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Evaluation of the Inhalation Carcinogenicity of Ethylene Oxide

APPENDICES

(CASRN 75-21-8)

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

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LIST OF ABBREVIATIONS

ADAF	age-dependent adjustment factor
AIC	Akaike information criterion
AIDS	acquired immune deficiency syndrome
AML	acute myeloid leukemia
AUC	areas under the curve
BEIR	Committee on the Biological Effects of Ionizing Radiation
CI	confidence interval
DSB	double-strand breaks
EC	effective concentration
EOIC	Ethylene Oxide Industry Council
EPA	U.S. Environmental Protection Agency
EtO	ethylene oxide
FRG	Federal Republic of Germany
GST	glutathione S-transferase
HAP	hazardous air pollutants
N7-HEG	N7-(2-hydroxyethyl)guanine
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IRIS	Integrated Risk Information System
LEC	lower confidence limit
MLE	maximum likelihood estimate
NCEA	National Center for Environmental Assessment
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
O^6-HEG	O^6-hydroxyethylguanine
OBS	observed number
OR	odds ratios
PBPK	physiologically based pharmacokinetic
POD	point of departure
RR	relative rate, i.e., rate ratio
SCE	sister chromatid exchanges
SE	standard error
SEER	Surveillance, Epidemiology, and End Results
SIR	standardized incidence ratio
SMR	standard mortality ratios
TWA	time-weighted average
UCC	Union Carbide Corporation
UCL	upper confidence limit
WHO	World Health Organization

APPENDIX A.
CRITICAL REVIEW OF EPIDEMIOLOGIC EVIDENCE

[EDITORIAL NOTE: Please note that in this assessment document the responses to the 2007 external peer review and public comments can be found in Appendix H. Responses to the 2013 public comments are in Appendix L.]

A.1. BACKGROUND

On the basis of studies indicating that EtO was a strong mutagen and that exposure to EtO produced increased chromosomal aberrations in human lymphocytes (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 completed (Gardner et al., 1989) on a cohort of workers from four companies and eight hospitals in Great Britain, and it was followed by a third independent study on a cohort of exposed workers in eight chemical plants from the Federal Republic of Germany (Kiesselbach et al., 1990). A follow-up study of the Gardner et al. (1989) cohort was recently conducted by Coggon et al. (2004).

Greenberg et al. (1990) was the first in a series of studies of workers exposed to EtO at two chemical manufacturing facilities in the Kanawha Valley (South Charleston, WV). The workers at these two facilities were studied later by Teta et al. (1993), 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 recently completed a follow-up of the mortality study (Steenland et al., 2004) and a breast cancer incidence study based in the same cohort (Steenland et al., 2003). The results of the Steenland et al. (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.

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.

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 excesses that are significant are due mainly to an increased risk of stomach cancer at one plant and an excess of blood and lymphatic malignancies at all three. Seven deaths from leukemia occurred, whereas only 0.8 were expected (standard mortality ratio [SMR] = 9.2). Ten deaths due to stomach cancer occurred versus only

1.8 expected (SMR = 5.46). The results tend to agree with those from clastogenic and short-term tests on EtO (Ehrenberg and Gustafsson, 1970). The authors believe 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 even at low-level and intermittent exposures to EtO should be "seriously considered by industry and regulating authorities."

The average air EtO concentrations in the three plants were as follows: In Plant 1 (Hogstedt et al., 1979a) in 1977, levels 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 and 1,500 ppm inside.

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, 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 allude to the fact that there was exposure to benzene, 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 gender 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 believed 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 were 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 between 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 in use 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, and it 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 experimental animal evidence demonstrating excess risks of hematopoietic cancer in animals exposed to EtO. But the authors note that since 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 other than benzene in these chemical workers cannot entirely be 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. (1981) 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 for the production of other chemicals. SMRs were calculated in comparison with the general U.S. population and the regional population. Results based on regional population death rates were found to be similar to those based on the U.S. general population. Follow-up began either on January 1, 1940, if exposure to EtO began sooner, or on the date when exposure began, if it occurred after January 1, 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 was comprised 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, e.g., 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 prior to 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 prior to 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 (55% female) 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 of the assumption 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 experimental 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. 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.

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

The Cox analysis produced a significantly positive trend with respect to lymphoid cell tumors (combination of lymphocytic leukemia and NHL) when EtO exposures were lagged 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, it 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.

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. This resulted 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, that is, 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, such as in Stayner et al. (1993). 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 gender. 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, OBS = 5). Even in the 10-years or more latency period, the risk was less than expected (SIR = 0.36, OBS = 2).

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 similar studies.

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% 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. The authors stated that one heavily exposed case was chiefly responsible for the high cumulative total for all the benzene-exposed cases; however, it was not statistically significant. Only a few studies have suggested that exposure to benzene could possibly 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 that there

were many different possible exposures to the chemicals produced in the workplaces of these employees, it is not likely that 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 data to support the latter observation by the authors were not presented in tabular form.

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 their cohort has not experienced a significant increased risk for pancreatic cancer and lymphopoietic and

hematopoietic cancer in ethylene chlorohydrin and propylene chlorohydrin workers. 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 their 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.

Another major weakness of this study is the lack of any actual airborne measurements of EtO and the chlorohydrin chemicals.

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). 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.)

[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; rather, lagged exposure was treated as an alternate exposure metric.

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.

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

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

Source: Steenland et al. (2004).

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

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

Source: Steenland et al. (2004).

The hematopoietic cancer trends were somewhat weaker in this analysis than were those reported in the earlier studies of the same cohort. This is not unexpected because most of the cohort was not exposed after the mid-1980s, and the workers who were exposed in more recent years were exposed to much lower levels because EtO levels decreased substantially in the early 1980s. No association was found in females, although average exposures were only twice as high in males (37.8 ppm-years) as in females (18.2 ppm-years), and there was enough variability

in female exposure estimates to expect to be able to see a similar trend if it existed. In later analyses conducted by Dr. Steenland and presented in Appendix D, the difference between the 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 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]

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 in the neighborhood of 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. Since there were no female workers in the chemical

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

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)

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 (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. Year of hire was reportedly included to adjust for potential cohort effects; however, it is unclear whether or not this covariate was a statistically significant factor in the regression. Furthermore, because age at hire is often correlated with exposure, 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, where there is 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. 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, especially for the highest exposures,

(see above) 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, it should also be noted that some of these UCC cases are occurring 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

[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).

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 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 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 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 EPA rejected as not providing a good representation of the low-exposure data (EPA's 2006 draft risk estimate is based on a linear model). In addition, Valdez-Flores et al. (2010) used maximum likelihood estimates, while EPA uses upper bounds on risk (or lower bounds on exposure). Valdez-Flores et al. (2010) also modeled down to 10^{-6} risk, whereas 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 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

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

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 EPA uses a cutoff of 85 years.

A further reason for differences between the risk estimates of Valdez-Flores et al. (2010) and EPA's 2006 draft result is that Valdez-Flores et al. (2010) estimated mortality risks, while EPA estimates incidence risks. In a separate publication, Sielken and Valdez-Flores (2009) disagree with the assumption of similar exposure-response relationships for lymphohematopoietic cancer incidence and mortality used by EPA in deriving incidence estimates and assert that the methods used by EPA in calculating these estimates were inappropriate. Sielken and Valdez-Flores (2009) 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 (2009) 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 EPA in calculating the incidence estimates, EPA used adjustments to the life-table analysis where warranted (U.S. EPA, 2006a). 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, 2009). On the other hand, for the breast cancer incidence analyses, where incidence rates (and the differentials between incidence and mortality rates) are higher, 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 (2009) about using life-table analyses to derive incidence estimates do not apply to EPA's calculations.

Finally, the risk estimates of Valdez-Flores et al. (2010) and EPA's 2006 draft also differ because Valdez-Flores et al. (2010), based on analyses in a separate publication by Sielken and Valdez Flores (2009), 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 (2009). 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 (2009). 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 (2009). One might conceivably incorporate the ADAFs into the lifetable 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 lifetable analysis each time.

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. These are chiefly cancers of the lymphohematopoietic system and include leukemia, lymphosarcoma, reticulosarcoma, and NHL. More recently, an association with breast cancer has also been suggested. However, the overall epidemiological evidence is not conclusive because of inadequacies and limitations in the epidemiological database. The main effects and limitations in the epidemiological studies of EtO are presented in Table A-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 in sterilizer room Plant 2: mean = 14 ppm in early years, less than 6 ppm later Plant 3: less than 8 ppm in early years, less than 2 ppm later	33 cancer deaths vs. 20 expected 7 leukemia deaths vs. 0.8 expected (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	No personal exposure information from which to estimate dose No latency analysis Mixed exposure to other chemicals
Sterilizing workers in 8 hospitals and users in 4 companies, Great Britain Gardner et al. (1989)	2,876 (1,864 men, 1,012 women)	In early years, odor threshold of 700 ppm noted; in later years, 5 ppm or less was noted	3 leukemia deaths vs. 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	Ibid.	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	Ibid.	Ibid. and, in addition, 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 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. (1981).	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, and, in addition, 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 two cases 5 breast cancer cases vs. 10.8 expected	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
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, Nah., 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 utilizing the chlorohydrin process (both ethylene and propylene) Olsen et al. (1997)	1,361 men	No actual measurements were taken	10 lymphohematopoietic cancer deaths vs. 7.7 expected (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 natural radium, 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 have generally fallen to 5 ppm and below. Some plants may have never sustained high levels of airborne EtO. Assuming that there is a true risk of cancer associated with exposure to EtO, then the risk is not evident at the levels that existed in these plants except under certain conditions, possibly due to a lack of sensitivity in the available studies to detect associated cancers at low exposures.

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 recent 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 Dr. 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 UCC 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.

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 strongest evidence of a cancer risk is with cancer of the hematopoietic system, 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, it attained significance only in the study by Hogstedt et al. (1979b), with 9 observed versus 1.27 expected. It was reported by Shore et al. (1993) that this excess may have been due to the fact that 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 his 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.

In addition to the cancer risks described above, some recent evidence indicates that exposure to EtO may increase 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), 3 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 4, making this a significant finding for cancer in this small cohort.

The most compelling evidence on breast cancer comes from the NIOSH cohort. In the recent update of this cohort, 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.

Several 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 any of 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.

A.4. CONCLUSIONS

Experimental evidence demonstrates that exposure to EtO in rodents produces lymphohematopoietic cancers; therefore, an increase in the risk of lymphohematopoietic cancer in humans should not be unexpected. An increase in mammary gland carcinomas was also observed in mice. Although several human studies have indicated the possibility of a carcinogenic effect from exposure to EtO, especially for lymphohematopoietic cancers, 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 and specificity in the elevated risk of lymphohematopoietic cancer as a single entity in the human studies. The earlier significant risk of leukemia seen in the Hogstedt

studies was supported in some studies and not in others. In fact, not all human studies of EtO have suggested an elevated risk of cancer and in those that do, the marginally elevated risks vary from one site to another within the lymphohematopoietic system. When combined under the rubric "lymphohematopoietic cancers," this loosely defined combination of blood malignancies produces a slightly elevated risk of cancer in some studies but not in all. There is evidence of a biological gradient in the significant dose-response relationship seen in the large, high-quality Steenland et al. (2004) study.

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.

There is also evidence that exposure to EtO increases the risk of breast cancer, based chiefly on the NIOSH studies (Steenland et al., 2004; Steenland et al., 2003) discussed earlier, with some corroborating support from the Norman et al. (1995) and Kardos et al. (2003) studies of breast cancer in women exposed to EtO. The risk of breast cancer was analyzed in a few other studies (Coggon et al., 2004; Hagmar et al., 1995; 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.

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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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 in (IARC, 2008; Kolman et al., 2002; Thier and 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; Thier and 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 (*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). 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 that EtO interacts with the genome from both in vitro studies and in vivo studies of laboratory animals and occupationally exposed humans. 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 regarding the mutagenic potential of EtO.

C.1. DNA ADDUCTS

Covalent binding 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 which 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 [ENU] and methylnitrosourea or [MNU]), while the S_N2-type agents exhibit an intermediate transition state (e.g., EtO and methyl methanesulfonate [MMS]). 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 MMS, 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 ENU 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-hydroxyethylguanine (N7-HEG) adduct, although minor adducts such as those forming at O^6 of guanine, N^1, N^3, and N^6 of adenine, and N^3 of cytosine, uracil and thymine are found in some instances (Segerbäck, 1994).

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 the main concern. These methods include immunochemical assays, fluorescence techniques, high pressure 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, respectively, when used in the direct and competitive enzyme-linked immunosorbent assay and in immunofluorescence microscopy. 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 single-ion monitoring electrospray mass spectrometry which has a detection limit of 1 fmol (10^{-10} M), allowing the detection of 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 tissues of rats. 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-hydroxyethyl-2'-deoxyadenosine (N1-HEA), O^6-hydroxyethyl-2'-deoxyguanosine (O^6-HEG), N6-hydroxyethyl-2'-deoxyadenosine (N6-HEA) and N3-hydroxyethyl-2'-deoxyuridine (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. 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 experimental 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.1.2. 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-(2-hydroxyethyl)adenine (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*-values 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 (HE) DNA adducts whose relative yields (nmol/mg DNA) were in the descending order: N7-HEG (330) > N3-HEA (39) > N1-HEA (28), N6-HEA (6.2) > N3-HE-Cyt (3.1) > N3-HE-dThd (2.0) > N3-HEU (0.8). This in vitro study did not detect the O^6-HEG adduct.

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.[5] 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.1.3. In Vivo Studies—Animal Experiments

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. Segerback (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 such as 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 WBCs) and nontarget (kidney, liver, lung, and testis) tissues with maximum levels (1.5 times 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

[5]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).

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. (1992a) 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. (1992a) 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 it was 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., 1992a).

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. (1992a) discussed above, which is possibly due to the use of a highly sensitive gas chromatography high-resolution mass spectrometry (GCHRS) 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, which clearly show a difference between 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* flies 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, 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 conclude that accumulation of AP sites is not likely a primary mechanism for mutagenicity and carcinogenicity of EtO, and further suggest 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., 1992a), steady-state concentrations of O^6-HEG were reported after 4 weeks of exposure with 300 ppm EtO, a finding which warrants further investigation.

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.

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.1.4. In Vivo Studies—Human Subjects

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 four-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, i.e., the ratio of unscheduled DNA synthesis and N-acetoxy-2-acetylaminofluorene (NA-AAF)-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.1.5. DNA Adducts—Summary

In summary, EtO predominantly forms N7-HEG adducts. Minor adducts are O^6-HEG adducts and reaction products with *N*1, *N*3 and N^6 of adenine and with *N*3 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., 1992a). 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., 1992a). 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. There are only two studies 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.1.6. 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., 1992a; Britton et al., 1991). Csanady 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; Thier and 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 Stahlberg (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; Thier and 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 10–2,000 µM at 22ºC 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 10–100 mM at 37ºC 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.1.2 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 issues pertaining to the study 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, this 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—Experimental 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 base-pair substitutions, transitions, and transversions. 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 preexisting 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 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 oncogenes (*Kras, Hras*) and in the *p53* 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 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 EtO-induced lung neoplasms compared to spontaneous lung neoplasms (27/108, 25%). EtO-induced lung neoplasms predominantly exhibited GGT-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 EtO-induced lung neoplasms 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 EtO-induced neoplasms compared to spontaneous tumors (2/27, 7%). The predominant mutations in EtO-induced harderian gland neoplasms 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 EtO-induced uterine neoplasms 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 this 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 *p53* (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. P53 protein expression was detected in 67% (8/12) of the EtO-induced mammary carcinomas and 42% (8/19) of the spontaneous tumors; however, expression levels were about 6-times higher in the EtO-induced than in the spontaneous tumors. *P53* mutations were observed in 67% (8/12) of the EtO-induced mammary carcinomas and 42% (8/19) of the spontaneous tumors. *Hras* mutations were detected in 33% (4/12) of the EtO-induced mammary carcinomas and 26% (5/19) of the spontaneous tumors of the samples. While the mutation levels for these two genes were not substantially elevated in the EtO-induced mammary carcinomas compared to the spontaneous tumors, a shift in the mutational spectrum was observed, with EtO-induced *Hras* mutations exhibiting a preference for A-to-G and A-to-T transversions while spontaneous *Hras* mutations exhibited a preference for C-to-A transversions and EtO-induced *p53* mutations exhibiting a base preference for guanine while spontaneous *p53* mutations exhibited a preference for cytosine. In addition, concurrent *Hras* and *p53* mutations were more common in the EtO-induced tumors 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, may present a greater risk to germ cell damage than cumulative, long-term exposure to lower levels.

Dominant-lethal mutations were investigated by Generoso et al. (1986) by conducting 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, since 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*

In 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 and corresponded to a 40-hour time-weighted average of 0.025 ppm in hospital workers and 5 ppm in factory workers. 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 in the respective sections below.

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 and one unexposed group (seven workers per group). Contrary to the earlier study, no significant differences in mutant frequencies were observed between the groups; however, the authors stated that about 50 subjects per group would have been needed to detect a 50% increase.

Major et al. (2001) measured *HPRT* mutations in female nurses employed in hospitals in Eger and Budapest, Hungary. This study was conducted to examine a possible causal

relationship between EtO exposure and a cluster of cancers (mostly breast) in nurses exposed to EtO in the Eger hospital. 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 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., 1984b; 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 dose levels of 50–100 ppm. Frequencies of both spontaneous and EtO-induced chromosomal aberrations (and other endpoints, such as micronucleus formation and SCEs, which are discussed in Sections 3.3.2.4 and 3.3.2.5) 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 recent 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., 1984b). 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 or to 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 dose levels 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 discussed in Section C.3, 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., 1984b; 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. (1984b). 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 chronically 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.

The ability of long-term exposures to inhaled EtO to induce SCEs in peripheral lymphocytes of monkeys was investigated by Lynch et al. (1984b). 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., Schulte et al., 1992; Galloway et al., 1986; Sarto et al., 1984a, b; Yager et al., 1983; Garry et al., 1979), 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 3 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 (Thier and Bolt, 2000; IARC, 1994a)]. Recently, Marsden et al. (2009) proposed that oxidative stress can induce the endogenous formation of ethylene, which can in turn be metabolized to EtO. Endogenous production of ethylene has been 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 would 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 experimental 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., 1992b; Cushnir et al., 1991; Föst et al., 1989). However, there is a wide variation in the levels of adducts detected in rodents and humans which appears 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 were below the level of detection. Also, some researchers consistently demonstrated higher background levels of DNA adducts (Wu et al., 1999a; Walker et al., 1992b). 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.

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

Species	Tissue	Detection method	Adduct levels reported	Adducts/10^7 nucleotides*	Reference
Human	Lymphocytes	GC/MS	8.5 pmol/mg DNA	28.05	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	10.56	Bolt et al. (1997)
Human	Lymphocytes	GC/MS	2–19 adducts per 10^7 nucleotides	2.0–19	Wu et al. (1999b)
Human	WBC	^{32}P/TLC/HPLC	0.6 adducts/10^7 nucleotides	0.6	Zhao et al. (1999)
Human	WBC	^{32}P/TLC/HPLC	2.9 adducts/10^7 nucleotides	2.9	Zhao et al. (1999)
Human	Lung	^{32}P/TLC/HPLC	4.0 adducts/10^7 nucleotides	4	Zhao et al. (1999)
Rat	Lymphocytes	GC/MS	5.6 pmol/mg DNA	18.48	Föst et al. (1989)
Mice/Rats	Control tissues	HPLC-fluorescence	2–6 pmol/mg DNA	8.58	Walker et al. (1992b)
Rat	Liver, kidney, spleen	^{32}P/GC/MS	0.4 to 1.1 adducts/10^7 nucleotides	0.4–1.1	Eide et al. (1999)
Mice/Rats	Spleen	GC/EC/NCI-HRMS	0.2 to 0.3 pmol/mmol guanine		Wu et al. (1999a)
Rat	Control tissues	^{32}P/TLC/HPLC	0.6 to 0.9 adducts/10^7 nucleotides	0.6–0.9	Zhao et al. (1999)
Rat	Liver	GC/MS	2.6 adducts/10^8 nucleotides	0.26	van Sittert et al. (2000)
Rat	Control tissues	LC-MS/MS	1.1–3.5 adducts/10^8 nucleotides	0.11–0.35	Marsden et al. (2007)
Rat	Liver	HPLC/ESI TMS	8 adducts/10^8 normal nucleotides	0.8	Tompkins et al. (2008)
Rat	Spleen	HPLC/LC-MS/MS	0.08 adducts/10^{10} nucleotides	0.00008	Marsden et al. (2009)

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

*Adduct levels are normalized using the formula: 1 pmol adducts/mg DNA = 3.3 adducts/10^7 normal nucleotides.
GC/MS, gas chromatography mass spectrometry; HPLC, high performance liquid chromatography; ^{32}P, ^{32}P-postlabeling assay; TLC, thin-layer chromatography; LC-MS, liquid chromatography mass spectrometry; ESI TMS, electrospray ionization tandem mass spectrometry; GC/EC/NCI-HRMS, gas chromatography/electron capture/negative chemical ionization high-resolution mass spectrometry.

Using sensitive detection techniques and an approach designed to separately quantify both endogenous N7-HEG adducts and "exogenous" N7-HEG adducts induced by EtO treatment in F344 rats, Marsden et al. (2009) recently reported increases in exogenous adducts in DNA of spleen and liver at the lowest dose administered (0.0001 mg/kg injected i.p. daily for 3 days). 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 below the limit of accurate quantitation. 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).[6]

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 (Section 3.4) supports low-

[6]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 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.

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 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 have a background lifetime incidence risk on the order of 3%, whereas the background lifetime incidence risk for breast cancer is on the order of 15%.

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 upon the likely role for DNA alkylation in the production of 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 AND INTERPRETATION OF ETHYLENE OXIDE EXPOSURE-RESPONSE DATA

Kyle Steenland

May 27, 2010

(EDITORIAL NOTE: This Appendix contains the report submitted by Dr. Steenland summarizing the results of analyses that he conducted under contract to U.S. EPA. The terminology originally used by Dr. Steenland to designate the different exposure-response model forms has been changed to be consistent with the terminology used in EPA's Ethylene Oxide Carcinogenicity Assessment. Models that are linear in log RR and which were previously referred to as "linear" models have been renamed "log-linear" models (except where it is stated that they are log RR models), and models of the form $RR = 1 + \beta \times$ exposure, which were previously referred to as "excess relative risk" (ERR) models have been renamed "linear" models. In addition, section headings, figures, and tables have been renumbered for the table of contents. Finally, some supplemental results received from Dr. Steenland after the original completion of this Appendix have been inserted in the relevant sections.)

This report contains the results of reanalyses of the National Institute for Occupational Safety and Health cohort of workers exposed to ethylene oxide conducted for the U.S. Environmental Protection Agency. The report begins with an overview of the modeling strategy used, followed by the results of 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 which is likely necessary before an exposure can result in (observable or fatal) cancer. We have relied primarily on Cox regression as a flexible method of modeling the log RR; however we have also included

some linear relative risk models. Cumulative exposure is typically the exposure metric of interest in predicting chronic disease.

For breast cancer incidence, we have relied principally on 2-piece linear models, in which log RR (in the log-linear model) or RR (in the linear model) is a function of two lines which join smoothly at a single point of inflection. Two-piece linear models may also be thought of as linear splines with one knot, or point of inflection. They 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 2-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 2 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 (often considered the default 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 has the disadvantage that the curve is usually highly supra-linear at low doses. Two-piece linear 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 2-piece linear models avoid this supra-linearity in the low-dose region (Steenland and Deddens, 2004).

The shape of the 2-piece linear 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 linear pieces 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 ppm-days (or 1000 ppm-days) 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

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

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 model, $\varphi(Z;\beta) = e^{(z\beta)}$.

While the 2-piece models work well for ETO breast cancer incidence, they do not for hematopoietic cancer (and to a lesser extent for breast cancer mortality) because the best knots are at very low doses and the resulting slopes for the first piece of the 2-piece model are very steep, resulting in the same problem which occurs using log transform models (i.e., where the exposure metric is the log of cumulative exposure). Risk for hematopoietic cancer in fact increases quite steeply with very low exposure versus no exposure, and then plateaus at higher exposures. This may be partly a result of the relatively small numbers of hematopoietic cancers and the overall instability of the results. In this case, EPA's original approach of a weighted regression through categorical RRs is a reasonable alternative to both the log transform and 2-piece models.

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

a. Distribution of exposure among ETO-exposed women in breast cancer incidence cohort with interviews (*n* = 5139)

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 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, again cumulative exposure is 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 below. 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-1a. 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
>0–355 ppm-days	157	17
356–842 ppm-days	580	16
843–1361 ppm-days	1097	17
1362–2187 ppm-days	1725	17
2188–3772 ppm-days	2899	17
3773–5522 ppm-days	4546	18
5523–7891 ppm-days	6554	16
7892–14483 ppm-days	14384	17
14484–25112 ppm-days	18859	17
>25112 ppm-days	48807	18

b.1. Results of Cox regression analysis of breast cancer incidence using a variety of (log RR) models

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

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a log transform model, a cubic spline model, and a square-root transform model. We also ran a number of analogous models using linear RR models.

The categorical analysis (log RR model) used deciles, as indicated in Table D-1b. 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-1b below.

Table D-1b. Categorical analysis of breast cancer incidence by deciles (log RR model)

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CAT1	-0.09015	0.29318	0.0945	0.7585	0.914
CAT2	-0.08363	0.30341	0.0760	0.7828	0.920
CAT3	0.18536	0.29757	0.3880	0.5333	1.204
CAT4	0.12606	0.29995	0.1766	0.6743	1.134
CAT5	0.07900	0.29968	0.0695	0.7921	1.082
CAT6	0.37651	0.29675	1.6097	0.2045	1.457
CAT7	0.38177	0.31168	1.5003	0.2206	1.465
CAT8	0.25179	0.30640	0.6753	0.4112	1.286
CAT9	0.57845	0.31120	3.4551	0.0631	1.783
CAT10	0.80396	0.30766	6.8284	0.0090	2.234

-2 LOG L 1936.910, df=15 (10 exposure terms, 5 covariates)

We then fit a cubic spline (restricted at the ends to be linear) which presents a description of the data similar to the categorical analyses but using a smooth curve. The exposure metric was cumulative exposure with a 15-year lag, which was found in earlier analyses to be the optimal lag (Steenland et al., 2003). Five knots for the cubic spline were chosen using every other midpoint from the categorical analysis (598, 1774, 4647, 11187, and 37668 ppm-days).

We then ran a 2-piece linear (log RR) model. The knot, or inflection point, was chosen to be the one where the model likelihood was highest, which was at 5,800 ppm-days. To choose this knot we looked at possible inflection points over the range 100 to 15,000 ppm-days by 100 ppm-day increments. Figure D-1a shows the –2 log likelihood graphed against the knots. In this figure the lower peak corresponds to the highest likelihood.[7]

Figures D-1b and D-1c show the results of the 2-piece linear, the categorical, the linear, and the cubic spline (log RR) models. In these figures the categorical points are the mid-points of the categories in Table D-1a, with final category assigned the final cut point plus 50%.

[7]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 chi-squared 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-1b 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-1c.

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 6000 ppm-days, which was close to the knot in the 2-piece log-linear model [5800 ppm-days]). The ratios of the slope (coefficient) for the linear term (log RR model) with these exclusions vs. the slope for the linear term (log RR model) with no exclusions were 1.5, 2.3, 3.2, 3.2, 2.5, 3.1, 6.1, 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-1d, along with the full log-linear curve from Figure D-1c. Nonetheless, even the log-linear curve from these truncated data has a markedly lower slope in the low-exposure region than the 2-piece log-linear (or spline) curves. For example, inspection shows that the RR for 6000 ppm-days is about 1.2 for the log-linear curve from the truncated data and 1.6 from the 2-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 2-piece log-linear model.

A 2-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 (Figure D-1e), 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-1f. This model also fit the breast cancer morbidity well (it did not fit the other outcomes well and is not shown for them), 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 2-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 between 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.

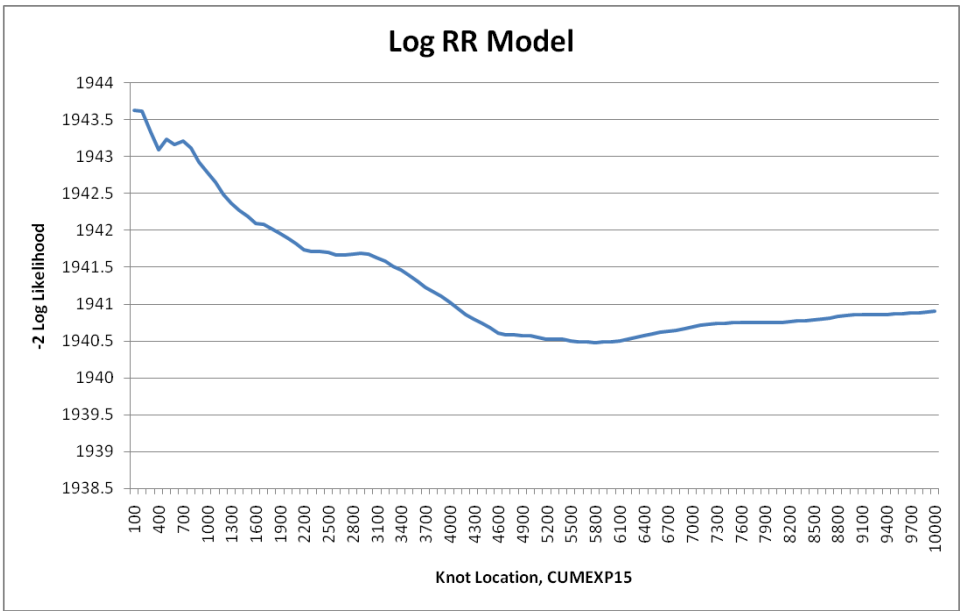

Figure D-1a. Likelihoods vs. knots, 2-piece linear log RR model for breast cancer morbidity.

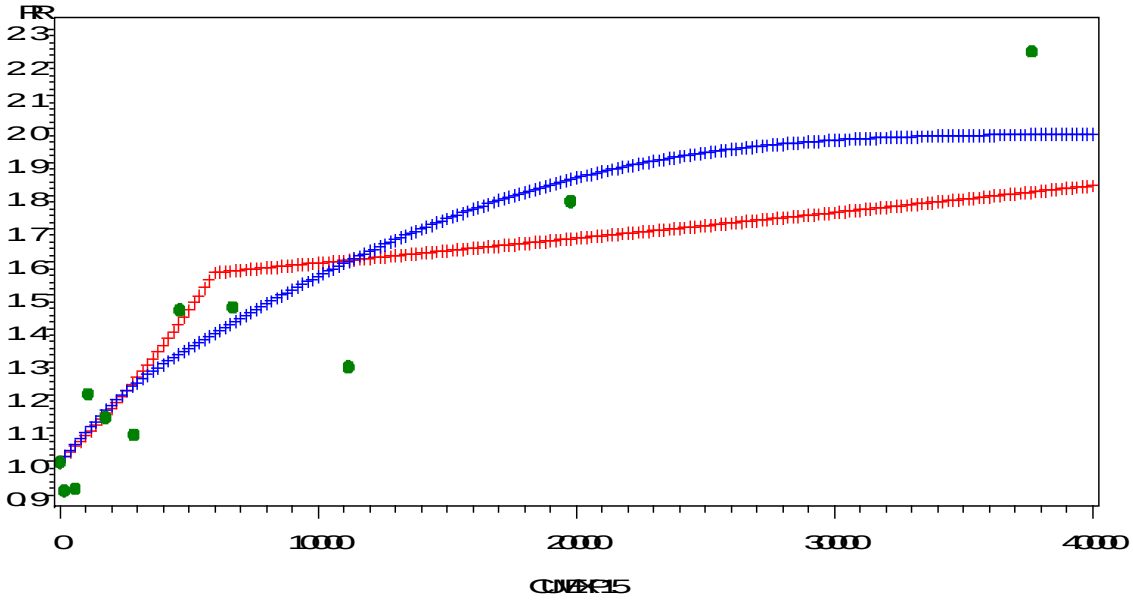

Figure D-1b. Breast cancer incidence. Plot of the dose-response relationship for continuous exposure generated using a 2-piece log-linear spline overlaid with a plot using restricted cubic (log RR) splines. Dots that represent the effect of exposure grouped in deciles (log RR categorical model) are also presented in the plot. Deciles formed by allocating cases approximately equally in ten groups, above lagged-out cases, see Table D-1a above. Y-axis is rate ratio, X-axis is cumulative exposure lagged 15 years, in ppm-days.

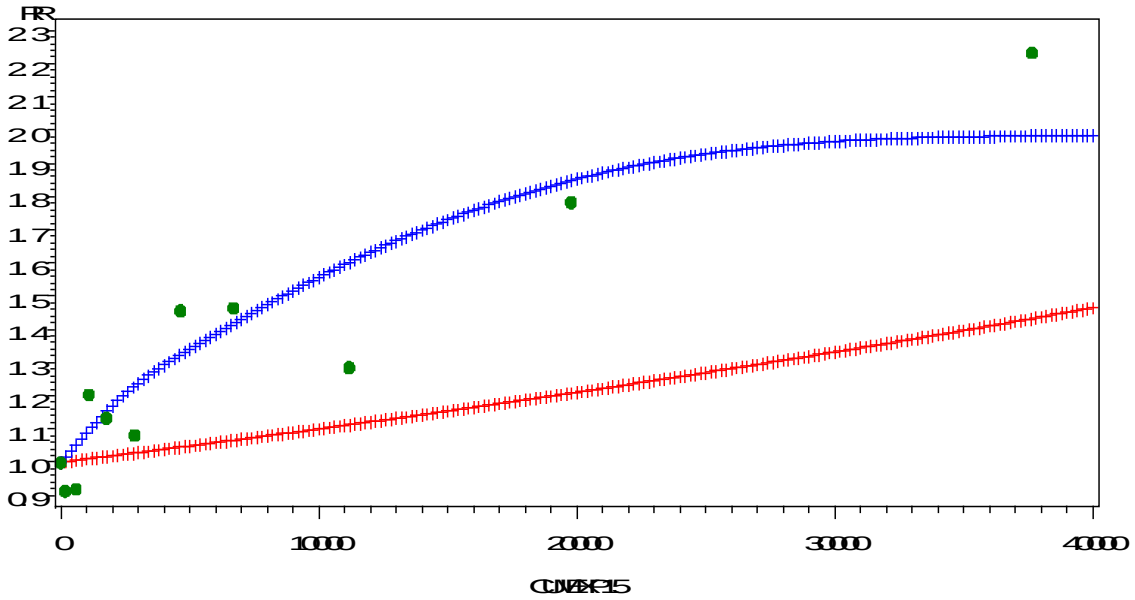

Figure D-1c. Breast cancer incidence. Plot of a log-linear dose-response relationship overlaid with a dose-response relationship generated using restricted cubic log RR model with continuous exposure. Dots that represent the effect of exposure grouped in deciles (log RR categorical model) are also presented in the plot. Deciles formed by allocating cases approximately equally in ten groups, above lagged-out cases.

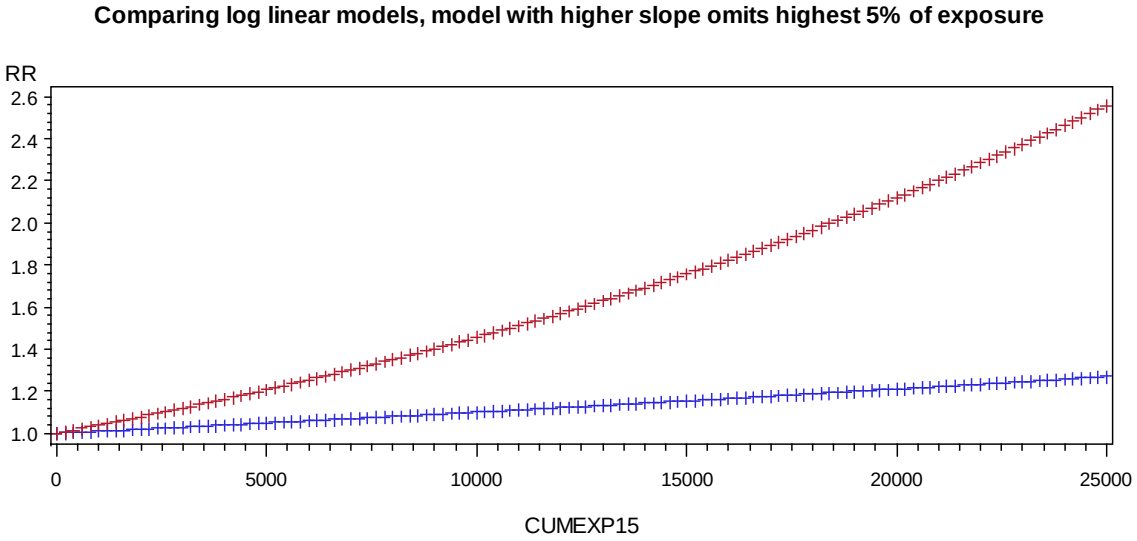

Figure D-1d. Breast cancer incidence. Comparison of log-linear curve (log RR = β × cumexp) with all the data and the log-linear curve (higher slope) after excluding those in the top 5% of exposure (>27,500 ppm-days).

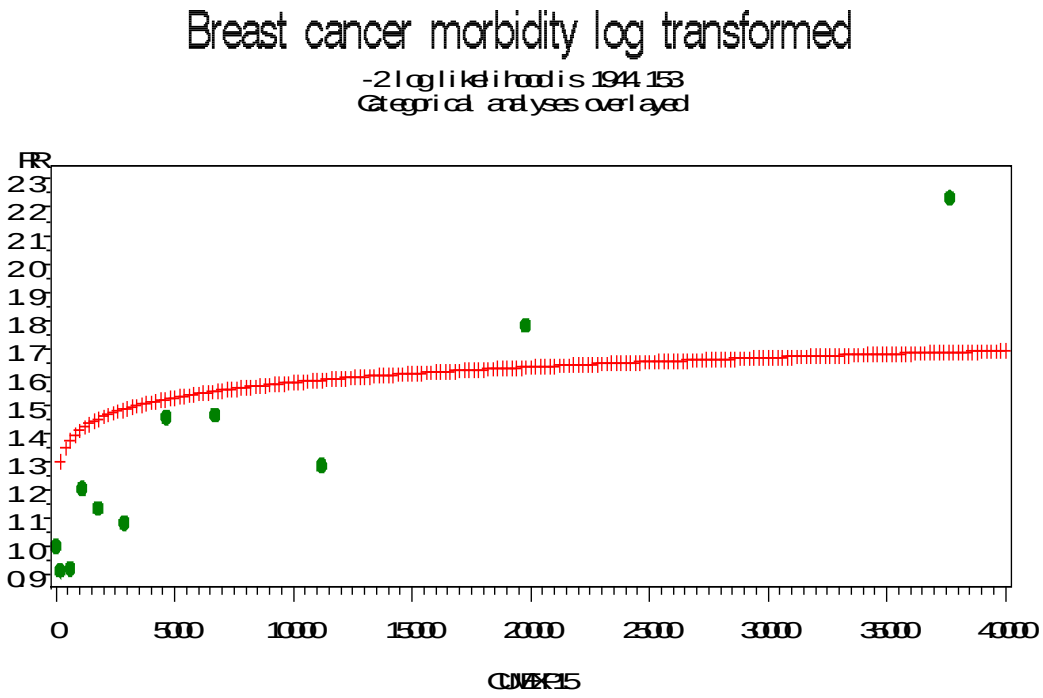

Figure D-1e. Breast cancer incidence. Plot of a logarithmic transformation log RR dose-response model (log RR = β × log(cumexp)) overlaid with a dose-response relationship generated using categorical log RR analyses (deciles). Deciles formed by allocating cases approximately equally in ten groups, above lagged-out cases.

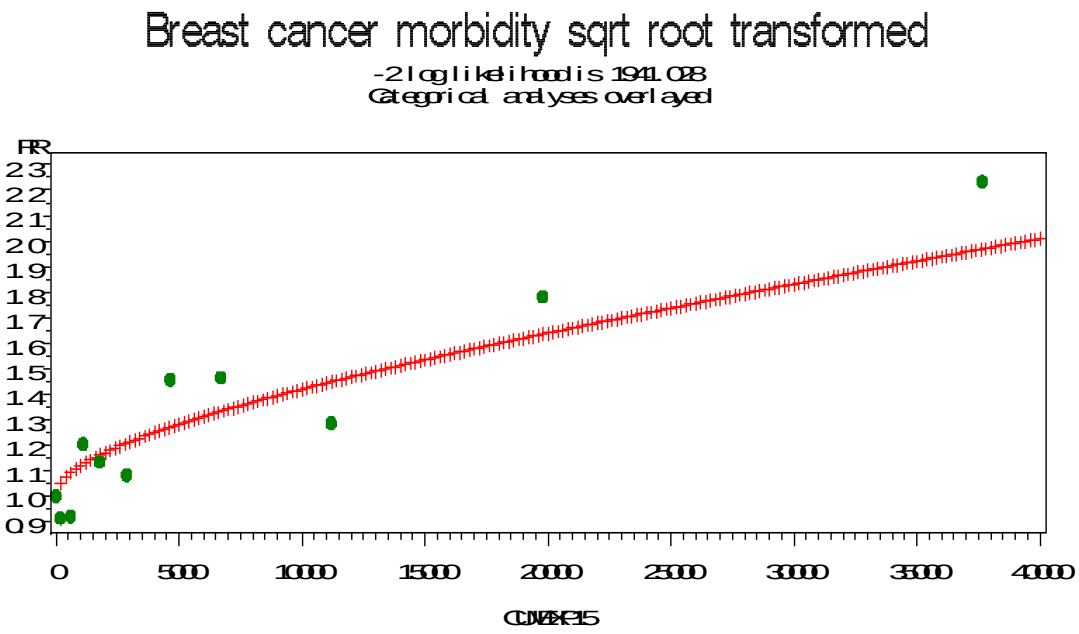

Figure D-1f. Breast cancer incidence. Plot of a square-root transformation log RR dose–response model overlaid with a dose-response relationship generated using categorical log RR analyses (deciles). Deciles formed by allocating cases approximately equally in ten groups, above lagged-out cases.

Tables D-1c, D-1d, D-1e, and D-1f below present the model fit statistics for the 2-piece log-linear, the log-linear, the square root log RR model, and the log transform log RR model seen above. Table D-1g summarizes the goodness-of-fit data with regard to the exposure term. Table D-1g shows that the addition of exposure terms to the various models results in similar model fits. The exposure terms in the 2-piece log-linear improve model fit marginally better than those in the other models except the square root log RR model, with which the 2-piece log-linear model is tied. If one adds a degree of freedom to the chi-square test for the 2-piece log-linear model, on the assumption that the choice of the knot is equivalent to estimating another parameter, the *p*-value increases to 0.04, in the same range as the log-linear and log-transform log RR models. Our argument here, however, is not that the 2-piece log-linear model fits the data dramatically better than other models in purely statistical terms. Rather we believe that the fit conforms to the categorical and cubic spline models well in the low-exposure region of interest, and that the nearly linear exposure-response relationship in that region (strictly linear with the linear RR model) is a reason to prefer the 2-piece log-linear model to the other models. In particular, among the parametric models, the log transform and square root log RR models are 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 the next sections (c, d, and e). In these sections we use some of the results from the exposure-response models to calculate EC_{01}s. We restrict these calculations to models which appear most reasonable based on our results above, namely the 2-piece log-linear model, the square root transform log RR model, and the cubic spline log RR model. 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 provides a good comparison to other parametric models.

Table D-1c. Fit of 2-piece log-linear model to breast cancer incidence data, Cox regression[8]

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1940.485
AIC	1967.813	1954.485
SBC	1967.813	1978.612

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	27.3281	7	0.0003
Score	29.0949	7	0.0001
Wald	28.4426	7	0.0002

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
LIN_0 (β1)	0.0000770	0.0000317	5.4642	0.0194	1.000
LIN_1	-0.0000724	0.0000334	4.1818	0.0409	1.000
DOB1	0.08770	0.21805	0.1618	0.6875	1.092
DOB2	0.41958	0.24430	2.9496	0.0859	1.521
DOB3	0.55168	0.29096	3.5950	0.0580	1.736
PARITY1	-0.23398	0.18793	1.5502	0.2131	0.791
FREL_BR_CAN1	0.47341	0.17934	6.9686	0.0083	1.605

Covariance lin0 and lin1 -1×10^{-9}

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

Table D-1d. Fit of log-linear model to breast cancer incidence data, Cox regression (RR = $e^{(\beta \times \text{exposure})}$)

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1944.675
AIC	1967.813	1956.675
SBC	1967.813	1977.356

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.1374	6	0.0008
Score	25.8389	6	0.0002
Wald	25.3594	6	0.0003

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CUMEXP15 (β)	9.54826E-6	4.09902E-6	5.4261	0.0198	1.000
DOB1	0.13558	0.21676	0.3912	0.5316	1.145
DOB2	0.53147	0.23741	5.0116	0.0252	1.701
DOB3	0.74477	0.27425	7.3748	0.0066	2.106
PARITY1	-0.23394	0.18882	1.5351	0.2154	0.791
FREL_BR_CAN1	0.46449	0.17928	6.7126	0.0096	1.591

Table D-1e. 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

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1941.028
AIC	1967.813	1953.028
SBC	1967.813	1973.708

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	26.7851	6	0.0002
Score	28.9446	6	<.0001
Wald	28.5277	6	<.0001

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq
dob1	1	0.09778	0.21756	0.2020	0.6531
dob2	1	0.43872	0.24177	3.2929	0.0696
dob3	1	0.58623	0.28404	4.2596	0.0390
sqrtcumexp15 (β)	1	0.00349	0.00118	8.7489	0.0031
PARITY1	1	-0.22539	0.18787	1.4393	0.2302
FREL_BR_CAN1	1	0.46937	0.17922	6.8589	0.0088

Table D-1f. 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	1967.813	1944.176
AIC	1967.813	1956.176
SBC	1967.813	1976.856

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.6371	6	0.0006
Score	24.0044	6	0.0005
Wald	23.5651	6	0.0006

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.08605	0.21943	0.1538	0.6949	1.090
dob2	1	0.38780	0.25363	2.3378	0.1263	1.474
dob3	1	0.47303	0.31528	2.2509	0.1335	1.605
LCUMEXP15 (β)	1	0.04949	0.02288	4.6787	0.0305	1.051
PARITY1	1	-0.25908	0.18638	1.9322	0.1645	0.772
FREL_BR_CAN1	1	0.47620	0.17923	7.0595	0.0079	1.610

Table D-1g. Change in –2 log likelihood for log RR models for breast cancer incidence, with addition of exposure term(s)[a]

Log RR model	Change (chi square)	d.f.	*p*-value
Log transform	4.8	1	0.03
Linear	4.2	1	0.04
Categorical	12.0	10	0.29
Cubic spline	8.8	4	0.07
2-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.

b.2. Linear relative risk models for breast cancer incidence

We also ran linear relative risk models for breast cancer incidence, using the techniques described recently by Langholz and Richardson (2010) to use SAS to fit these models, using the same data as used for the log RR models. The form of these linear RR models is $RR = 1 + \beta x$, where x can be cumulative dose, the log of cumulative dose, a 2-piece linear function of cumulative dose, etc.

Figure D-1g below shows the different curves with the linear RR model, using cumulative exposure lagged 15 years as the exposure metric. The categorical points in Figure D-1g come from the published categorical results for the log RR model (Steenland et al., 2003). The midpoints for the 5 categories (above the lagged out referent, at 0 exposure) are the medians of cumulative exposure (lagged 15 years), which were 253, 1193, 3241, 7741, and 26,597 ppm-days.

Figure D-1h shows the likelihood profile for different possible knots for the 2-piece linear spline, with the search conducted by using increments of 100 ppm-days. For the 2-piece linear spline model the best knot was 5800 ppm-days, as was the case for the 2-piece log-linear model.

Table D-1h shows the model fit statistics for the linear RR models. These models tend to fit slightly better than their log RR counterparts, although generally the improvement in the chi square does not attain significance at the 0.05 level. For the 2-piece linear model, the model

likelihood is 1936.9 vs a likelihood of 1940.5 for the 2-piece log-linear model. Among the linear RR models, the 2-piece spline model fits better than the other models, although not significantly so. Table D-1i gives the exposure parameter values for the linear RR models. Table D-1j presents the parameter estimates for the exposure variables for the categorical (decile) linear RR model.

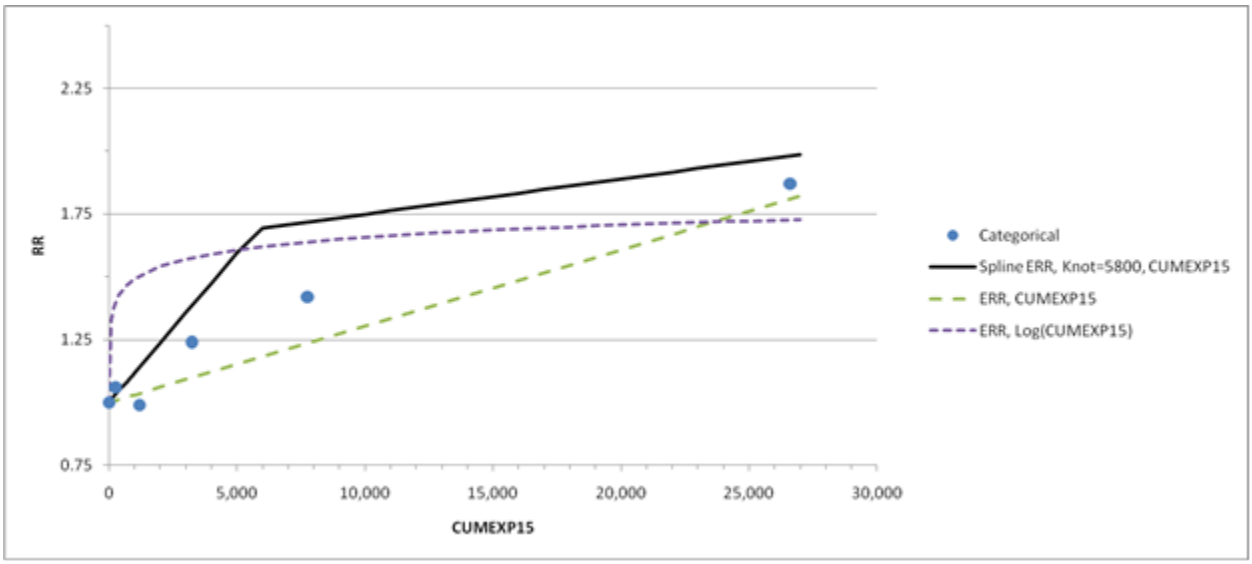

Figure D-1g. Breast cancer incidence exposure-response curves, linear RR models (units are ppm-days, 15-year lag). [Editorial note: "ERR" refers to linear RR models.]

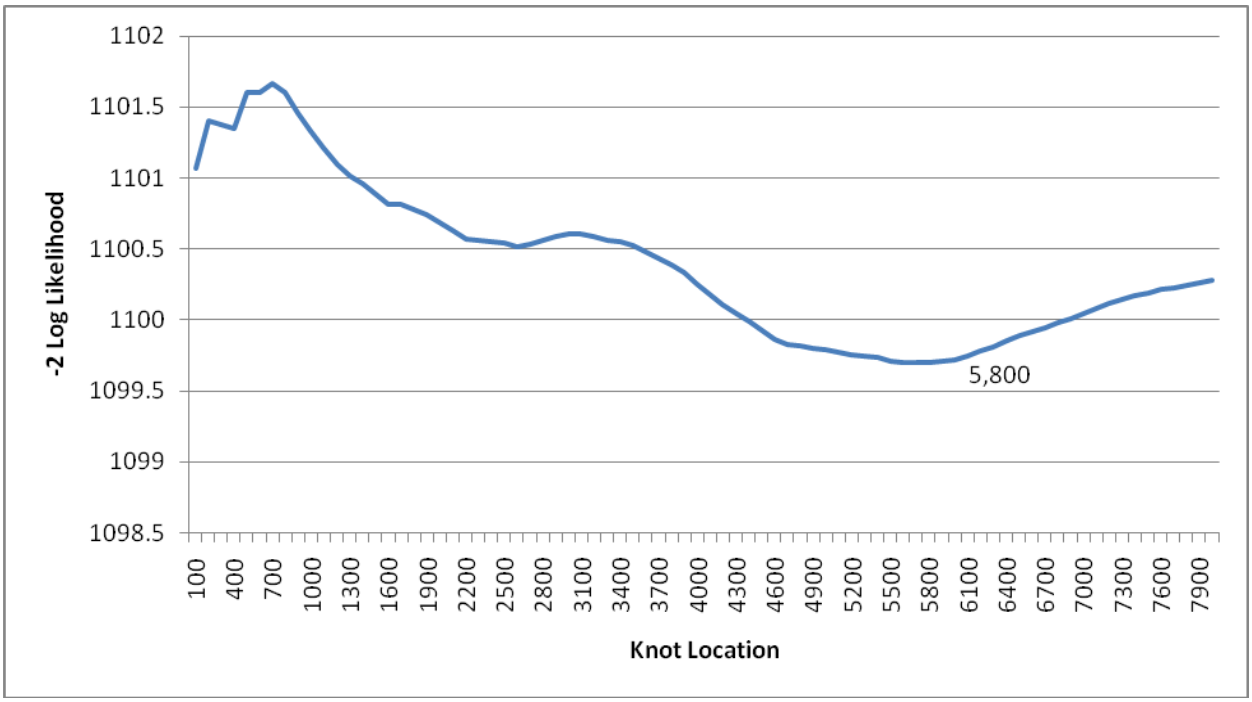

Figure D-1h. Knot location for Figure D-1g above, 2-piece linear spline model, breast cancer incidence (units are ppm-days, 15-year lag).

Table D-1h. Model fit statistics for linear RR models, breast cancer incidence[a]

Linear RR Model	d.f. (full model)[b]	–2 Log likelihood (full model)	–2 LL (model without exposure)	–2 LL (model without any covariates)	*p*-value (full model)	*p*-value (for addition of exposure terms)[c]
CUMEXP15	6	1940.260	1949.06	1967.813	< 0.0001	0.0030
Log(CUMEXP15)	6	1942.267	1949.06	1967.813	0.0003	0.0096
Spline, knot = 5,800, CUMEXP15	7	1936.935	1949.06	1967.813	< 0.0001	0.0023

[a]For the linear RR models, all covariates were included linearly (i.e., additively). Including the nonexposure covariates in the model multiplicatively instead did not improve model fit (e.g., for the 2-piece spline model, inclusion of the non-exposure covariates multiplicatively instead of additively gave a -2 LL of 1940.4 (vs. 1936.9 for additive inclusion).

[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).

Table D-1i. Model coefficients for linear RR models, breast cancer incidence

Linear RR Model	Parameter(s)	SE	Profile likelihood 95% (one-sided) upper and lower bounds[c]
CUMEXP15	B = 0.000030402	SE = 0.000017549	UB = 0.0000745 LB = 0.00000975
Log(CUMEXP15)	B = 0.071322	SE = 0.039227	
Spline, knot = 5,800, CUMEXP15[a, b]	B1 = 0.000119, B2 = –0.000105	SE1 = 0.000067727, SE2 = 0.000070478	UB1 = 0.000309 LB1 = 0.000032

[a]Covariance of 2 pieces of linear spline, -4.64×10^{-9}.

[b]For estimating risks from occupational exposures (Section 4.7 of the Carcinogenicity Assessment Document), both pieces of the 2-piece linear spline model were used. For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × (exp – knot)). For the 95% upper confidence limit, for exposures below the knot, RR = 1 + ((β1+ 1.645 × SE1) × exp); for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp-knot) + 1.645 × sqrt(exp^2 × var1 + $(exp\text{-}knot)^2$ × var2 + 2 × exp × (exp-knot) × covar)), where exp = cumulative exposure, var = variance, covar = covariance.

[c]Editorial note: As discussed in footnotes i and j of Table 4-7 in Section 4.1.2.3 of this assessment, confidence intervals were determined using the Wald approach. Confidence intervals for linear RR models, however, in contrast to those for the log-linear RR models, may not be symmetrical. EPA also evaluated application of a profile likelihood approach (Langholz and Richardson, 2010), which allows for asymmetric CIs, for selected linear RR models, for comparison with the Wald approach. 95% (one-sided) upper and lower bounds on the parameter estimate (regression coefficient) derived using the profile likelihood method are presented here. For the continuous linear model (CUMEXP15), the profile likelihood upper bound is about 29% higher than the upper bound obtained using the Wald approach. For the low-exposure segment of the linear spline model, the profile likelihood upper bound is about 34% higher than the upper bound obtained using the Wald approach. Calculating the profile likelihood bounds for the second spline segment parameter estimate is computationally difficult and was not pursued here.

Table D-1j. Supplemental Results: Parameter estimates for exposure variables for categorical (decile) linear RR model (RR = 1 + β), breast cancer incidence

N	Parameter	Estimate	Approx Std Err	t Value	Approx Pr > |t|	Gradient Objective Function
1	beta1a	0.039745	0.340310	0.116792	0.907133	2.4264E-10
2	beta1b	0.133788	0.371450	0.360177	0.719065	6.847537E-11
3	beta1c	0.304056	0.438525	0.693361	0.488824	1.119129E-10
4	beta1d	0.166744	0.402813	0.413950	0.679319	3.011577E-10
5	beta1e	-0.071617	0.347348	-0.206182	0.836842	7.617004E-11
6	beta1f	0.749956	0.583672	1.284893	0.200200	9.568004E-11
7	beta1g	0.919113	0.643333	1.428674	0.154536	7.337724E-11
8	beta1h	0.487590	0.505453	0.964660	0.335789	5.850301E-11
9	beta1i	0.985298	0.753167	1.308206	0.192187	1.207377E-10
10	beta1j	1.575028	0.960886	1.639141	0.102633	1.341514E-10

Value of Objective Function (log likelihood) = -966.9720784

c. Risk assessment for breast cancer incidence using the 2-piece log-linear spline

We used the 95% upper bound of the coefficient for the 1st piece of the 2-piece log-linear model from Table D-1c, which is 0.0000770 + 1.64 × 0.0000317 or 0.0001290, to calculate the LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their risk assessment. Here we used the same data on background breast cancer incidence and background all-cause mortality as used by EPA in their 2006 calculations. The rate ratio then, as a function of exposure, is $RR = e^{(0.0001290 \times cumexp15)}$. Note that the 2-piece log-linear model is linear for the log RR. Once this is exponentiated, it is no longer strictly linear, but is still approximately so, as can be seen in Figure D-1a.

Use of the function $RR = e^{(0.0001290 \times cumexp15)}$ in the life-table analysis results in an excess risk of 0.01 when the daily exposure is 0.0090 ppm, which is the LEC_{01}. This is slightly lower than the previous LEC_{01} of 0.0110 ppm in EPA's 2006 draft risk assessment [U.S. EPA (2006a), Table 14].

Similar calculations were done for the EC_{01}, which resulted in a value of 0.0152 ppm.

d. Risk assessment for breast cancer incidence using the square root transformation log RR model

Use of the 95% upper bound of the relative risk function, i.e., RR = $e^{((0.000349 + .00118 \times 1.64) \times \text{square root(cumexp15)})}$, in the life-table analysis results in an excess risk of 0.01 when the daily exposure is 0.00225 ppm, which is the LEC_{01}. This is about 5 times lower than the previous LEC_{01} of 0.0110 ppm in EPA's 2006 draft risk assessment [U.S. EPA (2006a), Table 14]. The EC_{01} is 0.0060 ppm, which is about four times lower than the EPA's 2006 EC_{01}. The reason these estimates are much lower than the EPA's is that the square root curve, as can be seen in Figure D-1d, rises very sharply (is supralinear) in the low-dose region. In this sense, it shares the disadvantage of the log transform model, and we recommend against using it as a basis for risk assessment for that reason.

e. 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 semi-parametric 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 2-piece log-linear model. The point is to show that the cubic spline log RR model

and the 2-piece log-linear spline give similar answers, not to use the cubic spline for 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 error 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 2-piece log-linear model.

f. Risk assessment for breast cancer incidence using the 2-piece linear spline model

Use of the function RR = 1 + (0.000119 + 1.64 × 0.000067) × cumexp15 in the life-table analysis results in an excess risk of 0.01 when the daily exposure is 0.0052 ppm, which is the LEC_{01}, which is about half of the value of 0.0110 ppm from the 2-piece log-linear spline model. The corresponding EC_{01} is 0.0100 ppm.

g. Supplemental results: results for cumulative exposure and log cumulative exposure Cox regression models with different lag times (no lag, 5 years, 10 years, 15 years, and 20 years)

(i) cumulative exposure model, no lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1946.492
AIC	1967.813	1958.492
SBC	1967.813	1979.172

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	21.3211	6	0.0016
Score	22.2448	6	0.0011
Wald	22.0301	6	0.0012

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.17056	0.21590	0.6241	0.4295	1.186
dob2	1	0.59054	0.23671	6.2242	0.0126	1.805
dob3	1	0.83494	0.27295	9.3573	0.0022	2.305
CUMEXP	1	5.93879E-6	3.52892E-6	2.8321	0.0924	1.000
PARITY1	1	-0.25022	0.18784	1.7746	0.1828	0.779
FREL_BR_CAN1	1	0.47120	0.17920	6.9144	0.0086	1.602

(ii) cumulative exposure model, 5-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1945.875
AIC	1967.813	1957.875
SBC	1967.813	1978.555

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	21.9381	6	0.0012
Score	23.1833	6	0.0007
Wald	22.9563	6	0.0008

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.16362	0.21604	0.5736	0.4488	1.178
dob2	1	0.57250	0.23698	5.8363	0.0157	1.773
dob3	1	0.80642	0.27311	8.7184	0.0032	2.240
CUMEXP5	1	6.8565E-6	3.59626E-6	3.6350	0.0566	1.000
PARITY1	1	-0.24489	0.18810	1.6951	0.1929	0.783
FREL_BR_CAN1	1	0.47063	0.17919	6.8981	0.0086	1.601

(iii) cumulative exposure model, 10-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1945.521
AIC	1967.813	1957.521
SBC	1967.813	1978.201

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	22.2922	6	0.0011
Score	23.9807	6	0.0005
Wald	23.6876	6	0.0006

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.15185	0.21633	0.4927	0.4827	1.164
dob2	1	0.55144	0.23733	5.3986	0.0202	1.736
dob3	1	0.77339	0.27377	7.9805	0.0047	2.167
CUMEXP10	1	7.75726E-6	3.80799E-6	4.1498	0.0416	1.000
PARITY1	1	-0.24110	0.18839	1.6379	0.2006	0.786
FREL_BR_CAN1	1	0.46864	0.17921	6.8385	0.0089	1.598

(iv) cumulative exposure model, 15-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1944.675
AIC	1967.813	1956.675
SBC	1967.813	1977.356

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.1374	6	0.0008
Score	25.8389	6	0.0002
Wald	25.3594	6	0.0003

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.13558	0.21676	0.3912	0.5316	1.145
dob2	1	0.53147	0.23741	5.0116	0.0252	1.701
dob3	1	0.74477	0.27425	7.3748	0.0066	2.106
CUMEXP15	1	9.54826E-6	4.09902E-6	5.4261	0.0198	1.000
PARITY1	1	-0.23394	0.18882	1.5351	0.2154	0.791
FREL_BR_CAN1	1	0.46449	0.17928	6.7126	0.0096	1.591

(v) cumulative exposure model, 20-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1946.040
AIC	1967.813	1958.040
SBC	1967.813	1978.720

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	21.7730	6	0.0013
Score	24.0576	6	0.0005
Wald	23.5506	6	0.0006

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.13721	0.21682	0.4005	0.5268	1.147
dob2	1	0.53985	0.23711	5.1839	0.0228	1.716
dob3	1	0.76037	0.27371	7.7177	0.0055	2.139
CUMEXP20	1	0.0000101	5.27041E-6	3.6890	0.0548	1.000
PARITY1	1	-0.23887	0.18905	1.5966	0.2064	0.788
FREL_BR_CAN1	1	0.46310	0.17935	6.6673	0.0098	1.589

(vi) log cumulative exposure model, no lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1943.662
AIC	1967.813	1955.662
SBC	1967.813	1976.343

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	24.1508	6	0.0005
Score	24.4372	6	0.0004
Wald	24.1563	6	0.0005

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.17618	0.21596	0.6655	0.4146	1.193
dob2	1	0.59516	0.23703	6.3045	0.0120	1.813
dob3	1	0.83783	0.27359	9.3780	0.0022	2.311
lcumexp	1	0.09294	0.04097	5.1458	0.0233	1.097
PARITY1	1	-0.25682	0.18640	1.8984	0.1683	0.774
FREL_BR_CAN1	1	0.47417	0.17923	6.9991	0.0082	1.607

(vii) log cumulative exposure model, 5-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1946.843
AIC	1967.813	1958.843
SBC	1967.813	1979.523

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	20.9703	6	0.0019
Score	21.0320	6	0.0018
Wald	20.7379	6	0.0020

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.15082	0.21658	0.4850	0.4862	1.163
dob2	1	0.53156	0.24038	4.8900	0.0270	1.702
dob3	1	0.72413	0.28191	6.5981	0.0102	2.063
LCUMEXP5	1	0.04458	0.03135	2.0222	0.1550	1.046
PARITY1	1	-0.26745	0.18630	2.0608	0.1511	0.765
FREL_BR_CAN1	1	0.47497	0.17922	7.0241	0.0080	1.608

(viii) log cumulative exposure model, 10-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1944.040
AIC	1967.813	1956.040
SBC	1967.813	1976.721

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.7728	6	0.0006
Score	23.5846	6	0.0006
Wald	23.1565	6	0.0007

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.11282	0.21798	0.2679	0.6048	1.119
dob2	1	0.43207	0.24800	3.0352	0.0815	1.540
dob3	1	0.53777	0.30203	3.1702	0.0750	1.712
LCUMEXP10	1	0.05654	0.02594	4.7498	0.0293	1.058
PARITY1	1	-0.26063	0.18629	1.9573	0.1618	0.771
FREL_BR_CAN1	1	0.47636	0.17921	7.0653	0.0079	1.610

(ix) log cumulative exposure model, 15-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1944.176
AIC	1967.813	1956.176
SBC	1967.813	1976.856

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.6371	6	0.0006
Score	24.0044	6	0.0005
Wald	23.5651	6	0.0006

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.08605	0.21943	0.1538	0.6949	1.090
dob2	1	0.38780	0.25363	2.3378	0.1263	1.474
dob3	1	0.47303	0.31528	2.2509	0.1335	1.605
LCUMEXP15	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

(x) log cumulative exposure model, 20-year lag

Criterion	Without Covariates	With Covariates
-2 LOG L	1967.813	1947.020
AIC	1967.813	1959.020
SBC	1967.813	1979.700

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	20.7930	6	0.0020
Score	21.5306	6	0.0015
Wald	21.1847	6	0.0017

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
dob1	1	0.10961	0.22008	0.2481	0.6184	1.116
dob2	1	0.46136	0.25203	3.3509	0.0672	1.586
dob3	1	0.61353	0.30969	3.9248	0.0476	1.847
LCUMEXP20	1	0.02970	0.02151	1.9068	0.1673	1.030
PARITY1	1	-0.26623	0.18642	2.0397	0.1532	0.766
FREL_BR_CAN1	1	0.47060	0.17925	6.8927	0.0087	1.601

D.2. BREAST CANCER MORTALITY

a. Exposure distribution among women and breast cancer deaths in the cohort mortality study (*n* = 9544)

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. Below is the distribution of the 102 breast cancer deaths used in the analysis. The cut points are those used in the published data (Steenland et al., 2004).

Table D-2a. 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
647–2779 ppm-days	16
2780–12321 ppm-days	15
>12321 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.

Regarding the women in the cohort as a whole, cumulative exposure at the end of follow-up, with no lag, had a mean of 8.2 ppm-years, with a standard deviation of 38.2. This distribution was highly skewed; the median was 4.6 ppm-years.

b. Results of Cox regression analysis of breast cancer mortality using a variety of log RR models

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

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, a log transform model, and a cubic spline model. We also ran a number of analogous models using linear RR models (Section D-2.c below).

The categorical log RR model for breast cancer mortality was run using the originally published cut points to form four categories above the lagged-out group, as shown in Table D-2a. To graph the categorical points, each category was assigned the mid-point of the category as its exposure level, except for the last one which was assigned 50% more than the last cut point 12,322 ppm-days.

For the 2-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 (Figure D-2a). We also explored knots beyond 7,000 ppm-days by looking at increments of 1,000 ppm-days from 0 to 25,000 (Figure D-2a shows the results for knots up to 15,000 ppm-days). None of these outperformed the knot at 700 ppm-days, although Figure D-2a' suggests a local maximum likelihood near 13,000 ppm-days.

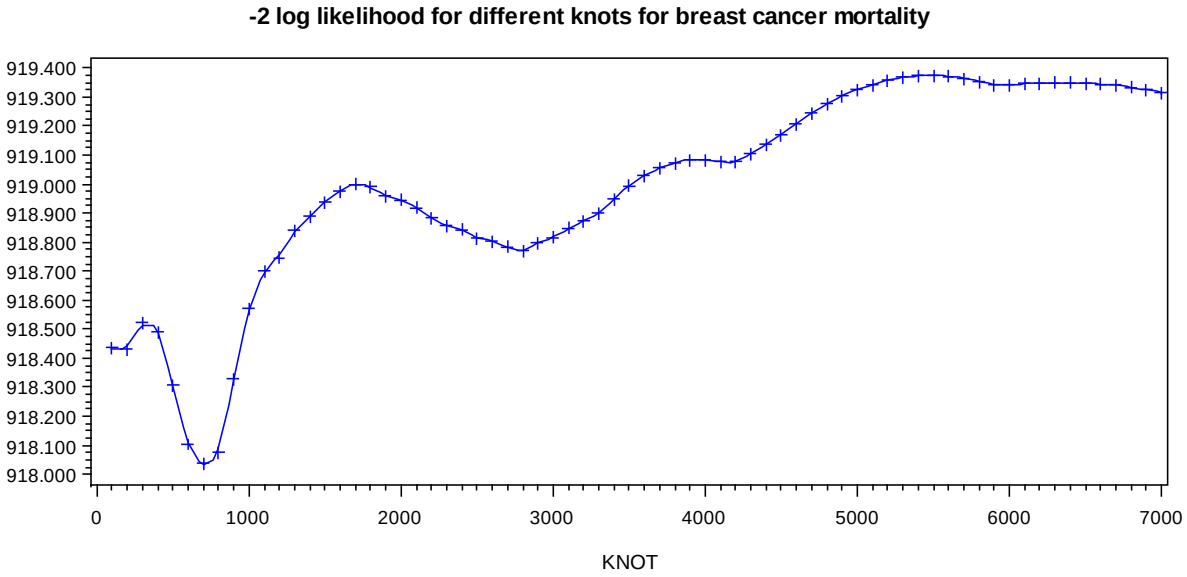

Figure D-2a. Likelihoods vs knots for the 2-piece log-linear model, breast cancer mortality.

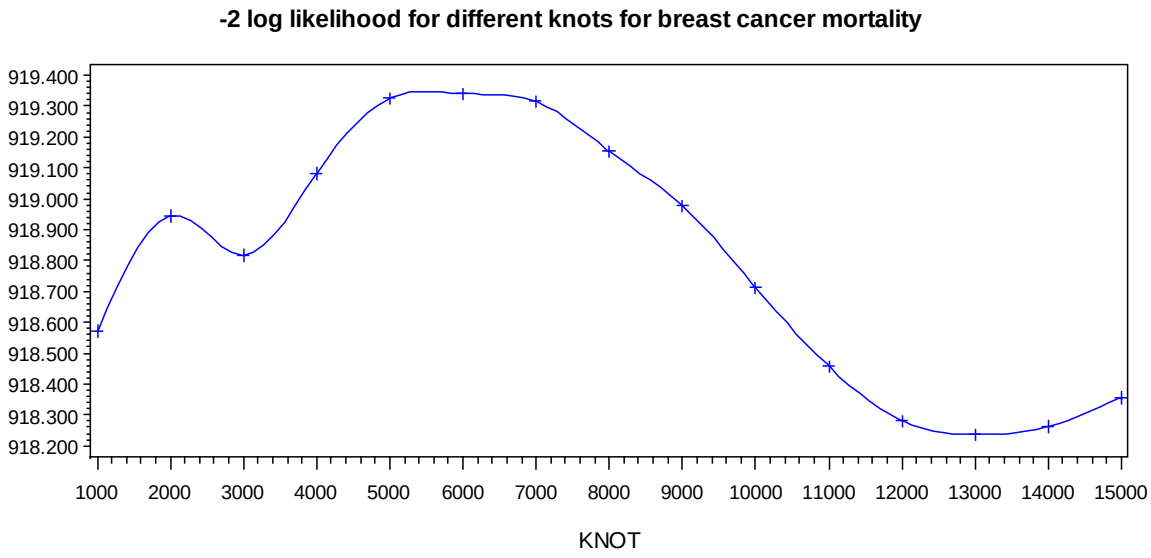

Figure D-2a′. Likelihoods vs knots for the 2-piece log-linear model, breast cancer mortality.

In Figure D-2b below, we show the categorical and 2-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 it is responsible for the attenuation seen in the exposure-response at high exposures.

The 2-piece log-linear spline model in Figure D-2b 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).

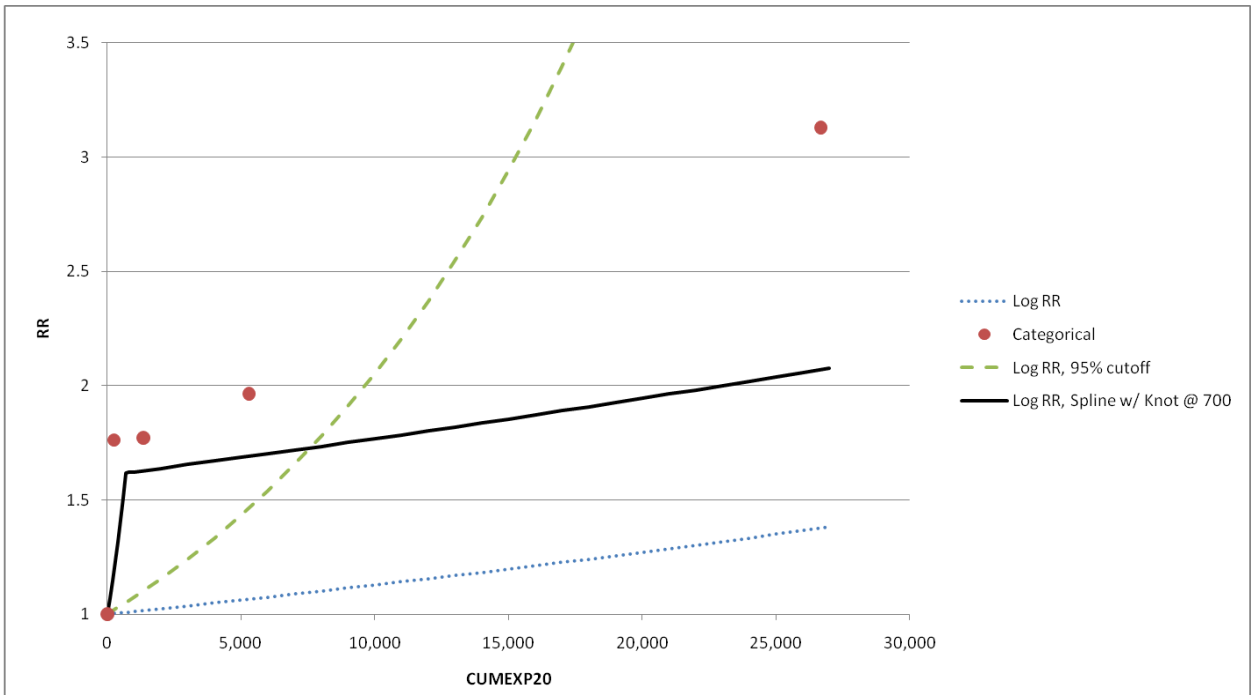

Figure D-2b. Plot of the dose-response relationship of continuous exposure (lagged 20 years) for breast cancer mortality, with the 2-piece log-linear spline, the categorical, and the log-linear RR models (labeled "log RR"). Also shown is the log-linear curve (log RR = β × cumexp20) after cutting out the top 5% of exposure subjects ('log RR 95% cutoff').

For comparison purposes, we also show the logarithmic transformation log RR model in Figure D-2c (which we have not used for risk assessment because it is supralinear in the low dose region).

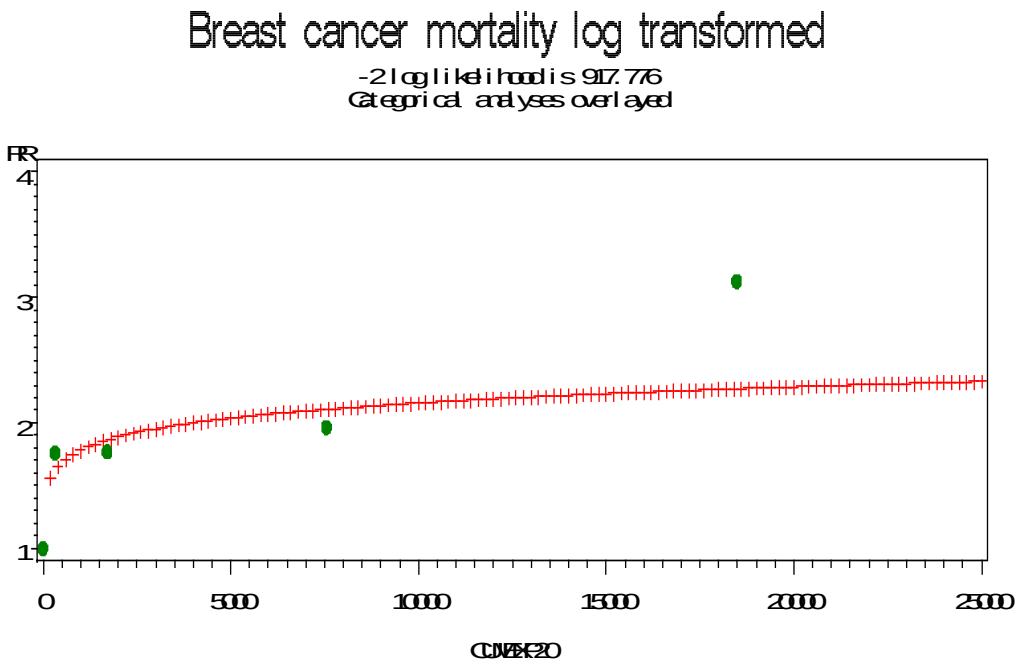

Figure D-2c. Plot of the dose-response relationship of continuous exposure (lagged 20 years) for breast cancer mortality, generated using a logarithmic transformation log RR model. Dots that represent the effect of exposure grouped in categories are also presented in the plot.

Outputs from the categorical, 2-piece log-linear spline, and log-linear RR models are given below. The 2-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, judged by conformity with categorical and cubic spline analysis, and may still be useful for risk assessment.

Table D-2b. Categorical output breast cancer mortality, 20-year lag (log RR model)

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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	7.9244	4	0.0944
Score	8.5160	4	0.0744
Wald	8.3993	4	0.0780

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	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-2c. 2-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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.3967	2	0.0673
Score	6.0153	2	0.0494
Wald	5.8857	2	0.0527

Analysis of Maximum Likelihood Estimates

Parameter	Parameter Estimate	Standard Error	Chi-Square	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

*covariance lin0 and lin1 -1.75×10^{-7}

Table D-2d. 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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	2.7865	1	0.0951
Score	3.7383	1	0.0532
Wald	3.6046	1	0.0576

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CUMEXP20	0.0000122	6.40812E-6	3.6046	0.0576	1.000

Table D-2e. 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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.6908	1	0.0171
Score	5.7676	1	0.0163
Wald	5.7688	1	0.0163

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
lcum20	1	0.08376	0.03487	5.7688	0.0163	1.087

Table D-2f. 2-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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.1963	2	0.0744
Score	5.9044	2	0.0522
Wald	5.7813	2	0.0555

Analysis of Maximum Likelihood Estimates

Variable	Parameter Estimate	Standard Error	Chi-Square	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

c. Linear relative risk models for breast cancer mortality

Finally, we also ran linear RR models for these data, as shown in Figure D-2d below (denoted "ERR" models), which also includes the RRs from the log RR categorical model as shown in other graphs. Again, the linear curve, highly influenced by the upper 5% tail of exposure, underestimates the categorical points, while the log transform and 2-piece spline capture better the initial increase in risk followed by an attenuation. Parameter estimates for these models can be found in Table D-2g.

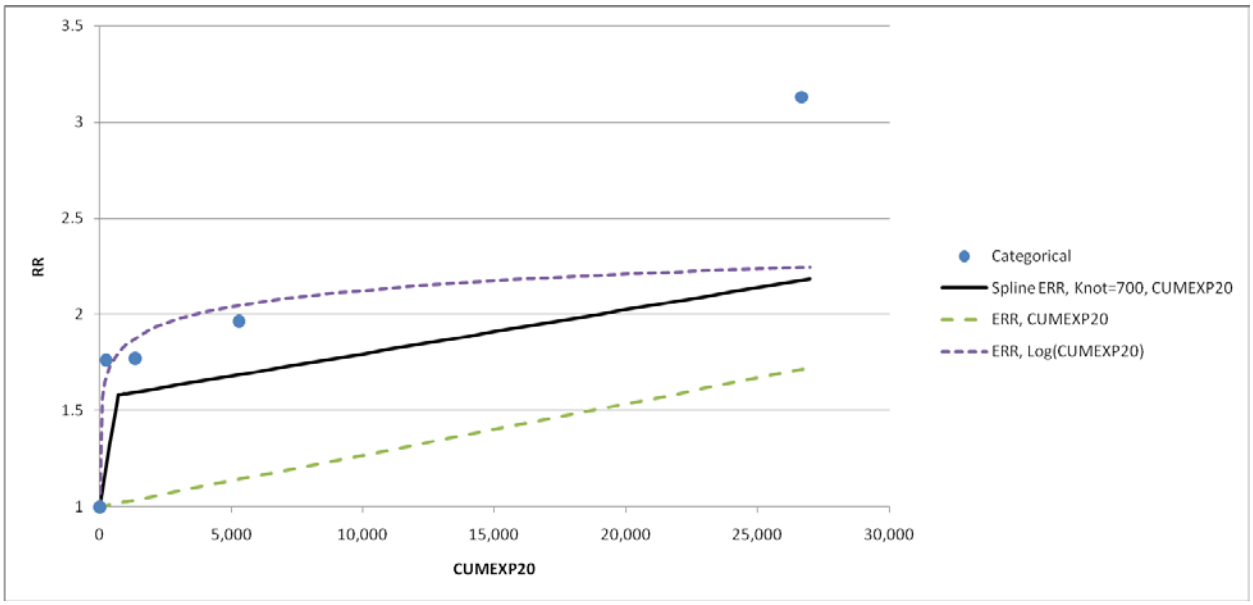

Figure D-2d. Linear RR models for breast cancer mortality. [Editorial note: "ERR" refers to linear RR models.]

Table D-2g. Model results for breast cancer mortality, linear RR models[b]

Linear RR Model	Parameter(s)	SE	–2 Log Likelihood
CUMEXP20	B = 0.000026779	0.000021537	920.122
Log(CUMEXP20)	B = 0.122090	SE = 0.061659	917.841
Spline, knot = 700, CUMEXP20[a]	B1 = 0.000830, B2 = –0.000807	SE1 = 0.000614, SE2 = 0.000619	918.058

[a]Covariance 2 pieces of spline, -3.80×10^{-7}.

[b]Editorial note: As discussed in footnotes i and j of Table 4-7 in Section 4.1.2.3, 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, EPA also evaluated application of a profile likelihood approach for the linear RR models (Langholz and Richardson, 2010), which allows for asymmetric CIs, for comparison with the Wald approach. The unit risk estimate for breast cancer mortality presented in this assessment does not rely on any of the linear RR models, thus revised CIs calculated using the profile likelihood method are not shown here.

d. Risk assessment for breast cancer mortality using the 2-piece log-linear spline model

We next used the 95% upper bound of the coefficient for the 1st piece of the 2-piece log-linear model from Table D-2c, which is 0.0006877 + 1.64 × 0.0004171, to calculate the LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their 2006 draft risk assessment. Here we used the same data on background breast cancer mortality and background all cause mortality as used by EPA in their 2006 calculations. The rate ratio, then, as a function of exposure, is $RR = e^{(0.00137 \times cumexp20)}$. Note that the 2-piece log-linear model is linear for the log of the rate ratio. Once this is exponentiated, it is no longer strictly linear, but is still approximately so, as can be seen in Figure D-2b.

Use of this function in the life-table analysis results in an excess risk of 0.01 when the daily exposure is 0.0048 ppm, which is the LEC_{01}. This is substantially lower than the previous LEC_{01} of 0.0195 ppm in EPA's 2006 draft risk assessment [U.S. EPA (2006a), Table 12].

Similar calculations were done to derive the EC_{01} which was 0.0095 ppm.

e. Risk assessment for breast cancer mortality using the 2-piece linear spline model.

The slope of the first segment of the 2-piece linear model was 21% higher than the slope of the corresponding 2-piece log-linear spline (knot at 700 ppm-days). The slope coefficient was 0.0008300, with a std. err. of 0.000614. The resulting EC_{01} and LEC_{01} were 0.0080 and 0.0037 ppm, respectively.

D.3. LYMPHOID CANCER MORTALITY (SUBSET OF ALL HEMATOPOIETIC CANCERS COMBINED) (*N* = 18,235).

a. 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.

As noted in Section D.1.a, 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.a 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 cut points as in the publication. Lymphoid cancer consists of nonHodgkin lymphoma, lymphocytic leukemia, and myeloma (ICD-9 200, 202, 203, 204). The distribution of cases for lymphoid cancer mortality is seen below.

Table D-3a. 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
>0–1200 ppm-days	2	8	10
1201–3680 ppm-days	4	7	11
3681–13,500 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.

b. Results of Cox regression analysis of lymphoid cancer mortality using categorical, 2-piece linear, log transform, and linear log RR models

While the published results in Steenland et al. (2004) focused on males [Table 7 in Steenland et al. (2004)], in fact males and females do not differ greatly in categorical results using a 15-year lag. A formal chunk test for four interaction terms between exposure and gender is not close to significance ($p = 0.58$), although such tests are not very powerful in the face of sparse data such as these. Table D-3b below shows the categorical odds ratio results for men

and women separately and combined. In the analyses presented here, males and female are combined.

Table D-3b. 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
>0–1200 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, gender, and race.

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, and a log transform model. We also ran a number of analogous models using linear RR models (Section D-3.c below).

The categorical log RR model for lymphoid cancer mortality was run using the originally published cut points to form four categories above the lagged-out group, as shown in Table D-3b. To graph the categorical points, each category was assigned the mid-point of the category as its exposure level, except for the last one which was assigned 50% more than the last cut point.

For the 2-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-3a below shows the likelihood vs the knots. Figure D-3a also suggests a local maximum likelihood near 1600 ppm-days. Figure D-3b shows the log RR models.

Model results for the categorical and 2-piece linear log RR models are shown in Tables D-3c and D-3d. Tables D-3e and D-3f give the results for the log transform model and linear log RR models; the latter does not fit the data well. Table D-3g shows the model results for the 2-piece log-linear spine model with the knot at the local maximum likelihood of 1600 ppm-days.

Figure D-3b shows the graphical results for the categorical, 2-piece linear, and log transform log RR models. There is a very steep increase in risk at very low exposures. The knot for the 2-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 (e.g., only 19 cases in the non-exposed lagged-out referent group and the lowest cumulative exposure group, up to 1200 ppm-days, combined).

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 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.

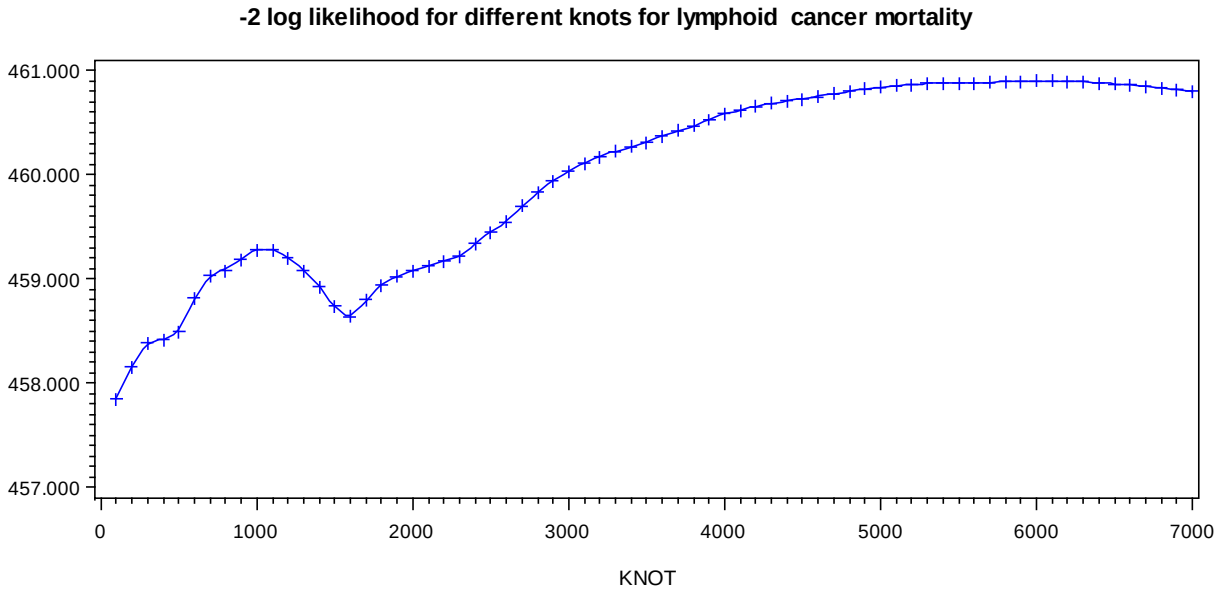

Figure D-3a. Likelihoods vs. knots for 2-piece log-linear model, lymphoid cancer mortality.

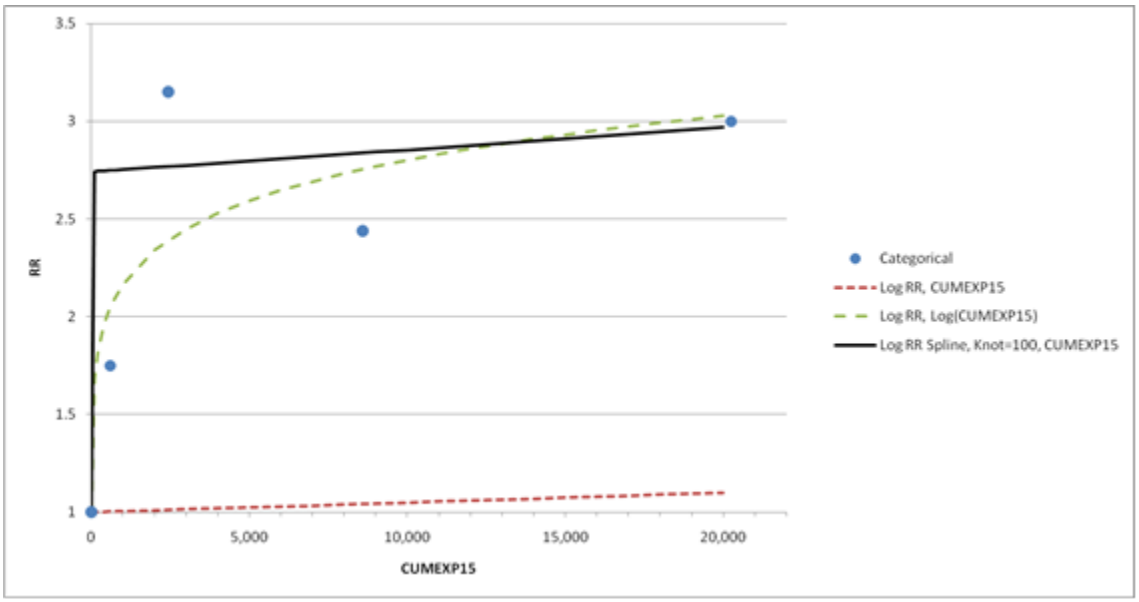

Figure D-3b. Plot of the exposure and lymphoid cancer mortality rate ratios generated using a 2-piece log-linear spline model overlaid with other log RR curves and categorical log RR model points.

Table D-3c. Categorical results for lymphoid cancer mortality (log RR model), men and women combined

Model Fit Statistics

Criterion	Without Covariates	With Covariates
-2 LOG L	463.912	458.069
AIC	463.912	466.069
SBC	463.912	473.950

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.8435	4	0.2111
Score	5.7397	4	0.2195
Wald	5.6220	4	0.2292

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	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

Table D-3d. Results of 2-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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	6.0658	2	0.0482
Score	5.9648	2	0.0507
Wald	5.8246	2	0.0544

Analysis of Maximum Likelihood Estimates

Parameter	Parameter Estimate	Standard Error	Chi-Square	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-3e. 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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.4868	1	0.0192
Score	5.3479	1	0.0207
Wald	5.2936	1	0.0214

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
lcum15	1	0.11184	0.04861	5.2936	0.0214	1.118

Table D-3f. 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

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	1.4998	1	0.2207
Score	2.0403	1	0.1532
Wald	1.9959	1	0.1577

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CUMEXP15	1	4.73679E-6	3.35285E-6	1.9959	0.1577	1.000

Table D-3g. Results of 2-piece log-linear spline model for lymphoid cancer mortality, men and women combined, knot at 1600 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

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	5.2722	2	0.0716
Score	5.2666	2	0.0718
Wald	5.1436	2	0.0764

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	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

c. Results for linear relative risk models

Table D-3h shows the model fit statistics and coefficients for the linear RR models (Supplemental Results). Results for linear RR models are seen in Figure D-3c (denoted as "ERR" models). They are quite similar to the log RR results in Figure D-2b. Again there is a very steep rise in the exposure-response curve at very low exposures. The knot for the 2-piece linear curve is again at 100 ppm-days.

Table D-3h. Supplemental Results: 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)	SE[d]
CUMEXP15	461.62	463.62	0.13	B = 0.00001226	SE = 0.00001214
Log(CUMEXP15)	458.54	460.54	0.02	B = 0.2083	SE = 0.1434
Spline, knot = 100, CUMEXP15[b,c]	457.48	461.48	0.04	B1 = 0.010090 B2 = –0.010086	SE1 = 0.004458 SE2 = 0.004458

[a]From likelihood ratio test.
[b]Covariance of 2 pieces of linear spline: -2.52×10^{-5}.
[c]For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × (exp – knot)). For the 95% upper confidence limit, for exposures below the knot, RR = 1 + ((β1+ 1.645 × SE1) × exp); for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp-knot) + 1.645 × sqrt(exp^2 × var1 + $(exp\text{-}knot)^2$ × var2 + 2 × exp × (exp-knot) × covar)), where exp = cumulative exposure, var = variance, covar = covariance.
[d]Editorial note: Confidence intervals for linear RR models, in contrast to those for the log-linear RR models, may not be symmetrical. 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.

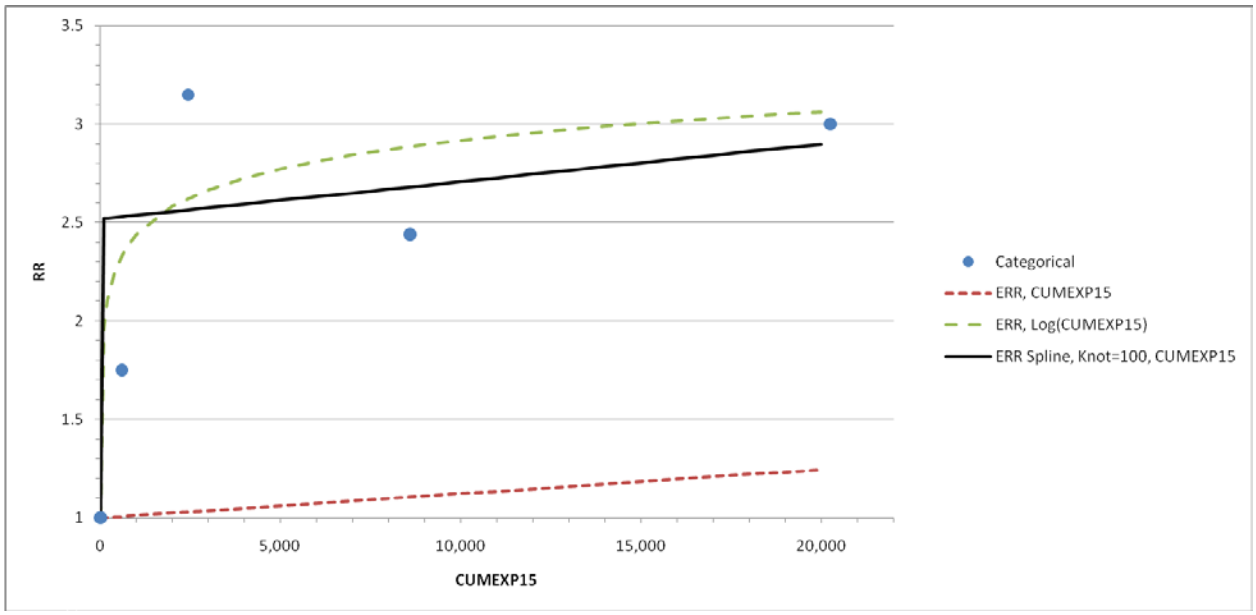

Figure D-3c. Linear RR models for lymphoid cancer. [Editorial note: "ERR" refers to linear RR models.]

d. Risk assessment for all lymphoid cancer mortality using the 2-piece log-linear spline model

We consider that none of the parametric models (either log RR or linear RR) generated for the lymphoid cancer data (and the same is true for all hematopoietic cancer) are suitable for EPA risk assessment because of the overly steep exposure-response relationship in the low-dose range for the 2-piece models and log transform models (highly influenced by the sparse number of deaths in the low-exposure region), and the overly shallow exposure-response relationship for the linear and log-linear models, which are influenced highly by the upper tail of exposures. A reasonable alternative approach is a weighted regression through the categorical points (excluding the highest exposure group), an approach adopted originally by EPA.

Nonetheless, we have used the 2-piece log-linear model to calculate the LEC_{01} and the EC_{01}, by way of illustrating the effect of the very steep exposure-response curve in the low-dose region.

We used the 95% upper bound of the coefficient for the 1st piece of the 2-piece log-linear model from Table D-3d, which is $0.01010 + 1.64 \times 0.00493$, to calculate the LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their 2006 draft risk assessment. Here we used the same data on lymphoid cancer mortality and background all-cause mortality as used by EPA in their 2006 calculations. The predicted rate ratio, then, as a function of exposure, is $RR = e^{((0.01010 + 1.64 \times 0.00493) \times cumexp15)}$. Use of this RR model in the life-table analysis results in an excess risk of 0.01 when the daily exposure (15-year lag) is 0.0006 ppm, which is the LEC_{01}. This is much lower than the previous LEC_{01} of 0.0165 ppm for lymphoid cancer mortality in EPA's 2006 draft risk assessment [U.S. EPA (2006a), Table 9].

A similar calculation was done for the EC_{01}, which resulted in a value of 0.0012 ppm.

e. Supplemental results: results for log cumulative exposure Cox regression models with no lag

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	Chi-Square	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	Chi-Square	Pr > ChiSq	Hazard Ratio
lcumexp	1	0.10230	0.07515	1.8530	0.1734	1.108

D.4. HEMATOPOIETIC CANCER MORTALITY (ALL HEMATOPOIETIC CANCERS COMBINED)

a. 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 cut points as in that publication. The distribution of cases for hematopoietic cancer mortality is seen below.

Table D-4a. Exposure categories and case distribution for hematopoietic cancer mortality

Cumulative exposure, 15 year lag	Male hematopoietic cancer deaths	Female hematopoietic cancer deaths	Total hematopoietic cancer deaths
0 (Lagged out)	9	4	13
>0–1200 ppm-days	4	13	17
1201–3680 ppm-days	5	10	15
3681–13,500 ppm-days	8	7	15
>13,500 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.

b. Results of Cox regression analysis of hematopoietic cancer mortality using categorical, 2-piece linear, linear and log transform log RR models

While the published results of these data in Steenland et al. (2004) focused on males [Table 8 in Steenland et al. (2004)], in fact males and females do not differ greatly in categorical results using a 15-year lag. A formal chunk test for four interaction terms between exposure and gender is not close to significance (chi square 4.5, 4 df; $p = 0.34$), although such tests are not very powerful in the face of sparse data such as these. Table D-4b 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-4b. All hematopoietic cancer mortality categorical results by sex (log RR model)

Cumulative exposure, 15 year lag	Odds ratio (95% CI) males	Odds ratio (95% CI) females	Odds ratio (95% CI) combined
0 (Lagged out)	1.00	1.00	1.00
>0–1200 ppm-days	1.23 (0.32–4.74)	3.76 (1.01–17.23)	2.33 (0.93–5.86)
1201–3680 ppm-days	2.53 (0.69–9.27)	4.93 (1.01–23.99)	3.46 (1.33–8.95)
3681–13,500 ppm-days	3.14 (0.95–10.37)	3.31,(0.64–17.16)	3.02 (1.16–7.89)
>13,500 ppm-days	3.42 (1.09–10.73)	2.11 (0.33–13.74)	2.96 (1.12–7.81)

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

Using log RR models, we used a categorical model, a linear model, a 2-piece linear model, and a log transform model. We also ran a number of analogous models using linear RR models (Section D-4.c below).

The categorical log RR model for hematopoietic cancer mortality was run using the originally published cut points to form four categories above the lagged-out group, as shown in Table D-4b. To graph the categorical points, each category was assigned the mid-point of the category as its exposure level, except for the last one which was assigned 50% more than the last cut point.

For the 2-piece log-linear model, the single knot was chosen based on a comparison of likelihoods assessed every 100 ppm-days from 0 to 7,000 ppm-days. The best likelihood was at 500 ppm-days (Figure D-4a). In Figure D-4b below we show the categorical, 2-piece linear spline and log transform log RR model results.

Model results for the categorical and 2-piece linear log RR models are shown in Tables D-4c and D-4d, and the results of the log transform and linear log RR models in Table D-4e and Table D-4f. Again the 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.

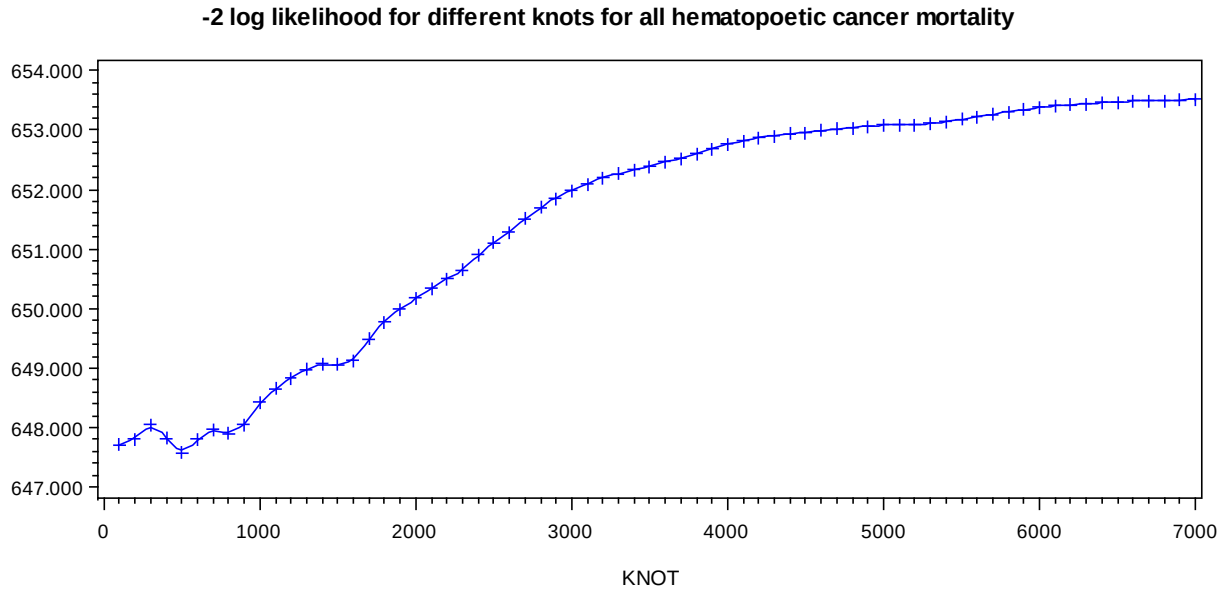

Figure D-4a. Likelihood vs. knots for 2-piece log-linear model, all hematopoietic cancer.

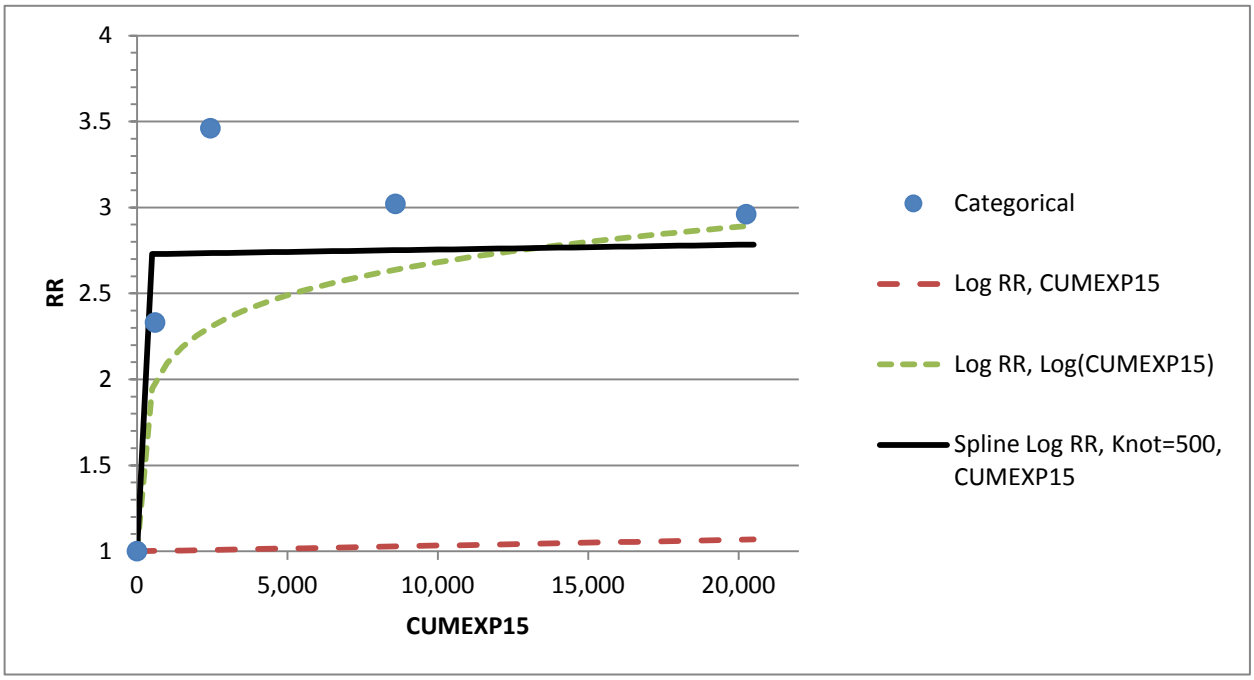

Figure D-4b. Plot of exposure and rate ratios for all hematopoietic cancer generated using a 2-piece log-linear spline model and log transform, linear, and categorical log RR models.

Table D-4c. Categorical results for all hematopoietic cancer mortality (log RR model), 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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	7.8371	4	0.0977
Score	7.3994	4	0.1162
Wald	7.2354	4	0.1240

Analysis of Maximum Likelihood Estimates

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	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-4d. Results of 2-piece log-linear spline 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	647.581
AIC	655.643	651.581
SBC	655.643	656.189

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	8.0615	2	0.0178
Score	7.5092	2	0.0234
Wald	7.3467	2	0.0254

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
spl1	1	0.00201	0.0007731	6.7457	0.0094	1.002
spl2	1	-0.00201	0.0007738	6.7249	0.0095	0.998

Table D-4e. 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	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	6.8177	1	0.0090
Score	6.6260	1	0.0100
Wald	6.5593	1	0.0104

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
lcum15	1	0.10706	0.04180	6.5593	0.0104	1.113

Table D-4f. 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	654.922
AIC	655.643	656.922
SBC	655.643	659.226

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	0.7213	1	0.3957
Score	0.8783	1	0.3487
Wald	0.8739	1	0.3499

Analysis of Maximum Likelihood Estimates

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio
CUMEXP15	1	3.26052E-6	3.48788E-6	0.8739	0.3499	1.000

c. Results for linear relative risk models for hematopoietic cancer mortality

For completeness, we also present the results of the linear RR models below (Table D-4g and Figure D-4c; 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 2-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-4g. Supplemental Results: 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[d]
CUMEXP15	654.64	656.64	0.32	B = 0.000006257	SE = 0.000008187
Log(CUMEXP15)	648.13	650.13	0.006	B = 0.2322	SE = 0.1437
Spline, knot = 100, CUMEXP15[b,c]	646.95	650.95	0.01	B1 = 0.003673 B2 = –0.003668	SE1 = 0.002345 SE2 = 0.002345

[a]From likelihood ratio test.
[b]Covariance of 2 pieces of linear spline: -5.70×10^{-6}.
[c]For the maximum likelihood estimate, for exposures below the knot, RR = 1 + (B1 × exp); for exposures above the knot, RR = 1 + (B1 × exp + B2 × (exp – knot)). For the 95% upper confidence limit, for exposures below the knot, RR = 1 + ((β1+ 1.645 × SE1) × exp); for exposures above the knot, RR = 1 + (β1 × exp + β2 × (exp-knot) + 1.645 × sqrt(exp^2 × var1 + (exp-knot)^2 × var2 + 2 × exp × (exp-knot) × covar)), where exp = cumulative exposure, var = variance, covar = covariance.
[d]Editorial note: Confidence intervals for linear RR models, in contrast to those for the log-linear RR models, may not be symmetrical. 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.

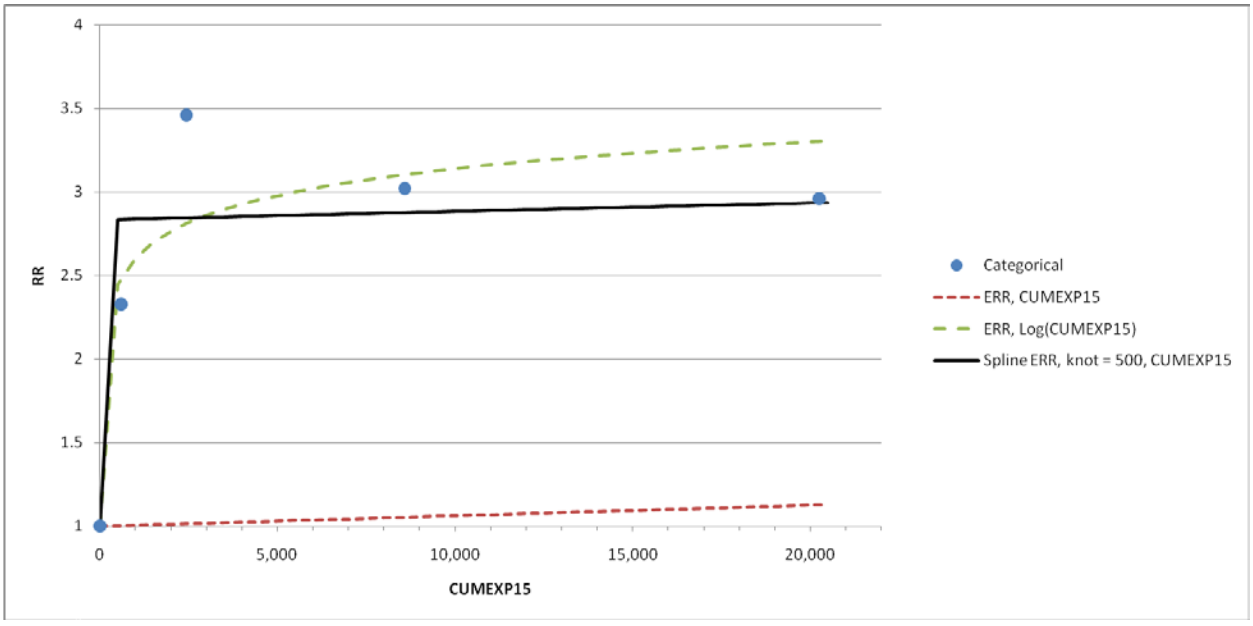

Figure D-4c. Linear RR models for hematopoietic cancer mortality.
[Editorial note: "ERR" refers to linear RR models.]

d. Risk assessment for all hematopoietic cancer mortality using the 2-piece log-linear spline model

As was the case for lymphoid cancer (which is a subset of the hematopoietic cancers), we consider that none of the parametric models (either log RR or linear RR) generated for the hematopoietic cancer data are suitable for EPA risk assessment because of the overly steep exposure-response relationship in the low-dose range for the 2-piece models and the log transform models (highly influenced by the sparse number of deaths in the low-exposure region), and the overly shallow exposure-response relationship for the linear models, which are influenced highly by the upper tail of exposures. A reasonable alternative approach is a weighted regression through the categorical points (excluding the highest exposure group), an approach adopted originally by EPA.

Nonetheless, we have used the 2-piece log-linear model to calculate the LEC_{01} and the EC_{01}, by way of illustrating the effect of the very steep exposure-response curve in the low-dose region.

We used the 95% upper bound of the coefficient for the 1st piece of the 2-piece log-linear model from Table D-4d, which is 0.00201 + 1.64 × 0.000773, or 0.003277, to calculate the predicted LEC_{01} via the life-table analysis of excess risk used by EPA in Appendix C of their 2006 draft risk assessment. Again, here we used the data on hematopoietic cancer mortality and background all-cause mortality as used in EPA's 2006 calculations. The predicted RR, then, as a function of exposure, is $RR = e^{(0.003277 \times cumexp15)}$ (up to the knot of 500 ppm-days).

This results in an excess risk of 0.01 when the daily exposure (15-year lag) is 0.0032 ppm, which is the LEC_{01}. This is notably lower than the previous LEC_{01} of 0.0109 ppm for hematopoietic cancer mortality in EPA's 2006 draft risk assessment [U.S. EPA (2006a), Table 7].

Similar calculations were done for the EC_{01}, which resulted in a value of 0.0043 ppm.

D.5. SUMMARY TABLE OF EC_{01}S FOR DIFFERENT OUTCOMES, USING 2-PIECE SPLINE MODELS

Table D-5 below provides a summary of the current findings for EC_{01} and the prior EPA findings for EC_{01}.

In general, findings are similar. As described above, the EC_{01} values based on the 2-piece spline models were obtained by multiplying the background cancer rate by $e^{(beta \times cumexp)}$ for log RR models or by (1 + beta × cumexp) for linear RR models, where the beta coefficient was for the first piece of the 2-piece linear models, and cumexp was determined such that a daily exposure would result in an excess risk of 1% above background, with risk calculated through age 85 years (BIER methodology, spreadsheet obtained from EPA). In the case of breast cancer incidence, following EPA's methods in the risk assessment, the life-table values for all-cause mortality (within each 5-year age interval) were adjusted to account for incident cases being withdrawn from the pool at risk entering the next age interval, by adding the breast cancer incidence rate to the all-cause mortality rate and then subtracting breast cancer mortality rate so that fatal breast cancer cases are not "counted" twice in this adjustment.

As noted above, we believe the 2-piece spline models (either log RR or linear RR versions are reasonable bases for risk assessment for the breast cancer incidence and mortality data. They also result in EC_{01} values that are lower than but in the ballpark of the previous EPA estimates using weighted regression for categorical points, excluding the highest exposure quintile. However, this is not the case for the hematopoietic/lymphoid cancer data.

Table D-5a. Summary of EC_{01} results (in ppm) in current analysis and previous EPA risk assessment

	U.S. EPA (2006a) EC_{01}[a]	Steenland[a] LEC_{01} 2-piece spline	Steenland EC_{01} 2-piece spline
Breast cancer incidence[b] (log RR model, 15-year lag)	0.0238	0.009	0.0152
Breast cancer incidence (linear RR model, 15-year lag)[b]	--	0.0052	0.0100
Breast cancer mortality (log RR model, 20-year lag)	0.0387	0.0048	0.0096
Breast cancer mortality (linear RR model, 20-year lag)	--	0.0037	0.0080
Hematopoietic cancer mortality (log RR model, 15-yr lag)[c]	0.0238	0.0032	0.0043[d]
lymphoid cancer mortality (log RR model, 15-yr lag)[c]	0.0427	0.0006	0.0012[e]

[a]EPA uses regression through categorical points (U.S. EPA, 2006a), Steenland uses 2-piece spline models.
[b]Breast cancer incidence for the subgroup with interviews, see Steenland et al. (2004).
[c]For hematopoietic and lymphoid cancer, EPA EC_{01} calculated for males only, Steenland includes both men and women.
[d]Using at knot at 500 ppm-days. 2-piece linear RR model results similar but not presented.
[e]Using knot at 100 ppm-days. 2-piece linear RR model results similar but not presented.

D.6. SENSITIVITY OF 2-PIECE SPLINE CURVES TO PLACEMENT OF KNOT

By way of sensitivity analysis, we ran 2-piece log-linear models for all breast cancer incidence with knots chosen at 5000, 5800 (optimal) and 7000 ppm-days, and for hematopoietic cancer mortality for knots of 500 (optimal) and 1000. Results show the relatively large sensitivity to the knot placement in the EC_{01}.

Table D-6a. Exposure-response coefficients and EC_{01}s based on selection of different knots, using 2-piece log-linear models

	Coefficient first piece	–2 log-likelihood[b]	EC_{01}
Breast cancer incidence knot at 5000 ppm-days	0.0000860	1940.6	0.0133
Breast cancer incidence knot at 5800 ppm-days[a]	0.0000770	1940.5	0.0151
Breast cancer incidence knot at 7000 ppm-days	0.0000653	1940.7	0.0176
Hematopoietic cancer mortality knot at 500 ppm-days	0.00201	647.6	0.0043
Hematopoietic cancer mortality knot at 1000 ppm-days	0.00089	648.4	0.0098

[a]Knot used in analysis.
[b]Lower numbers equal better fit, linear RR model likelihoods not comparable to log RR likelihoods and are not shown here.

D.7. POSSIBLE INFLUENCE OF THE HEALTHY WORKER SURVIVOR EFFECT

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

D.8. POSSIBLE INFLUENCE OF EXPOSURE MIS-MEASUREMENT

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, such as used here, 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.

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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 environmental exposure to 0.0114 ppm (the LEC_{01} for lymphoid cancer incidence)[b] using the weighted linear regression model based on the categorical cumulative exposure results of Steenland et al. (2004), reanalyzed by Steenland for both sexes combined (see Appendix D of this assessment), with a 15-year lag, as described in Section 4.1.1

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	Age interval	All cause mortality ($\times 10^5$/yr)	lymphoid cancer incidence ($\times 10^5$/yr)	All cause hazard rate (h*)	Prob of surviving interval (q)	Prob of surviving up to interval (S)	lymphoid cancer hazard rate (h)	Cond prob of lymphoid cancer incidence in interval (R0)	Exp duration mid interval (xtime)	Cum exp mid interval (xdose)	Exposed lymphoid cancer hazard rate (hx)	Exposed all cause hazard rate (h*x)	Exposed prob of surviving interval (qx)	Exposed prob of surviving up to interval (Sx)	Exposed cond prob of lymphoid cancer in interval (Rx)
1	<1	685.2	1.9	0.0069	0.9932	1.0000	0.0000	0.00002	0	0.00	0.00002	0.0069	0.9932	1.0000	0.00002
2	1–4	29.9	8.1	0.0012	0.9988	0.9932	0.0003	0.00032	0	0.00	0.00032	0.0012	0.9988	0.9932	0.00032
3	5–9	14.7	4.2	0.0007	0.9993	0.9920	0.0002	0.00021	0	0.00	0.00021	0.0007	0.9993	0.9920	0.00021
4	10–14	18.7	3.2	0.0009	0.9991	0.9913	0.0002	0.00016	0	0.00	0.00016	0.0009	0.9991	0.9913	0.00016
5	15–19	66.1	3.5	0.0033	0.9967	0.9903	0.0002	0.00017	2.5	31.64	0.00018	0.0033	0.9967	0.9903	0.00018
6	20–24	94	3.2	0.0047	0.9953	0.9871	0.0002	0.00016	7.5	94.92	0.00017	0.0047	0.9953	0.9871	0.00017
7	25–29	96	4.1	0.0048	0.9952	0.9824	0.0002	0.00020	12.5	158.20	0.00022	0.0048	0.9952	0.9824	0.00022
8	30–34	107.9	6.0	0.0054	0.9946	0.9777	0.0003	0.00029	17.5	221.49	0.00034	0.0054	0.9946	0.9777	0.00033
9	35–39	151.7	9.0	0.0076	0.9924	0.9725	0.0005	0.00044	22.5	284.77	0.00052	0.0077	0.9924	0.9724	0.00050
10	40–44	231.7	13.2	0.0116	0.9885	0.9651	0.0007	0.00063	27.5	348.05	0.00079	0.0117	0.9884	0.9650	0.00075
11	45–49	352.3	20.9	0.0176	0.9825	0.9540	0.0010	0.00099	32.5	411.33	0.00128	0.0179	0.9823	0.9538	0.00121
12	50–54	511.7	32.5	0.0256	0.9747	0.9373	0.0016	0.00150	37.5	474.61	0.00205	0.0260	0.9743	0.9369	0.00190
13	55–59	734.8	49.2	0.0367	0.9639	0.9137	0.0025	0.00221	42.5	537.90	0.00319	0.0375	0.9632	0.9128	0.00286
14	60–64	1140.1	70.1	0.0570	0.9446	0.8807	0.0035	0.00300	47.5	601.18	0.00467	0.0582	0.9435	0.8793	0.00399
15	65–69	1727.4	101.1	0.0864	0.9173	0.8319	0.0051	0.00403	52.5	664.46	0.00691	0.0882	0.9156	0.8296	0.00549
16	70–74	2676.4	128.7	0.1338	0.8747	0.7631	0.0064	0.00460	57.5	727.74	0.00902	0.1364	0.8725	0.7595	0.00640

Table E-1. Extra risk calculation[a] for environmental exposure to 0.0114 ppm (the LEC_{01} for lymphoid cancer incidence)[b] using the weighted linear regression model based on the categorical cumulative exposure results of Steenland et al. (2004), reanalyzed by Steenland for both sexes combined (see Appendix D of this assessment), with a 15-year lag, as described in Section 4.1.1 (continued)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Interval number (i)	**Age interval**	**All cause mortality ($\times 10^5$/yr)**	**lymphoid cancer incidence ($\times 10^5$/yr)**	**All cause hazard rate (h*)**	**Prob of surviving interval (q)**	**Prob of surviving up to interval (S)**	**lymphoid cancer hazard rate (h)**	**Cond prob of lymphoid cancer incidence in interval (R0)**	**Exp duration mid interval (xtime)**	**Cum exp mid interval (xdose)**	**Exposed lymphoid cancer hazard rate (hx)**	**Exposed all cause hazard rate (h*x)**	**Exposed prob of surviving interval (qx)**	**Exposed prob of surviving up to interval (Sx)**	**Exposed cond prob of lymphoid cancer in interval (Rx)**
17	75–59	4193.2	163.0	0.2097	0.8109	0.6675	0.0082	0.00491	62.5	791.02	0.01171	0.2132	0.8080	0.6627	0.00699
18	80–84	6717.2	179.8	0.3359	0.7147	0.5412	0.0090	0.00413	67.5	854.31	0.01323	0.3401	0.7117	0.5354	0.00601
							Ro =	0.02797						**Rx =**	0.03769
extra risk = (Rx−Ro)/(1−Ro) = 0.01001															

Column A: interval index number (i).
Column B: 5-yr age interval (except <1 and 1−4) up to age 85.
Column C: all-cause mortality rate for interval i ($\times 10^5$/yr) (2004 data from NCHS).
Column D: lymphoid cancer incidence rate for interval i ($\times 10^5$/yr) (2000−2004 SEER data).[c]
Column E: all-cause hazard rate for interval i (h^*_i) (= all-cause mortality rate × number of years in age interval).[d]
Column F: probability of surviving interval i (without being diagnosed with lymphoid cancer) (q_i) [= $\exp(-h^*_i)$]. 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).

Table E-1. Extra risk calculation[a] for environmental exposure to 0.0114 ppm (the LEC_{01} for lymphoid cancer incidence)[b] using the weighted linear regression model based on the categorical cumulative exposure results of Steenland et al. (2004), reanalyzed by Steenland for both sexes combined (see Appendix D of this assessment), with a 15-year lag, as described in Section 4.1.1 (continued)

Column J: exposure duration at midinterval (taking into account 15-yr lag) (xtime).
Column K: cumulative exposure midinterval (xdose) (= exposure level (i.e., 0.0114 ppm) × 365/240 × 20/10 × xtime × 365) [365/240 × 20/10 converts continuous environmental exposures to corresponding occupational exposures; xtime × 365 converts exposure duration in years to exposure duration in days).
Column L: lymphoid cancer incidence hazard rate in exposed people for interval i (hx_i) (= $h_i \times (1 + \beta \times xdose)$, where $\beta = 0.0002472 + (1.645 \times 0.0001854) = 0.0005522$) (0.0002472 per ppm × day is the regression coefficient obtained from the weighted linear regression model of the categorical results [see Section 4.1.1.2]). To estimate the LEC_{01}, i.e., the 95% lower bound on the continuous exposure giving an extra risk of 1%, the 95% upper bound on the regression coefficient is used, i.e., MLE + 1.645 × SE].
Column M: all-cause hazard rate in exposed people for interval i (h^*x_i) [= $h^*_i + (hx_i - h_i)$].
Column N: probability of surviving interval i (without being diagnosed with lymphoid cancer) for exposed people (qx_i) [= $\exp(-h^*x_i)$].
Column O: probability of surviving up to interval i (without having been diagnosed with lymphoid cancer) for exposed people (Sx_i) ($Sx_1 = 1$; $Sx_i = Sx_{i-1} \times qx_{i-1}$, for $i > 1$).
Column P: conditional probability of being diagnosed with lymphoid cancer in interval i for exposed people [= $(hx_i/h^*x_i) \times Sx_i \times (1-qx_i)$] (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]Background cancer incidence rates are used to estimate extra risks for cancer incidence under the assumption that the exposure-response relationship for cancer incidence is the same as that for cancer mortality (see Section 4.1.1.3).
[d]For the cancer incidence calculation, the all-cause hazard rate for interval i should technically be the rate of either dying of any cause or being diagnosed with the specific cancer during the interval, i.e., (the all-cause mortality rate for the interval + the cancer-specific incidence rate for the interval—the cancer-specific mortality rate for the interval [so that a cancer case isn't counted twice, i.e., upon diagnosis and upon death]) × number of years in interval. For the lymphoid cancer incidence calculations, this adjustment was ignored because the lymphoid cancer incidence rates are small when compared with the all-cause mortality rates. For the breast cancer incidence calculations, on the other hand, this adjustment was made in the all-cause hazard rate (see Section 4.1.2.3).
MLE = maximum likelihood estimate, SE = standard error.

APPENDIX F.
EQUATIONS USED FOR WEIGHTED LINEAR REGRESSION

[Source: Rothman (1986), p. 343−344]

linear model: $RR = 1 + bX$

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

b can be estimated from the following equation:

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

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):

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

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) mice 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 (chi-square goodness of fit)
Males						
Lung adenomas plus carcinomas	1	2.52×10^{-1}	1.52×10^{-2}			0.92
Females						
Lung adenomas plus carcinomas	2	3.87×10^{-2}	0.0	4.80×10^{-4}		0.39
Malignant lymphoma	3	1.74×10^{-1}	0.0	0.0	1.13×10^{-5}	0.18
Uterine carcinoma	2	0.0	0.0	9.80×10^{-5}		0.90
Mammary carcinoma	1[d]	2.27×10^{-2}	1.09×10^{-2}			–

[a]The exposure concentrations were at 0, 50 ppm, 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.
[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, (Lynch et al., 1984a; Lynch et al., 1984c) study of male F344 rats;[a] multistage model parameters

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

[a]The exposure concentrations were at 0, 50 ppm, 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.
[c]The 100-ppm dose was deleted; the fit was perfect with only two points to fit.

Table G-3. Analysis of grouped data, 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 (chi-square goodness of fit)
Males				
Splenic mononuclear cell leukemia	1	1.63×10^{-1}	8.56×10^{-3}	0.34
Testicular peritoneal mesothelioma	1	2.38×10^{-2}	4.74×10^{-3}	0.68
Primary brain tumors	1	5.88×10^{-3}	2.92×10^{-3}	0.46
Females				
Splenic mononuclear cell leukemia	1	1.08×10^{-1}	2.37×10^{-2}	0.75
Primary brain tumors	1	5.94×10^{-3}	1.65×10^{-3}	0.80

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

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

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

[a]The exposure concentrations were at 0, 50 ppm, and 100 ppm. These were adjusted to continuous exposure.
[b]$P(d, t) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ... + q_k d^k) \times (t - t_0)^z]$, 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 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 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 EPA's responses are also included in a separate section of this appendix.

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 (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 Panel 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, EPA is retaining the conclusion that the combined weight of the epidemiological, experimental 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]. EPA and the majority of the SAB Panel disagree that the epidemiological evidence is weak. EPA has strengthened the summary review of these data in the human evidence section (Section 3.1) and in the hazard characterization section (Section 3.5.1). In addition, the revised assessment specifically addresses the precursor data for rodents and humans, and while the databases for humans and rodents contain different types of studies, 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. 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, 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, EPA determined that the in-depth level of detail in Appendix A was not appropriate for the main body of the document. Instead, EPA has added two shorter summary tables of the lymphohematopoietic cancer (Table 3-1) and breast cancer (Table 3-2) findings in the various epidemiology studies to Section 3.1.1. 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.

EPA notes that the Panel agreed with 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 (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 thus 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 Panel 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 (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 (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) and 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 (Section 3.3.3.1 and Section C.1 of Appendix C). Section

C.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.

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.

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.

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

SAB Panel 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: EPA has expanded the discussion of DNA adduct formation (Section 3.3.3.1 and Section C.1 of Appendix C), mutagenicity (Section 3.3.3 and Sections C.2–C.5 of Appendix C), and possible mechanisms (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 Panel 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 (Section 3.5.1). To address the issue of precursor events, the genotoxicity (Section 3.3.3 and Appendix C) and mode of action (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, 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 (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 Panel 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: EPA reevaluated 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 (Appendix A, Section A.2.20) and NIOSH (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 co-exposures, 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, 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.

EPA requested that Professor Kyle Steenland, the principal investigator of the NIOSH study, respond to the following excerpt from this comment from the SAB Panel:

> "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 (e.g. log cumulative exposure with 15 year lag) that is the independent variable in that dose-response model."

***Professor Steenland's response**:*

"This comment is difficult to understand, but appears to be a concern that the 15-year lag in the exposure metric, which discounts the most recent exposure, may cause an over-reliance in the exposure-response analysis on exposures which were estimated prior to 1979, which possibly are less accurate. The reason they may be less accurate is because the NIOSH exposure model assumed that the effect of calendar year was constant before 1979. There are a couple of comments to be made here. First, it is certain the much higher exposures took place before the early 1980s when engineering controls were implemented, and that these exposures are likely to compose the majority of the metric "cumulative exposure." Second, such early exposures would often, but not always, also be more biologically relevant than later exposures, given that there is likely to be some latency period before a given exposure causes a cancer (the best fitting lag was 15 years in the analysis), and cancers occurred during the period 1980–2004, so that later lower exposures were often discounted by the lag. But were such early exposures estimated appreciably worse than later exposures by the NIOSH regression model? The NIOSH regression model was based on seven variables [addition by EPA: The seven variables were job, product type, sterilizer size, exhaust, aeration, time since product sterilization, and calendar year.], one of

which had 8 levels (job), one of which had 5 levels (product types), and one of which was time or year. All these variables were statistically significant at the $p < 0.05$ level except one (aeration), which had a p value of 0.10. Given that engineering controls were included in the model, the effect of calendar year was thought to reflect improved work practices which got better year by year as employees and managers became more conscious of the dangers of exposure. The effect of year only began in 1979, and was not apparent in the period 1975–1978 when there much less concern about the dangers of EtO. It would seem logical that prior to 1975 (when there were no sampling data to include in the model), work practices also would have changed little year to year, given that worker and management concern about the dangers of EtO was minimal or nonexistent. Furthermore, data for the other variables in the model were available for years before 1979, and hence were able to play a role in prediction of EtO prior to 1979, independent of the year effect, which was constant prior to 1979. Hence, the model would be expected to perform reasonably well in the period before sampling data were available, i.e., prior to 1975, regardless of the assumption that calendar year had no effect independent of the other variables in the model."

"In summary, there is obviously more uncertainty about the estimation of exposures prior to 1975 when there were no sampling data. This uncertainty is of some concern in the sense that the majority of cumulative exposure metric for most workers is probably contributed by earlier, higher exposures. The use of a 15-year lag does not, however, necessarily increase this uncertainty, given that exposure in the lagged out period for most workers would be appreciably lower than exposure before the lag came into effect. Furthermore, while the validity of the NIOSH estimates before 1975 cannot be tested against sampling data, the NIOSH model would be expected to permit reasonable estimation of exposure prior to 1975 based on other variables in the model (job, type of product, size of sterilizer, exhaust of sterilizer, etc.)."

"What if exposures prior to 1975 were estimated poorly? This raises the general question of measurement error, which is more likely to have occurred in years before sampling data existed. Measurement error is a complicated issue and its effects cannot be easily predicted. It does not seem likely that the use of the 15-year lag, however, would appreciably increase whatever measurement error occurred for early years of exposure before 1975. While it is possible that the EPA should formally evaluate the likely effect of measurement error, this is a large task which would take considerable amount of time and would necessarily depend on a large number of assumptions about the error in the period before sampling data existed (as I have argued, it is also largely independent of the use of a 15-year lag)."

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 Panel 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 EPA: more detailed comments provided by the SAB Panel about the linear regression approach and 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 EPA's responses to some of these issues are comparatively detailed; thus, 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, 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 EPA, in consultation with Dr. 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, 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 EPA's unit risk estimate for breast cancer incidence (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 (Section 4.1.1.2). In particular, the statistically significant models predicted extremely steep slopes in the low-dose region. Thus, 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 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, 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, 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, EPA again used a linear regression of the categorical data, excluding the highest exposure group (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 (Section 4.1.2.3).

EPA Response on the use of a nonlinear approach to low-exposure extrapolation: 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 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, multi-site 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. 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." 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 (Section 3.4.1).

EPA specifically considered a 2-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 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 (Section 3.3.3.1 and Section C.1 of Appendix C), and recent data from Marsden et al. (2009) specifically demonstrate increases of DNA adducts for very low exposures to exogenous EtO (Section 3.3.3.1). Thus, 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 non-linearity (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, recent data from Marsden et al. (2009) support a linear exposure-response relationship for EtO exposure and DNA adducts ($p < 0.05$) and demonstrate increases of DNA adducts from exogenous EtO exposure above those from endogenous EtO for very low exposures to exogenous EtO, as discussed in detail in the assessment (Section 3.3.3.1 and 4.5), providing strong 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, EPA's analysis of these data sets, summarized below, finds that they are in fact consistent with low-dose linearity. In summary, 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 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.

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 *hpr*t mutation frequencies between the values given in the slide and those reported by Walker et al. (2000). EPA performed statistical analyses using the data presented in slide 26 of Appendix C.

To examine these data, EPA first analyzed the EtO data set (Walker et al., 1997) using maximum likelihood estimation (MLE). 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 utilizing 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 results did not show measureable increases in *hprt* mutations is consistent with the modeled EtO results.

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, 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, EPA refit the data using an exponential model that provided a 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, 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, EPA concluded that combining the ethylene data with EtO data in evaluating dose-response relationships for the *hprt* mutations might not be appropriate.

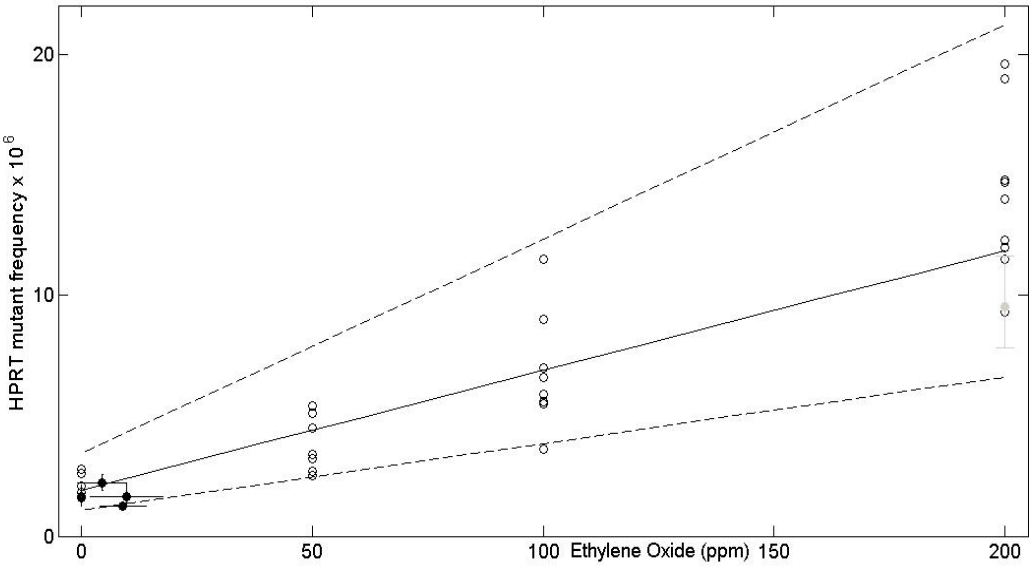

Figure H-1. Induction of *hprt* mutations by EtO (open circles and modeled fit) with data from ethylene (using estimated EtO equivalents) shown (solid circles). Source: SAB (2007), Appendix C (slides 25 and 26); original experiments of Walker et al. (1997).

Slide 27 of the SAB report presents data from Nivard et al. (2003) on the frequency of recessive lethal (RL) 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 non-log-transformed axes shows a downward curving (supralinear) relationship indicating greater potency of EtO (per unit exposure) at low exposures as compared with high exposures (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, 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, EPA's review finds these data to be supportive of the inferences made in the assessment [and more broadly in 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.

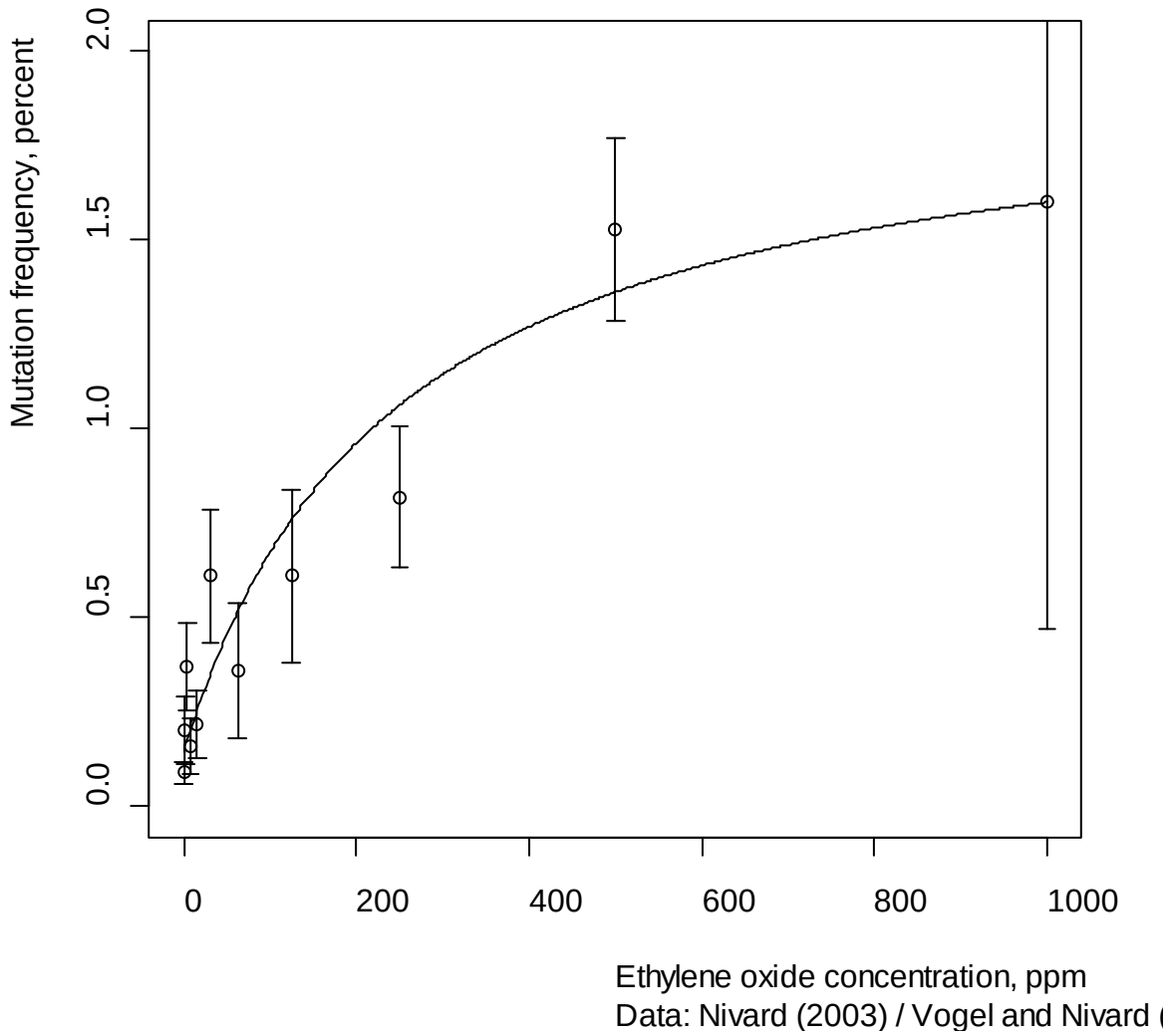

Figure H-2. Induction of recessive lethal mutations by EtO in *Drosophila* (wild-type). Standard deviations are calculated as the square root of the number of mutations, assuming a Poisson distribution, and plotted as ± (SD × percent mutation frequency).

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 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 (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 Dr. Steenland determined that there was not a statistically significant difference between the lymphohematopoietic cancer results for males and females (Sections D.3.b and D.4.b 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 EPA's responses:

SAB Panel 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 EPA has subdivided the response into separately titled subsections to make reading it easier.

EPA Response on using the BEIR approach to estimate incidence risks: In this assessment, 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, 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. 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 between cancer types is one of the uncertainties discussed (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 estimates might be overestimated would come primarily from the inclusion of multiple myeloma, because that subtype has the lowest incidence:mortality ratios (and, thus, if that subtype were driving the increased mortality observed for the lymphoid

cancer grouping, then including the incidence rates for the other subtypes, which have higher incidence:mortality ratios, 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, EPA has retained the application of the BEIR (life-table) approach, which it judges to provide a reasonable estimate of incidence risks. EPA recognizes the uncertainties and assumptions outlined by the Panel and has expanded the discussion of these in the carcinogenicity assessment (Section 4.1.1.3). However, EPA notes that deriving mortality estimates as the sole cancer risk estimates for lymphohematopoietic cancer would substantially underestimate cancer risk. In addition, 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, 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. 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 (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 (Section 4.1.1.2) and not for providing a realistic upper bound estimate for the EC_{01}.

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 2-step approach used by 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 (though 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 EPA did not undertake to develop any for this assessment. (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 Panel 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: EPA has added a new subsection (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 Panel 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 2-piece linear spline model is now recommended for the occupational exposure scenarios. The 2-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 2-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 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 Panel 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: 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)

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 Panel 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 Panel 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: 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, 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 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, 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, 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)].

EPA followed the Panel's recommendation and performed additional analyses of the individual data in collaboration with Professor Steenland. The work performed by Professor Steenland is described in Appendix D of the revised assessment. Working with Professor 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, 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. EPA does not believe, however, that these assumptions preclude the use of the Rothman model in the context of the EtO cancer risk estimation. 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. 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, EPA investigated the possible use of the "errors in variables" approach (page 27 of the Panel report). Professor 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) 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. 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 and, 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 (Sections 4.1.1.2 and 4.1.2.2).

Responses to SAB Panel 'bullet' comments (contained within the SAB comment on page H-27 above):

• Assumption that the risk within the category boundaries is constant.

EPA Response: 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 a 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 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.

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 EPA's responses (note that some duplicate comments were omitted).

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 EPA's position that as a result of the extensive development, review, reanalysis and revision, the revised assessment follows 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 Information Quality Guidelines.

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 data and the UCC data. The Union Carbide data and the publications that this public commentator referred to were evaluated and included in the assessment. 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, 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 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, 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 impossible an analysis of breast cancer, which was seen to have a significant increase among those with high EtO exposures in the NIOSH cohort. 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 EPA's response to comments on charge question 2.a above.

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 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 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 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 EPA to use the regression methodology with the published grouped data. EPA, in consultation with Professor Steenland, did perform analyses to fit additional models to the continuous exposure NIOSH data. The work performed by Professor Steenland is described in Appendix D of the revised assessment. Working with Professor Steenland, 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 EPA's response to comments on charge question 2.b above.

Comment 4.0: EPA Statistical Analysis of the Data Is Flawed and Other Incorrect Procedures Grossly Overestimate Risk. Key flaws include:

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 referred to 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).

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).

Comment 4.3: EPA's rationale and methodology for exclusion of the highest exposure group is inappropriate;

EPA RESPONSE: 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 Dr. Steenland excluding various percentages of the highest exposures confirmed that the highest exposures are attenuating the slopes in such models (Section D.3.b 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, in the draft assessment, EPA used a linear regression model and excluded the highest exposure group. 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. 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, 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**.**

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: EPA uses the narrower, less heterogeneous category of lymphoid cancer data for the primary risk estimates in the revised assessment.

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 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 EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a); (2), background incidence rates were used with mortality-based relative rates because 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 EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) (see also EPA's response to this issue under the further statistical issues subsection at the end of charge question 2.b above); (3), 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), EPA's application of adjustment factors for early life exposures in the EtO assessment was in accordance with the recommendations in 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, 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.

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.

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.

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: 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. And 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. However, Snellings et al. (1984) do 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) report that when the rats with unscheduled deaths were included in the evaluation, EtO exposure appeared to be related to an earlier occurrence of mesotheliomas.

Comment 9.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.

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 EPA has undertaken. Differences in relative potency across chemicals based on exposure levels may reflect differences in absorption, distribution, metabolism, excretion, or pharmacodynamics of the chemicals.

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.

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: 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.

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: EPA believes that it has made the best use of the available information in revising the assessment. EPA's evaluation of the weight of evidence concludes that the epidemiological evidence is strong (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" (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.

Comment 10.0: The Draft Cancer Assessment Does Not Use the Best Available Science as Required under the Information Quality Act and Cancer Guidelines.

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.

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: EPA considered all the available epidemiological data, including NIOSH data and the Union Carbide data and the publications that the ACC Panel 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.

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 EPA's response to that comment above.

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 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.

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 3 rodent bioassays, all but one show an increased incidence at the lowest exposure level, though not all the increases are statistically significant at that level.

Comment 12.0: EPA's Risk Estimates Do Not Pass Simple Reality Checks.

Comment 12.1: [This was the same as Comment 9.1 above.]

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.

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.
LIST OF REFERENCES ADDED AFTER THE 2006 EXTERNAL REVIEW DRAFT

Note: These references were added to the Carcinogenicity Assessment in response to the 2007 peer reviewers' and public comments, and for completeness. The added references have not changed the overall qualitative or quantitative conclusions. These references are also included in the reference list at the end of the main body of the assessment or the reference list at the end of this appendix volume; see those reference lists for the HERO links.

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APPENDIX J.
SUMMARY OF MAJOR NEW STUDIES SINCE THE LITERATURE CUTOFF DATE

A systematic literature search was conducted for the time frame from January 2006 to May 2013 to ensure that no major studies were missed from the time of the first external review draft in 2006 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 have been published, 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. The Appendix first provides a description of the systematic literature search that was conducted to identify relevant new studies (Section J.1) and then provides the reviews of the two major new studies identified in the May 2013 literature search (Section J.2) and of the two additional major studies identified from the 2013 public comment period (Section J.3).

J.1. SYSTEMATIC LITERATURE SEARCH

A systematic literature search was conducted in May 2013, covering the time frame from January 2006 to May 2013. The search was conducted using the LitSearch tool in 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 search identified 372 references, of which 56 were determined to be potentially relevant.[9] 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[10] or were reviews or other secondary source

[9]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.

[10]This refers to general exposure studies; exposure studies related to any of the epidemiological studies of EtO would be considered further.

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

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 et al. (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) 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.

Table J-1. Disposition of 56 new references identified as potentially relevant (continued)

Category	References	Disposition
Cancer studies (continued)	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 cases' processes.
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.
	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 utility 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. (2009) 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.
Other	Sielken and Valdez-Flores (2009) Sielken and Valdez Flores (2009) 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.

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Table J-1. Disposition of 56 new references identified as potentially relevant (continued)

Category	References	Disposition
Other (continued)	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., in the responses to SAB comments in Appendix H).

EtO: ethylene oxide.
N7-HEG: N7-(2-hydroxyethyl)guanine.

J.2. REVIEWS OF MAJOR NEW STUDIES PUBLISHED SINCE THE LITERATURE CUTOFF DATE

As discussed in Section J.1, a systematic literature search was conducted to determine whether any significant new 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 have been published since the cutoff date for literature inclusion. The two studies are epidemiology studies of occupational exposure to ethylene oxide (EtO). These studies are reviewed briefly here for transparency and completeness, and key features of the studies are summarized in Table J-2.

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 WHO 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 versus 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), so the study power was not large, 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-2. 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 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 (Fischer 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[11]), 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.

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

[11]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).

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 cause-, calendar year-, sex-, and 5-year age group-specific rates in the Swedish population (external referents). For cancer incidence (but not mortality), internal analyses were also conducted using Poisson regression analyses, adjusted for age group and calendar period, with no induction (latency) period. In the internal analyses, incidence rate ratios were calculated by comparing the incidence rates for the two highest cumulative exposure quartiles with that for the 50% of workers with cumulative exposures below the median of 0.13 ppm × years (internal referents). Internal analyses are generally preferred 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-2. 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 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. It should also be noted that data were not reported or analyzed for the subgrouping of "lymphoid" cancers.

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 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-2), despite having a low-exposed rather than an unexposed referent group.

In conclusion, 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 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. 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 average cumulative exposure was 67.16 ppm × years. In the more comparable NIOSH cohort of sterilization workers, cumulative exposures 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 were derived based on the Mikoczy et al. (2011) study, they would be higher than the estimates calculated from the NIOSH study. However, no such estimates were derived based on the Mikoczy et al. (2011) study because, in comparison to the NIOSH study, the Mikoczy et al. (2011) study had limitations that would have made the estimates more uncertain than those based on the NIOSH study. In particular, there was greater uncertainty about the exposure estimates [e.g., measurement data were available only from 1973 for one plant and 1975 for the other; 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), but this is a less sophisticated approach than that of the NIOSH exposure assessment, which used a detailed, validated regression model]; there were many fewer breast cancer cases (41 incident cases vs. 319 cases in NIOSH's full

incidence study cohort and 233 in the subcohort with interviews); and there was no information on potential breast cancer risk factors, as was available for the NIOSH subcohort.

Table J-2. 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 (# cases/# 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 since not 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

Table J-2. 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 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-2. 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 × yrs [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.3. REVIEWS OF MAJOR STUDIES IDENTIFIED AFTER THE MAY 2013 LITERATURE SEARCH

Although no new systematic literature search was performed after the May 2013 search, two additional major studies were later identified from public comments on the July 2013 public review draft of the EtO carcinogenicity assessment. The major studies identified since May 2013 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) criticize the approach employed by EPA in this and 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. Valdez-Flores and Sielken (2013) express concern, for example, that because all the categorical RR estimates share the same denominator, if the rate in the reference group differs from the true rate, the RR estimates will be systematically biased.

Valdez-Flores and Sielken (2013) also contend that, because the data for the unexposed group are random, the intercept should be estimated for any exposure-response model fit to the categorical RR estimates rather than being fixed at 1. To illustrate their arguments, Valdez-Flores and Sielken (2013) fit several models to the Steenland et al. (2004) breast cancer mortality data: a categorical log-linear model (model 1); a linear model fit to the categorical RR estimates, with the intercept fixed at 1 (model 2); a log-linear model fit to the categorical RR estimates, with the intercept unrestricted (model 3); and a continuous log-linear model (fit to the continuous exposure data; model 4). With four exposure groups (not counting the unexposed reference group)—the number Steenland et al. (2004) used for their categorical modeling—the RR estimates and the slopes for models 2 and 3 are similar, but the slope for model 4, the continuous log-linear model, is much shallower. With more exposure groups (20 and 61, the latter being chosen so that there was exactly one breast cancer death in each exposure group), the results for models 2 and 3 differ more appreciably and the slope for model 3 (unrestricted intercept) is more similar to the slope for model 4, the continuous log-linear model.

Valdez-Flores and Sielken (2013) further suggest that it is inappropriate to use comparisons with categorical RR estimates when evaluating models of the continuous individual data. Valdez-Flores and Sielken (2013) disregard the apparent supralinear shape of the

exposure-response relationship, suggesting it is the result of exposure measurement error, citing work by Crump (2005).

EPA notes that the Agency did use continuous exposure models for the breast cancer incidence data. Extensive modeling of the individual data based on continuous exposure for the data sets for lymphoid cancer mortality and breast cancer mortality, however, yielded no preferred models for the purposes of deriving unit risk estimates because of the strong supralinearity of the data (i.e., the response rate increases rapidly at low exposure levels and then attenuates or plateaus), which results in a very high slope in the low-exposure range (see Sections 4.1.1.2 and 4.1.2.2). Thus, EPA retains the approach of using the linear regression of the categorical results for those two cancer data sets (although EPA does not rely on the breast cancer mortality data set for the derivation of a unit risk estimate because the breast cancer incidence data are more suitable for the derivation of the desired incidence estimates). Valdez-Flores and Sielken (2013) state that "[u]nder no circumstances should an appropriate and relevant exposure-response model fit to individual epidemiological data be discarded in favor of an exposure-response model fit to summary data"; however, EPA did consider the continuous exposure log-linear model that Valdez-Flores and Sielken (2013) advocate and did not find it to be appropriate. For example, the log-linear model does not provide a statistically significant fit to either the lymphoid cancer mortality or breast cancer mortality data sets. Valdez-Flores and Sielken (2013) ignore the fact that when EPA modeled the continuous exposure data, the supralinear models (e.g., log-linear models with log-transformed exposure and two-piece spline models) fit the data better than linear and sublinear (e.g., log-linear) models, consistent with the supralinear shape suggested by the categorical results. The attenuation in the exposure-response relationship results from the influence of a small number of subjects with very high exposures. Such attenuation is commonly observed in occupational epidemiology studies of cancer (Stayner et al., 2003). Consideration of the categorical data can help avoid the influence of the subjects with very high exposures; in fact, EPA omits the highest exposure category in its linear regressions of the categorical results. The supralinear log-transformed exposure model similarly mitigates the influence of the subjects with very high exposures.

Moreover, EPA sees no reason to suppose that there is a problem with the reference group that would result in notable systematic bias. Rothman (1986) (p. 345) notes that the linear regression approach he presents for estimating a continuous slope based on categorical data can be inefficient if the reference category contains small frequencies; such is not the case for the Steenland et al. (2004) data, however. For the breast cancer mortality data set, the reference group contains 40% of the breast cancer deaths, and for the lymphoid cancer data set, the reference group contains about 17% of the deaths. Furthermore, the reference groups are highly

comparable internal comparison groups made up of other workers in the same facilities with short follow-up times since the time of first exposure (e.g., with exposures all within 20 years of follow-up for the breast cancer mortality analyses) who were "lagged out" in the analyses (i.e., assigned zero exposure). There is no reason to expect that these workers differ from those with non-zero exposure estimates with respect to background cancer rates.

In addition, EPA disagrees with the contention that the intercept in the linear regression model should be estimated. In conducting its weighted linear regressions, EPA used the approach of Rothman (1986), in which the RR at the intercept is explicitly fixed at 1 (see Appendix F). The categorical rate for the unexposed reference group, although not defined explicitly, is the best estimate available for the rate in that group, and it is appropriate to fix the baseline rate for the RR estimates at that level. When the underlying exposure-response relationship is supralinear, as is the case for these two data sets (see Sections 4.1.1.2 and 4.1.2.2), using the data for all the categorical groups to "estimate" the rate in the unexposed group – as proposed by Valdez-Flores and Sielken (2013) – would result in the data with the high exposures in the plateau region receiving a lot of weight in the linear regression. This would "pull" the linear model down at the higher exposures and concomitantly "pull" the model up at the lower exposures, thus "overestimating" the implicit rate in the unexposed group. In other words, the results for the higher exposure levels would unduly influence the estimate for the unexposed group, yielding a flawed estimate of the baseline rate. Moreover, Valdez-Flores and Sielken (2013) appear to be using an unweighted linear regression model, which undervalues the fact that a lot of data are reflected in that group (40% of deaths for breast cancer mortality) relative to the exposed groups and exacerbates the over-influence of the high-exposure results. (How one would obtain a weight for the reference group in their approach is unclear because the variability in the RR estimates is built into the estimates for the exposed groups and there is no SE or CI for the RR = 1 value of the reference group.) This over-influence of the high-exposure results in the Valdez-Flores and Sielken (2013) approach is illustrated in their findings. In their Table 2, for example, the increasing intercept and decreasing slope values obtained for model 3 with the increasing number of exposure intervals reflect the very high exposure values getting more weight. This is visually depicted in their Figures 2 and 3, which show the high-exposure values "pulling" model 3 down to a greater extent than model 2. Valdez-Flores and Sielken (2013) find affirmation in the fact that the slope of model 3 (the unrestricted log-linear model fit to the categorical results) approaches the slope of the log-linear model fit to the continuous exposure data, but they are aspiring to a (sublinear) continuous exposure model that does not provide a good fit to the data. In EPA's analyses, the log cumulative exposure model provides a better fit to the continuous exposure data, reflecting the underlying supralinear pattern of the exposure-

response relationship. The Breslow and Day (1980) quote cited by Valdez-Flores and Sielken (2013) recognizes that although the scale of the relative risk estimates is arbitrary, the shapes of the curves have meaning; Valdez-Flores and Sielken (2013), however, largely ignore the underlying shape.

EPA's analyses of the effects of excluding the top 5% of exposures confirm the impact of these high-exposure results in dampening the slopes of the log-linear models. For example, Figure D-2c in Appendix D shows the considerable increase in the slope of the log-linear model when the top 5% of exposures are omitted from the breast cancer mortality data set. As discussed above, the unrestricted intercept approach used by Valdez-Flores and Sielken (2013) is similarly susceptible to the undue influence of a small number of subjects with very high exposures. For unit risk estimation, it is the lower exposure region that is of greatest interest for low-exposure extrapolation, and EPA's approach of fixing the RR for the unexposed group at 1 and omitting the highest exposure group (due to the plateauing of the responses at high exposure levels) from the linear regression of the categorical results provides a better representation of the exposure-response relationship in the lower exposure range than the approach advocated by Valdez-Flores and Sielken (2013).

Furthermore, EPA disagrees that the categorical results are not useful depictions of the underlying exposure-response relationship. Valdez-Flores and Sielken (2013) themselves note that because there is a separate beta parameter estimate for each exposure group in the categorical model, there is considerable flexibility for the beta parameters to represent changes from the background hazard rate. For this reason, categorical models can be useful to show underlying exposure-response patterns (shapes) in the data. Such patterns can be obscured with single-parameter continuous models, such as the log-linear model, which presupposes a sublinear shape.

EPA also disagrees with the discounting by Valdez-Flores and Sielken (2013) of the apparent supralinear shape of the exposure-response relationship as the result of exposure measurement error based on work by Crump (2005). Exposure misclassification error is a complicated issue, and Crump's (Crump, 2005) conclusions appear to rely on the simplifying assumptions that the measurement error is classical and multiplicative. However, in epidemiological studies, such as the NIOSH ethylene oxide study, in which job-exposure matrices are used to estimate individual worker exposures, exposure measurement error is generally considered to be largely of the Berkson type and is often treated as additive (Armstrong, 1990). Heid et al. (2004) have demonstrated that different assumptions about exposure measurement error can have different impacts on the observed exposure-response relationship. For a specified log-linear relationship, Heid et al. (2004) found that multiplicative

classical error could make the observed exposure-response relationship appear supralinear, consistent with the findings of Crump (2005); however, additive classical error dampened the log-linear relationship, multiplicative Berkson error intensified the log-linear relationship, and additive Berkson error had no impact on the log-linear relationship, although the precision would be reduced. Moreover, NIOSH conducted an extensive exposure assessment that included the development of a regression model that had high validity when tested against independent measurement data (Section A.2.8 of Appendix A); thus, the existence of substantial exposure measurement error in the Steenland et al. (2004) data is speculative.

J.3.2. Parsons 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) present 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 preferentially cause G:C→T:A mutations, an early increase of the GGT→GTT (and/or GGT→TGT) mutation relative to the GGT→GAT 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 between 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) report 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) note, 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. 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 no further analyses were performed. Parsons et al. (2013) also report 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) report 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 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) propose the following biphasic response. Parsons et al. (2013) hypothesize 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.

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 MoA 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) propose 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) propose, 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 MoA 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, thus far these hypotheses have no independent support. In fact, this proposal disagrees with a recent (Nagy et al., 2013) study 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) report 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 report 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).

In addition, 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 note 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).

APPENDIX K.
DOCUMENTATION OF IMPLEMENTATION OF THE 2011 NATIONAL RESEARCH COUNCIL RECOMMENDATIONS

Background: On December 23, 2011, The Consolidated Appropriations Act, 2012, was signed into law.[12] The report language included direction to EPA for the Integrated Risk Information System (IRIS) Program related to recommendations provided by the National Research Council (NRC) in its review of EPA's draft IRIS assessment of formaldehyde.[13] The report language included the following:

> The Agency shall incorporate, as appropriate, based on chemical-specific data sets and biological effects, the recommendations of Chapter 7 of the National Research Council's Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde into the IRIS process…For draft assessments released in fiscal year 2012, the Agency shall include documentation describing how the Chapter 7 recommendations of the National Academy of Sciences (NAS) have been implemented or addressed, including an explanation for why certain recommendations were not incorporated.

The NRC's recommendations, provided in Chapter 7 of the review report, offered suggestions to EPA for improving the development of IRIS assessments. Consistent with the direction provided by Congress, documentation of how the recommendations from Chapter 7 of the NRC report have been implemented in this assessment is provided in the tables below. Where necessary, the documentation includes an explanation for why certain recommendations were not incorporated.

The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that, "the committee recognizes that the changes suggested would involve a multiyear process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others."

The IRIS ethylene oxide carcinogenicity assessment is in Phase 1 of implementation, which focuses on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to

[12]Pub. L. No. 112−74, Consolidated Appropriations Act, 2012.

[13]NRC (2011). Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde.

present information and data in assessments. Phase 1 also focuses on assessments near the end of the development process and close to final posting. Chemical assessments in Phase 2 of the implementation will address all of the short-term recommendations from Table K-1. Chemical assessments in Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC as outlined below in Table K-2. In May 2014, the NRC released their report reviewing the IRIS assessment development process. As part of this review, the NRC reviewed current methods for evidence-based reviews and made several recommendations with respect to integrating scientific evidence for chemical hazard and dose-response assessments. In their report, the NRC states that EPA should continue to improve its evidence-integration process incrementally and enhance the transparency of its process. The committee did not offer a preference but suggests that EPA consider which approach best fits its plans for the IRIS process. The NRC recommendations will inform the IRIS Program's efforts in this area going forward. This effort is included in Phase 3 of EPA's implementation plan.

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
General recommendations for completing the IRIS formaldehyde assessment that the EPA will adopt for all IRIS assessments (p. 152)	
1. To enhance the clarity of the document, the draft IRIS assessment needs rigorous editing to reduce the volume of text substantially and address redundancies and inconsistencies. Long descriptions of particular studies should be replaced with informative evidence tables. When study details are appropriate, they could be provided in appendices.	**Partially Implemented.** The EtO Assessment uses more tables, figures, and appendices to present information and data. For example, the main body of the EtO Assessment contains only abbreviated study summaries and study summary tables; the longer descriptions of the epidemiology studies and the genotoxicity studies are contained in appendices (Appendices A and C, respectively), along with a detailed summary table of the epidemiology studies in Appendix A. The main text of the hazard identification chapter (see Chapter 3) is comparatively streamlined.

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment (continued)

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
2. Chapter 1 needs to be expanded to describe more fully the methods of the assessment, including a description of search strategies used to identify studies with the exclusion and inclusion criteria articulated and a better description of the outcomes of the searches and clear descriptions of the weight-of-evidence approaches used for the various noncancer outcomes. The committee emphasizes that it is not recommending the addition of long descriptions of the EPA guidelines to the introduction, but rather clear concise statements of criteria used to exclude, include, and advance studies for derivation of the reference concentrations (RfCs) and unit risk estimates.	**Partially Implemented.** The EPA's literature search strategy is described in Chapter 2 of the EtO Assessment. To update the Assessment, a systematic literature search was done covering the time span from the year of the 1st external review draft (2006) to May 2013; this search is described in detail in Appendix J. In addition, the text has been expanded to include more description of the considerations made in evaluating the epidemiology studies (p. 3-1) and in selecting the study that formed the basis for the quantitative cancer risk estimates (p. 4-1−4-3).
3. Standardized evidence tables for all health outcomes need to be developed. If there were appropriates tables, long text descriptions of studies could be moved to an appendix or deleted.	**Partially Implemented.** The EtO Assessment contains a detailed summary table of the epidemiology studies in Appendix A (see Table A-5) along with the longer text study descriptions. Less detailed tables of the results are presented in the main text (see Tables 3-1 and 3-2).
4. All critical studies need to be thoroughly evaluated with standardized approaches that are clearly formulated and based on the type of research: for example, observational epidemiologic or animal bioassays. The findings of the reviews might be presented in tables to ensure transparency.	**Partially Implemented.** All critical studies were thoroughly evaluated in Chapter 3 and Appendix A. As discussed above, the text has been expanded to include more description of the considerations made in evaluating the epidemiology studies (see p. 3-1), and the epidemiology studies are summarized in a detailed table in Appendix A (see Table A-5). Standardized approaches for evaluating studies are under development as part of Phases 2 and 3.
5. The rationales for the selection of the studies that are advanced for consideration in calculating the RfCs and unit risks need to be expanded. All candidate RfCs should be evaluated together with the aid of graphic displays that incorporate selected information on attributes relevant to the database.	**Implemented.** As discussed above, the text has been expanded to include more description of the considerations made in selecting the study that formed the basis for the quantitative cancer risk estimates (see p. 4-1−4-3). The selection considerations are also summarized in a table (see Table 4-1). The EtO Assessment is a carcinogenicity assessment; thus, no RfCs or reference doses (RfDs) are derived.

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment (continued)

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
6. Strengthened, more integrative and more transparent discussions of weight of evidence are needed. The discussions would benefit from more rigorous and systematic coverage of the various determinants of weight of evidence, such as consistency.	**Implemented.** The weight-of-evidence discussion in the EtO Assessment has been substantially enhanced (see Section 3.5.1), and two tables have been added addressing consistency in the epidemiology study results (see Table 3-1 for lymphohematopoietic cancer and Table 3-2 for breast cancer).
General Guidance for the Overall Process (see p. 164)	
7. Elaborate an overall, documented, and quality-controlled process for IRIS assessments.	**Partially Implemented.** A team approach was used for the development of the EtO Assessment to help ensure that the necessary disciplinary expertise was available for assessment development and review. Because EtO is a post-peer review, phase one chemical, the EtO team did not have access to the "overall, documented, and quality-controlled process" that is now being developed in response to the NRC recommendations.
8. Ensure standardization of review and evaluation approaches among contributors and teams of contributors; for example, include standard approaches for reviews of various types of studies to ensure uniformity.	
9. Assess disciplinary structure of teams needed to conduct the assessments.	
Evidence Identification: Literature Collection and Collation Phase (see p. 164)	
10. Select outcomes on the basis of available evidence and understanding of mode of action.	**Implemented.** The EtO Assessment has detailed discussions of genotoxicity (see Section 3.3.3 and Appendix C) and mode of action (see Section 3.4) . The cancer outcomes selected are consistent with that mode-of-action finding as well as the available hazard evidence.
11. Establish standard protocols for evidence identification.	**Partially Implemented.** This is being implemented by the IRIS program as part of Phase 2. The EPA's literature search strategy is described in Chapter 2 of the EtO Assessment. More details of the original search were not retained at that time and are no longer available for this Assessment, which was largely finalized before the release of the NRC recommendations. To update the Assessment, a systematic literature search was done covering the time span from the year of the 1st external review draft (2006) to May 2013; this search is described in detail in Appendix J.
12. Develop a template for description of the search approach.	This is being implemented by the IRIS program as part of Phase 2.

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment (continued)

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
13. Use a database, such as the Health and Environmental Research Online (HERO) database, to capture study information and relevant quantitative data.	**Implemented.** HERO links were incorporated for all citations.
Evidence Evaluation: Hazard Identification and Dose-Response Modeling (see p. 165)	
14. Standardize the presentation of reviewed studies in tabular or graphic form to capture the key dimensions of study characteristics, weight of evidence, and utility as a basis for deriving reference values and unit risks.	**Partially Implemented.** The EtO Assessment includes a detailed summary table of key characteristics of the epidemiology studies (see Table A-5 of Appendix A) and a table summarizing the considerations made in selecting the study that formed the basis for the quantitative cancer risk estimates (see Table 4-1).
15. Develop templates for evidence tables, forest plots, or other displays.	This is being implemented by the IRIS program as part of Phase 2.
16. Establish protocols for review of major types of studies, such as epidemiologic and bioassay.	**Partially Implemented.** This assessment was developed using standard protocols for evidence evaluation that are provided in existing EPA guidance.

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment (continued)

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
Selection of Studies for Derivation of Reference Values and Unit Risks (see p. 165)	
17. Establish clear guidelines for study selection. a. Balance strengths and weaknesses. b. Weigh human vs. experimental evidence. c. Determine whether combining estimates among studies is warranted.	**Partially Implemented.** As discussed above, the text has been expanded to include more description of the considerations made in selecting the study that formed the basis for the quantitative cancer risk estimates (see p. 4-1−4-3). The selection considerations are also summarized in a table (see Table 4-1). Consideration was given to combining data from the Union Carbide Cohort (UCC) and NIOSH cohort studies, and discussion is provided for why the UCC data were ultimately not used (see Section 4.1). The EtO Assessment is a carcinogenicity assessment; thus, no RfCs or RfDs are derived.
Calculation of Reference Values and Unit Risks (see pp. 165−166)	
18. Describe and justify assumptions and models used. This step includes review of dosimetry models and the implications of the models for uncertainty factors; determination of appropriate points of departure (such as benchmark dose, no-observed-adverse-effect level, and lowest observed-adverse-effect level), and assessment of the analyses that underlie the points of departure.	**Implemented.** The EtO Assessment has a detailed discussion of model selection for the epidemiological data sets (see Section 4.1) and the laboratory animal data sets (see Section 4.2), including a discussion of cross-species scaling (see Section 4.2.2). The EtO Assessment is a carcinogenicity assessment; thus, no reference values are derived.
19. Provide explanation of the risk-estimation modeling processes (for example, a statistical or biologic model fit to the data) that are used to develop a unit risk estimate.	**Implemented.** The EtO Assessment has a detailed discussion of model selection for the epidemiological data sets (see Section 4.1) and the laboratory animal data sets (see Section 4.2).

Table K-1. The EPA's implementation of the National Research Council's recommendations in the ethylene oxide (EtO) carcinogenicity assessment (continued)

NRC recommendations that the EPA is implementing in the short term	Implementation in the EtO carcinogenicity assessment
20. Provide adequate documentation for conclusions and estimation of reference values and unit risks. As noted by the committee throughout the present report, sufficient support for conclusions in the formaldehyde draft IRIS assessment is often lacking. Given that the development of specific IRIS assessments and their conclusions are of interest to many stakeholders, it is important that they provide sufficient references and supporting documentation for their conclusions. Detailed appendixes, which might be made available only electronically, should be provided, when appropriate.	**Implemented.** The EtO Assessment includes, as an appendix (see Appendix D), more detailed fit statistics and modeling results for the epidemiological cancer data sets. Appendix G provides results of the laboratory animal tumor modeling. The EtO Assessment is a carcinogenicity assessment; thus, no reference values are derived.

Table K-2. National Research Council recommendations that the EPA is generally implementing in the long term

NRC recommendations that the EPA is implementing in the long term	Implementation in the EtO Carcinogenicity Assessment
Weight-of-Evidence Evaluation: Synthesis of Evidence for Hazard Identification (see p. 165) **1.** Review use of existing weight-of-evidence guidelines. **2.** Standardize approach to using weight-of-evidence guidelines. **3.** Conduct agency workshops on approaches to implementing weight-of-evidence guidelines. **4.** Develop uniform language to describe strength of evidence on noncancer effects. **5.** Expand and harmonize the approach for characterizing uncertainty and variability. **6.** To the extent possible, unify consideration of outcomes around common modes of action rather than considering multiple outcomes separately.	As indicated above, Phase 3 of EPA's implementation plan will incorporate the longer-term recommendations made by the NRC. On May 16, 2012, EPA announced that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. In addition, EPA held a workshop in August 2013 on issues related to weight of evidence to inform future assessments.
Calculation of Reference Values and Unit Risks (see pp. 165−166) **7.** Assess the sensitivity of derived estimates to model assumptions and end points selected. This step should include appropriate tabular and graphic displays to illustrate the range of the estimates and the effect of uncertainty factors on the estimates.	**Implemented.** The EtO Assessment is a carcinogenicity assessment; thus, no reference values are derived. Chapter 4 presents derivations of unit risk estimates for multiple data sets, species, and models. Many of these derivations are summarized in tables and figures; for example, for the breast cancer incidence subcohort, Figure 4-5 depicts the range of relative risk estimates for different exposure-response models considered and Table 4-13 summarizes unit risk estimates derived from different models.

APPENDIX L.
SUMMARY OF PUBLIC COMMENTS RECEIVED ON THE JULY 2013 PUBLIC COMMENT DRAFT AND EPA RESPONSES

EPA's Science Advisory Board (SAB) reviewed an external review draft of the ethylene oxide (EtO) carcinogenicity assessment in 2007. Following that review, a revised draft was developed and released on 23 July 2013 for a 45-day public comment period. In response to requests from the American Chemistry Council's Ethylene Oxide Panel (ACC), the Ethylene Oxide Sterilization Association (EOSA), and Balchem Corporation, the public comment period was extended from 5 September to 11 October 2013.

During the public comment period, 16 comments were received, not including the 3 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 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 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.

<u>Comments from ARASP (#0055), EOSA (#0056), and ACC (#0057)</u>

1. EPA failed to comply with multiple guidelines, including Information Quality Act guidelines and 2011 National Academy of Sciences (NAS) 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: EPA has complied with applicable guidelines. The EtO assessment was largely developed before the IRIS program started implementing the 2011 NAS 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. Major new 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, EPA's Information Quality Act guidelines do not require that all underlying raw epidemiology data be publicly available; they allow for confidentiality constraints.

2. 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 # 7 in Section 3.5.1).

3. Lymphohematopoietic and lymphoid cancers should not be grouped because they are derived from different cells of origin. (ARASP, ACC, EOSA)

EPA Response: 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. The evidence for breast cancer is too weak. (ACC, EOSA)

EPA Response: Although the data base for breast cancer is more limited (i.e., few studies with sufficient numbers of female breast cancer cases) than that for lymphohematopoietic cancers, EPA determined that the available evidence is sufficient to consider breast cancer a potential hazard from EtO exposure. The 2007 SAB Panel did not object to the derivation of unit risk estimates based on the available breast cancer evidence.

5. EtO is a weak mutagen. (ACC, EOSA)

EPA Response: EPA agrees that EtO is a relatively weak mutagen compared to the anti-cancer agents and the other reactive epoxides investigated in the Vogel and Nivard (1998) paper. Vogel and Nivard (1998) compared 37 anti-cancer agents, which are generally highly mutagenic by design, and 4 epoxides, including EtO, one of which was a cross-linking diepoxide.

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. 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 EPA's conclusion at that time that a mutagenic MoA was operative in the carcinogenicity of EtO. In its 2013 public review draft, 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 recent 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) investigate 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) hypothesize 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 recent 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 acknowledge, the results Parsons et al. (2013) present 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 (Section 3.3.3.1 and Appendix C), even though they were not statistically significant, increases in exogenous adducts were observed at the lowest dose in liver and spleen, and Marsden et al. (2009) note 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 recent 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 since 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) propose 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 acknowledge 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. 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, EPA did consider using the UCC data and, for 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), determined that the UCC data were not of sufficient quality to add useful information to the NIOSH study's data for the derivation of unit risk estimates. Thus, 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. 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), EPA investigated multiple models based on the individual continuous exposure data, including a log-linear model. For the breast cancer incidence data, 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 (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 (Section 4.1.1.2). In particular, the statistically significant models predicted extremely steep slopes in the low-dose region. Thus, 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. 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, EPA again used a linear regression of the categorical data, excluding the highest exposure group, as the basis for the preferred estimate (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. EPA used a non-peer-reviewed supralinear spline model. (ACC, EOSA)

EPA Response: The spline model EPA used for the breast cancer incidence data was the best-fitting model 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. 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: EPA did not find that Valdez-Flores and Sielken (2013) or Valdez-Flores et al. (2010) provided convincing evidence of flaws in the modeling. EPA addressed the issues presented by Valdez-Flores et al. (2010) in the July 2013 assessment (Section A.2.20 of Appendix A). Discussion of the new Valdez-Flores and Sielken (2013) study has been added to Appendix J (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, so the specific claims could not be evaluated. EPA notes, however, that the ACC is no longer claiming that the observed number of cancer mortalities is over-predicted "by more than 60-fold." In Appendix I of the ACC comments, the claim is made that the lymphoid cancer mortality is over-predicted by "1.87 to 3.26 fold" and breast cancer mortality is over-predicted 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 the ACC's Figure E.1 in 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. EPA should present both linear and nonlinear extrapolation approaches. (ARASP, ACC, EOSA)

EPA Response: EPA notes that some members of the 2007 SAB Panel recommended that 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. EPA considered available information and opinions presented by SAB members and concluded that there was not sufficient 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 EPA's responses to the comments of the first SAB Panel, including 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 EPA's response to comment #6 above).

12. 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. 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 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 (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-scale 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 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 (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. EPA should not derive occupational exposure limits for EtO. (ACC, EOSA)

EPA Response: EPA's Office of Pesticide Programs (OPP) has a regulatory interest in occupational exposures resulting from sterilization uses of EtO, as 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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This reference list includes all the references cited in the document except for Appendix B, which is a reference list pertaining to Figure 3−3, and Section J.1 of Appendix J, which discusses the disposition of some more recent references identified in a May 2013 literature search. References added after the 2007 external peer review are also listed separately in Appendix I.

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