Table J-7. Evaluation of reported measurements of various DNA adducts; averages (SD) [*n* = 5 analytical samples; Zhang et al. (2015b)][a]

Group (ppm)	O^6-HEdG (A/dG)[b]	8-OHdG (A/dG)[b]	CrotondG (A/dG)[b]	N1-HEdA (A/dG)[b]	N^6-HEdA (A/dG)[b]
0	0.229 (0.167)[c,d]	42.0 (7.78)	0.120 (0.0302)[d]	< LOQ	< LOQ
100	0.467 (0.365)	39.3 (8.47)	0.147 (0.0324)	1.96 (0.152)	1.39 (0.0897)
200	0.743 (0.118)*	46.0 (3.97)	0.190 (0.0165)**	6.97 (1.79)**	4.27 (0.540)***

[a]Authors presented no statistical analysis of results. Average (standard deviation: SD) data reported in Table 4 of Zhang et al. (2015b) were evaluated within each column by the EPA using one-way ANOVA for 0^6-HEdG, 8-OHdG, and CrotondG, and student's t-test with Welch's correction for N1-HEdA and N^6-HEdA. Significant changes compared with the 0-ppm group by Dunnett's multiple comparison posttest, or between the 100- and 200-ppm groups for N1-HEdA and N^6-HEdA by unpaired student's t-test with Welch's correction for unequal variance, are indicated by *($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).
[b]Adducts × 10^6/dG levels = A/dG.
[c]Levels were < LOQ in 2/5 samples; for purpose of average (SD) calculations, samples < LOQ were considered to be equal to the LOQ for the O^6-HEdG (0.00125 ng/mL), and A/dG were calculated using this LOQ value × 10^6/dG concentration presented in Table 4 for each sample.
[d]Means significantly differed amongst the treatment groups for each endpoint by 1-way ANOVA ($p < 0.05$), and a linear trend was present between means and row number, $p < 0.01$, using test for linear contrast (posttest for trend) in Graphpad (http://www.graphpad.com/guides/prism/6/statistics/index.htm?stat_posttesttrend.htm).

While neither reactive oxygen species nor oxidized lipids (e.g., malondialdehyde or thiobarbituric acid reactive substances [tBARs] levels), were measured directly in either study, the lack of decrease in the ratio of GSH:GSSG (Zhang et al., 2015a) or increase in 8-OHdG levels (Zhang et al., 2015b), both routinely evaluated as markers of cellular oxidative stress, coupled with the limited increase in CrotondG adducts and the inability to detect N2,3-ethenodG, both formed following lipid peroxidation, suggest to the EPA that oxidative stress is not induced in the lungs of mice following 4–12 weeks of exposure to ≤ 200 ppm EtO (Zhang et al., 2015b; Zhang et al., 2015a). In addition, the EPA finds that the Zhang et al. (2015b) study supports the identification of O^6-HEdG as a direct product of EtO reactivity, consistent with previous in vitro and in vivo reports (see Section 3.3.3.1), and adds coherence to the available database by observing an exposure-related increase in lung O^6-HEdG 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. Furthermore, the significant increases in other potentially mutagenic purine adducts (i.e., CrotondG, N1-HEdA, and N^6-HEdA) is consistent with the novel mutational spectra preferentially affecting purine nucleotides reported in lung and other tumors from EtO-exposed male and female B6C3F_1 mice [e.g., NTP (1987); Hong et al. (2007); Houle et al. (2006); see Section 3.3.3.4] and the conclusion that EtO-induced rodent tumors likely arise via a mutagenic mode of action following the direct formation of mutagenic EtO-DNA adducts.

APPENDIX K. SUMMARY OF PUBLIC COMMENTS RECEIVED ON THE JULY 2013 PUBLIC COMMENT DRAFT AND EPA RESPONSES

The EPA's Science Advisory Board (SAB) reviewed an external review draft of the ethylene oxide (EtO) carcinogenicity assessment in 2007 (see Appendix H). Following that review, a revised draft was developed and released on July 23, 2013 for a 45-day public comment period. In response to requests from the American Chemistry Council's (ACC's) Ethylene Oxide Panel, the Ethylene Oxide Sterilization Association (EOSA), and Balchem Corporation, the public comment period was extended from September 5 to October 11, 2013.

During the public comment period, 16 sets of comments were received, not including the three requests to extend the public comment period. The major substantive science comments came from four groups. The first of these, the Breast Cancer Fund (docket #0043), expressed agreement with the EPA's hazard and mode of action (MOA) conclusions. The comments from the remaining three groups [American Chemistry Council's Center for Advancing Risk Assessment Science and Policy (ARASP) (#0055), EOSA (#0056), and ACC (#0057)] largely overlapped. A summary of the substantive science comments from these latter three groups and the EPA's responses is provided below. The comments have been synthesized and paraphrased and are organized roughly to follow the order of the carcinogenicity assessment. The complete set of public comments is available in Docket ID No. EPA-HQ-ORD-2006-0756-0035 at http://www.regulations.gov.

The July 2013 draft was further revised in response to the public comments and submitted for additional SAB review in August 2014. Comments on the 2014 SAB review draft and the EPA's responses are presented in Appendix I.

1. **COMMENT:** EPA failed to comply with multiple guidelines, including Information Quality Act guidelines and 2011 National Academy of Sciences [NRC] recommendations. Specifically, EPA failed to apply a transparent and systematic weight-of-evidence approach in both qualitatively and quantitatively assessing the cancer risks, did not base the assessment on the best available science, and used National Institute for Occupational Safety and Health (NIOSH) breast cancer incidence data that are not available to the public. (ACC, EOSA)

   EPA RESPONSE: The EPA has complied with applicable guidelines. The EtO assessment was largely developed before the IRIS program started implementing the 2011 NRC recommendations and formalizing approaches to conducting and documenting systematic review. Although not presented in the formalized manner IRIS has been developing, the EtO assessment provides a valid and transparent weight-of-evidence analysis based on

the best available science. Considerations used in assessing the epidemiological studies are summarized at the beginning of Section 3.1, and the considerations used in the weight-of-evidence analysis for carcinogenic hazard are detailed in Section 3.5.1, culminating in a synopsis of how the evidence fits the lines of evidence for the characterization of "carcinogenic to humans" laid out in the EPA's 2005 *Guidelines for Carcinogen Risk Assessment*. Considerations used in selecting the epidemiology study(ies) for quantitative risk estimation are summarized in Section 4.1, along with considerations used in selection of exposure-response models. A systematic literature search was conducted from January 2006. Major new studies identified in the literature search as well as even more recent studies noted by the ACC in its public comments have been added to Appendix J. The charge to the SAB includes questions addressing adequacy, transparency, and clarity of the assessment and completeness of the appendix on new studies. With respect to the breast cancer incidence data, the EPA's Information Quality Act guidelines do not require that all underlying raw epidemiology data be publicly available; they allow for confidentiality constraints.

2. **COMMENT:** Data quality evaluation should clearly describe the criteria used to deem a study as high quality. (ARASP)

   EPA RESPONSE: The EtO assessment discusses general characteristics used to evaluate epidemiology studies and notes numerous characteristics that supported the determination that the NIOSH study was a "high-quality" study, for example, high-quality exposure estimates (as discussed in Section A.2.8 of Appendix A), large size, adequate follow-up, inclusion of males and females, absence of other occupational exposures, and use of internal comparisons. The assessment has been revised to summarize these characteristics clearly in one location (see Footnote 13 in Section 3.5.1).

3. **COMMENT:** Lymphohematopoietic and lymphoid cancers should not be grouped because they are derived from different cells of origin. (ARASP, ACC, EOSA)

   EPA RESPONSE: The EPA did appropriately combine lymphoid cancers, as the "lymphoid" cancer category is a grouping of cancers with a common lymphohematopoietic cell lineage (multiple myeloma and most lymphocytic leukemias and non-Hodgkin lymphomas develop from B-lymphocytes). The 2007 SAB panel supported the use of this grouping. The larger lymphohematopoietic cancer grouping is provided solely for comparison because many of the epidemiologic studies do not present data for a lymphoid cancer grouping.

4. **COMMENT:** The evidence for breast cancer is too weak. (ACC, EOSA)

EPA RESPONSE: Although the epidemiological database for breast cancer is more limited (i.e., few studies with sufficient numbers of female breast cancer cases) than that for lymphohematopoietic cancers, the EPA determined that the available evidence is sufficient to consider breast cancer a potential hazard from EtO exposure. In addition, the epidemiological database is strengthened by the follow-up study (Mikoczy et al., 2011) of the Swedish cohort of sterilizer workers first reported on by Hagmar et al. (Hagmar et al., 1995; Hagmar et al., 1991) (see Section J.2.2 of Appendix J), and the epidemiological evidence is supported by the finding of mammary gland carcinomas in female mice exposed to EtO by inhalation (NTP, 1987) and by mechanistic data (see Section 3.4.1.3). The 2007 SAB panel did not object to the derivation of unit risk estimates based on the available breast cancer evidence.

5. **COMMENT:** EtO is a weak mutagen. (ACC, EOSA)

EPA RESPONSE: The EPA agrees that EtO is a relatively weak mutagen compared to the anticancer agents and the other reactive epoxides investigated in the Vogel and Nivard (1998) paper. Vogel and Nivard (1998) compared 37 anticancer agents, which are generally highly mutagenic by design, and four epoxides, including EtO, one of which was a cross-linking diepoxide.

The EPA notes, however, that there is generally no strong correlation between potency in short-term mutagenicity and genotoxicity tests and carcinogenic potency. For example, for the Ames assay, Fetterman et al. (1997) found a "very weak" relationship between quantitative mutagenic and carcinogenic potencies. In addition, EtO is highly volatile and concentrations can become much reduced over the course of an in vitro assay, making potency from such assays difficult to determine.

6. **COMMENT:** A mutagenic MoA is not supported by the most recent scientific evidence; other MoAs, specifically oxidative stress and cell proliferation, should be considered. (ACC)

EPA RESPONSE: The 2007 SAB panel concurred with the EPA's conclusion at that time that a mutagenic MOA was operative in the carcinogenicity of EtO. In its 2013 public review draft, the EPA presented more recent information and found this information to be supportive of the earlier conclusion of a mutagenic MOA. New information presented by the ACC is not sufficient to alter that conclusion. Other MOAs proposed by the ACC are speculative.

As evidence against a direct mutagenic MOA, the ACC cites a paper by Parsons et al. (2013). This study and its limitations are discussed in detail in Section J.3.2 of Appendix J. In brief, Parsons et al. (2013) investigated only one type of mutation (base substitution mutations) in one codon (12) of one gene (K-*ras*) in one tissue (mouse lung) for exposure durations up to 12 weeks. Given that carcinogenesis is a multifaceted

process, involving numerous genes, and that EtO can induce a variety of different types of mutation and other genotoxic effects, one cannot infer too much about the MOA for EtO-induced mouse lung carcinogenesis from this one study. In addition, the high degree of variability in the mutant fraction results for most of the dose groups and the instability of the control results across different exposure durations suggest that the assay results might be unreliable. To attempt to explain the irregular time- and dose-response patterns that they observed (e.g., statistically significant increases in specific K-*ras* mutant cells at 4 weeks but statistically significant decreases at 8 and 12 weeks compared to controls), Parsons et al. (2013) hypothesized that EtO causes a low level of oxidative stress that modifies Ras signaling, resulting first in the amplification and then in the death of K-*ras* mutant cells (the erratic control results are still unexplained). That hypothesized explanation for the irregular results, however, does not constitute a complete MOA for the EtO-induced lung carcinogenicity observed in the NTP mouse cancer bioassay and does not explain the observations by Hong et al. (2007) of later-occurring K-*ras* codon 12 mutations in all lung tumors evaluated from EtO-exposed mice in the NTP 2-year cancer bioassay. A more straightforward explanation for the highly variable dose group results, the erratic control group results, and the irregular time- and dose-response patterns is measurement error associated with the assay.

Thus far, there is no independent support for the hypotheses of Parsons et al. (2013). In fact, the proposed hypotheses are at odds with a Nagy et al. (2013) study of human cells in vitro. Using the sensitive comet assay, Nagy et al. (2013) found that lung epithelial cells are relatively susceptible to the DNA alkylating effects of EtO and relatively resistant to oxidative DNA damage (induced by hydrogen peroxide) compared to peripheral blood lymphocytes and keratinocytes. In addition, Nagy et al. (2013) found no evidence that EtO induced oxidative DNA damage in the examined cells at the applied concentrations.

Furthermore, as Parsons et al. (2013) and the ACC acknowledged, the results Parsons et al. (2013) presented do not preclude direct genotoxic effects of EtO. Direct effects of EtO could include K-*ras* mutations as well as genotoxic effects elsewhere in the DNA.

Moreover, any inferences about K-*ras* mutations that one can draw from the Parsons et al. (2013) study are not necessarily generalizable to other cancer types. Codon 12 of the K-*ras* gene was selected for investigation because Hong et al. (2007) had observed mutations in this K-*ras* codon in all 23 lung tumors they evaluated from EtO-exposed mice in the NTP 2-year cancer bioassay. However, Hong et al. (2007) observed other patterns of K-*ras* mutations, involving other codons, in other tumors (Harderian gland and uterine tumors) from the NTP mouse bioassay.

In support of an oxidative stress MOA, the ACC also cites work by Marsden et al. (2009). Marsden et al. (2009) used sensitive detection techniques and an approach designed to quantify endogenous N7-HEG adducts and exogenous N7-HEG adducts

separately to measure the amounts of endogenous and exogenous N7-HEG adducts occurring in rat liver, spleen, and stomach following EtO treatment (see also Section 3.3.3.1 and Appendix C). In addition to direct DNA adduct formation via alkylation observed in the liver, spleen, and stomach, Marsden et al. (2009) observed an indirect effect of EtO exposure on endogenous N7-HEG adduct formation in the liver and spleen and hypothesized that EtO also could cause adduct formation indirectly by inducing oxidative stress, which might in turn induce the endogenous formation of ethylene, which can be metabolized to EtO.

As discussed in the EtO assessment (see Section 3.3.3.1 and Appendix C), although not statistically significant, increases in exogenous adducts were observed at the lowest dose in liver and spleen, and Marsden et al. (2009) noted that the exogenous adduct data are consistent with a linear dose-response relationship ($p < 0.05$) in all three tissues examined. In addition, more substantial relative increases in exogenous adducts appear to be occurring at lower doses than for endogenous adducts [see Table 1 of Marsden et al. (2009)]. Thus, even if the speculative oxidative stress MOA is also operative in liver and spleen at higher doses, it does not rule out direct genotoxic effects of EtO. Moreover, liver and spleen (the parenchymal tissue) are not known target organs for EtO-induced carcinogenicity and the results do not seem to be generalizable to other tissues, as there was no evidence of increased endogenous adducts in the stomach, where there were clear, statistically significant increases in exogenous adducts for all but the lowest dose.

Regarding cell proliferation, the ACC offers no solid evidence that such an effect is induced by EtO exposure. The ACC acknowledges that no generalized mitogenesis occurred in the lung in the Parsons et al. (2013) study. Nor was cytotoxicity or apoptosis detectable (Parsons et al., 2013). Similarly, in the Nagy et al. (2013) study mentioned above, all observed genotoxic effects occurred at subcytotoxic doses. Cytotoxicity also has not been an issue in other toxicity and genotoxicity studies of EtO; thus, regenerative proliferation resulting from EtO-induced cytotoxicity is not credible as a key component of a MOA for EtO-induced carcinogenesis.

The ACC suggests that the observation of early increases in the GAT K-*ras* codon 12 mutation in the Parsons et al. (2013) study supports a mitogenesis MOA because the GAT mutation is the most common K-*ras* mutation observed in spontaneous mouse lung tumors. G:C→A:T mutations do not just occur spontaneously, however; they can be induced by a variety of agents, including EtO (see Section J.3.2). Furthermore, as discussed above and in Section J.3.2, there is considerable uncertainty pertaining to the Parsons et al. (2013) results, and to explain some of the irregular time- and dose-response patterns observed, Parsons et al. (2013) proposed first amplification *and then death* of K-*ras* mutant cells, so how the Parsons et al. (2013) findings support mitogenesis as a MoA for EtO-induced carcinogenesis is unclear. The ACC also proffers the claim by Parsons et al. (2013) that no single type of DNA adduct correlates with the K-*ras* codon

12 mutations observed as evidence of a mitogenesis MOA; however, EtO induces multiple types of DNA adducts and Parsons et al. (2013) themselves acknowledged that it could "be postulated that a combination of different types of DNA damage could lead to the profile of induced K-*ras* mutation..."

7. **COMMENT:** EPA failed to incorporate the Union Carbide Corporation (UCC) data into the dose-response assessment. The NIOSH exposure assessment also suffered from limitations. (ACC, EOSA)

EPA RESPONSE: As recommended by the 2007 SAB panel, the EPA considered using the UCC data and determined that they were not of sufficient quality to add useful information to the NIOSH study's data for the derivation of unit risk estimates (see the reasons discussed in detail in the assessment [e.g., Section A.2.20 of Appendix A] and in the responses to the SAB comments [p. H-6 to H-8]). Thus, the EPA decided to use the NIOSH data as the basis for the exposure-response modeling (see also Section 4.1).

Although no exposure assessment is without limitations, the NIOSH regression model includes a number of relevant variables and had a high validity when tested against independent data (see Section A.2.8 for details). The approach used to derive the UCC exposure estimates was much less rigorous and there is considerable uncertainty in the resulting estimates. The 2007 SAB panel supported the use of the NIOSH study as a basis for risk estimates.

8. **COMMENT:** Despite SAB recommendations, EPA used summary data rather than the individual data in the modeling of breast cancer mortality and lymphoid cancer. (ACC, EOSA)

EPA RESPONSE: As documented in the assessment and in the responses to SAB comments (p. H-12 and H-13), the EPA investigated multiple models based on the individual continuous exposure data, including a log-linear model. For the breast cancer incidence data, the EPA was able to develop several continuous models that provided reasonable fits to the individual-level exposure data across the entire range of the data (see Section 4.1.2.3), consistent with the SAB recommendations.

For lymphoid cancer, however, despite the extensive modeling efforts, the various alternative continuous models investigated—including the two-piece spline models—proved problematic, as explained in detail in the text (see Section 4.1.1.2). In particular, the statistically significant models predicted extremely steep slopes in the low-dose region. Thus, the EPA has retained the approach of using a linear regression of the categorical data, excluding the highest exposure group, as the basis for the preferred unit risk estimates for lymphoid cancer. The EPA notes that modeling of grouped data is also an important and well-recognized statistical methodology and its use is consistent with EPA guidance, policy, and past practice. The breast cancer mortality data were similarly

difficult to model due to extreme supralinearity, and the optimal two-piece spline model yielded an unrealistically steep low-dose slope estimate; thus, the EPA again used a linear regression of the categorical data, excluding the highest exposure group, as the basis for the preferred estimate (see Section 4.1.2.2). (The breast cancer mortality data are not critical to the assessment because the breast cancer incidence data set is preferred.)

Since the July 2013 public comment draft, however, unit risk estimates for lymphoid cancer and breast cancer mortality from the most suitable alternative models based on the continuous-exposure data were developed and added to the assessment for comparison purposes.

9. **COMMENT:** EPA used a non-peer-reviewed supralinear spline model. (ACC, EOSA)

EPA RESPONSE: The spline model the EPA used for the breast cancer incidence data was the best fitting of the continuous models considered, and others have used this model with similar data sets to estimate risk. The breast cancer modeling work was published in a peer-reviewed journal (Steenland et al., 2011), and the EtO spline model will receive further SAB review. Moreover, the two-piece spline model used is not inherently supralinear; it is a flexible model that can accommodate sublinear or supralinear (or linear) exposure-response relationships. The EtO two-piece spline models become supralinear models because the underlying exposure-response relationships of the data to which they are being fitted are supralinear.

10. **COMMENT:** There are a number of modeling issues in addition to those mentioned in other comments, specifically flaws discussed in Valdez-Flores and Sielken (2013) and Valdez-Flores et al. (2010) and over-predictions of the cancer deaths in the NIOSH study. (ACC, EOSA)

EPA RESPONSE: The EPA did not find that Valdez-Flores and Sielken (2013) or Valdez-Flores et al. (2010) provided convincing evidence of flaws in the modeling. The EPA addressed the issues presented by Valdez-Flores et al. (2010) in the July 2013 assessment (see Section A.2.20 of Appendix A). Discussion of the new Valdez-Flores and Sielken (2013) study has been added to Appendix J (see Section J.3.1). In light of issues raised by Valdez-Flores and Sielken (2013), text was added to the assessment clarifying the model comparisons in some of the figures of Chapter 4.

How the predicted numbers of deaths for the cohort study are being calculated is unclear from the submitted comments; thus, the specific claims could not be evaluated. The EPA notes, however, that the ACC is no longer claiming that the observed number of cancer mortalities is overpredicted "by more than 60-fold." In Appendix I of the ACC comments, the claim is made that the lymphoid cancer mortality is overpredicted by "1.87- to 3.26-fold" and breast cancer mortality is overpredicted by "1.24- to 1.84-fold."

These estimates are based on the upper confidence limits on the models, however; a more suitable basis for comparison with the observed deaths is the maximum likelihood estimates (MLEs) of the models. According to Figure E.1 in the ACC's Appendix I, the best estimate from the MLE of the model for lymphoid cancer mortality is only about a 1.6-fold difference, and Figure A.1 suggests less than a 1.3-fold difference for breast cancer mortality.

11. **COMMENT:** EPA should present both linear and nonlinear extrapolation approaches. (ARASP, ACC, EOSA)

EPA RESPONSE: The EPA notes that some members of the 2007 SAB panel recommended that the EPA include a nonlinear approach; this view was not a consensus position—some panel members thought that such an approach should be included, but others thought a nonlinear approach was not warranted. The EPA considered available information and opinions presented by SAB members and concluded that there was insufficient evidence for a nonlinear approach. This conclusion and its basis are discussed in detail in the responses to SAB comments in Appendix H of the draft assessment (p. H-13 to H-18). Part of the charge for the second SAB review will be to consider the EPA's responses to the comments of the first SAB panel, including the EPA's judgment not to include a nonlinear approach. New information presented by the ACC is not sufficient to alter the determination not to include a nonlinear approach (see the EPA's response to Comment 6 above).

12. **COMMENT:** Combining breast cancer and lymphoid cancer unit risk estimates is not justified, and EPA did not discuss competing risks, different background populations, incidence vs. mortality, and the use of different exposure-response models. (ACC, EOSA)

EPA RESPONSE: When combining cancer types in a dose-response model, it is desirable that the cancer types have a common origin. In contrast, when combining *unit risk estimates* (for cancer types that have been modeled separately) to derive a total cancer unit risk estimate, it is desirable that the cancer types be independent. Thus, in the EtO assessment, breast cancer and lymphoid cancers were modeled separately, and then the unit risk estimates were combined to develop a total cancer unit risk estimate. It is standard practice in IRIS assessments to estimate total cancer risk and not just the risk from individual cancer types, and this practice is consistent with EPA guidelines (U.S. EPA, 2005a) and National Research Council recommendations (NRC, 1994).

In terms of extra risks (above background) from environmental exposure levels of EtO, the likelihood of co-occurrence of EtO-induced breast and lymphoid cancers is negligible. In addition, considering the risks from both cancer types occurring in a single

individual is not "double-counting" if the cancer types are independent with respect to EtO exposure.

The total cancer unit risk estimate is intended to apply to the general population, of which females comprise a substantial portion. For a risk estimate for males only, the unit risk estimate for lymphoid cancer alone is presented in the assessment also. The issue of different background populations (male and female) is now addressed in the assessment.

The unit risk estimates that are being combined are for cancer incidence, so no inconsistency exists with respect to cancer status. Similarly, the unit risk estimates that are being combined are linear slopes, so no inconsistency exists with respect to the model form being combined, either (the exposure-response models used to derive the unit risk estimates are irrelevant to the combing of the unit risk estimates).

13. **COMMENT:** EPA should reexamine its risk determination given background and endogenous levels of EtO; EPA's risk estimates are unrealistically high. (ARASP, ACC, EOSA)

EPA RESPONSE: The unit risk estimates the EPA developed are for extra risk (i.e., above background); background and endogenous levels of EtO, which would be relevant to (the true) background risk, are not integral to the development of the estimates of extra risk. As discussed in the assessment (see Section 4.5), given the high background rates of lymphoid and breast cancers (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%), EPA does not consider the risk estimates for exogenous exposure to be inconsistent with the data on background and endogenous levels.

According to EPA's 2005 National Air Toxics Assessment data, the average exposure concentration of EtO from all sources (including background) in the United States is 0.0062 $\mu g/m^3$; the average background concentration is 0.0044 $\mu g/m^3$. Using the EPA's draft unit risk estimates, adjusted for assumed increased early-life susceptibility, upper-bound estimates of the cancer risk resulting from a lifetime exposure to the average concentration from all sources are roughly 1 lymphoid cancer case for every 220,000 people and 1 breast cancer case for every 120,000 women; the upper-bound estimates resulting from a lifetime exposure to the average concentration above background (i.e., from known sources) (0.0018 $\mu g/m^3$) are roughly 1 lymphoid cancer case for every 770,000 people and 1 breast cancer case for every 410,000 women. The calculations the ACC provided were for an unrealistic exposure concentration of 1 ppb (1.8 $\mu g/m^3$).

14. **COMMENT:** EPA should not derive occupational exposure limits for EtO. (ACC, EOSA)

EPA RESPONSE: The EPA does not set "occupational exposure limits" for EtO; however, the EPA's Office of Pesticide Programs (OPP) has a regulatory interest in occupational exposures resulting from sterilization uses of EtO, as the EPA has the legal authority to consider occupational risks in pesticide labeling and registration decisions under FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act). Typically, OPP uses the IRIS unit risk estimates for its risk assessments of occupational exposures, which is valid when the exposure-response model is reasonably linear over the relevant range of exposures. With the models used for the EtO cancer data, however, the unit risk estimate is not appropriate in the full range of the occupational exposure scenarios of interest to OPP. Thus, the assessment provides sample risk estimates for exposure scenarios of interest to OPP for its risk assessment of sterilization uses of EtO. These estimates are not "occupational exposure limits," and OPP will conduct its own risk assessment based on current exposure estimates. OSHA and NIOSH had the opportunity to review an earlier draft EtO assessment during the interagency review phase of the IRIS assessment process.

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