PART B

HEALTH EFFECTS OF ETHYLENE OXIDE

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16 September 1986

Revised August 31, 1987

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The editorial assistance of L. Cullen Phillips and the word processing skills of Rose Findley, Thelma Brown, Susan Freeman, Velinda Knowles and Mary Erickson are gratefully acknowledged.

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1. EXECUTIVE SUMMARY

The health effects of ethylene oxide have been reviewed and evaluated to determine if ethylene oxide may be a toxic air contaminant as defined by California Health and Safety Code Section 39655. At ambient temperatures ethylene oxide is a gas. In vivo ethylene oxide is rapidly distributed throughout the body. Acute and chronic exposure leads to respiratory tract irritation and central nervous system depression, as well as other pathologic changes. At high doses ethylene oxide can induce dominant lethal mutations and cause embryotoxicity in rodents. One epidemiologic study suggested an increase in spontaneous abortions due to ethylene oxide exposure. At current ambient levels of ethylene oxide, however, no acute or noncarcinogenic chronic effects are expected.

Ethylene oxide, presumably due to its ability to alkylate DNA, causes gene mutations in both prokaryotic and eukaryotic cells and leads to sister chromatid exchanges and chromosomal damage, including the formation of aberrations and micronuclei. Several types of tumors have been induced in rats by ethylene oxide. Gavage induced tumors of the forestomach, i.e., at the site of application. Inhalational exposure of rats led to increases in a variety of tumors. The strongest dose-dependent response was seen for peritoneal mesotheliomas in males and for mononuclear cell leukemias in females. There was also an increase in brain tumors, which are rarely seen in control animals. Epidemiologic studies of people occupationally exposed to ethylene oxide suggest increased incidences of stomach cancer and leukemia.

The International Agency for Research on Cancer concluded that there is sufficient evidence for the carcinogenicity of ethylene oxide in animals; in humans, the evidence for carcinogenicity is limited. Overall, based on both the animal and human data, IARC considers that ethylene oxide is probably carcinogenic in people. DHS staff concurs with these conclusions. In addition, DHS staff has found no evidence for a carcinogenic threshold level for ethylene oxide.

The DHS staff recommends that the range of risks for ambient exposures to ethylene oxide be based on the maximum likelihood estimate and upper 95% confidence limit predicted from fitting a multistage model to the animal data. The range of estimated excess lifetime cancer risk, the risk from 24-hour-per-day exposure for a 70 year lifetime to average ambient airborne concentrations, estimated to be 0.09 $\mu g/m^3$, is 6 to 8 cases per million persons exposed. These values were also obtained by EPA using the same data. When the models are applied to the epidemiologic data to estimate excess cancers, the estimates are compatible with what has actually been observed; therefore, the risk assessment based on animal data is compatible with the epidemiologic evidence. Exposure to the ambient value of 0.09 $\mu g/m^3$ (50 ppt) estimated by the Air Resources Board for the Los Angeles basin could result in up to 55 excess lifetime cancers (Upper 95% Confidence Limit) among the 7 million residents of that area. Using several others models for cancer risk estimation, an upper limit range of 49 to 497 excess lifetime cancers was estimated.

The range of risk values represents several sources of uncertainty, including statistical uncertainty due to the relatively small number of

animals used in the bioassay. Other general sources of uncertainty include the choice of the animal-to-human scaling factors, the choice of the extrapolation model, and the large range of extrapolation (four orders of magnitude) from the ethylene oxide concentrations used in the animal experiments to current ambient levels.

Based on the findings of carcinogenicity and the results of the risk assessment, DHS staff find that ambient ethylene oxide is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

2. PHARMACOKINETICS AND METABOLISM

2.1. Summary

The absorption, distribution, metabolism and excretion of ethylene oxide have been reviewed elsewhere (EPA 1985, SRI 1983). Only limited data are available on the pharmacokinetic behavior of ethylene oxide in mammals. After administration to mice and rats by various routes, ethylene oxide is rapidly distributed throughout the body and quickly excreted. The metabolic fate of ethylene oxide has not been completely elucidated. Urinary metabolites that have been identified are: ethylene glycol in dogs; two glutathione derivatives in rats; and 7-hydroxyethylguanine in mice. The observation of calcium oxalate crystals in mice kidney tubules following ethylene oxide exposure is also suggestive evidence of ethylene glycol formation. Alkylation of tissue macromolecules has also been observed. The formation of ethylene oxide adducts with DNA and protein may suggest a mechanism for its genotoxicity in biological systems.

2.2. Absorption, Distribution, and Elimination

Two minutes after [^{14}C]ethylene oxide was administered intravenously to mice, whole-body autoradiography indicated high concentrations of radioactivity in the liver, kidney and pancreas at levels 2-3 times the level observed in the blood. Between 20 minutes and 4 hours after injection, radioactivity was detected in the liver, kidney, intestinal mucosa, lung, epididymis, testis and cerebellum (Appelgren et al. 1977).

Autoradiographic studies have also demonstrated the affinity of ethylene oxide for the cerebellar cortex of mice after intravenous injection (Bergman et al. 1981).

Inhalation studies in mice indicated that ethylene oxide is widely distributed throughout the body and rapidly excreted in the urine. Within 48 hours, 78% of the radioactivity absorbed was excreted in the urine. High levels of radioactivity (form unspecified) were found in proteins isolated from the lungs, kidney and liver, and lower levels were found in the brain and spleen. The biological half-life in mice was reported to be approximately 9 minutes (Ehrenberg et al. 1974).

Comparable findings have been made in rats. After intraperitoneal injection of 2 mg/kg [1,2-^{14}C]ethylene oxide to Sprague-Dawley rats, 43% of the radioactive dose was excreted in the urine over a period of 50 hours. Most was excreted within 18 hours. Within 6 hours of dosing, 1.5% of the administered dose was exhaled as $^{14}CO_2$ and 1% as unchanged ethylene oxide. These may not be maximum amounts since exhaled radioactivity was not sampled at later post-exposure periods (Jones and Wells 1981).

Brugnone and coworkers (1985, 1986) have studied people working in a hospital sterilizer unit during and at the end of the workshift. Mean environmental concentrations were usually near 2 ppm (approximately 4 mg/m ^{3}) but ranged up to 10 ppm. They found a high positive correlation between the environmental and alveolar concentrations of ethylene oxide, substantial alveolar retention (absorption) of ethylene oxide (75-80% of that inhaled), and a high positive correlation between alveolar and blood

concentrations. Concentrations in the blood were 12 to 17 times the environmental concentration.

Martis et al. (1982) concluded that the rate of elimination is not dose dependent. They administered 25 or 75 mg/kg ethylene oxide intravenously to dogs. Despite variation in dose, both the rate of clearance from plasma and the percent ethylene oxide excreted remained fairly constant. Mean plasma half-lives were 29.3 ± 5.7 minutes for the low dose and 36.5 ± 18.5 minutes for the high dose. The percentage of the dose excreted in the urine as ethylene glycol within 24 hours ranged from 7 to 24. The mean percentages of the low and high doses excreted were 13.5 ± 3.5% and 14.2 ± 8.1%, respectively.

2.3. Metabolic Pathways

Ethylene glycol. The above study by Martis et al. (1982) indicated that a portion of ethylene oxide is converted to ethylene glycol in dogs. SEC/R_1 mice exposed to airborne concentrations of 150 or 250 ppm ethylene oxide had refractive calcium oxalate crystals in their kidney tubules (Niemann et al. 1986). The authors suggested that ethylene oxide may form ethylene glycol and subsequently be oxidized to oxalic acid.

Glutathione. Two glutathione derivatives were detected in the urine of Sprague-Dawley rats injected intraperitoneally with 2 mg/kg of [1,2-^{14}C]ethylene oxide. S-(2-hydroxyethyl)cysteine represented 9% of the administered dose and N-acetyl-S-(2-hydroxyethyl)cysteine 33% of the dose (Jones and Wells 1981). The last metabolite has been detected in the urine

of rats after 6-hour inhalational exposures down to 1.2 ppm (Gerin et al 1986). The amount excreted was a nearly linear function of exposures between 1.2 and 47 ppm.

DNA adducts. The only urinary metabolite characterized when mice were exposed by inhalation was 7-hydroxyethylguanine (Ehrenberg et al. 1974). This represented 0.007% of total urinary radioactivity. An N-7-alkylguanine derivative was found in DNA from the liver and testes of rats injected intraperitoneally with ethylene oxide (Osterman-Golkar et al. 1983). Cumming et al. (1981) found large differences in the initial alkylation patterns and removal of DNA adducts in different male mouse tissues after inhalational administration. The liver was reported as the tissue with the highest degree of initial alkylation, while the testis had the lowest degree.

Protein alkylation. Significant alkylation of proteins by ethylene oxide was first noted by Ehrenberg et al. (1974) during inhalation studies in mice. In later studies, ethylene oxide bound covalently to several amino acids in hemoglobin (Segerback 1983). The increased level of hydroxyethylation of the N-terminal valine of hemoglobin in cigarette smokers has been attributed to ethylene oxide formed by in vivo oxidation of ethene in inhaled smoke (Tornquist et al 1986).

3. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

The toxic effects from acute, subchronic, and chronic exposure to ethylene oxide have been reviewed previously (Hine et al. 1981, Glaser 1979, EPA 1985). Relevant findings will be summarized below.

3.1. Summary

The primary effects of acute, subchronic, or chronic exposure to ethylene oxide are similar for humans and animals: central nervous system depression and respiratory tract irritation. Skin or eye contact with either liquid or gaseous ethylene oxide causes burning. Sensitization and cataract formation are also associated with repeated exposure in humans.

Repeated inhalational exposure to high concentrations of ethylene oxide is associated with neuropathy. Observations of neurotoxicity in humans have been confirmed in some, but not all, studies by histopathologic observation. In laboratory animals, pathologic changes have been observed in the lungs, kidney, liver, testicles, and blood.

Epidemiologic studies have not revealed any toxic, nonmalignant effects after long-term exposure to levels of ethylene oxide below 10 ppm. In laboratory animals, depression in weight gain and decreased survival has been observed in some studies.

In 1977, the National Institute for Occupational Safety and Health (NIOSH) recommended a level for occupational exposure to ethylene oxide that was designed to protect workers against the acute and chronic nonmalignant health effects of ethylene oxide (NIOSH 1977, 1981). They recommended continued observation of the then-current occupational standard of 50 ppm as a time-weighted average (TWA) for an eight-hour shift. No noncarcinogenic effects were expected below this exposure level. However, in 1984, an occupational standard of 1 ppm as a permissible exposure limit (PEL) for an 8-hour TWA exposure was promulgated, based on evidence of ethylene oxide's carcinogenicity (OSHA 1984). This PEL was also adopted in California (California Administrative Code 1986). Current ambient exposures are estimated to be five orders of magnitude below this PEL.

3.2. Acute Toxicity

A variety of symptoms resulting from acute exposure of rats, mice, guinea pigs, dogs and humans to ethylene oxide have been reported, such as mucus membrane irritation, central nervous system (CNS) depression, lacrimation, nasal discharge, salivation, nausea, vomiting, diarrhea, respiratory irritation, loss of coordination, and convulsions (EPA 1985). The threshold for occurrence of all of these symptoms appears to be at least 100 ppm. It is not likely that persons exposed to ambient levels of ethylene oxide, which are estimated to be orders of magnitude less than this level of exposure, will experience acutely toxic symptoms.

Lethality. The LD_{50} of ethylene oxide administered intragastrically to rats and guinea pigs has been reported to be 330 mg/kg and 270 mg/kg,

respectively. By inhalation, the four-hour LC_{50} for rats, mice, and dogs was determined to be 1460 ppm, 835 ppm, and 960 ppm, respectively. The lungs, liver, and kidney were some of the organs reportedly showing toxicity after acute exposures of ethylene oxide (IARC 1985, EPA 1985).

Dermal toxicity. Skin irritation caused by contact with liquid ethylene oxide has been reported in rabbits, guinea pigs, and humans (EPA 1985, Glaser 1979). In rabbits, when ethylene oxide was applied dermally in concentrations of 0-5% in aqueous solutions, tissue irritation was observed above 1%. By subcutaneous administration, concentrations above 0.1% were observed to cause local inflammation.

In humans, treatment with aqueous solutions of ethylene oxide applied to the skin caused dermatological effects, including burns and blisters, which were related to duration of treatment and concentration.

Exposure to high concentrations of gaseous ethylene oxide also apparently causes burns. In one instance in which an outbreak of ethylene oxide burns resulting from wearing hospital gowns was reported in women, residues of ethylene oxide varying from 3,600 to 10,800 ppm were measured in the gowns (Fisher 1984).

Shupack et al. (1981) observed that the reaction of human skin exposed to ethylene oxide-permeated materials (fabric, rubber, and PVC materials) was correlated with total dose. Most reactions occurred after 4-8 hours of skin contact, with slowly airing materials containing levels of ethylene oxide

equal to 1000 ppm. A delayed skin reaction after exposure to 100 ppm ethylene oxide occurred in one subject who had been previously exposed.

Allergic contact dermatitis in humans in response to ethylene oxide has been demonstrated experimentally, and there have been several case reports about allergic reactions occurring during hemodialysis and peritoneal dialysis (Marshall et al. 1984) and cardiac catheterization (EPA 1985) when the equipment had been sterilized with ethylene oxide.

3.3. Subchronic and Chronic Toxicity

The literature contains limited information on the toxic effects of subchronic or chronic exposure to ethylene oxide in humans. Case reports indicate that neurotoxic effects, including incoordination, dizziness, and peripheral neuropathy, are associated with recurrent exposure to moderate to high levels (up to 500 ppm) (Garry et al. 1979, Schroeder et al. 1985). Subchronic exposure of a variety of different animal species to ethylene oxide by inhalational, oral, or subcutaneous administration produced symptoms similar to those resulting from acute exposure (summarized in Table 3.1). Neurotoxic effects occurred in rats after exposure to concentrations greater than 350 ppm for 6 months, but no significant effects were observed below that level (Hollingsworth et al. 1956, Jacobsen et al. 1956). Histopathological evidence of peripheral neuropathy has been documented after rats were exposed to 500 ppm for 13 weeks (Ohnishi et al. 1985).

Table 3.1 Chronic Toxicity of Ethylene Oxide

Species	Dose	Time	Observations	References
Rats	>350 ppm	6 months	Neurotoxic effects	Hollingsworth et al. 1956 Jacobson et al. 1956
Rats	500 ppm	13 weeks	Peripheral neuropathy	Ohnishi et al. 1985
Rats	50 ppm 100 ppm	2 years	Decreased body weight and various inflammatory lesions Decreased survival at 100 ppm	Lynch et al. 1984b
Rats	10 ppm 33 ppm 100 ppm	2 years " "	No effect Decreased weight gain Decreased weight gain	Snellings et al. 1984a
Mice	50 ppm 100 ppm 250 ppm	6 months " "	Decreased neuromuscular function at all levels; decreased weight of testicles and spleen at 250 ppm	Snellings et al. 1984b
Monkeys	357 ppm 354 ppm 204 ppm	Several weeks 38-94 exposures 176-276 days	Reversible neurologic effects Decreased sensory and motor function Paralysis and muscular function	Hollingsworth et al. 1956
Dogs	292 ppm	6 weeks	Decreased red blood cells	Woodward and Woodward 1971
Dogs	6-36 mg/kg	30 days 21 days	Anemia No change	Woodward and Woodward 1971 Balaz 1976

Inhalation studies in rodents. Two studies evaluated the chronic toxicity of inhaled ethylene oxide in rats (Lynch et al. 1984b, Snellings et al. 1984a). Lynch et al. administered 50 or 100 ppm ethylene oxide to male rats 7 hours/day, 5 days/week for two years. Survival in the high-dose group was significantly decreased ($p < 0.01$), and statistically significant decreases in body weights were seen in both exposed groups. Exposed rats had a higher incidence of inflammatory lesions of the lungs, nasal cavity, trachea, and internal ear, in addition to an increased incidence of bronchiectasis and bronchial epithelial hyperplasia; these findings were consistent with the manifestations of chronic respiratory disease complex in rodents. As discussed in Section 5.1.4, these findings may have been affected by an outbreak of Mycoplasma pulmonis. There was a high incidence of proliferative and degenerative lesions of the adrenal cortex in exposed rats at both dose levels and skeletal muscle myopathy was observed in the group exposed to the higher dose. The myopathy was not accompanied by any neurological change detectable by light microscopy. Multifocal mineralization of the posterior layers of the choroid/sclera portion of the eye was noted more often in ethylene oxide-exposed rats than in controls.

Snellings et al. (1984a) reported no significant, noncarcinogenic effects other than a significant depression in weight gain in male and female rats exposed to 100 ppm and females exposed to 33 ppm 6 hours/day, 5 days/week for two years. Weight curves of rats exposed to 10 ppm were essentially similar to those of the controls. The carcinogenicity of ethylene oxide reported in these two studies is reviewed in Section 4.

In mice, Snellings et al. (1984b) reported increased liver weight, decreased testicular weight and decreased spleen weight after exposure to 250 ppm of ethylene oxide 6 hours/day, 5 days/week for 10 to 11 weeks. Neuromuscular testing demonstrated a dose-related decrease in neuromuscular function in the animals treated with 50, 100, or 250 ppm, but no histopathological changes were observed in nerve or muscle tissue.

Neurotoxicity in monkeys. Hollingsworth et al. (1956) reported results of a subchronic inhalation study in monkeys. Neurotoxic effects (hindlimb paralysis, muscular atrophy, etc.) were observed in monkeys following inhalation of 357 ppm vapor for several weeks; the effects were reversible by 100-132 days after termination of exposure. Additional studies in which 357 ppm was administered 38-94 times resulted in impairment of both sensory and motor function of the lumbar and sacral level of the spinal cord. Exposure of monkeys to 204 ppm for 176-226 days caused partial paralysis and muscular atrophy of the hindlimbs.

Hematologic effects. Several researchers have reported significant hematological effects in ethylene oxide-exposed dogs (EPA 1985). Decreased red blood cell counts, hemoglobin, and hematocrit in 3 beagles were observed after inhalation of 292 ppm for 6 hours/day, 5 days/week for 6 weeks. After 30 daily subcutaneous injections of 6-36 mg/kg to dogs, dose-related anemia associated with hyperplastic bone marrow and ectopic hematopoiesis was observed (Woodward and Woodward 1971). These results were not reproducible in a 21-day test (Balaz 1976). Mononuclear cell leukemia was significantly increased in rats after lifetime exposure to 10 to 100 ppm ethylene oxide (Snellings et al. 1984a, Lynch et al. 1984b). Several epidemiological

studies indicate an association between ethylene oxide exposure and leukemia (see Section 4.2 below).

4. CARCINOGENICITY

4.1. Animal Studies

4.1.1. Summary

The International Agency for Research on Cancer (IARC) has recently evaluated the evidence for the carcinogenicity of ethylene oxide and concluded that there was "sufficient evidence for the carcinogenicity of ethylene oxide in experimental animals" (IARC 1985). Ethylene oxide induced local tumors in a dose-dependent manner after intragastric administration to rats. Two independent inhalation studies in Fischer 344 rats, one in males and females, and the other in males only, found similar types of treatment-related tumors. The incidence of mononuclear cell leukemia was significantly increased in females in one study and in males in the second study. In both studies the incidence of peritoneal mesotheliomas and brain gliomas was increased in male rats. Several types of tumors have also been detected in mice.

4.1.2. Topical Administration

Mice. The clipped dorsal skin of thirty 8-week-old female ICR/Ha Swiss mice was painted with 0.1 ml of a 10% solution of ethylene oxide in acetone, three times a week for life. The authors suggested that the negative results obtained resulted from rapid evaporation of the compound (Van Duuren et al. 1965).

4.1.3. Subcutaneous Administration

Rats. Subcutaneous administration in rats also produced negative results. Twelve rats were given subcutaneous injections of ethylene oxide dissolved in arachis oil for 94 days (maximum total dose, 1 gm/kg), and were observed for their lifetime. No local sarcomas were observed. Since the dosing schedule, the amount administered, and the ages and sex of the animals were not specified in the reports, these negative results cannot be evaluated (Walpole 1958).

Mice. A low, but statistically significant, incidence of injection site sarcomas was induced in groups of 100 female NMRI mice given subcutaneous injections of 0.1, 0.3, or 1.0 mg/mouse ethylene oxide in tricaprylin, weekly for 95 weeks (Dunkelberg 1981). The survival rate was lower in the highest dose group than in the two lower dose and control groups. The incidence of subcutaneous sarcomas (mostly fibrosarcomas) was 0/200, 4/200, 5/100, 8/100, and 11/100 in untreated controls, vehicle controls, 0.1, 0.3 and 1 mg dose groups, respectively ($p < 0.001$, Cochran-Armitage test for trend). Distant tumors could not be related to ethylene oxide exposure.

4.1.4. Oral Administration

Rats. No increase in tumor incidence was observed in male or female rats (strain unspecified) fed for two years on a diet fumigated with ethylene oxide (Baer and Griepentrog 1969). On the other hand, a dose-related increase in local tumors, mainly squamous cell carcinomas of the forestomach, was observed in female Sprague-Dawley rats given doses of 7.5

or 30 mg/kg body weight of 99.7% pure ethylene oxide twice a week by gastric intubation for 107 weeks (average total dose of 1186 and 5112 mg/kg body weight, respectively)(Dunkelberg 1982, EPA 1985). Rats treated with the high dose of ethylene oxide showed increased tumor-related mortality as well as decreased tumor latency compared to the low dose or the control groups. The incidence of local tumors was 0/50 in both control groups, 8/50 in the low-dose group, and 31/50 in the high-dose group. The frequency of tumors at other sites was not increased by ethylene oxide treatment (Dunkelberg 1982, EPA 1985).

4.1.5. Inhalational Administration

4.1.5.1. Snellings et al. 1984a

In a two-year inhalation study in rats, Snellings et al. (1984a) found that ethylene oxide increased the incidence of mononuclear cell leukemia in animals of both sexes, and peritoneal mesotheliomas in males. Tumor frequency among female rats was greater in all exposed groups than in controls. In addition, brain gliomas were observed in male and female exposed rats. Since this tumor has an historically rare background occurrence in F344 rats, it is one of the tumors that is appropriate for use in risk evaluation.

Eight-week-old Fischer 344 rats were exposed in inhalation chambers to 10, 33, or 100 ppm of 99.9% pure ethylene oxide 6 hours/day, 5 days/week, for two years. Initially, 120 males and 120 females were exposed per dose. Two

control groups (CI and CII) of 120 rats per sex were exposed in inhalation chambers to room air.

Necropsy studies. Planned terminations of 10 rats per sex per dose were performed at 6 and 12 months of exposure and of 20 rats per sex per dose at 18 months. The remainder of the females were sacrificed at 24 months and the males at 25 months. Postmortem examinations were performed on all rats. Histopathologic examinations of about 50 tissues were performed on rats from the 100 ppm and two control groups that were killed at the 6 month and final intervals and on rats in any group that died or were killed in a moribund state. About 15 major organs and tissues from rats in the 100 ppm and both control groups were examined microscopically at 12 and 18 months. At 6, 12, and 18 months, only tissues with gross lesions were examined from the 10 and 33 ppm groups. At the end of the study about 20 major organs and tissues from the rats in the 10 and 33 ppm groups were examined.

Virus infection. During the 15th month of exposure, rats in all groups became infected with sialodacryoadenitis virus. This resulted in a loss in body weight in all groups and increased mortality in the females exposed to 100 ppm compared with the other groups. Exposure to ethylene oxide was stopped for 2 weeks after which time body weights, clinical signs, and mortality rates returned to preinfection status. The authors concluded that this outbreak was unlikely to have affected the results of the study.

Mortality. Cumulative percentage mortality did not increase significantly in the 10 or 33 ppm dose groups, but did increase in the 100 ppm dose groups, after 22 months exposure for males and after 21 months for females.

Tumor incidence. Tumor incidence was not significantly increased at 18 months of exposure; however, increased incidences of several types of tumors were observed in groups sacrificed at 24 and 25 months, the end of the study for female and male rats, respectively (Table 4.1).

Mononuclear cell leukemia. The incidence of mononuclear cell leukemia (MNCL) was increased for both sexes in all dose groups, but was statistically significant only for females treated with 100 ppm ethylene oxide. A positive dose-related increase in MNCL incidence in females was observed ($p < 0.01$). A statistically significant trend was not observed for males. When the incidence of MNCL in rats killed at the end of the study is combined with the incidence in the rats dying spontaneously or euthanized when moribund, a statistically significant increase also occurs in females exposed to 33 ppm ($p < 0.01$) and 100 ppm ($p < 0.001$) (Table 4.2). A significant increase was not observed in males. A mortality-adjusted trend analysis revealed a significant positive trend for females ($p < 0.005$) and males ($p < 0.05$). The time to first tumor was not significantly decreased for MNCL in the exposed rats (Table 4.3), but trend analysis indicated earlier tumor development.

Peritoneal mesotheliomas. The increased incidence of peritoneal mesotheliomas observed in males treated with ethylene oxide for 24 months (Table 4.1) was not statistically significant; however, when the rats that died spontaneously or were euthanized when moribund are included, a

Table 4.1 Selected Tumor Incidence in F344 Rats Which Survived the Entire Period of Exposure to Ethylene Oxide and Were Killed After 24 Months

	ppm of Ethylene Oxide				
Organ/Sex	100	33	10	CI	CII
Total number animals examined grossly:					
Males	30	39	51	48	49
Females	26	48	54	60	56
Number with tumor[a]:					
Spleen mononuclear cell leukemia					
Males	9	12	9	5	8
Females	15[b]	14	11	5	6
Peritoneal mesothelioma					
Males	4	4	2	1	1
Pituitary adenoma					
Males	12	13	15	16	13

a. Number of those animals in which histological examination of tissues was performed that had the tumor indicated.

b. $p < 0.001$ for comparison to CI and CII

SOURCE: Adapted from Snellings et al. 1984a

Table 4.2 Selected Tumor Incidences in F344 Rats Exposed to Ethylene Oxide Which Died Spontaneously, Were Euthanized When Moribund or Were Killed at 24 Months of Exposure*

	ppm of Ethylene Oxide				
	100**	33**	10**	CI	CII
Spleen Mononuclear Cell Leukemia					
Males	25/80*** (31%)	23/80 (29%)	21/80 (26%)	20/80 (25%)	18/80 (23%)
Female	27/80(c,a,c) (34%)	24/80(b,a,b) (30%)	14/80 (18%)	9/80 (11%)	13/76 (17%)
Peritoneal Mesothelioma					
Male	21/80(c,c,c) (26%)	6/80)(-,-,a) (8%)	3/80 (4%)	1/80 (1%)	2/80 (3%)

* These data were taken from EPA (1985). EPA questioned whether microscopic examination of all tissues or only tissues with gross lesions was performed on animals that died spontaneously or were killed when moribund. Information in the paper published by Snellings et al. (1984a) indicates that histopathology was performed on all 50 tissues from these animals.

** Superscripts denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (CI plus CII).
a = $0.05 > p > 0.01$; b = $0.01 > p > 0.001$; c = $p < 0.001$; - = not significant

*** Numerator equals number of rats with specified finding. Denominator equals number of rats for which specified tissues were examined. Forty of the 120 animals had already been sacrificed before the first tumors of these types appeared.

SOURCE: Adapted from Snellings et al. 1981 and cited in EPA 1985.

Table 4.3 Time to Tumor for F344 Rats Exposed to Ethylene Oxide for 2 Years

Ethylene oxide concentration ppm	Time in months to: First tumor	Median tumor(a)
Mononuclear cell leukemia - Males		
100	19	24
33	13	25
10	20	25
0-I	18	23
0-II	21	25
Mononuclear cell leukemia - Females		
100	18	24
33	18	24
10	19	25
0-I	19	24
0-II	18	23
Peritoneal mesothelioma - Males		
100	15	23
33	18	25
10	20	--
0-I	18	--
0-II	20	--
Pituitary adenoma - Males		
100	15	25
33	15	25
10	18	25
0-I	17	25
0-II	18	25
Pituitary adenoma - Females		
100	10	24
33	17	25
10	16	24
0-I	15	25
0-II	18	25

(a)Medians were not presented if the total number of a particular tumor was three or less.

SOURCE: Adapted from Snellings et al. 1981 and cited in EPA 1985.

statistically significant increase ($p < 0.001$) for the high-dose group compared with controls was observed (Table 4.2, data from EPA, 1985). A mortality-adjusted trend analysis showed a highly significant relationship ($p < 0.005$) between ethylene oxide exposure and induction of peritoneal *mesotheliomas*. Snellings and co-workers state that this observation indicates that exposure to ethylene oxide was associated with this earlier occurrence of mesotheliomas. Data of time to tumor are reported in Table 4.3 (data from EPA 1985).

Pituitary adenomas. Although the incidence of pituitary adenomas was not significantly increased in either sex, exposure to ethylene oxide significantly decreased the time to tumor in males ($p < 0.01$) and females ($p < 0.0001$)(Table 4.3, data from EPA 1985).

Brain tumors. From the time of the 18-month sacrifice until the end of the study, the incidence of brain tumors, including gliomas (twelve *astrocytomas*, one oligodendroglioma, two mixed gliomas), granular cell tumors, and malignant reticulosis was increased in both sexes. The classification of brain tumors was based on light microscopic cytomorphologic features and on patterns of growth and infiltration. Immunohistochemical staining was not done; thus, the cellular origin of these tumors remained unresolved.

Data on tumors for rats killed at 18 or 24 months and those who died spontaneously or were sacrificed due to morbidity were further evaluated by Garman et al. (1985, 1986). The first brain tumors were noted in animals

killed at 18 months. The combined incidence of all three tumor types is shown in Table 4.4. The incidence in the 100 ppm and 33 ppm groups was significantly increased ($p = 0.004$ and $p = 0.027$, respectively) compared with the controls. In females a statistically non-significant, dose-related increase was noted in the combined incidence of these three tumor types (Table 4.4). The IARC working group noted that combining the three different histological types of tumors precluded a proper evaluation of the effects of ethylene oxide on the brain. (The IARC working group did not have the 1985 paper by Garman et al. available and based their results on Snellings et al. 1984a). However, even when only the gliomas are considered, a dose-related increase in tumor frequency is also observed (Table 4.4).

When the data are adjusted for early deaths, the Cox test statistic for adjusted trends in males is significant ($p < 0.001$) for gliomas or the combination of the three tumor types. In females, $p = 0.023$ for gliomas only and $p = 0.001$ for the three tumor types combined. Comparison of the controls with historical controls indicates that the concurrent group and the 10 ppm groups had the expected incidence of primary brain tumors.

Multiple neoplasms. The frequency of multiple primary (benign plus malignant) neoplasms was significantly greater than the controls in the 100 ppm-exposed male rats. For females all three exposed groups had significantly more multiple primary neoplasms than controls ($p<0.05$).

Conclusion. Snellings and co-workers (1984a) concluded that one or more biologically significant adverse effects were demonstrated in rats exposed

for 2 years to all three doses of ethylene oxide, based on the numerically increased incidence of MNCL in females at 10 ppm and the statistically significant increase in the number of rats with multiple primary neoplasms.

4.1.5.2. Lynch et al. 1984b

The results of this National Institute for Occupational Safety and Health (NIOSH) study in male Fischer 344 rats confirmed the findings of Snellings et al. (1984a). An increased incidence of mononuclear cell leukemia and of peritoneal mesothelioma was found in male rats. Brain gliomas were also found.

Groups of 80 weanling rats were exposed to 0 (filtered air), 50 or 100 ppm 99.7% pure ethylene oxide, 7 hours/day, 5 days/week, for two years. Histopathology was performed on standard sets of 34 tissues plus all gross lesions for all rats that died or were sacrificed.

Mycoplasma infection. At approximately 8, 16, and 20 months into the study, rats were treated for 2 to 3 weeks with tetracycline for pulmonary infections. (Mycoplasma pulmonis was confirmed by serology during the 16th month outbreak.) Exposure to ethylene oxide was stopped only for 14 days during the 16th month.

Mortality and morbidity. The median survival time and body weight gain were decreased in animals exposed to both concentrations of ethylene oxide compared with controls, survival time in the high-dose group was significantly decreased ($p < 0.01$). The authors concluded that mortality

Table 4.4 Statistical Analyses on Adjusted Ratios of Primary Brain Tumor Frequencies in F344 Rats Exposed to Ethylene Oxide for Two Years

	ppm of Ethylene Oxide			
Tumor/Sex	100	33	10	0 (CI & CII)
Gliomas				
Males	6/87*(1) (6.9%)	3/85(2) (3.5%)	0/92 (0%)	1/181 (0.6%)
Females	2/78(3) (2.6%)	2/90 (2.2%)	1/94 (1.1%)	0/187 (0%)
Gliomas, Malignant Reticulosis and Granular Cell Tumors				
Males	7/87(4) (8.0%)	5/85(5) (5.9%)	1/92 (1.1%)	1/181 (0.6%)
Females	4/80(6) (5.0%)	3/92 (3.3%)	1/94 (1.1%)	1/188 (0.5%)

* Adjusted ratios = number of rats with tumor/number alive at time first tumors observed in any group.

Fisher's Exact 2-Tailed Probability Levels:

(1) 0.011; (2) 0.195; (3) 0.172; (4) 0.004; (5) 0.027; (6) 0.058

SOURCE: Adapted from Garman et al. 1985.

was affected by ethylene oxide treatment as well as by the M. pulmonis infection. Rats exposed to 50 or 100 ppm had a higher incidence than controls of inflammatory lesions of the lungs, nasal cavities, trachea and internal ear as well as an increased incidence of bronchiectasis and bronchial epithelial hyperplasia. These findings are consistent with the manifestations seen in chronic respiratory disease complex in rodents.

Mononuclear cell leukemia. The incidence of MNCL in animals dying during the study plus the terminal sacrifices was significantly greater ($p = 0.03$) in the 50 ppm group, but not the 100 ppm group, than in the controls (Table 4.5). Survival in the 100 ppm group was 19% compared to 49% in controls. If the incidence of MNCL of only the terminally sacrificed rats is compared, a statistically significant increased incidence of MNCL ($p < 0.01$) is observed for the 100 ppm group.

Peritoneal mesotheliomas. Peritoneal mesotheliomas were significantly increased in the 100 ppm group ($p = 0.002$), but not the 50 ppm group, compared with controls, even in the presence of excess mortality. Use of the Armitage test for trend suggested a proportional increase in the incidence of mesotheliomas with increased exposure.

Other neoplasms. The incidence of brain gliomas was increased in the 100 ppm dose group ($p < 0.05$) compared with controls (Table 4.5). Trend analysis suggested a significant increase in gliomas with increased exposure to ethylene oxide. Two additional rats exposed to 50 ppm and four additional rats exposed to 100 ppm had increased numbers of glial cells,

Table 4.5 Selected Tumor Incidence in Male F344 Rats Exposed to Ethylene Oxide for 2 Years

Organ	ppm of Ethylene Oxide		
	100	50	Control
Spleen Mononuclear Cell Leukemia	30/76[a] (39%)	38/79[b] (48%)	24/77 (31%)
Peritoneal Mesothelioma	21/79[c] (27%)	9/79 (11%)	3/78 (4%)
Brain			
Glioma (Mixed cell)	5/79[b] (6%)	2/77 (3%)	0/76 (0%)
Astrocytoma	0/79	0/77	0/76

a Groups consisted of 80 male rats at beginning of study. Denominators less than 80 reflect tissues accidentally lost on that could not be examined histologically due to autolysis.

b Statistically significant difference versus controls: $p < 0.05$.

c Statistically significant difference versus controls: $p < 0.01$.

SOURCE: Adapted from Lynch et al. 1984b.

termed "gliosis." The authors suggest that these cases of gliosis may represent incipient gliomas.

The incidence of other neoplasms was generally comparable among treated groups and controls and was not related to ethylene oxide exposure. Although never seen in the controls, a high incidence of proliferative lesions of the adrenal cortex, including nodules that depressed the adjacent tissue, was noted in treated animals. These lesions were classified as nonneoplastic changes.

Conclusion. The authors concluded that a no-observed-effect level (NOEL) was not found for ethylene oxide in this study. Although the M. pulmonis outbreak affected the study, the authors and the DHS staff believe the findings are still valid because (1) the same types of exposure-related tumors were found in F344 rats by Snellings et al. (1984a), and (2) Mycoplasma infection has not been associated with carcinogenesis.

4.1.5.3. Mice

The National Toxicology Program has completed a two-year inhalational study of ethylene oxide effects at 0, 50, and 100 ppm in male and female $B6C3F_1$ mice (NTP 1986). Statistically significant, increased incidences of both benign and malignant lung tumors and of Harderian gland tumors in both sexes and of uterine, mammary gland, and hematopoeitic system (e.g., malignant lymphoma) tumors in females were observed. The incidence data for several tumors are shown in Table 4.6. Since human exposure includes inhalation,

Table 4.6. Selected Tumor Incidences in NTP Study of Mice Exposed to Ethylene Oxide for 2 Years

Organ/Sex		ppm of Ethylene Oxide 100	50	Control
Alveolar/Bronchiolar Adenoma or Carcinoma				
Male	Overall	26/50[c] (52%)	19/50 (38%)	11/50 (22%)
	K-M Adjusted	68.3%	55.4%	33.2%
Female	Overall	22/49[c] (45%)	5/48 (10%)	2/49 (4%)
	K-M Adjusted	58.6%	20.8%	7.7%
Malignant Lymphoma				
Female	Overall	22/49[c] (45%)	6/48 (12%)	9/49 (18%)
	K-M Adjusted	48.3%	19.0%	26.4%
Uterine Adenoma or Adenosarcoma				
Female	Overall	5/49[b] (10%)	2/47 (4%)	0/49 (0%)
	K-M Adjusted	14.3%	7.6%	0%
Mammary Gland Adenosarcoma or Adenosquamous Carcinoma				
Female	Overall	6/49 (12%)	8/48[b] (17%)	1/49 (2%)
	K-M Adjusted	17.1%	24.8%	2.9%

a Exposure groups consisted of 50 male and 50 female mice at the beginning of the study. Denominators less than 50 in the overall incidence category reflect tissues accidentally lost or that could not be examined histologically due to autolysis. K-M Adjusted incidences are Kaplan-Meier tumor incidences at the end of the study after adjusting for intercurrent mortality.

b Statistically significant difference versus controls: $p < 0.05$ (Fisher exact).

c Statistically significant difference versus controls: $p < 0.01$.

SOURCE: Adapted from NTP 1986.

the detection of lung tumors is especially interesting. Calculations using the several data sites from this study with the multistage model gave values for carcinogenic potency comparable to those calculated using the published data for inhalation by rats in the Bushy Run Research Center study (Snellings et al. 1984a).

In a more limited study in mice (Adkins et al 1986), strain A/J female mice (6- to 8-weeks old) were exposed to 0, 70, and 200 ppm ethylene oxide for 6 hours/day, 5 days/week for only 6 months in one study and to 0 and 200 ppm in the same protocol in a second study. There were 30 animals in each exposure group and at least 28 animals in each group survived. In each study 28% of the control animals developed pulmonary adenomas. At 70 ppm 56% developed adenomas. At 200 ppm 87% had adenomas in the first study; in the second study only 42% of the animals exposed to 200 ppm ethylene oxide developed pulmonmary adenomas.

4.2. Epidemiologic Studies: Leukemia

Epidemiologic evidence for the carcinogenic effects of ethylene oxide is based on five longitudinal studies of occupational cohorts in Sweden, the United States and West Germany. Together the studies demonstrate an association between exposure to ethylene oxide and cancer. Table 4.7 contains a summary of these studies. Two additional studies, which were cross-sectional in design, evaluated leukemia incidence as part of a health evaluation of two separate occupational cohorts (Joyner 1964, Ehrenberg and

Hallstrom 1967). Neither of these studies was adequate to evaluate the carcinogenic effect of ethylene oxide since they were not designed to study this outcome.

The five longitudinal studies examined cancer outcomes for all sites and site-specific cancers, with leukemia as a focus for all studies. Four studies reported excesses in leukemia, while one study found no cases of leukemia. A discussion of these studies follows.

Hogstedt and co-workers. Hogstedt et al. (1979a) reported that three cases of leukemia had occurred between 1972 and 1977 among 230 Swedish workers exposed to 50% ethylene and 50% methyl formate at a factory that sterilized hospital equipment. Exposure at the plant began in 1968 and measurements taken in 1977 indicated concentrations of ethylene oxide of approximately 20 $\pm$ 10 ppm (TWA); exposure levels prior to 1977 are not known. The expected number of leukemia cases at this factory for 1968-1977 was 0.2 cases, based on national rates. Three cases were observed, including two myelogenous leukemias (4 and 8 years exposure) and one primary macroglobulinemia (6 years exposure)(Table 4.7).

In order to replicate these findings, Hogstedt et al. (1979b) conducted a cancer mortality study of the cohort of ethylene oxide production workers originally studied by Ehrenberg and Hallstrom (1967). The findings were similar, demonstrating elevated rates of leukemia and other cancers (Tables 4.7 and 4.8). The cohort was, however, exposed to other carcinogens, such as ethylene dichloride and bis(2-chloroethyl)ether. Exposure to ethylene oxide was between 5.5 and 27.5 ppm (1 to $5 \times 10^4 \mu g/m^3$) in the 1960s and 0.55

Table 4.7 Mortality and Cancer Incidence Studies of Workers Exposed to Ethylene Oxide

AUTHORS		ETO EXPOSURE	OTHER EXPOSURE	STUDY DESIGN	STUDY POPULATION	LEUKEMIA OBS	LEUKEMIA EXP	LEUKEMIA SMR	STOMACH TUMOR OBS	STOMACH TUMOR EXP	STOMACH TUMOR SMR	COMMENTS
Hogstedt et al (1979a,b and 1986)												
Plant one	1968-77	20 ppm(TWA)	methyl formate	case series	229 (69, fulltime exposure; 160, intermittent exposure)	3	.2[1]	1500	--			Leukemia was only disease reported
	1978-82			retrospective cohort mortality and cancer incidence	203	1	.05	2000	--			
Plant two	1961-77	6-28 ppm[3]	ethylene dichloride	retrospective cohort mortality and cancer incidence	89 fulltime exposure	2	.14[2]	1429	3	.4[2]	750	Other carcinogens present; follow-up period did not result in statistically significant excess of leukemia; combination with earlier data resulted in significant excess
		0.6-6 ppm[4]	propylene oxide		86 intermittent exposure	1	.13	769	1	.4	250	
			bis (2-chloro-ethyl) ether									
			ethylene									
	1978-82					1	.14	714	3	.65	462	
Plant three	1963-1982	3.2 ppm(TWA)	propylene oxide	retrospective cohort mortality	128 exposed to ETO	1	.16	625	--	--	--	
			other		69 exposed to ETO and propylene oxide	--	--	--	--	--	--	
					158 exposed to mix of ETO and others	--	--	--	--	--	--	

Table 4.7 (continued)

AUTHOR	ETO EXPOSURE	OTHER EXPOSURE	STUDY DESIGN	STUDY POPULATION	LEUKEMIA OBS	LEUKEMIA EXP	LEUKEMIA SMR	STOMACH TUMOR OBS	STOMACH TUMOR EXP	STOMACH TUMOR SMR	COMMENTS
Morgan et al (1981)	generally <.2 ppm; where detected <10 ppm		retrospective cohort mortality	767 potentially exposed	0	0.7	0	--	--	--	no statistically significant excess from any specific cause; 10.5 fold excess in leukemia needed for detection in this study
Thiess et al (1982)	--	propylene oxide and other chemicals too numerous to list	retrospective cohort mortality	602 exposed 1662 styrene workers as comparison	1	.15	667	4	2.7	148	no excess in overall cancer mortality; strong healthy worker effect seen; used comparison with styrene workers to eliminate this effect but styrene may be a carcinogen.

* All studies used SMR analysis.
1 p<.05
2 p<.01
3 1950-1963
4 1963-1979

to 5.5 ppm (0.2 to $1x10^4 \mu g/m^3$) in the 1970s. Exposed workers developed 9 tumors (including leukemias) where 3.4 would have been expected (SMR = 265)($p<0.01$).

Hogstedt et al. (1986) published findings from follow-up studies of the two cohorts described above and a group of production workers from a third plant that had not been studied previously. This last group included workers exposed only to ethylene oxide, exposed to ethylene oxide and propylene oxide, or exposed to a mixture of chemicals. All workers from this third plant had been exposed for at least one year. One leukemia was detected where 0.16 was expected.

Leukemia cases from the three plants are shown in Table 4.7. An excess of leukemia cases was reported in plants 1 and 2 during the 1978-1981 follow-up study but not in plant 3. Eight cases of leukemia were observed among 733 workers in the three plants where 0.83 were expected (SMR = 964)(Table 4.8).

In addition to excess leukemia among the three groups of workers, six stomach cancers were reported among plant 2 workers where 0.65 were expected. Neither the leukemia nor stomach cancer cases follow a dose-response pattern when analyzed by years of employment. However, years of employment can be a poor surrogate for exposure if, for example, highly exposed workers tended to shift to jobs with lower exposure or terminate their employment early.

Table 4.8 Leukemia Cases Observed and Expected in the Studies of Hogstedt et al. (1986)

Plant	Workers Studied	Time Interval: 1960's*-1977 Obs.	1960's*-1977 Exp.	1978-1981 Obs.	1978-1981 Exp.	1960's*-1981 Obs.	1960's*-1981 Exp.
1	203	2**	0.09	1	0.05	3	0.14
2	175	3	0.38	1	0.14	4	0.52
3	355	1	0.09	0	0.07	1	0.16
Total	733	6	0.56	2	0.27	8	0.83

* The initial year was 1968 for plant 1, 1961 for plant 2, and 1964 for plant 3.

** One of the original cases (macroglobulinemia) was later reclassified as a non-Hodgkin's lymphoma.

SOURCE: Adapted from Table 5 of Hogstedt et al. (1986).

Several comments need to be made about these studies in regard to size of populations studied, types of cancer induced, and multiple chemical exposures. First, although these three studies (Hogstedt 1979a, 1979b, 1986) include many of the ethylene oxide-exposed workers in Sweden, the number of workers involved in the above calculations are relatively small, resulting in large variability in the estimates of the ratios of observed to expected cases. On the other hand, the SMRs obtained are quite large. A second consideration in the finding of an association between ethylene oxide exposure and leukemia is that the leukemias were not limited to any one particular type. However, a single agent can induce a spectrum of cytogenetic aberrations in hematopoietic tissues resulting in the pattern of leukemias reported by Hogstedt et al. For example, while most data suggest that leukemia induced by benzene exposure tends to be myelocytic, one study examining rubber workers showed an excess of lymphocytic cases (McMichael et al. 1975). In addition, ethylene oxide induces cellular proliferation, including tumors in several rat tissues (see Tables 4.3 and 4.4). Third, in these studies all production workers experienced exposure to other chemicals, including carcinogens, in addition to ethylene oxide; however, the chemical common to all was ethylene oxide.

Morgan and co-workers. Two other epidemiologic studies have been conducted. Morgan et al. (1981) conducted a cohort study of ethylene oxide production workers at a Texas plant which had been in continuous operation since 1948. To be included in the study cohort, workers had to have been employed for at least five years. Unlike the previous studies, most of the employees worked outdoors. Although Morgan et al. did not report specific levels, ethylene oxide exposure levels were generally below 0.2 ppm, the limit of

detection of the analytical instrument used, since the authors state that in most areas sampled in a 1977 industrial hygiene survey, virtually no ethylene oxide was detected. A modified lifetable program was used to compare the mortality experience of 767 workers with the pattern expected on the basis of U.S. vital statistics. A reduced overall mortality (SMR = 58) was reported. Morgan and his collegues observed an excess of pancreas, bladder, and brain cancers and of Hodgkin's disease, but these excesses were not statistically significant. No leukemia cases were seen; however, the cohort size was sufficient to detect only very large increases, i.e., a 10.5-fold increase in leukemia, with 80% power. A 10.5-fold excess would not be expected, given the low levels of ethylene oxide exposure, when compared to Hogstedt's series. Also, this study, by excluding workers employed at the plant for less than five years, could have excluded a significant fraction of exposed workers, since entry level jobs are often associated with higher exposure to chemicals. In a recent letter (Divine and Amanollahi 1986) one of the authors of this study has attempted to use the above data to refute the studies of Hogstedt. The reasons that the study is inadequate for such a refutation are pointed out above.

Thiess and co-workers. Thiess et al. (1982) reported on the mortality experience of 602 production workers exposed to alkylene (ethylene plus propylene) oxide and other chemicals in nine West German plants. Ninety-two percent of workers employed between 1928 and 1980 were followed. Overall observed mortality and cancer deaths for the total cohort and for those with a minimum of 10 years of observation were lower than expected, based on mortality for either the local area or for West Germany. This indicates a strong healthy worker effect. A second comparison was made using a cohort

of 1662 styrene workers at the same plant in order to eliminate the healthy worker effect. However, the choice of styrene-exposed workers as a comparison group may have been inappropriate, since styrene monomer has been shown to be carcinogenic in animals (IARC 1979) and has been associated with an excess in lymphocytic leukemia among styrene-exposed workers (Ott et al. 1980). Nevertheless, in older workers, age 65 to 75, the relative risk of malignant tumors was 2.78 in ethylene oxide workers compared to styrene workers ($p<0.05$). The increased relative risks in younger age groups in the cohort were not statistically significant.

Conclusion. The evidence supporting an association between working with ethylene oxide and leukemia comes from 4 out of 5 occupationally exposed cohorts. The overall evidence cannot be considered conclusive due to the small numbers of workers involved and to the possibility that other workplace carcinogens may have been confounders. Nevertheless, the high estimates of risk are striking; standardized incidence and mortality ratios for leukemia ranged from 6 to 21. Furthermore, the replication of the early findings in other plants and in the followup of these same cohorts reduces the probability that the observed excesses of leukemia were chance findings. Since worker recall was not used to determine exposure, bias about exposure from that source is not present . The magnitude of the effect argues against the findings being due to confounding, particularly since the other carcinogens were different for the different plants. Though not conclusive, these studies provide substantial evidence of ethylene oxide's carcinogenicity in humans.

5. GENOTOXICITY

5.1. Summary

The genotoxicity of ethylene oxide has been extensively reviewed (EPA 1985, IARC 1985, Wolman 1979, NIOSH 1981, Kolman et al 1986). Ethylene oxide has been found to cause genetic damage in many major test systems in bacteria, fungi, higher plants, in vitro in mammalian cell systems, and in vivo in Drosophila, rodents, and monkeys. In lower prokaryotic and eukaryotic systems, and in cultured mammalian cell systems, ethylene oxide induced gene mutations and enhanced virally induced cell transformation in the absence of an exogenous metabolic activation system, thus indicating that it is a direct acting mutagen. Furthermore, in several test systems, there was a dose-dependent mutagenic response. There is also evidence of genetic damage to somatic cells caused by ethylene oxide in mice, rats, monkeys and humans. This consistent evidence of the genetic toxicity of ethylene oxide suggests that its reported carcinogenicity proceeds via genotoxic mechanisms. The available data are summarized below.

5.2. Mutagenicity

Ethylene oxide has been extensively tested in most major short-term tests and has been demonstrated to be a direct-acting mutagen.

5.2.1. Bacterial Assays

Several studies have been conducted in the Ames/Salmonella typhimurium assay (IARC 1976, 1985, De Flora 1981, Pfeiffer and Dunkelberg 1980, EPA 1985). These studies indicate that ethylene oxide is a direct-acting mutagen, producing base-pair substitutions. Typically, ethylene oxide has been found to be active in a dose-dependent manner in S. typhimurium strains TA1535 and TA100, but inactive in strains TA1538, TA1537, and TA98, both with and without activation by rat liver S-9 fraction. Positive responses have been reported using concentrations as low as 50 ppm when tested in the gaseous phase in strains TA1535 and TA100 (IARC 1985).

Similarly, a mutagenic response after exposure to ethylene oxide has been observed in the sporulation test, using his⁻ strains of Bacillus subtilis, and in the induction of Lambda bacteriophage in Escherichia coli. (EPA 1985, IARC 1985).

5.2.2. Eukaryotic Systems

Ethylene oxide has been demonstrated to induce revertants in Neurospora crassa and forward mutations in Schizosaccharomyces pombe (both with and without metabolic activation using phenobarbitone-induced mouse liver S-9 fraction) (EPA 1985). Ethylene oxide is also a recognized mutagen in higher plants (EPA 1985).

5.2.3. Cultured Mammalian Cell Assays

The mutagenicity of ethylene oxide has been evaluated in three different mammalian cell systems. In a study reported in abstract form, mouse lymphoma L5178Y (TK+/-) cells were exposed to a piece of polymethylmethacrylate that had been sterilized with ethylene oxide. The mutation frequency was increased 2 to 15 times by exposure to 0.01-0.05 mmol ethylene oxide (Brown et al. 1979).

In the CHO/HGPRT system, when 1-10 mmol ethylene oxide (99.7% pure) were added to one million cells in suspension, a linear, dose-related mutagenic response, with no apparent threshold, was observed. At the highest dose level not resulting in excessive cell toxicity, the mutation frequency was roughly ten times greater than background (Tan et al. 1981).

The frequency of ouabain- and 6-thioguanine-resistant mutants in V79 Chinese hamster lung cells was increased in a concentration-related fashion when these cells were treated with four concentrations of ethylene oxide ranging from 625 to 7,500 ppm in closed treatment chambers for 2 hours (Hatch et al. 1986). The mutation frequencies produced by ethylene oxide were approximately 32 to 94 and 7 to 128 times the spontaneous background levels for thioguanine- and ouabain-resistant mutants respectively.

5.2.4. In Vivo Mutagenicity Assays

Ethylene oxide has been found to induce an increased frequency in the number of lethal mutations, chromosome deletions, and chromosome breaks in

Drosophila melanogaster by oral or inhalational administration (EPA 1985). The mutation frequency in male D. melanogaster was shown to be directly related to the level of germ cell DNA alkylation, which in turn was proportional to the level of exposure to ethylene oxide (Lee et al. 1983).

No increase in gene mutations was observed in the stem- and poststem-spermatogonial cells of mice after inhalational exposure to 250 to 300 ppm ethylene oxide for 16 to 23 weeks. Litter size and frequency of mating were depressed in the early intervals after the end of exposure, an effect that the authors did not attribute to a dominant lethal effect but to the direct toxicity of ethylene oxide (Russell et al. 1984).

5.3. Chromosomal Damage

Ethylene oxide causes chromosomal damage in vitro and in rats and mice in vivo. Much of the work has been reviewed previously (IARC 1985, EPA 1985). Relevant findings are reviewed and summarized below.

5.3.1. Dominant Lethal Tests

Ethylene oxide has been tested for dominant lethal effects in both mice and rats, by different routes of administration, with positive responses in both species. In Long-Evans rats, a single four-hour inhalational exposure to 1000 ppm (which is close to the LC_{50}) caused a statistically significant increase in the number of post-implantation deaths. This observation was true for up to 5 weeks post-treatment, but not thereafter, indicating an effect on post-meiotic cells (Embree et al. 1977). Generoso et al. (1983)

observed an effect on post-meiotic cells of male (101XC3H)F_1 mice exposed to 255 ppm ethylene oxide for 6 hours/day for 5 days per week for two or eleven weeks.

In a follow-up study Generoso et al. (1986) examined what effect varying the inhaled dose-rate or the rate of exposure would have on the induction of dominant lethals. They not only reported a dose-related increase in dominant lethals, but also observed a marked dose-rate effect. For example, when mice were exposed to 1800 ppm-h on each of 4 consecutive days, mice that inhaled 1200 ppm for 1.5 h produced 64% dominant lethals while mice that inhaled 300 ppm for 6 h produced only 11% dominant lethals.

Just as ethylene oxide is more toxic to SEC/R_1 male mice than to C57BL/6 male mice, the germinal tissues of the former are more sensitive to ethylene oxide: dominant lethal mutations occurred in 6.9% of SEC mice and in none of the C57BL/6 mice (Niemann et al. 1986, Popp et al. 1986).

Results of other dominant-lethal tests by intraperitoneal injection in mice were consistent with these results except for one study using intramuscular injection and a dose level of 100 mg/kg (Appelgren et al. 1978). The reason for the difference in results is not immediately obvious but could be attributed to the different route of administration or the lower dose. However, results of a heritable translocation test in mice (Generoso et al. 1980) confirm the observation that ethylene oxide causes chromosomal damage.

5.3.2. Chromosomal Aberrations/Sister Chromatid Exchange Studies

Ethylene oxide has been shown to induce both chromosomal aberrations and sister chromatid exchanges (SCE) in a variety of in vitro and in vivo experimental systems. Ethylene oxide causes breaks, rings, inversions, and other types of chromosomal aberrations in rats and mice (Strekalova 1971). Kligerman et al. (1983) reported a dose- and treatment duration-related increase in SCE frequency but not in chromosomal aberrations in rats. Poirier and Papadopoulo (1982) observed a dose dependent induction of chromosomal aberrations in a FL cell line from human amniotic cells. Other investigators have reported SCE in monkeys and rabbits in vivo and in human skin fibroblasts in vitro (Yager and Benz 1982, Lynch et al. 1984a, Star 1980). Exposures as low as 36-59 ppm induced increased SCE or chromosomal aberrations.

The epidemiological literature indicates an association between occupational exposure to ethylene oxide and increased frequency of chromosomal aberrations and sister chromatid exchanges, even at very low exposure levels, as low as 1 ppm (Table 5.1). In most cases, however, exposure data were insufficient to make comparisons between air concentrations of ethylene oxide and cytogenetic findings, especially when no information on exposure prior to the time of the biological sampling was available. In addition, it has not been possible to measure frequencies of SCE and chromosomal aberrations in workers prior to their exposure to ethylene oxide, thus, cause and effect cannot be pinpointed. These studies have been reviewed previously (EPA 1985, IARC 1985, Sheikh 1984, Landrigan et al. 1984) and are

Table 5.1 Observations of SCE and Chromosomal Aberration (CA) Induction in Peripheral Lymphocytes of Occupationally Exposed Human Populations

Reference	Test System	Exposure Information	Results	Comments
Garry et al. 1979.	Induction of SCE in hospital workers.	Maximum exposures: 36 ppm as 8 hour TWA.	Statistically significant increased SCE frequency in exposed workers.	One individual exposed one time to 1500 ppm showed significantly increased SCE frequency.
Hansen et al. 1984.	Induction of SCE in hospital sterilizer workers.	All were exposed to < 5 ppm TWA as determined during 5 weeks of monitoring during the time of blood sampling.	No significant differences in SCE levels between exposed and control groups.	1. Controls matched for age and sex. 2. Smoking did not increase SCE frequency.
Hogstedt et al. 1983.	Induction of CA and SCE in lymphocytes and micronuclei in bone marrow cells of industrial sterilizer workers.	Work room concentrations did not exceed 1 ppm at time of blood sampling, but were measured as high as 28 ppm 2.5 years prior to blood sampling.	1. No significant difference in frequency of SCE. 2. Increased frequency of cells with breaks, gaps, and other aberrations significantly related to exposure. 3. Nonsignificant elevation in frequency of micronuclei.	1. Data corrected for age and smoking habits. 2. Culture methods deviated from standard procedures.

Reference	Test System	Exposure Information	Results	Comments
Johnson & Johnson 1982. (cited in EPA 1985)	Induction of SCE and CA in industrial workers.	Estimated relative exposures: Low (< 1 ppm) Moderate (1-10 ppm) High (5-200 ppm).	Suggestive dose-related increase in SCE and CA frequency.	Preliminary data only.
Laurent et al. 1982, 1983, 1984.	Induction of SCE in hospital workers chronically exposed to high levels of ethylene oxide.	Cumulative 2-year exposure was between 500 (low exposure) and 5800 mg (high exposure).	Significantly increased SCE frequency in exposed workers.	
Richmond et al. 1985.	Induction of CA and SCE in sterilizer workers.	1-40 ppm for 8-hour TWA; occasional short-term exposure to 75 ppm. Exposure monitored from 1977-1980; exposure prior to 1977 not known.	1. SCE increased in some, but not all, exposed persons. 2. CA were consistently increased in those persons with highest exposure.	1. Mild, nonspecific clinical symptoms of toxicity reported in exposed workers. 2. Insufficient data available to make comparison between air concentrations and cytogenetic findings.

Reference	Test System	Exposure Information	Results	Comments
Sarto et al. 1984.	Induction of SCE and CA in hospital workers.	High exposure group: 10.7 +/- 4.9 ppm as 8-hour TWA for 6.8 +/- 3.5 years. Low exposure group: 0.35 +/- 0.12 ppm as 8-hour TWA for 3.0 +/- 1.1 years.	1. Significant increases in SCE frequency observed at both exposure levels ($p < 0.001$). 2. Significantly increased frequency of CA in high-exposure group ($p = 0.05$). 3. Significantly increased frequency of CA in low exposure group only when gaps were excluded.	
Stolley et al. 1984. Galloway et al. 1986.	Induction of SCE in sterilizer workers.	Exposure levels measured at 3 worksites at time of blood sampling: Low: 0.5 ppm as TWA Moderate: 5-10 ppm as TWA High: 5-20 ppm; lowered from 50-200 ppm in previous years.	1. Significant increase in SCE and CA in those exposed to highest levels of ethylene oxide. 2. Some increase in CA for those exposed to lowest levels of ethylene oxide; no increase in SCE	1. High levels of SCE persisted for up to 24 months after cessation of treatment.

Reference	Test System	Exposure Information	Results	Comments
Theiss et al. 1981.	*Induction of CA in* industrial workers.	Exposure levels: 1. Long-term (> 20 years) 2. <20 years 3. Long-term plus accidental exposure. 4. Accidental exposure only.	Increased frequency of CA (excluding gaps) with longest inferred exposure.	Workers had been exposed to other alkylene oxides and breakdown products in addition to chemicals such as benzene.
van Sittert et al. 1985.	Induction of CA in workers in an ethylene oxide manufacturing plant.	Exposure levels were mostly below the detection limit of 0.05 ppm, with occasional intervals of 8 ppm recorded. Exposure monitored at intervals from 1974 to 1981. Exposure prior to 1974 unknown.	No significant difference observed between exposed group and control.	1. Controls matched for age, sex, and smoking habits. 2. Smoking and recent exposure to radiography had no effect on frequency of CA in either test group or controls.
Yager et al. 1983.	Induction of SCE in hospital workers.	Cumulative exposures determined by individual monitoring over a 6-month period: High: >100 mg Low: <100 mg	Significantly increased frequency in high-exposure group only.	Controls matched for age, sex, and personal habits.

summarized in Table 5.1. From these studies it is not possible to determine whether the presumed ethylene oxide effect on chromosomes results from continuous exposure to low, moderate, or high levels of ethylene oxide or from occasional, very high, brief exposures.

5.3.3. Micronucleus Tests

An increased number of micronuclei have been observed in bone marrow cells of mice and rats treated either by intraperitoneal or intravenous administration (Appelgren et al. 1978, EPA 1985) An elevated frequency of micronucleated cells has also been observed in bone-marrow smears from persons occupationally exposed to ethylene oxide (Table 5.1, Hogstedt et al. 1983).

5.3.4. Unscheduled DNA Synthesis (UDS) Tests

Several other studies suggest that ethylene oxide has the ability to damage DNA and cause unscheduled DNA synthesis (UDS). In an inhalation study, male mice were exposed for 8 hours daily to 300 ppm. Measurement of UDS in germ cells before and after exposure showed that the response increased in a nonlinear fashion with successive exposures, indicating accumulation of damage in germ cell line DNA. Normally there should be no DNA synthesis during sperm maturation. Different response patterns were observed for each successive 8-hour exposure to higher concentrations of ethylene oxide (presumably 400 to 500 ppm), which the authors considered evidence of a concentration-dependent inhibition of the capacity of germ cells to repair lesions in DNA (Cumming and Michaud 1979).

The ability of ethylene oxide to initiate UDS was verified in vitro by biochemical and autoradiographic studies of human blood cells exposed at 37°C for 1 hour to concentrations ranging up to 100 mmol. A linear increase in UDS was observed up to 0.5 mmol; above this concentration there was an inhibition (Pero et al. 1981, 1982).

5.4. Mammalian Cell Transformation Assays

One study has reported that ethylene oxide can transform mammalian cells in vitro. Syrian hamster embryo cells were treated for 2 or 20 hours with 625, 1250 or 2500 ppm ethylene oxide gas in sealed chambers. Cells treated for 2 hours, but not 20 hours, showed a concentration-related increase in sensitivity to SA-7 virus transformation. The 20-hour exposure was probably ineffective because the ethylene oxide concentrations decreased with time (Hatch et al. 1986). Although the assay is considered a transformation assay, in this case the chemical may enhance virus transformation rather than induce transformation by itself, yet it is another indication that ethylene oxide causes perturbations in DNA (Heidelberger et al. 1983).

6. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

6.1. Summary

Ethylene oxide has been evaluated for its potential to cause teratogenic and reproductive effects in four animal species by two routes of administration. Based on these results, exposure to ethylene oxide appears to be a risk for the developing embryo. Ethylene oxide was teratogenic in mice by intravenous injection at 150 mg/kg, a dose that was not maternally toxic. A teratogenic effect was not induced in the rat by inhalation in two studies, but embryotoxicity occurred at 150 mg/kg, a dose level that was marginally toxic to the mother. Rabbits administered ethylene oxide via injection or inhalation during organogenesis displayed no evidence of teratogenicity and no embryotoxicity was observed in the absence of maternal toxicity.

The risk of ethylene oxide to male and female reproductive systems has been demonstrated in laboratory animals; however, mechanisms by which ethylene oxide exerts these health effects are not clear. There is evidence that inhalational exposure may interfere with oocyte maturation or ovulation in the rat. Evidence of a mechanism for the reproductive toxicity of ethylene oxide comes from the experimental induction of dominant lethal mutations in male mice and rats.

The results of one epidemiologic study are suggestive of a toxic effect of ethylene oxide on human reproduction. The findings reviewed in the EPA

Health Assessment Document are summarized below, as well as other recent reports.

A NOEL for the reproductive toxicity of ethylene oxide in experimental animals has not been unequivocally established. However, based on laboratory studies which show no effects below 10 ppm, no adverse effects would be expected at ambient concentrations of ethylene oxide, estimated to be approximately 50 ppt.

6.2. Teratogenic Effects in Animals

6.2.1. Inhalation Studies

Maternal and fetal toxicity in rats. Snellings and coworkers (1982a) reported no developmental defects in the offspring of pregnant Fischer 344 rats exposed to 10, 33, or 100 ppm of ethylene oxide by inhalation 6 hours/day on days 6 to 15 of gestation. However, fetal body weights were significantly reduced at the high dose. Clinical signs of toxicity were not noted in the mothers, but it could not be determined whether this dose was also maternally toxic since maternal body weights were not recorded.

Hardin and co-workers (1983) exposed female Sprague-Dawley rats by inhalation to 150 ppm ethylene oxide on gestational days 7 to 16 or 1 to 16; a third group of rats was exposed to 150 ppm for 3 weeks prior to mating and throughout gestational days 1 to 16. Embryotoxicity was noted, as indicated by a significant increase in the incidence of resorptions occurring in the rats exposed before mating. This exposure regimen caused a reduction in

maternal body-weight gain. Rats under all exposure regimens displayed more subtle evidence of fetal toxicity. Fetal body weight, crown-rump length and the degree of skeletal ossification were reduced. Evidence of maternal toxicity in the treated rats included increased spleen and kidney weights, both absolute and relative to body weight.

Lack of teratogenicity in rabbits. Hardin and coworkers (1983) exposed pregnant female New Zealand white rabbits to 150 ppm via inhalation on gestational days 7 to 19 and found no evidence of either maternal or fetal toxicity or teratogenicity.

Fetal toxicity in mice. Cytostatic effects of ethylene oxide on fetal tissue have been reported in abstract form by Popp et al.(1985). Pregnant female C57BL/6 mice were exposed to 255 ppm ethylene oxide by inhalation, 6 hours/day for 4 days, beginning 13.5 and 14.5 days postcopulation. Fetal body weights were reduced and an adverse effect on the hematopoietic system of the fetal liver, as evidenced by a depressed hematocrit, reduced hemoglobin levels and decreased red cell number. Lock (1986) exposed pregnant BALB/c mice to 1500 ppm ethylene oxide by inhalation for either 2 or 3 successive days for 10 minutes twice a day between days 10 and 16 of gestation. Susceptibility to ethylene oxide exposure was noted in fetuses exposed on gestation days 12 to 14. A fetal mortality of 51% was observed in animals sacrificed on day 15.

6.2.2. Intravenous Studies

Reduction of fetal body weight in mice. The teratogenic effect of intravenous administration of 75 or 150 mg/kg ethylene oxide in CD-1 mice was investigated at four periods during gestation: days 4 to 6; 6 to 8; 8 to 10; or 10 to 12. At 150 mg/kg, some maternal toxicity was noted, especially at days 6 to 8, and a significant reduction in mean fetal body weight was observed at all treatment periods. Teratogenic effects, primarily skeletal defects including malformations of vertebrae, were observed as a result of exposure to 150 mg/kg ethylene oxide on days 6 to 8. Although not statistically significant, the frequency of malformations was also increased in groups given intravenous injections of ethylene oxide at 75 or 150 mg/kg on days 8 to 10 and 10 to 12 (LaBorde and Kimmel 1980).

Lack of a teratogenic effect in rabbits. Jones-Price et al. (1985) conducted a study of intravenous injection of ethylene oxide in New Zealand White rabbits. Doses of 9, 18, and 36 mg/kg were administered on days 6 to 14 of gestation, which was the entire period of organogenesis, and concentrations of 18 and 36 mg/kg were administered to other rabbits on days 6 to 9. An increase in the resorption rate was noted at the highest dose level, a dose that also induced maternal toxicity when administered on days 6 to 14. No evidence of a teratogenic effect was noted.

6.3. Other Reproductive Effects in Animals

6.3.1. Inhalation Studies

Reduction in number of offspring in rats. Snellings et al. (1982b) exposed both male and female Fischer 344 rats to 10, 33, and 100 ppm of ethylene oxide by inhalation, 6 hours/day, 5 days/week for 12 weeks before breeding. Females continued to be exposed 6 hours/day, 7 days/week through day 19 of gestation. In females exposed to 100 ppm, a reduction in the fertility index that was not statistically significant was observed, and there were significantly fewer offspring.

Hardin et al. (1983) administered 150 ppm of ethylene oxide by inhalation, 7 hours/day for 3 weeks, before mating and through gestation day 16. The percent of females that became pregnant was not altered, but the animals did have a small, but not statistically significant, reduction in corpora lutea. These data suggest that prolonged exposure to ethylene oxide may reduce female reproductive potential by impairing oocyte maturation or ovulation.

Reduction of fertility in male mice. Popp et al. (1986) reported strain differences in the effects of inhalation exposure of male C57BL/6 and SEC mice to ethylene oxide. Males were exposed to 150 ppm, 6 hours/day, 5 days/week for 4 weeks. The animals were mated to untreated females at the end of each week. The C57BL/6 strain was found to be much more resistant than the SEC strain to the toxic effects of ethylene oxide. SEC males were sterile during the exposure period and for 4 weeks after treatment. Extensive damage to the testes was confirmed histologically. The C57BL/6 strain remained fertile. However, the compound induced dominant lethal mutations, affecting primarily the mid- and late-stage spermatids.

Adverse effects on sperm of monkeys. Lynch et al. (1983) exposed cynomolgus monkeys to 50 and 100 ppm of ethylene oxide by inhalation 7 hours/day, 5 days/week for 2 years. Sperm concentration and sperm motility were significantly reduced in animals at both dose levels. Two of the eight monkeys exposed to 100 ppm were azoospermic. No adverse effects on spermhead morphology were observed.

6.4. Epidemiologic Studies of Reproduction: Spontaneous Abortions

Epidemiologic studies evaluating reproductive effects of ethylene oxide exposure are limited to one study by Hemminki et al. (1982) of spontaneous abortions among hospital sterilizing staff. A second study (Hemminki et al. 1985) has been cited in the literature; however, it looked at spontaneous abortions and malformations among offspring of nurses exposed to a variety of potential hazards in hospitals, rather than ethylene oxide specifically, and is, therefore, not considered further here.

In the 1982 study, current sterilizing staff in approximately 80 general hospitals in Finland were identified by supervising nurses and sent questionnaires regarding number of pregnancies and their outcomes, occupation at the time of each pregnancy, employer, children's health, smoking habits and the intake of coffee, tea, and alcohol. Information about exposure to sterilizing chemicals was obtained from supervising nurses in order to prevent the sterilizing staff from knowing the purpose of the study. Supervising nurses selected an equal number of nursing auxiliaries in the same hospital to serve as controls.

The crude rate of spontaneous abortion was 11.3% (of total pregnancies) for sterilizing staff and 10.6% for the control group. When the pregnancies of sterilizing staff were analyzed according to employment at the time of conception, the rates were 16.7% for the exposed and 6.0% for the non-exposed.

The effect of different sterilizing agents on the frequency of spontaneous abortions was analyzed. The use of ethylene oxide was associated with an increase in the rate of spontaneous abortions from 7.8% in women not exposed during pregnancy to 16.1% among exposed ($p<0.01$). Glutaraldehyde and formaldehyde use during pregnancy were not associated with an increase in spontaneous abortions. Rates were adjusted for age, parity, decade of pregnancy, smoking, alcohol and coffee consumption. The findings of this study have been questioned for a number of reasons (Gordon and Mienhardt 1983). For example, nurses were asked to recall pregnancies dating back to 1951. No exposure measurements were taken at the study sites. Data from a general survey of Finnish hospitals were used instead as an estimate of exposure. Since nursing supervisors were familar with the purpose of the study, there is a question of biased misclassification of exposure. This study considered all pregnancies in the analyses and therefore included non-independent events among women with repeat pregnancies. The data suggest an association between ethylene oxide and spontaneous abortion, but cannot stand alone as basis for a causal inference. An increase in spontaneous abortions would be consistent with the increased resorptions noted by Hardin et al. (1983) and the findings in other animal studies cited above.

7. QUANTITATIVE RISK ANALYSIS

7.1. Noncarcinogenic risks

At 0.09 $\mu g/m^3$ (50 ppt), the annual average ambient air level of ethylene oxide estimated in part A of this document by the Air Resources Board, noncarcinogenic effects risks are not expected to occur (see Sections 3 and 6).

7.2. Carcinogenic risks

The EPA Health Assessment Document for Ethylene Oxide (1985) includes quantitative risk assessments for cancer based on both animal and epidemiologic data. DHS staff members have relied on the EPA document, including the risk assessment, in the preparation of this report. When using animal data from inhalational studies in rats, EPA combined incidences from two anatomical sites to calculate risk. DHS staff, however, uses the most sensitive sex, site and species for risk assessment. Therefore, DHS staff has used the female rat mononuclear cell leukemia data from Snellings et al. (1984a) in the multistage model. Cancer risk at ambient levels was estimated by extrapolating five orders of magnitude from this data, by means of the best fitting linearized multistage model. This model provides a reasonably health-conservative risk estimate due to its property of being linear at low doses (California Department of Health Services 1985). In addition we have fit several other models to the data for comparison.

7.2.1. Thresholds

A threshold dose of a toxicant is one below which a specified outcome does not occur. While threshold models for carcinogenesis (based on, for example, saturation of detoxification enzymes, the existence of DNA repair mechanisms, or recurrent toxicity) have been proposed, none has been convincingly demonstrated.

An "epigenetic" mechanism that could in theory embody threshold doses has been invoked to explain the carcinogenic action of substances that do not directly produce genetic damage in short-term tests. However, for ethylene oxide there is compelling evidence of genotoxicity because of binding to DNA and mutagenicity (Chapter 5). There is also experimental evidence for ethylene oxide acting as an initiator of tumorigenesis (Chapter 4). Therefore, DHS staff treats ethylene oxide-induced carcinogenesis as a nonthreshold phenomenon.

7.2.2. Rat Leukemia and the Multistage Model.

The data used to calculate cancer risk from the female rat mononuclear cell leukemia observed in the Bushy Run Research Center study (Snellings et al. 1984a) are given in Table 7.1 (EPA 1985). The table differs from Table 4.2 presented above. The denominators in Table 7.1 include only those animals whose tissues were examined, that were alive at the time the first leukemia was detected (see Table 4.3) and were thus at risk for the tumor. There is

Table 7.1 Incidence of Mononuclear Cell Leukemia in Female Rats Among Survivors to First Tumor

Exposure (ppm)	Number with leukemia/ Corrected / number exposed	Equivalent human lifetime dose (mg/kg/day)
0	22/186 (11.8%)	0
10	14/71 (19.7%)	0.28
33	24/72 (33.3%)	0.75
100	28/73 (38.4%)	2.11

Adapted from EPA 1985 (Table 9-33, p. 9-150)

an extra tumor in the 100 ppm group numerator because it was found in one of the rats removed for quality control at 18 months and was therefore excluded from Table 4.2. We confirmed the EPA numbers by analysis of Table A-73 in the Appendices of the Bushy Run study (Snellings et al 1981). Denominators obtained by approaches other than that used by the EPA do not differ significantly from their approach.

Using the computer software Global 82 (Crump and Howe 1982), a linearized multistage model was fit to the female leukemia dose-response data. Doses were first converted to human equivalents (Tyler and McKelvey 1980, EPA 1985). The multistage model may be expressed as:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)}$$

where P(d) is the lifetime probability of cancer for a given dose d of carcinogen, q_0 is a constant that accounts for the background incidence of cancer occurring in the absence of carcinogen, and q_1, q_2, ... q_k are coefficients that allow the data to be expressed to various powers of the dose of carcinogen to obtain the best fit of the model to the data. The female rat leukemia data yielded a maximum likelihood estimate (MLE) for q_1 (the linear or slope term, which relates the probability of cancer to the dose of carcinogen administered in the equation for the multistage model) of 0.20 $(mg/kg/day)^{-1}$. "The multistage model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data" (EPA 1985). In the present case, however, no higher order terms, i.e., coefficients multiplied by dose raised to a power greater than

one, were obtained. For the rat leukemia data, the equation therefore reduces to:

$$P(d) = 1 - e^{-(q_0 + q_1 d)} = 1 - e^{-(0.14 + 0.20\ d)}$$

An Upper 95% Confidence Limit (UCL) on q_1 of 0.29 $(mg/kg/day)^{-1}$ was also obtained from the data . Such UCLs are calculated because they are health conservative, i.e., there is only a 5% chance that the true value of q_1 is greater than the UCL. [The values for q presented here are the same values EPA obtained using the same data].

Assuming that the percentage of ethylene oxide absorbed by inhalation is similar for rats and humans and using an average human body weight of 60 kg and an average air intake of 18 m^3 per day (California Department of Health Services 1985), a dose of 1 mg/kg/day ethylene oxide is equivalent to 3300 $\mu g/m^3$. Using the latter units, the MLE for q_1 equals $6.1 \times 10^{-5} (\mu g/m^3)^{-1}$ and the 95% UCL for q_1 equals $8.8 \times 10^{-5} (\mu g/m^3)^{-1}$. Since for ethylene oxide 1 ppm = 1800 $\mu g/m^3$, additional calculations yield a MLE of $q_1 = 0.11\ (ppm)^{-1}$ and a 95% UCL $q_1 = 0.16\ (ppm)^{-1}$.

7.2.3. Risk Estimate using the Gaylor-Kodell Approach and Other Models

The application of the multistage model requires the use of a computer program to fit the model to the data and extrapolation into the range below the lowest dose tested. A simpler approach is the technique introduced by Gaylor and Kodell (Gaylor and Kodell 1979, Williams and Burson 1985). In

this method the observed responses are fit to a model, then the upper confidence limit (UCL) is determined on the predicted value from the model at the lowest dose of chemical for which cancer risk is increased over background. This UCL is then extrapolated linearly ("interpolated") to the background incidence to determine an upper boundary line on risk. Under the assumption of strict linearity (not just at low doses), the true risk is predicted to be at or below this line with 95% probability . For mononuclear cell leukemia in female rats this 95% UCL on the 10 ppm dose was determined to be 0.31 using the model which gave the best fit to the data. Subtracting the background incidence of 0.1183 (22/186) yielded an additional risk of 0.1917. Dividing this by 0.28 mg/kg human equivalent dose yielded a potency of 0.685 $(mg/kg/day)^{-1}$. This converts to 0.374 $(ppm)^{-1}$. For an ambient level of 50 ppt ($50x10^{-6}$ ppm), the risk is 0.374 x $50x10^{-6}$ = $1.9x10^{-5}$. Risk values, both best estimates and 95% upper confidence limits, obtained using the multistage model, the Gaylor-Kodell approach, and several mathematical models of cancer risk (California Department of Health Services 1985, Kovar and Krewski 1981) are shown in Table 7.2. All risk estimates are within one order of magnitude.

Usually the multistage model predicts the highest risk at low dose levels. The probit generally predicts the least, followed in order of increasing risk by the gamma multihit, logit, and Weibull models; but, in this case, the multistage model appears to predict lower risks than the gamma multihit, logit, and Weibull models.

The gamma multihit and Weibull models predict higher risks than the multistage model (at low doses) because the best-fitting curve to the data

is supralinear. This occurs when the exponential factor is less than one, but greater than zero, but such a number would not be consistent with the biological basis of the models (Crump 1985). Therefore, the staff of DHS considers the multistage model the most appropriate to use in this case.

The data from the NTP study of ethylene oxide (NTP 1986) were also used in the multistage model. Both overall incidence and incidence adjusted for intercurrent mortality were used with animal ppm (Table 4.6). The q_1^* was highest for alveolar/bronchiolar adenomas and adenocarcinomas in males. The q_1^* was slightly less than twice that from the rat female mononuclear leukemia data. Since there were more animals and more dose groups including lower dose levels in the rat study and since the the data would be extrapolated over five orders of magnitude, it was decided to use the rat data.

7.2.4. Estimate of Cancer Incidence in the Los Angeles Basin

Air modeling of ethylene oxide in the Los Angeles Basin has yielded an estimated mean ambient concentration of 0.09 $\mu g/m^3$ (50 ppt). With an estimated population of $7x10^6$ and an upper 95% confidence limit on individual risk of $7.8x10^{-6}$ derived using the multistage model (Table 7.2), the upper 95% confidence limit estimate of excess cancers over a lifetime due to exposure to ethylene oxide would be:

$$7x10^6 \times 7.8x10^{-6} \approx 55 \text{ excess cancers.}$$

Using the risk of 1.9×10^{-5} calculated using the approach of Gaylor and Kodell, an estimate of 133 lifetime excess cancers in the population of 7 million people is obtained. Using the 95% UCLs from the several models in Table 7.2, the range of lifetime excess cancers is approximately 49 (One hit) to 497 (Weibull).

The risk estimate is based on extrapolating downward over four orders of magnitude from the doses used in the animal bioassay. While a small portion of the population is exposed to concentrations of ethylene oxide greater than 0.09 $\mu g/m^3$, others will be exposed to less.

7.2.5. Correlation of Animal and Epidemiologic Data

Hogstedt et al. (1986) observed 8 cases of leukemia in their population of 733 workers exposed or potentially exposed to ethylene oxide, where 0.83 cases would be expected. The compatibility of the animal-based risk assessment with these epidemiologic data was evaluated by comparing the observed leukemia mortality with the cancer deaths predicted by the extrapolation model (Hertz-Picciotto et al 1987). The predicted excess cancer deaths for the Hogstedt cohort were obtained by applying the linearized multistage model, with the slope estimated from the animal data, to the Hogstedt cohort. The model is of the form

$$P(d) \simeq 1\text{-}\exp(-q_1 \times d)$$

where P(d) is the excess risk for an individual exposed to a low-level constant lifetime dose d, and q_1 is the slope estimated as described above.

Summing the risks for all workers yields a total excess lifetime cancer risk for the cohort. The value for d was based on the assumption that exposures were 8 hours/day, 5 days/week, 48 weeks/year. Since the length of occupational exposures was given as 1 to 20 years, a mid-value of 10 years was assumed for all workers, and a human lifetime was assumed to be 70 years. The exposure data for plant 3 showed average levels of 3.2 ppm for the 355 workers exposed between 1963 and 1982. The conversion from the occupational to a lifetime ambient exposure yields

$$3.2 \text{ ppm} \times (8/24) \times (5/7) \times (48/52) \times (10/70) = .100 \text{ ppm}.$$

Assuming the exposures in plants 1 and 2 were similar, on average to the levels in plant 3, the model yields an individual lifetime excess cancer risk of

$$P = 1 - \exp (-.11 \times .100)$$

$$P = .011$$

For 733 workers at risk for a lifetime, this yields 8.18 excess cancer deaths. Adding the predicted excess to the expected leukemia deaths yields a total predicted leukemia mortality of 9.01. This is remarkably close to the observed eight leukemia deaths, though these represented only a partial lifetime of follow-up. Nevertheless, given the comparatively short latencies for hematopoietic malignancies, if these workers received no further exposure and were then followed until death or age 70, it is highly probable that the number of leukemia deaths observed would not be significantly different from the number predicted.

While these calculations involve uncertainties, particularly in regard to the human exposure estimates, they do indicate that the carcinogenic

potencies of ethylene oxide in animals and humans are comparable. Carrying out a similar calculation for a subset (n = 69) of the plant 1 employees who received exposures of 20 ± 10 ppm, (= .63 ppm lifetime ambient exposure) the predicted leukemia cancer deaths are 4.8 while 2 were observed. Assuming leukemia deaths in this cohort follow a Poisson distribution, the predicted deaths are well within the 0.95 confidence interval of the observed (0.24, 7.2). Similarly, by limiting this analysis to the plant 3 employees, 4.1 leukemia deaths are predicted while one (0.03, 5.6) was observed.

Finally, these same calculations were applied to the data of Morgan et al. (1981). These authors state that their analytical instrument, which had a detection limit of 0.2 ppm, "...detected virtually no ethylene oxide in the area." Where there were readings, they "..were less than 10 ppm." Suppose all workers were exposed to 0.2 ppm ethylene oxide for their work careers. The average years of exposure cannot be determined from the published paper. A minimum and a maximum can be derived. The upper limit on length of exposure is 18.2 years (the average length of exposure plus follow-up). The estimated lifetime dosage would be:

$$0.2 \text{ ppm} \times 18.2/70 \times 48/52 \times 5/7 \times 8/24 = 0.011 \text{ ppm}$$

The risk per worker at this plant would therefore be:

$$1 - \exp(-.11 \times .011) = 0.0012$$

yielding a predicted excess of 0.93, or total of 1.63 leukemia deaths, compared to zero observed.

The minimum predicted excess risk is based on the lower limit of exposure which yields an estimated lifetime dosage of 0.003 ppm exposure. The risk per worker would be .0003, the predicted excess would be 0.25, and the total leukemia deaths would be 0.95. Since the true predicted risk is between the minimum (0.95) and the maximum (1.63), and since these lie well within the 95% confidence interval of the observed (0, 3.69), the model is compatible with results from this epidemiologic study.

Table 7.2 Excess Lifetime Cancer Risk Predicted by Various Models for an Environmental Exposure of 0.09 $\mu g/m^3$ (50 ppt) of Ethylene Oxide

Model	Excess Lifetime Risk		
	MLE[1]	UCL[2]	p value[3]
Multistage[4]	5.5×10^{-6}	7.8×10^{-6}	0.17
One hit (linear regression)	5.3×10^{-6}	7.0×10^{-6}	ND
Gamma Multihit	1.1×10^{-5}	2.9×10^{-5}	0.20
Probit	1.8×10^{-5}	5.4×10^{-5}	0.38
Logit	1.9×10^{-5}	6.0×10^{-5}	0.38
Weibull	2.2×10^{-5}	7.1×10^{-5}	0.36
Gaylor-Kodell	--	1.9×10^{-5}	NA

1. Maximum Likelihood Estimate
2. Upper 95% Confidence Limit
3. p value for goodness of fit. In this case the higher the p value, the better the fit.
4. For the multistage model, the excess risk stated is the extra risk which makes use of Abbott's correction and equals $[P(d) - P(0)] / [1 - P(0)]$.

ND = not determined; NA = not applicable

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