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United States Environmental Protection Agency
Office of Pollution Prevention and Toxics

ETHYLENE OXIDE

PROPOSED ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

"PUBLIC DRAFT"

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, which authorizes development of Acute Exposure Guideline Levels (AEGLs), the National Advisory Committee to develop Acute Exposure Guideline Levels (AEGLs) has been established to identify, review and interpret relevant toxicologic and other scientific data, and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent ceiling exposure values for the general public and are applicable to emergency exposure periods ranging from less than 1 hour to 8-hours. Three AEGLs will be developed for each of four exposure periods (30 minutes, 1 hour, 4 hours, and 8 hours) and will be distinguished by varying degrees of severity of toxic effects. The three AEGLs have been defined as follows:

AEGL-1 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience notable discomfort. Airborne concentrations below AEGL-1 represent exposure levels that could produce mild odor, taste, or other sensory irritations.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience irreversible or other serious, long-lasting effects or impaired ability to escape. Airborne concentrations below the AEGL-2 but at or above AEGL-1 represent exposure levels that may cause notable discomfort.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance at or above which it is predicted that the general population, including "susceptible" but excluding "hypersusceptible" individuals, could experience life-threatening effects or death. Airborne concentrations below AEGL-3 but at or above AEGL-2 represent exposure levels that may cause irreversible or other serious, long-lasting effects or impaired ability to escape.

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EXECUTIVE SUMMARY

Ethylene oxide is a highly flammable gas produced in very large quantities in the U.S. (5.3-6.3 billion pounds). It is very reactive with nucleophiles, such as water, alcohols, halides, amines, and sulfhydryl compounds. Ethylene oxide is used as an intermediate in the production of ethylene glycol and nonionic surfactants; a small amount is used as a fumigant for sterilizing foods and heat-sensitive medical equipment. The odor detection level for ethylene oxide is 260 ppm (468 mg/m^3) to 700 ppm (1260 mg/m^3).

The database of toxicity to ethylene oxide vapor in humans and experimental animals is very extensive including data on all aspects of toxicity except lethality in humans. Pharmacokinetics data show that ethylene oxide is readily absorbed from the respiratory tract of both humans and animals. It alkylates proteins and DNA, and it is metabolized by hydrolysis and glutathione conjugation.

In humans, inhaled ethylene oxide vapor affects the eyes, respiratory tract, central and peripheral nervous systems, gastrointestinal tract (probably secondary effects to nervous system toxicity), hematopoietic system, and possibly the reproductive system, and fetus. Acute exposure to ethylene oxide at the odor detection level ($\geq$ 260 ppm) causes eye and upper respiratory tract irritation and signs and symptoms of effects on the central and peripheral nervous system. Acute exposure to a calculated concentration of 500 ppm for 2 to 3 minutes caused hematologic effects and more severe effects on the central nervous system than those noted at the odor detection level. Effects observed after acute exposure are reversible, including severe nervous system effects. Peripheral nervous damage is exacerbated by repeated exposures. Human studies have provided suggestive evidence of reproductive toxicity, some evidence of an association between exposure to ethylene oxide and genetic damage to somatic cells and limited evidence of carcinogenicity.

Acute lethality studies in experimental animals showed that mice are the most sensitive species (4-hour LC_{50} = 660-835 ppm) (Jacobson et al., 1956), followed by the dog (4-hour LC_{50} = 960 ppm) (Jacobson et al., 1956) and rat (4-hour LC_{50} = 1537-1972 ppm; 1-hour LC_{50} = 4439-5748 ppm) (Jacobson et al., 1956). Immediate deaths were due to respiratory failure and delayed deaths were due to secondary respiratory infections. Experimental animals exposed to lethal and nonlethal concentrations of ethylene oxide showed evidence of eye and respiratory irritation and effects on the central and peripheral nervous system (Embree et al., 1977). Additional studies in animals exposed to ethylene oxide for various durations up to 6 hours/day provided evidence of reproductive toxicity at $\geq$ 50 ppm, developmental toxicity at $\geq$ 50 ppm, genetic toxicity in germ cells at $\geq$ 75 ppm, and carcinogenicity at 100 ppm.

Data were available for deriving AEGL-2 and -3 values. Values for AEGL-1 were not derived because the odor threshold and concentrations causing mild sensory irritation would be above the AEGL-2 levels.

The AEGL-2 values were based on a rat study showing central nervous system depression, diarrhea, and eye and respiratory tract irritation after exposure to 1000 ppm of ethylene oxide for 4 hours (Embree et al., 1977); genetic toxicity (dominant lethality) was also seen at this concentration in this same study. An uncertainty factor of 10 was applied for intraspecies variability, because of the steep slope of the dose response relationship from severe irritation and central nervous system depression to the lethality threshold. An uncertainty factor of 3 was applied for interspecies sensitivity, because modes of action are likely to be similar between rodents and humans and systemic uptake of ethylene oxide is similar across species. The time-scaling approach used ten Berge's equation in which $C^n \times t = k$, and n = 1.2 based on analysis of rat lethality data.

AEGL-3 values were derived from lethality data in the rat. An LC_{01} value (628 ppm), which is considered an approximation of the lethality threshold, was estimated from data in a 4-hour acute inhalation study with rats reported by Jacobson et al. (1956). An uncertainty factor of 10 for intraspecies sensitivity was applied to the LC_{01} estimated values and this was followed by scaling to the different AEGL exposure periods based on ten Berge's equation ($C^n \times t = k$, where n = 1.2 based on reported lethality data for 1- and 4-hour exposures). An interspecies uncertainty factor of 3 was applied because systemic uptake, distribution, and modes of action are likely to be similar between rodents and humans. There are differences in metabolism kinetics, but they are unlikely to affect responses to high acute exposures. Assessment of carcinogenicity data (lung adenomas/carcinomas in female mouse) (NTP, 1987) showed that extrapolating the total cumulative exposure over a 2-year period to single exposures and estimating a 10^{-4} risk resulted in AEGL-3 values of 2764, 1382, 346, and 173 ppm for 0.5-, 1-, 4-, and 8-hour exposures. These values exceed those derived from lethality data.

AEGL values derived for ethylene oxide are summarized below:

PROPOSED AEGL VALUES FOR ETHYLENE OXIDE

Classification	Exposure Periods				Endpoint (Reference)
	30 minutes	1 hour	4 hours	8 hours	
AEGL-1	No values derived				
AEGL-2	190 ppm (342 mg/m^3)	110 ppm (198 mg/m^3)	33 ppm (59 mg/m^3)	19 ppm (34 mg/m^3)	Central nervous system effects Embree et al., 1977
AEGL-3	360 ppm (648 mg/m^3)	200 ppm (360 mg/m^3)	63 ppm (113 mg/m^3)	35 ppm (63 mg/m^3)	Lethality threshold Jacobson et al., 1956

References

Embree, J.W.; Lyon, J.P.; Hine, C.H. 1977. The mutagenic potential of ethylene oxide using the dominant-lethal assay in rats. Toxicol. Appl. Pharmacol. 40:261-267.

Jacobson, K.H.; Hackley, E.B.; Feinsliver, L. 1956. The toxicity of inhaled ethylene oxide and propylene oxide vapors. Arch. Ind. Health. 13: 237-244.

1. INTRODUCTION

Ethylene oxide (a monoepoxide) is a gas at room temperature and normal atmospheric pressure; the vapor density indicates that it is heavier than air. The vapor is highly flammable at concentrations ranging from 3 to 100%, and it may undergo explosive decomposition (WHO, 1985; Gardiner et al., 1993). Ethylene oxide is very reactive with nucleophiles such as water, alcohols, halides, amines, and sulfhydryl compounds (WHO, 1985, U.S. EPA, 1985). Physicochemical properties of ethylene oxide are presented in Table 1.

Ethylene oxide is produced in very large quantities in the United States and in other countries. Estimated U.S. production was 5.3 to 6.2 billion pounds in 1990 (Gardiner et al., 1993; IARC, 1994) and 5.6 billion pounds in 1992 (IARC, 1994). Worldwide production exceeds 12 billion pounds (IARC, 1994) and may be as high as 16.5 billion pounds (Gardiner et al., 1993). Ethylene oxide is an intermediate used in the production of ethylene glycol (antifreeze), which accounts for about 60% of its use; nonionic surfactants, which account for about 16%; ethanolamines, which account about 8.5%; and glycol ethers, diethylene glycol, triethylene glycol and other chemicals, which account for the remaining 16% (IARC, 1994). A small amount of ethylene oxide is used as a fumigant for sterilizing heat-sensitive medical and dental equipment and foods, such as spices, and nuts (Gardiner et al., 1993; IARC, 1994).

Ethylene oxide is not persistent in the environment; estimated degradation rate in the atmosphere is 37% in 5.8 days. The half-life is 12 to 14 days in fresh water and 4 days in salt water (U.S. EPA, 1985; IARC, 1994).

The database for ethylene oxide is very large; humans and experimental animals studies on acute toxicity, developmental and reproductive toxicity, genetic toxicity (somatic and germ cells), carcinogenicity, and pharmacokinetics and metabolism were available. These data are used to derive the AEGL values.

Table 1. Physical/chemical Data		
Chemical Name	**Ethylene oxide**	
Synonym	1, 2-epoxyethane, oxirane, dimethylene oxide, ethene oxide	
CAS Registry No.	75-21-8	
Chemical Formula	C_2H_4O	
Molecular Weight	44.05	Budavari et al., 1996
Physical State	colorless, flammable gas	Budavari et al., 1996
Vapor Pressure	1.50 atm; 152 kPa, 1.52 bar @ 21°C	Braker and Mossman, 1980
Density (vapor)	1.49 @ 40°C	Gardiner et al., 1993
Density (liquid)	0.8824 @10/10°C	IARC, 1994
Specific gravity	0.8966 @ 0/4°C; 0.8711 @ 20/20°C	Gardiner et al., 1993
Critical Temperature	468.95°K, 195.8°C, 384.4°R	Braker and Mossman, 1980
Boiling/Freezing point	10.4°C/-112.5°C	Gardiner et al., 1993
Autoignition tempt.	702°K, 429°C, 804°F	Braker and Mossman, 1980
Flammability Limit	3.0 to 80.0%	Braker and Mossman, 1980
Solubility	soluble in water, acetone, acetone, benzene, ethanol, and diethyl ether	IARC, 1994
Conversion	1ppm = 1.8 mg/m^3@ 25°C, 1 atm	Gardiner et al., 1993

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No studies were available on lethality attributable to ethylene oxide in humans. Marchland et al (1956) reported the accidental death of three workers involved in the manufacture of ethylene oxide. They experienced vomiting, abdominal pain, diarrhea, headache, and severe nervous system effects that progressed to coma, circulatory collapse, and respiratory failure. Pulmonary edema and congestion of the meninges and brain was observed at the postmortem examination of one victim. The workers were exposed to glycol chlorohydrin, dichloroethane, and ethylene oxide; the deaths were attributed to glycol chlorohydrin and dichloroethane exposure and not ethylene oxide.

2.2. Nonlethal Toxicity

2.2.1. Experimental Studies, Case Reports, and Anecdotal Data

Several experimental studies on ethylene oxide exposure were available in the literature. In one study, Jacobson et al. (1956) used an osmoscope to determined the concentration at which human volunteers detected the odor of ethylene oxide; no other details were provided concerning experimental procedure. The subjects reported the odor was pleasantly to sickeningly sweet, fruity, alcoholic, or acetone- or ether like. The median detectable concentration was 700 ppm (1260 mg/m^3) with a 95% confidence interval of 317 to 1540 ppm (571 to 2772 mg/m^3).

Hellman and Small (1974) conducted a study in which a trained panel of subjects ("odor panel") characterized the sensory odor properties of 101 petrochemicals, one of which was ethylene oxide. The properties were defined as (1) absolute odor threshold: the concentration at which 50% of the panel observed an odor, (2) 50% odor recognition threshold: the concentration at which 50% of the panel defined the odor as being representative of the odorant, (3) 100% odor recognition threshold: the concentration at which 100% of the panel defined the odor as being representative of the odorant, and (4) hedonic tone: the pleasure or displeasure associated with the odor quality as judged by the panel. They also derived an "odor index", which is the vapor pressure (ppm)/100% odor recognition threshold (ppm). The absolute odor threshold for ethylene oxide was 260 ppm (468 mg/m^3), and the 50% and 100% odor recognition threshold was 500 ppm (900 mg/m^3). The odor index was 2000, which placed ethylene oxide in a category of low odor potential. The odor was considered to be sweet or olefinic and judged to be neutral with respect its odor pleasantness or unpleasantness. Hellman and Small (1974) did not report the number of subjects involved in this study or provide additional information on the "training" received by the subjects. Cawse et al. (1980) reported that olfactory fatigue occurs upon repeated exposure to ethylene oxide, thus rendering the warning properties of smell as ineffective.

In another study, Walker and Greeson (1932) exposed four human subjects to ethylene oxide vapor diluted to a concentrations of 1:80 or 1:400 (13,349 or 2670 ppm equivalent to 24,028 or 4806 mg/m^3). [The authors reported that 2 lb/1000ft^3 is equal to a 1:60 dilution]. At the 1:80 dilution, ethylene oxide was definitely irritating to the nasal passages within 10 sec, but caused no lacrimation. At the 1:400 dilution, the subjects described the odor as acetic acid-like, but not unpleasant; this concentration was slightly irritating to the nasal passages; the duration of exposure was not reported. These exposure concentrations cannot be considered reliable, because the study authors did not discuss the means by which the concentrations were verified (calculations based on chamber size and quantity of ethylene oxide released or measurement of ethylene oxide in air samples).

The following case studies describe signs and symptoms of ethylene oxide intoxication and the concentrations and exposure durations at which they occurred.

Salinas et al. (1981) reported that a female nurse accidentally exposed to ethylene oxide vapor for 2 to 3 minutes showed immediate signs and symptoms of intoxication including repeated episodes of nausea, stomach spasms, paleness, lightheaded, short periods of unconsciousness, convulsive movements of her arms and legs, and periods of apnea (cessation of breathing). Muscle twitching, nausea, and malaise continued for 24 hours after exposure; malaise and an inability to perform minor motor tasks continued for up to 1 week after exposure. Chest X-rays, laboratory studies, and arterial blood gases were normal. The patient was asymptomatic 2 months after exposure. The authors estimated maximum exposure as 500 ppm based on the release of 17 g of ethylene oxide into the sterilizer bag. Her exposure could have been considerably greater than the calculated concentration of 500 ppm.

Five hospital workers were exposed 30 minutes to ethylene oxide vapors emitted from a leaky sterilizer at concentrations high enough to be detected by its odor ($\geq$ 260 ppm) (Deleixhe et al. 1986; in French, cited from IARC, 1994; Laurent, 1988). Two workers experienced only headache and diarrhea, which disappeared within 70 hours after exposure. Three workers suffered more serious signs of toxicity including irritation of the upper respiratory tract, dry mouth and thirst, conjunctival irritation, severe headache, and intense generalized pruritus, along with muscular weakness in one worker and dizziness in another (Laurent, 1988). Muscular weakness may have been a sign of toxicity to the peripheral nervous system. Nausea, vomiting, and diarrhea started 20 hours after exposure, lasted for 14 days, and cleared up by 21 days. Hemolysis noted on days 9-11 persisted until day 16. The concentrations experienced by the sterilizer workers could have been higher than the odor threshold of 260 ppm (1260 mg/m^3); measured concentrations were 15 to 50 ppm (27 to 90 mg/m^3) 2.5 hours after the accident and about 5 ppm (9 mg/m^3) the next day.

Garry et al. (1979) described the symptoms experienced by 15 nurses exposed to ethylene oxide. The frequency of upper respiratory irritation indicated that exposure was intermittent showing a bimonthly cycle over a 5-month period. During a 2-month period, 12 nurses experienced sore throat and dry mouth (most prominent symptoms), diarrhea, conjunctival irritation, headache, nausea, speech difficulty, recent memory loss, weakness, dizziness, and incoordination. The maximum ethylene oxide concentrations ranged from 36 ppm (64.8 mg/m^3) about 15 feet (breathing zone) from the sterilizer to 1500 ppm (2700 mg/m^3) at the floor level. Garry et al. (1979) also reported than an investigator was exposed to 1500 ppm for 5 minutes; symptoms of intoxication were not described. The signs and symptoms cannot be attributed to a single exposure.

Finelli et al. (1983) described the signs and symptoms experienced by three sterilizer operators accidentally exposed to ethylene oxide for 4 months to 1½ years. Symptoms of intoxication included numbness,

tingling, cramps, weakness, and incoordination in the lower extremities and cramps in the hands. In addition, there were frequent complaints of eye irritation, headaches, smelling of fumes, sleeplessness, and nervousness. Neurological examination showed distal abnormalities in the legs and feet (reflex, vibratory sensation, and flexion), but no abnormalities in cranial nerves. An abnormal gait was noted in one patient and bilateral footdrop in two patients. Nerve conduction studies showed abnormalities in motor and sensory conduction potential in the lower extremities in two patients and normal conduction potential of the third, and electromyograms showed abnormal potentials in the lower extremities. The resulting diagnosis was distal axonal neuropathy (peripheral neuropathy).

The National Institute for Occupational Safety and Health (NIOSH) conducted a survey to assess the effects of exposure to ethylene oxide on ten hospital workers (Zey et al., 1994). The workers complained of headache, dizziness, mucous membrane irritation, nasal bleeding, vomiting, diarrhea, facial flushing and swelling, fatigue, nervousness, and a "sweet"-like odor. The 8-hour TWA concentration in the breathing zone of the workers ranged from 0.23 to 0.56 ppm, with short-term excursions reaching 77 ppm for one area of the breathing zone and 11 ppm in another. The authors believed the concentrations were higher than those measured in the present investigation, because the employers noticed the ethylene oxide odor, which has a detection threshold higher than the measured concentration. The effects also suggest higher exposures.

Deschamps et al. (1992) described a case of persistent nonimmunological asthma and slight peripheral neuropathy that developed in a worker exposed to ethylene oxide 4 h/day for 4 days. The worker was about 18 meters from an ethylene oxide leak and he wore no protective equipment. The worker noticed an odor suggesting that the concentration was $\geq$260 ppm. Signs and symptoms after the 4-day exposure included coughing, shortness of breath, and wheezing. Respiratory symptoms persisted and 1 year after the accident, pulmonary function tests showed bronchial obstruction and bronchial hyper-reactivity. The forced vital capacity (FVC) was 93% of the predicted value, forced expiratory volume in 1 second (FEV_1) was 74% of predicted, mid-expiratory flow rate (FEF_{25-75}) was 44% of predicted, and the FEV_1 after 600 μg acetylcholine showed a 20% decrease. The respiratory effects persisted for at least 3 years after exposure. Immunological tests showed no formation of IgE antibody to ethylene oxide. In addition, a neurological examination showed signs of proprioceptive axonal neuropathy. Another five workers including one asthmatic were exposed because of the leak; none experienced respiratory effects.

Gross et al. (1979) reported on three workers accidentally exposed for 2 weeks to 2 months to ethylene oxide vapor from a leaky sterilizer. Symptoms experienced by the workers included irritation of the conjunctiva and mucous membranes, decreased sense of smell and taste, headaches, nausea, vomiting, and lethargy. Recurrent major motor seizures occurred in one patient, but there was no evidence of peripheral neuropathy. A

second worker experienced muscle weakness and increased fatigue and showed evidence of peripheral neuropathy. Problems with memory and thinking, difficult swallowing, cramps, numbness, and weakness in the arms and legs occurred in a third worker, along with clinical signs that included slurred speech, confusion, weakness of facial and distal muscles, and muscular incoordination. A neurological test also showed evidence of peripheral neuropathy. The exposure concentrations for these workers were not monitored; however, intermittent odor detection of ethylene oxide suggested excursions greater than 260 ppm during workshifts.

2.2.2. Epidemiologic studies

Bryant et al.(1989) surveyed hospital sterilizer workers from 27 different hospitals who were potentially exposed to ethylene oxide. Short-term symptoms were identified by means of a questionnaire sent to 241 workers; 182 responded, 165 of whom worked with ethylene oxide. The age of the cohort ranged from less than 20 years (1%) to greater than 60 years (9%). Ethylene oxide concentrations ranged from peaks of 11 to 23.5 ppm decreasing to <1ppm within 60 sec or from 8.5 ppm decreasing to 1 ppm within 160 sec depending on the type of sterilizer used. The total exposure concentration per sterilizer cycle ranged from undetectable to 10.7 ppm with exposure times per cycle ranging from 166 sec (2.77minutes) to 705 sec (11.75 minutes). The mean concentration per cycle was 3.4 ppm. The most prevalent symptoms other than the odor of ethylene oxide included headaches, skin and eye irritation, dry mouth, and sore throat. The detection of the ethylene oxide odor suggests that the concentrations exceeded 260 ppm, at least briefly. Other symptoms included skin rash, runny nose, loss of sense of smell, shortness of breath, nausea, numbness in fingers, and drowsiness. A larger number of workers exposed to concentrations above the mean reported more symptoms than did workers exposed to concentrations below the mean, suggesting a concentration effect. Some of the symptoms may have been due to daily peak exposures and some were likely due to repeated exposures over a prolonged period of time.

2.3. Developmental/Reproductive Toxicity

Hemminki et al. (1982) conducted a cross-sectional study on the spontaneous abortion rate (number of spontaneous abortions/number of pregnancies) among the staff of 80 Finnish hospitals who used ethylene oxide to sterilize heat-sensitive equipment. Ethylene oxide-exposed and control groups were identified by the hospital nursing staff who also distributed the questionnaires to each individual. The return rate for the questionnaires was about 91% for both groups. Specific exposure data were not reported in this study, but the mean 8-hour time-weighted average (TWA) ranged from 0.1 to 0.5 ppm, with the peak concentration reaching 250 ppm at Finnish hospitals. Data from about 24 hospitals showed that concentrations varied between 5 and 10 ppm for about 20 minutes when the sterilizer door was open (Hemminki et al., 1983). The data as summarized in Table 2 are presented as crude and adjusted rates (age, parity, decade of reported pregnancy, coffee and alcohol consumption, and smoking habits). Crude and adjusted spontaneous abortion rates were significantly elevated

for female staff exposed to ethylene oxide compared with the unexposed control group. Data obtained from hospital discharge records produced similar results for the spontaneous abortion rates: 22.5% ($p<0.05$, compared with for controls) for the ethylene oxide-exposed staff and 9.2% for the control. The abortion ratio (number of spontaneous abortions/number of births) based on hospital records was also higher in ethylene oxide exposed workers (33.3% compared with 11.8% for controls, $p<0.05$). The findings of this study are not conclusive; several weaknesses are evident. Both the exposed and control populations were identified by the nursing staff without corroborating exposure data. Hospital discharge records confirmed the results for only about one-third of the respondents. There are inherent recall biases when results are based on memory of the respondents. The number of sterilizing staff exposed to ethylene oxide alone during pregnancy was very small compared with the other groups.

Table 2. Spontaneous Abortion Rates Among Hospital Sterilizing Staff And Controls

Group	Total Number of pregnancies	Crude Rate (%)	Adjusted. Rate[a] (%)
Sterilizing staff[b]	1443	11.3	9.7
Exposed during pregnancy	545	16.7*	15.1*
Not exposed during pregnancy	605	6.0	4.6
Uncertain	293	123*	11.3*
Ethylene oxide alone			
Exposed during pregnancy	82	20.7**	16.1*
Not exposed during pregnancy	1068	10.3	7.8
Control	1179	10.6	10.5

Source: Hemminki et al., 1982.
[a]adjusted for age, parity, decade of reported pregnancy, coffee and alcohol consumption, and cigarette smoking.
[b]Includes staff exposed to ethylene oxide, glutaraldehyde, and formaldehyde sterilants.
*$p<0.05$, **$p<0.01$; exposed versus nonexposed pregnancies.

Rowland et al. (1996) conducted a cross-sectional epidemiologic study on the reproductive outcomes in California dental assistants potentially exposed to ethylene oxide and showed an increased risk of adverse reproductive outcome associated with exposure to ethylene oxide. The exposed population consisted of respondents who listed ethylene oxide as the method used to sterilize instruments at the last menstrual date of their last pregnancy. Adverse pregnancy outcomes included spontaneous abortion (<21 weeks), preterm delivery (21-36 weeks), and postterm deliveries ($\geq$ 42 weeks). Thirty-two women reported exposure to ethylene oxide;

spontaneous abortion occurred in five, preterm birth in three, and postterm birth in five. Of the 1,288 unexposed women in the study; 88 reported a spontaneous abortion, 56 a preterm birth, and 141 a postterm birth. The adjusted relative risk of adverse outcomes and relative risk are presented in Table 3. The small number of respondents exposed to ethylene oxide reduced the statistical power of the study and limited the analysis of confounding factors (exposure to nitrous oxide, mercury, and cigarette smoking). Further analysis by the study authors suggested that these confounders did not significantly affect the risk of an adverse reproductive outcome. This study has a number of limitations and weaknesses. Exposure measures were not reported, but the authors noted that high concentrations were likely because of the type of sterilization system used by dental technicians. The exposure status of the respondents was not confirmed, and the reproductive outcome of the respondents was not verified through hospital records. Although this study suggests that exposure to ethylene oxide can adversely affect the outcome of pregnancy and that the effect can occur at any stage during pregnancy, the results are suggestive and not conclusive.

Table 3. Adverse Pregnancy Outcomes among Female Dental Assistants Exposed to Ethylene Oxide Compared with Unexposed Females

Reproductive Outcome	Number Exposed	Relative Risk	95% Confidence Interval
Spontaneous abortion[a]	32	2.5	1.0 - 6.3
Preterm birth[a]	21	2.7	0.8 - 8.8
Postterm birth[a]	17	2.1	0.7 - 5.9
Spontaneous abortion/preterm[a]	32	2.6	1.3 - 5.4
Spontaneous abortion/preterm[b]	26	2.3	1.0 - 5.4
Spontaneous abortion, preterm, or postterm[a]	25	2.7	1.2 - 6.1
Spontaneous abortion, preterm, or postterm[b]	20	2.5	1.0 - 6.1

Source: Rowland et al., 1996
[a]Adjusted for age only.
[b]Adjusted for age and potential nitrous oxide and mercury exposure.

2.4. Carcinogenicity

2.4.1. Epidemiologic Studies

Several epidemiologic studies have been conducted on the mortality experience of workers potentially exposed to ethylene oxide. Types of cancer that are of concern among workers exposed to ethylene oxide include lymphohematopoietic cancers (combined), leukemia, non-Hodgkin's lymphoma, and cancer of the brain, stomach, and pancreas.

Hogstedt et al. (1979a) reported three leukemia cases among 230 workers potentially exposed to ethylene oxide in a factory where hospital equipment was sterilized, whereas only 0.2 cases were expected based on a rough estimate of the person-years of observation and sex- and age-specific rates in Sweden. Exposure concentrations ranged from 2 to 70 ppm with 8-hour TWA concentrations of 20±10 ppm in the breathing zone and 150 ppm at floor level. Hogstedt and coworkers followed these workers and two additional cohorts engaged in the production of ethylene oxide; one group produced ethylene oxide by the chlorohydrin method and another by direct oxidation of ethylene. The three cohorts comprised a total of 709 Swedish workers with total follow up extending from 1961 to 1985 for mortality and 1983 for cancer (Hogstedt et al., 1979a,b; Hogstedt, 1988). Ethylene oxide exposures varied over the years (Hogstedt et al., 1979b), ranging from a high of 1300 mg/m^3 (~260 ppm, odor threshold) to averages <25 mg/m^3 (14 ppm) during the 1940s, 10 to 50 mg/m^3 (6 to 28 ppm) during the 1950s and early 1960s, and 1 to 10 mg/m^3 (0.6 to 6 ppm) during the 1970s. Confounding exposures included ethylene chlorohydrin, ethylene dichloride, bis(2-chloroethyl) ether, other chlorinated chemicals, and ethylene glycol. The risks of all cancers combined, stomach cancer, and blood and lymphatic cancer, particularly the risks of stomach cancer and leukemia among male workers were increased . The risk of cerebrovascular diseases among male workers exposed to ethylene oxide was also increased (Hogstedt, 1988). Because of confounding exposures to other chemicals, the observed effects cannot be attributed to ethylene oxide alone.

Steenland et al. (1991) conducted a retrospective mortality study on 18,254 U.S. workers (55% female and 45% male) employed for at least 3 months at 14 facilities producing sterilized medical supplies and spices. The average follow up was 16 years. The average 8-hour TWA concentration was 4.3 ppm (7.7 mg/m^3) for sterilizer operators and 2.0 ppm (3.6 mg/m^3) for other exposed workers. No statistically significant increases were observed for deaths due to all causes, all cancers, all hematopoietic cancers, leukemia-aleukemia, non-Hodgkin's lymphoma, or stomach cancer compared with mortality rates for the general U.S. population. However, deaths due to hematopoietic cancers showed a significant positive trend ($p=0.03$) with increasing time since first exposure (latency), and deaths due to kidney cancer were significantly increased ($p<0.05$) when the latency was >20 years. Significant increases in the mortality rates for all hematopoietic cancers and

lymphosarcoma and/or reticulosarcomas were noted for male workers. Steenland et al. (1991) noted that their study was limited by the small number of cases and short follow-up.

Wong and Trent (1993) analyzed the data on the same cohort consisting of 18,728 workers. They also showed no statistically significant increases in mortality rates except for deaths due to non-Hodgkin's lymphoma among male workers; this increase did not show a trend associated with duration of employment or latency. However, the number of cases was very small. This study was also reported by UCCPC (1991).

Bisanti et al. (1993) conducted a study on 1971 male Italian chemical workers: 637 licensed for at least one year to handle only ethylene oxide and 1334 licensed for at least one year to handle ethylene oxide and other chemicals. The license was a qualitative indication of exposure. No quantitative exposure estimates were available. Follow-up of the entire cohort from 1940 to 1984 showed six deaths due to hematopoietic cancer and four due to lymphosarcoma or reticulosarcoma ($p<0.05$). Five hematopoietic cancers ($p<0.05$) and three lymphosarcomas/reticulosarcomas ($p<0.001$) occurred in the subcohort licensed to handle only ethylene oxide.

Teta et al. (1993) followed the mortality experience of 1896 chemical workers potentially exposed to ethylene oxide from 1940 to 1988. These investigators did not find a statistically significant increase in mortality due to all malignant neoplasms or lymphohematopoietic, stomach, brain, or pancreatic cancers.

Hagmar et al. (1995) analyzed the mortality experience of a cohort consisting of 2170 workers (1309 women and 861 men) employed for at least one year in facilities producing ethylene oxide-sterilized medical supplies. Ethylene oxide exposure that was initially about 40 ppm at one facility and 75 ppm at the other decreased throughout the years such that only sterilizer operators were exposed to concentrations greater than 0.2 ppm at later years. These investigators failed to find statistically significant increases in the risks of malignant neoplasms, lymphohematopoietic neoplasms, or leukemia.

Shore et al. (1993) evaluated available epidemiologic studies and conducted a meta-analysis of 10 cohorts that included 29,800 workers with potential exposure to ethylene oxide. A total of 2540 deaths were recorded. No association was found between ethylene oxide exposure and risk of leukemia, pancreatic cancer, brain and nervous system cancer, or total cancer. A suggestive increased risk was observed for non-Hodgkin's lymphoma and stomach cancer; however evaluations of intensity, frequency, and duration of exposure and latency did not support the conclusion. This study was also reported by UCC (1993).

2.4.2. Risk Assessment

In 1984 the Occupational Safety and Health Administration (OSHA) reported the results of a quantitative cancer risk assessment on occupational exposure to ethylene oxide. For a 45-year working lifetime exposure to 1 ppm, OSHA estimated 12 to 23 excess deaths due to cancer per 10,000 workers. OSHA (1984) reported that Crump (no date provided) estimated 3.7 to 23 deaths per 10,000 workers, the Ethylene Oxide Industry Council

(EOIC) estimated 18 to 79 per 10,000 workers and Sielken (no date provided) estimated 1 to 6 per 10,000 workers

EPA reported a 95% upper bound on slope or q_1^* of 1×10^{-4} $\mu g/m^3$ based on the total incidence of leukemias and brain gliomas in female Fischer 344 rats (from data reported by Snellings et al., (1981))(U.S. EPA, 1985). No estimates are available for single exposures to ethylene oxide.

2.5. Genetic Toxicity

2.5.1. Epidemiologic and Case Studies

Various endpoints of genetic toxicity have been studied extensively in humans receiving accidental high acute exposures and long-term low level exposures to ethylene oxide. The populations receiving the most attention are sterilizer operators and chemical manufacturers. The literature has been reviewed recently by Rhomberg et al. (1990), Dellarco et al. (1990), and IARC (1994). These reviews described both positive and negative associations between exposure to ethylene oxide and increased frequencies of sister chromatid exchanges (SCE) and chromosome aberrations in peripheral lymphocytes. Because the literature is quite extensive, only a few studies will be described in this report.

Although most of the studies involve long-term exposure to ethylene, two acute exposure studies with mixed results were located in the literature. Laurent (1988) reported increased SCE frequencies in peripheral lymphocytes of three sterilizer workers accidentally exposed for 30 minutes to ethylene oxide concentrations exceeding the odor threshold (260 ppm or 1260 mg/m^3). Clinical symptoms were described in section 2.2 of this document. SCE frequencies analyzed in the peripheral lymphocytes 5 days and 2 years after the accident were compared with a group of control or chronically-exposed workers. Five days after the accident, the mean SCE frequency was significantly elevated by 160% compared with the control group and 144% compared with the chronically-exposed group. The mean SCE frequency in the chronically- exposed group was significantly elevated (112%) compared with the control group. A significant increase in the proportion of high frequency cells (cells with more than 15 SCEs/cell) was observed in the exposed subjects 5 days after the accident; this increase accounted for the increased frequency of SCE. By 2 years after the accident, SCE frequencies had returned to the preaccident level.

Tates et al. (1995) compared the frequencies of several endpoints of genetic damage in seven chemical workers incidentally exposed to ethylene oxide at concentrations ranging from 52 to 785 mg/m^3 (29 to 436 ppm, 8-hour TWA concentrations) with a group of seven unexposed controls. Frequencies of SCEs, *hprt* mutants, and micronuclei were evaluated in peripheral lymphocytes harvested 89 to 180 days after exposure. The level of hemoglobin adducts also indicated very high exposures to ethylene oxide; nevertheless, the various genetic tests showed no positive results compared with the control group. These results differ from those obtained by

Laurent (1988). However, Tates et al. (1995) did not conduct their genetic tests until 89 to 180 days (3 to 6 months) after exposure and it is possible that any genetic lesions were repaired before this time. Tates et al. (1995) also did not see increases in the same genetic parameters in workers chronically exposed to ethylene oxide at average concentrations ranging from <0.01 mg/m^3 (0.006 ppm) to <0.1 ppm (0.18 mg/m^3), which may have been too low to induce measurable genetic damage.

Garry et al. (1979) reported that four sterilizer operators exposed for 2 months to ethylene oxide at concentrations high enough to cause respiratory and neurologic symptoms had elevated SCE frequencies 3 and 8 weeks after the last exposure. A concentration of 36 ppm (64.8 mg/m^3) was measured 15 feet from the sterilizer and 1500 ppm (2700 mg/m^3) at floor level. The mean SCE frequency was 9.75 ± 0.75 per metaphase cell three weeks after exposure, 10.34 ± 2.55 eight weeks after exposure, compared with 5.98 ± 0.31 in the control group (eight subjects). In four asymptomatic workers incidentally exposed to ethylene oxide, including one subject exposed to 1500 ppm (2700 mg/m^3) for 5 minutes, the frequency of SCEs for the group was elevated (mean = 9.73 ± 0.98 SCEs per metaphase cell) 7 to 9 weeks after the last known exposure to ethylene oxide.

In a recent study, Major et al. (1996) compared genetic damage in two groups of ethylene oxide exposed nurses with control groups. One group comprised 9 exposed nurses (5 to 20 mg/m^3 [2.8 to 11 ppm]) and 14 controls and the other group comprised 10 exposed nurses (5 to 100 mg/m^3 [2.8 to 55.6 ppm]) and 27 controls; a group of 48 "historical" controls was also used for comparison. Compared with their respective hospital controls, both groups of nurses showed increases in the frequency of SCEs, chromosome aberrations, or lectin-stimulated labeling index. Aberrations seen in exposed nurses included deletions, dicentrics, chromatid exchanges, and rings. The background rates in the two control populations varied, indicating differences in confounding factors (alcohol consumption, smoking, age). Overall this study showed genetic damage in both exposed groups.

2.5.2. Risk Assessment

Rhomberg et al. (1990) calculated risk estimates for heritable translocations in offspring by fathers exposed to ethylene oxide; they used data reported by Generoso et al. (1990) for their estimates. For an exposure of 10 ppm for 8 h/day for 3 weeks or 15 days of exposure (1200 ppm•hours), 16 translocations carriers are expected among 10,000 live offspring. Preston et al. (1995) reviewed the Rhomberg et al. (1990) assessment and concluded that the genetic risk for induction of reciprocal translocations would be negligible at low doses. They further noted that Rhomberg estimated the risk by a factor of 10. Natarajan et al. (1995) used a parallelogram approach to assess genetic risk in humans based on dominant mutations. Their assessment considered genetic endpoints in germ cells and somatic cells in both animals and humans. They estimated a risk of 4×10^{-4} above background from occupational exposure to 1 ppm of ethylene oxide for 1 year.

2.6. Occupational Exposure

Workers have been exposed to ethylene oxide at concentrations ranging from undetectable to peaks at moderately high levels. Occasionally, very high concentrations have been experienced during accidental exposures, but not in the routine working environment. Data on some occupational exposures to ethylene oxide are presented in Table 4. Additional information was presented by IARC (1994).

Table 4. Occupational Exposure to Ethylene Oxide

Industry	Duration of exposure	Concentration (ppm)	Signs/Symptoms of Exposure	Reference
Hospital/sterilizer operation	5-minutes TWA	62.5 ±46 (13 - 160)	not reported	Sarto et al., 1984
	1 cycle	15.8 ±9.8 (3.7 - 35.5)	not reported	
	8-hr TWA	10.7±4.9 (3.7 - 20)	not reported	
Hospital/sterilizer operation	8-hr TWA	0.1 - 0.5	not reported	Hemminki et al.,1982
Hospital/sterilizer operation	peak	up to 250	not reported	
	20 minutes	5 - 10	not reported	Hemminki et al., 1983
Hospital/sterilizer operation	purge cycle	up to 36	upper respiratory and neurologic symptoms	Garry et al., 1979
Hospital sterilizer operation	8-hr TWA	ND - 6.3	not reported	Elliot et al., 1988
	2 - 30 minutes	ND to 103	not reported	
Hospital/folding & packing	8-hr TWA	ND - 6.7	not reported	

2.7. Summary

No adequate data are available on lethality of ethylene oxide in humans. Nonlethal effects of ethylene oxide and the exposure concentrations at which the effects occur are summarized in Table 5. Primary targets for nonlethal effects include the eyes, respiratory tract, and the central and peripheral nervous systems. Experimental studies, case reports, and epidemiologic studies have documented noncancer effects on the respiratory tract, eyes, central and peripheral nervous system, gastrointestinal tract (probably due to nervous system toxicity), hematopoietic system, and possibly the reproductive system and fetus. The absolute odor detection level for ethylene oxide is 260 ppm as reported by one author and the median odor threshold is 700 ppm as reported by another. The odor recognition level is 500 ppm. As noted in Table 5, nonlethal effects occur after exposure to ethylene oxide concentrations approximating the

Table 5. Summary of Nonlethal Effects of Ethylene Oxide in Humans				
Concentration		**Exposure duration**	**Effects**	**Reference**
ppm	**mg/m^3**			
13349	24028	10 sec	definitely irritating to nasal passages	Walker and Greeson, 1932
2670	4806	not reported	slightly irritating to nasal passages, acetic acid-like odor	Walker and Greeson, 1932
≥260	1260	30 minutes	odor, headache, gastrointestinal effects, eye and upper respiratory tract irritation, pruritus, muscle weakness, dizziness, hemolysis	Deleixhe et al., 1986; Laurent, 1988
≥260	≥1260	4 hr/day for 4 days	coughing, shortness of breath, wheezing, slight peripheral neuropathy, nonimmunological asthma	Deschamps et al., 1992
excursions ≥260	≥1260	2 weeks to 2 months	eye and mucous membrane irritation, difficult swallowing, headache, gastrointestinal effects, lethargy, fatigue, problems with memory and thinking, major motor seizures, peripheral neuropathy	Gross et al., 1979
≤500	900	2 to 5 minutes	gastrointestinal effects, unconsciousness, apnea, muscle twitching, malaise, incoordination for up to 1 week	Salinas et al., 1981
not reported	not reported	4 months to 1½ years	eye irritation, headaches, smelling of fumes, distal axonal neuropathy	Finelli et al., 1983
up to 36 ppm	65	cyclic for 2 to 5 months	upper respiratory irritation, eye irritation, sore throat and dry mouth, gastrointestinal effects, headache, speech difficulty, recent memory loss, weakness, dizziness, and incoordination	Garry et al., 1979

Table 5. Continued

Concentration		Exposure duration	Effects	Reference
ppm	**mg/m^3**			
0.23 to 0.56 ppm (TWA); excursions of 11 or 77 ppm	0.4 to 1mg/m^3 and 19.8 to 139.6 mg/m^3	chronic	sweet-like odor, headache, dizziness, irritation of mucous membranes, gastrointestinal effects, fatigue, nervousness	Zey et al., 1994
peak = 23.5 total up to 10.7 average 3.4	42.3 19.3 6.1	up to 1 minutes up to 11.75 minutes not reported	odor, headache, skin and eye irritation, dry mouth, sore throat, runny nose, shortness or breath, nausea, numbness in fingers, drowsiness	Bryant et al., 1989
peak 250 ppm; 5 to 10 ppm (20 minutes daily)	0.18 to 0.9 (TWA); 450 9 to 18	during pregnancy	increased risk of spontaneous abortion	Hemminki et al., 1982
not reported	not reported	any duration during pregnancy	increased risk of spontaneous abortion, preterm birth, or postterm birth	Rowland et al., 1996

odor threshold (≥ 260 ppm) for short periods (2 to 30 minutes) or repeatedly for a few days. Genetic damage to somatic cells occurs at concentrations below the 700-ppm level. Chronic exposure to low 8-hour TWA concentrations is associated with the same effects as acute exposure, probably due to daily high level excursions.

Toxic effects occurring after short-term exposure to ethylene oxide include eye and upper respiratory tract irritation, nausea, vomiting, diarrhea, headache, dizziness, malaise, fatigue, muscle weakness, and signs and symptoms of peripheral neuropathy. Other effects noted in some studies include dry mouth, sore throat, runny nose, shortness of breath, apnea, memory loss, and seizures. Nonimmunological asthma was reported in one study; this effect has not been confirmed and may not be due to ethylene oxide exposure. Two epidemiologic studies presented suggestive evidence that exposure to ethylene oxide is associated with adverse reproductive outcomes: spontaneous abortions, preterm births, and postterm births. An increase in the rate of spontaneous abortions was reported for a cohort exposed to ethylene oxide at concentrations ranging from 0.1 ppm (8-hour TWA), 5 to 10 ppm for 20-minutes intervals, to peaks of 250 ppm.

Epidemiologic studies conducted to assess the effect of exposure to ethylene oxide on mortality due to malignant neoplasms in chemical factories or sterilizer facilities have produced mixed results regarding increased cancer risk. Some studies showed increased risks for lymphohematopoietic cancer in the entire cohort or in male subcohorts, whereas other studies showed no increased risk. IARC (1994) concluded that the evidence of carcinogenicity based on human studies is limited.

Humans studies also showed that the frequency of SCEs are increased in peripheral lymphocytes of workers exposed for 30 minutes to ethylene oxide at concentrations approximating the odor threshold (260 ppm), to concentrations high enough to cause respiratory and neurologic symptoms for 2 months, incidentally concentrations of 36 to 1500 ppm, or chronic exposure to 2.8 to 55.6 ppm. The frequency of chromosome aberration were also increased by chronic exposure. Increases in frequencies of genetic damage were not associated with exposures to high incidental concentrations of 29 to 436 ppm (8-hr TWA) when cells were analyzed 89 to 180 days following exposure suggesting that repair had occurred. No damage was seen after chronic exposures to concentrations less than 0.1 ppm.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Rats

Three rats (strain not specified) exposed for 30 minutes to ethylene oxide vapor diluted to 1:80 with air (12,500 ppm or 22,500 mg/m^3) became dyspneic (labored breathing) within 3.75 hours and died within 6 hours after exposure (Walker and Greeson, 1932). Gross findings included marked lung congestion and acute pneumonia (secondary effect); however, death was due to paralysis of the respiratory center. Exposure to a 1:100 dilution (10,679 ppm or 19,222 mg/m^3) for 30 minutes was also lethal to the rats, but exposure to a 1:150 dilution (7119 ppm or 12,815 mg/m^3) for 30 minutes was not lethal to rats. These exposure concentrations cannot be considered reliable, because the study authors did not discuss the means by which the concentrations were verified (calculations based on chamber size and quantity of ethylene oxide released or measurement of ethylene oxide in air samples).

Jacobson et al. (1956) exposed groups of ten male white rats to 2298, 1992, 1843, 1648, 1343, or 882 ppm (4140, 3590, 3320, 2970, 2420, or 1590 mg/m^3) of ethylene oxide vapor for 4 hours and observed the animals for signs of toxicity and death during the next 14 days. Toxic signs included frequent movement and preening, clear nasal discharge, lacrimation, occasional salivation, diarrhea, gaspingthat increased in severity during exposure, and death. Mortality occurred in all groups. The mortality data are summarized in Table 6. The LC_{50} was 1460 ppm (2630 mg/m^3) (C.I. = 620 - 2550 ppm). Signs of upper respiratory tract irritation, tracheal congestion and petechial hemorrhages, and mild edema in the lungs and peribronchial region were seen upon gross examination. In addition, a secretion was noted around the eyes and nose, and the stomach was distended.

In another 4-hr acute inhalation study, groups of five male and five female Sprague-Dawley rats received exposures to ethylene oxide vapor at concentrations of 1850, 1443, or 1021 ppm (3330, 2597, 1838 mg/m^3); groups of five males also received exposures to 2182 or 2026 ppm (3928 or 3647 mg/m^3) and five females were exposed

Table 6. Mortality in Male White Rats Exposed to Ethylene Oxide Vapor for 4 Hours		
Concentration		**Mortality (%)**
ppm	**mg/m^3**	
2298	4140	10/10 (100)
1992	3590	10/10 (100)
1843	3320	9/10 (90)
1648	2970	4/10 (40)
1343	2420	2/10 (20)
882	1590	2/10 (20)

Source: Jacobson et al. (1956)

to 1637 ppm (2947 mg/m^3) (Nachreiner, 1991). Surviving animals were observed for 14 days following exposure. This study is summarized in Tables 7 and 8. The LC_{50} was 1972 ppm (C.I. = 1887 - 2061) for male rats, 1537 ppm (C.I. = 1391 - 1698 ppm) for female rats, and 1741 (C.I. = 1655 - 1831 ppm) for the combined sexes. During exposure, signs of eye, nasal, and oral irritation (blepharospasm, wetness and encrustation around the eyes, nose, and mouth, swollen eye tissue, hypoactivity, and increased or shallow respiration) and respiratory distress (audible respiration, mouth breathing, and gasping) were noted (Table 7). Clinical signs immediately following exposure included tremors and an absence of tail and toe pinch reflex in some groups. Clinical signs indicative of eye and respiratory tract irritation and neurological effects were observed during the first 3 or 4 days after exposure. No clinical signs were observed after the day of exposure in the 1021-ppm group or after day 4 in the other exposure groups. Gross findings included brain hemorrhage, lung discoloration and hyperinflation, crusts and scabbing in the oral cavity and pharynx, and abnormal contents in the nose.

Table 7. Lethality and Clinical Signs in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide Vapor for 4 Hours

Effects	Concentration (ppm)								
	Males					Females			
	2182	2026	1850	1443	1021	1850	1637	1443	1021
Mortality (%)	4/5	4/5	0/5	0/5	0/5	5/5	4/5	1/5	0/5
During Exposure									
Blepharospasm	+	+	+	+	+	+	+	+	+
Wetness around eyes and nose	+	+	+	+	+	+	+	+	+
Hyperactivity	+	+	+	+	+	+	+	+	+
Mouth breathing	+					+			
After Exposure									
Unkempt fur	+	+	+			+	+		
Wetness or encrustation around eyes, nose, and mouth	+	+	+	+	+	+	+	+	+
Swollen tissue around eyes					+				+
Mouth breathing	+	+	+	+	+	+	+	+	
Audible respiration	+	+	+			+	+	+	
Gasping	+	+	+			+			
Decreased, increased or shallow respiration	+	+	+		+[a]	+	+		+[a]
Absence of tail and toe pinch reflex		+					+		
Hypoactivity	+	+	+	+		+	+	+	
Tremors		+		+				+	

Source: Nachreiner, 1991

[a]Increased respiration rate and shallow respiration only.

Table 8. Gross Findings in Male and Female Sprague-dawley Rats Exposed to Ethylene Oxide for 4 Hours									
Effects	**Concentration (ppm)**								
	Males					**Females**			
	2182	**2026**	**1850**	**1443**	**1021**	**1850**	**1637**	**1443**	**1021**
Brain: hemorrhage	3	0	0	0	0	–	–	–	–
Lungs: Discoloration, diffuse or focal/multifocal	3	4	0	3	2	5	4	1	3
Lungs: hyperinflated	3	3	0	0	0	0	0	0	0
Nose: abnormal contents						3	0	0	0
Oral/Pharyngeal: crust, scab, scale						3	0	0	0

Source: Nachreiner, 1991
[a]Number of animals with lesions; five animals per group were exposed.

In a 1-hour acute inhalation study, groups of five male Sprague-Dawley rats were exposed to ethylene oxide at measured concentrations of 6161, 5546, or 4827 ppm and groups of five females were exposed to concentrations of 4287, 4202, 4064, 3966, or 3609 ppm (Nachreiner, 1992). All surviving animals were observed for 14 days. Mortality and clinical signs are summarized in Table 9 and gross findings in Table 10. No deaths occurred in the male group exposed to 4827 ppm or in the female group exposed to 3609 ppm. The LC_{50} was 5748 ppm (95% C.I. = 5276 - 6262 ppm,) for males, 4439 ppm (C.I. = 4034 - 4884 ppm) for females, and 5029 ppm (95% C.I. = 4634 - 5459 ppm) for the combined sexes. Because of extreme variations in the measured concentrations (3584 to 4432 ppm), which probably caused the unusual mortality rate, the 4064-ppm female group was not included in the calculation for the LC_{50}. Clinical signs of toxicity were observed in all groups during and after the 1-hour exposure up to day 3 or 4 postexposure. Restlessness was observed in all groups during the first 10 minutes of exposure. In all groups of males and in the 4827-ppm

Table 9. Clinical Signs in Male and Female Sprague-dawley Rats Exposed to Ethylene Oxide for 1 Hour								
Effects	**Concentration (ppm)**							
	Males			**Females**				
	6161	**5546**	**4827**	**4827**	**4202**	**4064**	**3966**	**3609**
Mortality (%)	4/5 (80)	1/5 (20)	0/5 (0)	5/5 (100)	1/5 (20)	5/5 (100)	2/5 (40)	0/5 (0)
During exposure								
Restlessness	+	+	+	+	+	+	+	+
Wetness around eyes	+	+	+	+	+	+	+	+
Lacrimation	+	+	+	+				
Mouth breathing	+							
Hypoactivity	+	+	+	+	+	+	+	+
No acoustic startle reflex	+	+	+	+				
After exposure								
Unkempt fur	+	+		+	+	+	+	+
Encrustation/wetness: eyes, mouth, nose			+	+	+			+
Decreased respiration	+	+		+	+	+	+	
Hypoactivity	+	+		+		+	+	+
Ataxia	+				+	+	+	+
Tremors	+	+			+	+	+	

Source: Nachreiner, 1992

Table 10. Gross Findings in Male and Female Sprague-Dawley Rats Exposed to Ethylene Oxide for 1 Hour								
Effects	**Concentration (ppm)**							
	Male			**Female**				
	6161	**5546**	**4827**	**4827**	**4202**	**4064**	**3966**	**3609**
Encrustation in the nose	2[a]	1	0	2	1	3	2	–
Lungs: Discoloration, diffuse or focal/multifocal	4	1	0	5	4	5	3	–
Lungs: hyperinflated	1	0	0	0	1	1	0	–
Kidneys: diffuse color change	–	–	–	0	0	3	0	–

Source: Nachreiner, 1992
[a]Number of animals with lesions; five animals per group were exposed.

female group, only lacrimation was observed on the same day of exposure; periocular wetness was observed in the remaining female groups. These findings suggest that ethylene oxide was irritating to the eyes and the respiratory tract and toxic to the nervous system. Gross examination showed effects in the nose, lungs, and kidneys (Table 10). Lung weights appeared to be elevated in animals dying before study termination compared with the lungs of animals surviving to termination, particularly in the male groups.

Hollingsworth et al. (1956) conducted several experiments in which rats and others species were exposed to ethylene oxide vapor for various durations. Controls were included but not described. The investigators reported that all ten male and ten female rats died after exposure to ethylene oxide at a concentration of 841 ppm (1510 mg/m^3) for 7 h/day, 5 days/week for eight exposures. Gross and microscopic effects were assessed on rats receiving two or three exposures to ethylene oxide and killed 1 or 3 days after exposure. Toxic effects occurred in the lungs (interstitial edema, congestion, alveolar hemorrhage), liver (fatty degeneration), kidneys (congestion and cloudy swelling of the convoluted tubules), and adrenal glands (fat vacuoles). Renal effects were more severe 3 days after exposure than on the first day after exposure. Exposure to 357 ppm (640 mg/m^3) for 7 h/day, 5 days/week for seven exposures resulted in the death of 2/20 rats (10 males and 10 females exposed). Severe lung irritation and secondary pulmonary effects were observed in these animals. In another experiment, 10 male and 10 female rats were exposed to ethylene oxide vapor at 357 ppm for 33 to 59 exposures in 48 to 85 days. Growth was retarded, and by the 38th exposure, 18 rats (90%) had died because of secondary respiratory effects. Near the end of the exposure period, neuromuscular impairment at the lumbar and sacral region manifested as paralysis

and muscular atrophy of the hindlimbs was observed. The two surviving rats (males) were allowed to recover after receiving 42 exposures.

Jacobson et al. (1956) exposed 20 male white rats to 440 ppm (720 mg/m^3) of ethylene oxide vapor, 6 h/day, 5 days/week for 6 weeks; they included an equal number of unexposed animals as controls. There were 13 deaths (65%) among the 20 exposed rats . Clinical signs observed in the exposed rats included a reddish discharge from the nose, diarrhea, labored breathing, hindlimb weakness followed by hind limb paralysis during the last 2 weeks of exposure, and progressive weight loss. No significant pathologic effects were noted except for marked hemosiderosis in the spleen of a few animals. The weight loss and paralysis were reversible in five rats observed for several months after terminating exposure.

3.1.2. Mice

Three female white mice exposed for 30 minutes to ethylene oxide vapor diluted 1:80 with air (13,349 ppm or 24,028 mg/m^3) died within 2.75 hours (Walker and Greeson, 1932). Gross findings included marked lung congestion and acute pneumonia; however, death was due to respiratory paralysis. Exposure to a 1:100 dilution (10,679 ppm or 19,222 mg/m^3) and a 1:150 dilution (7119 ppm or 12,815 mg/m^3) for 30 minutes was also fatal to mice. These exposure concentrations cannot be considered reliable, because the study authors did not discuss the means by which the concentrations were verified (calculations based on chamber size and quantity of ethylene oxide released or measurement of ethylene oxide in air samples).

Jacobson et al. (1956) exposed groups of ten female white mice to 1365, 1343, 960, 882, 860, or 533 ppm (2460, 2420, 1730, 1590, 1550, 960) of ethylene oxide for 4 hours and observed the mice for 14 days or until death. The mice showed toxic signs similar to those of the rat, which included frequent movement and preening, clear nasal discharge, lacrimation, occasional salivation, gasping followed by severe dyspnea, and death. Mortality data are summarized in Table 11. The LC_{50} for mice was 835 ppm (1504 mg/m^3) (C.I. = 623 - 1040 ppm). The only gross finding reported for mice was distention of the stomach.

Table 11. Mortality in Female White Mice Exposed to Ethylene Oxide Vapor For 4 Hours		
Concentration		Mortality (%)
ppm	mg/m^3	
1365	2460	10/10 (100)
1343	2420	10/10 (100)
960	1730	7/10 (70)
882	1590	3/10 (30)
860	1550	6/10 (60)
533	960	1/10 (10)

Source: Jacobson et al. (1956)

In an NTP (1987) inhalation study, groups of five male and five female $B6C3F_1$ mice were exposed to ethylene oxide vapor at concentrations of 0, 100, 200, 400, 800, or 1600 ppm (180, 360, 720, 1440, 2880 mg/m^3) for 4 hours and observed for 14 days. Measured concentrations were within 5% of target concentrations. Mortality data are summarized in Table 12. There were no deaths among animals of either sex exposed to 100 to 400 ppm. All males exposed to 800 ppm died 2 to 6 days after exposure and four females exposed to 800 ppm died 1 to 3 days after exposure. All male and female mice exposed to 1600 ppm died within 4 hours after exposure. Lacrimation and dyspnea were observed at 800 ppm; severe dyspnea, incoordination, semiconsciousness, and diarrhea were observed in animals exposed to 1600 ppm. An LC_{50} value of 660 ppm (95% C.I. = 509-856 ppm) was calculated for females mice; an LC_{50} value was not calculated for male mice. Postmortem examinations were not conducted on these animals.

Table 12. Mortality in Male and Female B6C3F$_1$ Mice Exposed to Ethylene Oxide Vapor for 4 Hours			
Concentration		**Mortality (%)**	
ppm	**mg/m^3**	**Male**	**Female**
100	180	0/5	0/5
200	360	0/5	0/5
400	720	0/5	0/5
800	1440	5/5 (100%)	4/5 (80%)
1600	2880	5/5 (100%)	5/5 (100%)

Source: NTP, 1987

NTP (1987) also conducted a 14-day study in which male and female B6C3F$_1$ mice were exposed to ethylene oxide at concentrations of 0, 50, 100, 200, 400, or 800 ppm (90, 180, 360, 720, or 1440 mg/m^3), 6 h/day, 5 days/week. All five male and five female mice exposed to 800 ppm died within 1 day of exposure except for one female that died within 2 days of exposure; thus confirming the lethality of 800 ppm in the single exposure study. Clinical signs at 800 ppm included hunched posture and listlessness. All animals exposed to 50 to 400 ppm survived except for two females exposed to 200 ppm; these deaths were not related to exposure.

Hollingsworth et al. (1956) reported that all five female mice died after exposure to ethylene oxide at a concentration of 841 ppm (1510 mg/m^3), 7 h/day, 5 days/week for 8 exposures. Four of ten female mice died after seven exposures to 357 ppm (640 mg/m^3) of ethylene oxide. Moderate body weight loss and severe lung injury indicative of irritation and secondary pulmonary effects were observed in these animals. Another ten female mice similarly exposed to 357 ppm for 33 to 59 exposures in 48 to 85 days showed growth retardation and died due to secondary respiratory infection after 33 exposures.

In a 6-week inhalation study, Jacobson et al. (1956) exposed 30 female white mice to 400 ppm (720 mg/m^3) of ethylene oxide vapor 6 h/day, 5 days/week for 6 weeks. An equal number of unexposed animals were included as controls. They observed a slight weight loss in exposed animals relative to that of controls. Twenty-four (80%) mice died during the study compared with three (10%) of the controls. No significant pathologic changes were reported.

3.1.3. Guinea Pig

Waite et al. (1930) exposed guinea pigs to ethylene oxide vapor at concentrations of 8.5, 6.3-6.4, 5.1, 4, 1.4-2.5, 0.7, 0.3, 0.13, 0.05, 0.025% for various times ranging from 1 to 480 minutes. The concentrations correspond to 85,000; 63,000- 64,000; 51,000; 40,000; 14,000-25,000; 7,000; 3,000; 1,300; 500; and 250 ppm of ethylene oxide vapor in air. One to four guinea pigs were exposed to each concentration. Twenty-four guinea pigs from the same colony were used as controls. Death occurred during exposure to 8.5% ethylene oxide for 33 minutes; death occurred within 24 hours after exposure to concentrations of 6.3-6.4% for 10 or 20 minutes, 2.5% for 60 minutes, 1.4% for 60 or 107 minutes, 0.7% for 150 minutes, and 0.3% for 330 minutes. Death occurred between 1 and 8 days in animals exposed to 5.1% for 6 minutes, 4% for 20 minutes, 1.4% for 20 minutes, 0.7% for 60 minutes, 0.3% for 190 minutes, and 0.13% for 480 minutes. No deaths occurred in the groups exposed to 1.4% for 10 minutes, 0.7% for 20 minutes, 0.3% for 70 minutes, 0.13% for up to 290 minutes, and 0.025 or 0.05% for 480 minutes. Clinical signs of toxicity included irritation to the nose and eyes, profuse lacrimation, bloody and frothy nasal exudate, dyspnea and gasping, staggering, unsteadiness, and prostration. The onset of these signs varied depending on the concentration of ethylene oxide. Nasal and eye irritation were reversible upon termination of exposure. Gross pathologic examination showed lung congestion and edema, frothy serous exudate from the trachea and bronchi, hyperemia of the liver and kidneys of animals dying during exposure or within 24 hours after exposure. Animals killed immediately after exposure showed evidence of lung congestion, whereas those surviving for 2 to 4 days also showed changes in the kidneys. Animals surviving for more than 4 days showed slight lung congestion and slight changes in the kidneys, which cleared by 8 days after exposure.

Walker and Greeson (1932) reported that three guinea pigs exposed to ethylene oxide vapor at a concentration of 1:80 (13,349 ppm or 24,028 mg/m^3) for 30 minutes had bloody mucus streaming from the nose and mouth and showed marked dyspnea (time not reported); 7 hours after exposure, two guinea pigs were killed because they were severely "ill". The third guinea pig survived until study termination at 3 days. Gross findings were similar to those described for mice and rats; generally lung congestion and acute pneumonia. These exposure concentrations reported in this study cannot be considered reliable, because the study authors did not discuss the means by which the concentrations were verified (calculations based on chamber size and quantity of ethylene oxide released or measurement of ethylene oxide in air samples).

Hollingsworth et al. (1956) reported that all eight male and eight female guinea pigs died after exposure to 841 ppm of ethylene oxide at (1510 mg/m^3) for 7 h/day, five days/week for 8 exposures. Gross and microscopic effects were assessed on guinea pigs given two or three exposures and killed 1 or 3 days after exposure. Toxic effects occurred in the lungs (interstitial edema, congestion, alveolar hemorrhage), liver (fatty

degeneration), kidneys (congestion and cloudy swelling of the convoluted tubules), and adrenal glands (fat vacuoles). The renal effects were more severe after 3 days than after only 1 day. A control group was included but not described.

3.1.4. Dogs

Three male beagle dogs per group were exposed to ethylene oxide vapor at concentrations of 2830, 1393, 710, or 327 ppm (5100, 2510, 1282, or 590 mg/m^3) for 4 hours followed by a 14-day observation period (Jacobson et al., 1956). Death occurred only in the groups exposed to 2830 and 1393 ppm, and all deaths occurred within 24 hours after exposure. The LC_{50} for dogs was 960 ppm (1730 mg/m^3). Mortality data are summarized in Table 13. Clinical signs observed at 2830 ppm included lacrimation; clear nasal discharge; frothy, colorless, mucous vomitus; diarrhea; convulsions; dyspnea; and death. Dogs exposed to 1393 ppm showed similar signs except for diarrhea, convulsion, and dyspnea. No clinical signs were observed at 710 or 327 ppm. Pathologic changes included moderate lung congestion, dilation of perivascular lymphatic spaces, perivascular edema, and distention of the stomach.

Table 13. Mortality in Male Beagle Dogs Exposed to Ethylene Oxide Vapor for 4 Hours

Concentration		Mortality (%)
ppm	mg/m^3	
2830	5100	3/3 (100)
1393	2510	3/3 (100)
710	1280	0/3 (0)
327	590	0/3 (0)

Source: Jacobson et al., 1956

3.1.5. Other Species

One female rabbit, one male rabbit, and one female monkey died after exposure to ethylene oxide at 841 ppm (1510 mg/m^3), 7 h/day, 5 days/week for 8 exposures (Hollingsworth et al.,1956). One rabbit of each sex and one female monkey were similarly exposed to ethylene oxide for 33 to 59 exposures in 48 to 85 days. The male rabbit died after 48 exposures. In both rabbits and monkeys, neuromuscular impairment of the lumbar and sacral region manifested as paralysis and muscular atrophy of the hindlimbs occurred during the later part of the exposure period. Complete recovery was attained 100 to 132 days after terminating exposure.

3.2. Nonlethal Toxicity

Section 3.1 contains data on effects occurring at concentrations not causing death of the animals on study. These data may be used to further assess nonlethal toxicity in laboratory animals.

3.2.1. Rats

Embree et al. (1977) reported that 15 Long-Evans male rats exposed by inhalation to 1000 ppm (1800 mg/m^3) of ethylene oxide for 4 hours showed signs of toxicity including "central depression", diarrhea, and eye and respiratory tract irritation. These animals were used in a dominant lethal study and were not further investigated for general toxicity.

Ohnishi et al. (1985) studied the effect of inhalation exposure to ethylene oxide vapor on neuropathy in rats. Five male Wistar rats received exposures to ethylene oxide at a concentration of 500 ppm, 6 h/day, 3 days/week for 13 weeks. Five pair-fed animals exposed to ambient air served as controls. Clinical signs in the exposed rats included an awkward gait at week 5 to 8 and slight to moderate hindlimb ataxia starting at week 9 or 10. Light and electron microscopic examination of peripheral nerves showed axonal degeneration of myelinated fibers in the fasciculus gracilis and hindlimb nerves. The degenerative changes accounted for the ataxia observed in these animals.

3.2.2. Mice

Snellings et al. (1984a) reported the results of a subchronic inhalation study using $B6C3F_1$ mice exposed to ethylene oxide vapor. Groups of 30 male and 30 female mice were exposed to ethylene oxide vapor at target concentrations of 0, 10, 50, 100, or 250 ppm, 6 h/day, 5 days/week for 10 weeks (males) or 11 weeks (females). Measured concentrations were within 6% of target concentrations. No treatment-related clinical signs of toxicity or body weight changes were observed in animals exposed to ethylene oxide. Erythrocyte parameters were depressed suggesting a slight anemia in males and females at 250 ppm. Spleen weights were depressed in both sexes and testes weights were depressed in males; no corresponding histopathologic effects were observed suggesting that the organ weight changes were not treatment related. Neuromuscular screening tests performed on five females at 6 weeks and on five mice of each sex at study termination showed treatment-related effects on five parameters (toe and tail pinch reflex, righting reflex, gait, and locomotor activity) at 250 ppm and on two parameters (gait and locomotor activity) at 50 and 100 ppm.

Groups of ten male and ten female $B6C3F_1$ mice were exposed to ethylene oxide at concentrations of 0, 50, 100, 200, 400, or 600 ppm (90, 180, 360, 720, or 1080 mg/m^3), 6 h/day, 5 days/week for 14 weeks (NTP, 1987). All mice exposed to 400 or 600 ppm died within the first 4 weeks of the study. Clinical signs observed in mice exposed to 600 ppm of ethylene oxide included anorexia, dyspnea, decreased activity, bloatedness, and listlessness. One male in each of the remaining groups died before the end of the experiment. Treatment-related

histopathologic effects were observed in the kidney of males (≥ 100 ppm) and females (≥ 200 ppm), thymus of males and females (≥ 200 ppm), nasal cavity of both sexes (≥ 200 ppm), and spleen of both sexes (600 ppm). No treatment-related effects occurred at 50 ppm.

3.2.3. Dogs

Two of three male beagle dogs exposed to 290 ppm (523 mg/m^3) ethylene oxide vapor 6 h/day, 5 days/week for 6 weeks showed clinical signs of toxicity including vomiting, occasional tremors, and transient weakness in the hindlimbs (Jacobson et al., 1956). A mild anemia developed in all three dogs. Pathologic effects included lung congestion and moderate alveolar collapse, which was probably due to irritant effects of the ethylene oxide, and muscular atrophy (fat replaced muscle fibers), which caused the weakness in the hind limbs. No deaths occurred among the exposed or control animals.

3.2.4. Rabbits

The only effect observed in rabbits (3/group) exposed for 30 minutes to ethylene oxide vapor at concentrations of 1:80 (13,349 ppm or 24,028 mg/m^3), 1:100 (10,679 ppm or 19,222 mg/m^3), or 1:150 (7,119 ppm or 12,815 mg/m^3) was a slight weakness in the hindlimbs after exposure to the highest concentration (Walker and Greeson, 1932). These exposure concentrations reported in this study cannot be considered reliable, because the study authors did not discuss the means by which the concentrations were verified (calculations based on chamber size and quantity of ethylene oxide released or measurement of ethylene oxide in air samples).

3.3. Reproductive/Developmental Toxicity

3.3.1. Rats

Groups of 17 to 22 pregnant Fischer 344 rats were exposed to ethylene oxide vapor at concentrations of 0 (two control groups), 10, 33, or 100 ppm (18, 59, or 180 mg/m^3) for 6 h/day on gd 6-15 inclusive (Snellings et al., 1982a; Dow Chemical Co. 1982). The dams were killed on gd 20 for evaluation of maternal, reproductive, and developmental parameters. No effects were noted on maternal body weight gain, preimplantation loss, resorptions, or fetal deaths. The weights of male (3.1 g vs 3.3 or 3.4 g for controls) and female fetuses (2.9 g vs 3.0 or 3.1 for controls) were significantly ($p<0.05$) reduced at the 100-ppm exposure level; crown-to-rump length was not affected. There were no gross external or visceral malformations. The incidences of litters with split or poorly ossified sternebrae or bilobed vertebrae centra were elevated at the 100-ppm, but not significantly. These effects suggest a mild growth retardation with no corresponding effect on maternal body weight gain. No effects occurred at 33 ppm.

In another developmental toxicity study, groups of 25 pregnant CD rats (Sprague-Dawley stock) were exposed to ethylene oxide vapor at concentrations of 0 (control), 50, 125, or 225 ppm (90, 225, or 405 mg/m^3), 6 h/day on gd 6-15 inclusive (BRRC, 1993). Measured concentrations were within 3% of target concentrations.

The dams were killed on gd 21 for evaluation of maternal and developmental parameters. No treatment-related clinical signs of toxicity or maternal mortality occurred. Absolute maternal body weights and body weight gain showed statistically significant decreases at 125 and 225 ppm. Food consumption during the exposure period also was significantly reduced at 225 ppm. Mean fetal weights were significantly reduced in male (96, 95, and 90% of control weights) and female fetuses (97, 95, and 90% of control weights) at 50, 125, and 225 ppm, respectively. The incidences of litters with skeletal variations (primarily unossified or poorly ossified areas) in the head region, phalanges, forelimbs and hind limbs, and sternum were increased at 125 and 225 ppm. Twelve different types of variations were observed at 225 ppm group, and three were observed at 125 ppm. No increase in the incidence of delayed ossification occurred at 50 ppm. Therefore, the minimal effect on fetal weight at 50 ppm and the lack of effect of ossification suggest that reduced fetal weight is of minimal biological significance or it approximates the threshold for growth retardation.

In the most recent study, Saillenfait et al. (1996) exposed pregnant Sprague-Dawley rats to 0, 400, 800, or 1200 ppm of ethylene oxide for 0.5 hours once a day on gd 6-15 inclusive. They also exposed pregnant rats to 0, 200, or 400 ppm or 0, 800, or 1200 ppm for 0.5 hours three times a day on gd 6-15 inclusive. Measured concentrations were within 17% of the target concentrations. The animals were observed daily, and body weights were recorded on gd 6, 11, 16, and 21. All surviving dams were killed on gd 21 for evaluation of maternal and developmental parameters. There were no treatment-related clinical signs of toxicity in animals exposed to any concentration once daily; one dam exposed to 1 × 800 ppm died due to causes unrelated to exposure. Maternal body weight gain in the 3 × 1200-ppm group was 26% ($p<0.01$) of the control value between gd 6-11 and 32% ($p<0.01$) of the control values between gd 11-16. Other differences in body weight gain were not attributed to ethylene oxide exposure. Compared with control weights, absolute maternal weight gain (less gravid uterine weight) was reduced by 59% ($p<0.01$) in the 3 × 1200-ppm group and 18% in the 3 × 800-ppm group (not significant, N.S.). The lack of statistical significance in absolute body weight of the 3 × 800-ppm group, may be due to the large standard deviations. No treatment-related effects were observed on reproductive parameters. Body weights of male and female fetuses exposed 3 × 800 ppm or 3 × 1200 ppm were significantly ($p<0.01$) decreased (90-94% of control values) when compared with controls. Mean fetal weight at 3 × 200 ppm, but not at 3 × 400 ppm, was also significantly reduced compared with its control. The mean number of live fetuses per litter was very low in the corresponding control group (N.S.) compared with all other exposed and control groups. Further, the percent resorptions were very high (N.S.) in the 3 × 400-ppm group compared with the 3 × 200-ppm group and the corresponding control group.

The Saillenfait et al. (1996) study also showed a significant increase in the incidences of litters with dilated renal pelvis (13/18 vs 4/18 for controls, $p<0.01$) and dilated ureter (14/18 vs 7/18 for controls, $p<0.01$)

in the group exposed to 1 × 1200 ppm. Considering the wide variations that can occur in renal development (Woo and Hoar, 1972), it is doubtful that the the variations in the renal pelvis and ureter are of biological significance.

Hackett et al. (1982) (also reported by Hardin et al., 1983) reported on a study in which female Sprague-Dawley CD rats were exposed to filtered air (control) or 150 ppm (270 mg/m^3) of ethylene oxide vapor, 7 h/day, 5 days/week for 3 weeks before mating. After the 3-week premating period, rats exposed to filtered air were subdivided into the following: (group 1) received filtered air throughout gestation, (group 2) received filtered air from gd 1-6 and ethylene oxide exposure from gd 7-16, (group 3) received ethylene oxide exposure from gd 1-16. The rats exposed to ethylene oxide for 3 weeks continued on the same treatment throughout gestation (group 4). There were 41 pregnant rats in groups 1, 2, and 3 and 39 in group 4. The results are summarized in Table 14. Mean body weight of female rats exposed to ethylene oxide vapor before mating and throughout gestation (group 4) was significantly lower (4-6%) than that of controls (group 1) during the latter part of the premating period and throughout gestation. The investigators reported no other treatment-related or biologically significant maternal effects. No statistical differences occurred in the pregnancy rate or the number of live fetuses/litter. Fetal parameters showing treatment-related effects included decreases in male and female fetal weights and increases in the incidence of litters with reduced ossification of the skull and sternebrae in all groups exposed during gestation (2, 3, and 4) compared with controls. No treatment-related malformations were observed. These results showed that fetal effects were produced by ethylene

Table 14. Maternal, Reproductive, and Developmental Effects of Exposure to Ethylene Oxide Vapor by Inhalation in Rats

Parameter	Exposure Group[a]			
	Group 1	Group 2	Group 3	Group 4
Maternal Body Weight (g)				
Day 21, premating	278[b]	277	280	267*
gd 6	298	298	293	279*
gd 11	315	314	308	295*
gd 16	339	335	328	317*
gd 21	382	381	378	360*
Reproductive				
No. live litters/No. pregnant	41/41	41/41	41/41	38/39
No. implantation sites/dam	14.7	14.0	14.8	14.3
No. resorptions/litter	0.75	0.71	0.92	1.60*
No. live fetuses/litter	13.9	13.5	13.8	12.7
Fetal Parameters				
Weight of female (g)	3.56	3.35*	3.23*	3.12*
Weight of male (g)	3.73	3.53*	3.47*	3.34*
Crown-to-rump length (mm), female	36.1	35.3*	34.7*	34.8*
Crown-to-rump length (mm), male	36.5	36.1*	35.8*	35.6*
Morphologic Alterations[c]				
Reduced ossification, skull	3/2 (4.9)	16/9 (22.0)*	10/9 (22.0)*	14/10 (26.3)*
Reduced ossification, sternebrae	69/23 (56.1)	145/36 (87.8)*	159/36 (87.8)*	155/33 (85.8)*

Source: Hackett et al., 1982

[a]Group 1 - unexposed during gestation; Group 2 - exposed gd 7-16; Group 3 - exposed gd 1-16; Group 4 - exposed from premating through gd 1-16.

[b]Mean values except when presented as incidence.

[c]No. Fetuses/no. litters; numbers in parenthesis are percentages relative to controls.

*$p \leq 0.05$ compared with controls.

oxide exposure whether administered over a prolonged period or during organogenesis, and the effects were indicative of growth retardation.

In a one-generation reproduction study, groups of 30 male and 30 female Fischer 344 rats were exposed to ethylene oxide vapor at concentrations of 0 (control), 10, 33, or 100 ppm (18, 59,or 180 mg/m^3) for 6 h/day for 12 weeks prior to mating (5 days/week), during mating (7 days/week), during gd 0 to 19 and day 5 to 21 postpartum (7 days/week) (Snellings et al., 1982b). Two control groups were included in the study. The survival rates and fertility indices of male and female rats were not affected by exposure to ethylene oxide. The length

of gestation was increased in 7/14 dams at 100 ppm compared with controls. In addition, the median number of pups born (4 versus 10 for controls), median number of implantation sites (6 versus 10 for controls), and the median number of fetuses born/implantation site (0.57 versus 1-0.92 for controls) were significantly lower in dams at 100 ppm. No effect occurred on pup survival, but at 100 ppm, mean body weights were lower in F_1 pups on day 4 postpartum than in control groups. On day 21 postpartum, mean body weights were reduced at 33 ppm, but not at 100 ppm.

Mori et al. (1991) studied the effects of inhaled ethylene oxide on spermatogenesis in Wistar rats. Groups of six male rats were exposed to 50, 100, or 250 ppm (90, 180, or 450 mg/m^3) of ethylene oxide vapor 6 h/day, 5 days/week for 13 weeks and killed 40 hours after the last exposure; the control group consisted of 12 males rats exposed to clean air. No treatment-related effects were observed on mean body weight, testicular weights, testicular lactate dehydrogenase, or the sperm count in the epididymal head. The following treatment-related effects were observed at 250 ppm: degenerative changes in the seminiferous tubules, decreased epididymal weight (20% compared with the controls), decreased sperm count in the epididymal tail, and increased incidence of sperm head abnormalities (immature and teratic types combined and immature types separately). When analyzed separately, the incidence of teratic types was significantly elevated in all exposure groups. No other changes were observed in groups exposed to 50 and 100 ppm of ethylene oxide.

Mori et al. (1989) conducted another study in which groups of six to eight male Wistar rats were exposed to clean air or 500 ppm (900 mg/m^3) of ethylene oxide vapor, 6 h/day, 3 days/week for 2, 4, 6, or 13 weeks. Endpoints evaluated included body weights (controls were pair-fed), weight and histopathology of the testes and epididymides, and testicular enzyme activities. No significant effect was observed on body weight at any time during the study. The testes of exposed rats were atrophic after 13 weeks of treatment and the relative testicular weights showed a corresponding decrease (82, 59, and 46% of control weight) after 4, 6, and 13 weeks, respectively. Relative epididymal weights were decreased (86, 71, and 59% of control weight) at the same time points. Histopathological examination of the testes showed degenerative changes in the seminiferous tubules manifested as mild degeneration of germ cells at 2 weeks, conspicuous degeneration at 4 weeks, exfoliation of germ cells at 6 weeks, and a marked reduction in germ cells in about 50% of seminiferous tubules, which contained only Sertoli cells at 13 weeks. Plasma testosterone levels were not affected. Testicular glutathione reductase activity was reduced at all time points, glutathione peroxidase activity was decreased at 2 weeks and increased at 6 and 13 weeks, and glutathione-S-transferase activity was increased at 4-13 weeks.

3.3.2. Mice

Generoso et al. (1987) and Rutledge and Generoso (1989) showed that exposure to ethylene oxide before mating or within 24 hours of mating can have pronounced effects on mouse fetal development.

In the study to assess the effect of inhaled ethylene oxide on preovulatory oocytes, Generoso et al. (1987) exposed female mice to 0 or 1200 ppm (2160 mg/m^3) for 1.5 h/day during 4 consecutive days before mating or to 300 ppm (540 mg/m^3) for 6 h/day for 10 exposures during a 14-day premating period. The dams were killed on gestation day (gd) 17 to assess the effect on resorptions, midgestational deaths, and late fetal deaths. The number of implants/female was significantly reduced at 300 ppm but not at 1200 ppm.However, the percentage of resorptions in both groups of females exposed before mating was significantly elevated, 10.8% (3.0% in controls) and 41.1% (6.4% in controls) at 1200 ppm and 300 ppm, respectively; Midgestational deaths and late fetal deaths were slightly elevated but not significantly; the induced loss of conceptuses was 15.7% at 1200 ppm and 58.2% at 300 ppm, showing that exposure to the lower concentration for a longer duration was more effective than the high concentration for a short duration.

Rutledge and Generoso (1989) exposed groups of female (C3H × C56BL)F_1 and (SEC × C57BL)F_1 mice to 0 (control) or 1200 ppm (2160 mg/m^3) of ethylene oxide vapor for 1.5 hours beginning 1, 6, 9, or 25 hours after a 30-minutes mating period; the mice were killed on gd 17. The exposure times corresponding to different developmental stages of the zygote are: 1h – sperm entry; 6 hours – early pronuclear stage, before DNA synthesis; 9 hours – pronuclear DNA synthesis stage; and 25 hours – early 2-cell stage. Additional experimental groups were included to study the effects of ethylene oxide exposure on the preovulatory oocytes. Two additional groups of female mice were exposed similarly to 0 or 1800 ppm (3240 mg/m^3) 6 hours after mating and killed serially on gd 11-15 to determine the effect on midgestational development. A marked reduction was observed in the number of live fetuses from female mice exposed to ethylene oxide vapor 1 hour after mating (6 fetuses/dam vs 9.72 for controls) and 6 hours after mating (1.81 fetuses/dam vs 10.11 for controls). In addition, there was a marked increase in the incidence of abnormal fetuses when females were exposed 1 hour (14.7% vs 0.2% for controls) and 6 hours (39.2% vs 1.7% for controls) after mating. The predominant types of abnormalities were hydrops (varying degrees of edema ranging from thick neck to a “balloon-like fetus”) and eye defects. Defects in the limbs and tail occurred in females exposed 6 hours after mating. Other abnormalities included abdominal wall defect, cleft palate, exencephaly, and small size. Generoso et al., (1987) reported that the percentage of resorptions was significantly elevated at all times, but the greatest effect occurred in females exposed 6 hours (52.9%) after mating; the induced loss of conceptuses was 82.25%. Significant, but less severe effects occurred when females were exposed at 9 and 25 hours after mating. Analysis of the uterine content of females exposed to 1800 ppm and killed on gd 11-15 showed significant increases in fetal deaths, particularly on gd 15 (late deaths). There were also significant increases in the number of defective living fetuses per dam and decreases in the number of living fetuses per dam. Most of the dead fetuses were hydropic (Rutledge and Generoso, 1989).

Ribeiro et al. (1987) evaluated the effect of inhaling 0, 200, or 400 ppm of ethylene oxide vapor on sperm morphology in mice. Male Swiss Webster mice were exposed 6 h/day for 5 days, and killed 1, 3, and 5 weeks after exposure. The results showed that ethylene oxide induced concentration-related increases in the incidences of abnormal spermatozoa, spermatids, and preleptotene spermatogonial cells compared with the incidences in controls. The increases were statistically significant at both doses.

3.3.3. Rabbits

Groups of 30 female New Zealand white rabbits were artificially inseminated and exposed to 150 ppm of ethylene oxide vapor (99.7% purity) for 7 h/day from gd 1-19 or gd 7-19 (Hackett et al., 1982). Another group of 30 inseminated rabbits were exposed to filtered air throughout the study. All rabbits were killed on gd 30. No statistically significant effects were observed on mean food consumption, mean body weight, organ weights, histopathological lesions, or maternal, reproductive, or developmental parameters.

3.4. Carcinogenicity

Inhalation carcinogenicity studies have been conducted in mice and rats. These studies are summarized in Table 15. Adkins et al. (1986) conducted a 6-month lung tumor bioassay in female A/J mice exposed to ethylene oxide at concentrations of 0, 70, or 200 ppm (126 of 360 mg/m^3) and showed an increase in the incidence of lung adenomas (Adkins et al., 1986). There was also a statistically significant increase in the number of lung tumors per tumor-bearing mouse (1.62, 1.53, and 2.47, for 0, 70, and 200 ppm, respectively) at the 200-ppm level.

A study reported by the NTP (1987) using B6C3F$_1$ mice exposed to 50 or 100 ppm of ethylene oxide vapor for 102 weeks showed statistically significant increases in the incidences of alveolar/bronchiolar adenomas or carcinomas and Harderian gland tumors in male and female mice and increases in uterine tumors and malignant lymphomas in female mice. Snellings et al. (1984b) and Garman et al. (1985) reported on a study using groups of 120 male and 120 female F344 rats exposed to ethylene oxide concentrations of 0, 10, 33, 100 ppm for 2 years. Ten rats per group were killed at 6 and 12 months; 20 rats per group were killed at 18 months and all survivors were killed at study termination. The results showed statistically increased incidences in mononuclear cell leukemia in males and females and in subcutis fibromas in males. Garman et al. (1985) specifically analyzed the brain tumors and reported

Table 15. Inhalation Exposure to Ethylene Oxide: Summary of Carcinogenicity Studies

Animal Description			Exposure Protocol	Response	
Species/strain	Sex	No./group		Tissue/Tumor Type	Incidence[a]
Mouse/A/J	F	30	0, 70, or 200 ppm, 6 h/d, 5 d/wk, for 6 mo	Lung adenoma	28, 56, and 87%; 0.46, 0.86*, 2.14* tumors/mouse

Mouse/B6C3F$_1$	M	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Alveolar/bronchiolar adenoma or carcinoma	11/50, 19/50, and 26/50*	NTP, 1987
	M	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Harderian gland papillary cystadenoma	1/43, 9/44*, and 8/42*	NTP, 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Alveolar/bronchiolar adenoma or carcinoma	2/49, 5/48, and 22/49*	NTP, 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Harderian gland papillary cystadenoma	1/46, 6/46, and 8/47*	NTP, 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Uterus, adenoma or adenocarcinoma	0/49, 4/47, and 5/49*	NTP, 1987
	F	50	0, 50, or 100 ppm, 6 h/d, 5 d/wk, for 102 wks	Malignant lymphoma	9/49, 6/48, and 22/49*	NTP, 1987
Rat, F344	M	50	0, 0, 10, 33, or 100 ppm, 6h/d, 5 d/wk, 2 yr	Spleen, mononuclear cell leukemia[c]	5/48, 8/49, 9/51, 12/39*[b], and 9/30*[b]	Snellings et al., 1984b
	M	100	0, 0, 10, 33, or 100 ppm, 6h/d, 5 d/wk, 2 yr	Brain, gliomas[d]	1/181, 0/92, 3/85, 6/87*	Garman et al., 1985
	M	50	0, 0, 10, 33, or 100 ppm, 6h/d, 5 d/wk, 2 yr	Skin, subcutis fibroma[c]	2/49, 1/48, 9/51*, 1/39, and 11/30*	Snellings et al., 1984b
	F	50	0, 0, 10, 33, or 100 ppm, 6h/d, 5 d/wk, 2 yr	Spleen, mononuclear cell leukemia[c]	6/56, 5/60, 11/54, 14/48*, and 15/26*	Snellings et al., 1984b
	F	100	0, 0, 10, 33, or 100 ppm, 6h/d, 5 d/wk, 2 yr	Brain, gliomas[d]	2/78, 2/90, 1/94, 0/187	Garman et al., 1985

Table 15. Continued

Animal Description			Exposure Protocol	Response		Reference
Species/strain	**Sex**	**No./group**		**Tissue/Tumor Type**	**Incidence[a]**	
Rat, F344	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 weeks	Brain, mixed cell glioma	0/76, 2.77, and 5/79*	Lynch et al., 1984a

	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 weeks	Body cavity, peritoneal mesothelioma	3/78, 7/79, and 21/79*	Lynch et al., 1984a
	M	80	0, 50, or 100 ppm 7 h/d, 5 d/wk, 104 weeks	Spleen, mononuclear cell leukemia	24/77, 38/79*, and 30/76	Lynch et al., 1984a

[a]Tumor incidence presented in order of exposure groups as shown in “Exposure Protocol” column.

[b]Statistically significant when compared with the combined control groups.

[c]incidences based on number of rats killed at 24 months.

[d]Incidences based on number of animals at risk.

*$p<0.05$ test group compared with control

increased incidences at 33 and 100 ppm and a dose-related trend for both male and female rats. The incidence of gliomas showed a statistically significant increase at 100 ppm in male rats. These data are summarized in Table 15. Another study using male F344 rats of the same strains exposed to 50 or 100 ppm of ethylene oxide showed increases in the incidences of mixed cell gliomas of the brain at 50 ppm and peritoneal mesotheliomas at 100 ppm (Lynch et al., 1984a).

3.5. Genetic Toxicity

Ethylene oxide readily alkylates DNA and other macromolecules; it has been studied extensively in numerous genetic toxicity systems employing both procaryotic and eukaryotic cells in vitro and in vivo. The results of these tests showed that ethylene oxide is genotoxic in bacteria, yeast, fungi, *Drosophila melanogaster*, and in rodent and human cells, causing gene mutations, gene conversions, sex-linked lethal mutations, and heritable translocations in nonmammalian systems; unscheduled DNA synthesis, gene mutations, SCEs, chromosomal aberrations, and micronuclei are induced in cultured mammalian cells (Golberg, 1986, Dellarco et al., 1990; IARC, 1994). The formation of DNA adducts with ethylene oxide in several mammalian system shows that ethylene oxide alkylates genetic material. There is also a wealth of information showing that inhalation exposure to ethylene oxide causes genetic damage in somatic and germ cells in species such as rodents, monkeys, and rabbits. A few of these studies will be presented in this report; reviews by Golberg (1986), Dellarco et al. (1990) and IARC (1994) are sources for additional information.

3.5.1. Germ Cells

Table 16 summarizes the data on genetic toxicity in germ cells in rats and mice.

Sega et al. (1988) exposed male (C3H × B/10)F_1 mice to ethylene oxide at concentrations of 450 ppm (810 mg/m^3) for 4 h, 900 ppm (1620 mg/m^3) for 2 h, or 1800 ppm (3240 mg/m^3) for 1 hour and showed increased DNA strand breaks and unscheduled DNA synthesis as measured by incorporation of tritiated thymidine into DNA. An exposure-rate effect was observed; 1800 ppm for 1 hour was more effective than 900 ppm for 2 h, which was more effective than 450 ppm for 4 h.

Table 16. Genotoxic Effects of Inhaled Ethylene Oxide on Germ Cells in Male Rodents

Species/Strain	Assay	Experimental Protocol	c × t	Results	Reference
Rat/Long-Evans	Dominant lethality[a]	1,000 ppm for 4 h; mated with females weekly for 10 weeks	4,000 ppm•hrs	Positive: increase in dead implants per pregnancy (wks 2, 3, 5) and dead implants per total implants (wks 1, 2, 3, 5)	Embree et al., 1977
Mouse/(C3H × B110)F_1	DNA strand breaks and UDS	450 ppm for 4 h, 900 ppm for 2 h, or 1,800 ppm for 1 h	1,800 ppm•hrs	Positive: DNA strand breaks and UDS; exposure-rate effect: 1800 ppm>900 ppm>450 ppm	Sega et al., 1988
Mouse/(C3H × B110)F_1	DNA alkylation of sperm and hemoglobin	75 ppm for 4 h, 150 ppm for 2 h, or 300 ppm for 1 h	300 ppm•hrs	DNA alkylation of epididymal and vas sperm and hemoglobin	Sega et al., 1991
Mouse/(101 × C3HF_1)	Dominant lethality[b]	255 ppm, 6 h/day, 5 d/wk for 2 or 11 wks	15,300 ppm•hrs or 84,150 ppm•hrs	Positive: dominant lethals produced after 2 (39%) and 11 weeks (55%)	Generoso et al., 1983
Mouse/(C3H × 101)F_1	Dominant lethality	control, 300, 400, or 500 ppm, 6 h/d for 4 d	7,200 ppm•hrs, 9,600 ppm•hrs, 12,000 ppm•hrs	Positive: exposure-related increase; 4, 27, and 62% dominant lethals	Generoso et al., 1986
Mouse/(C3H × 101)F_1	Dominant lethality	control, 300 ppm for 6 h/d, 600 ppm for 3 h/d, or 1,200 ppm for 1.5 h/d for 4 d	1,800 ppm•hrs	Positive: exposure-rate increase; 11, 32, and 64% dominant lethals	Generoso et al., 1986
Mouse/(C3H × 101)F_1	Dominant lethality	control, 165, 204, 250, or 300 ppm 6 h/d, 5 d/wk for 6 wks, then 7 d/wk for 2.5 wks.	47,025 - 85,500 ppm•hrs	Positive: dose-related increase; 6-8, 13-14, 23-24, and 45-60% dominant lethals	Generoso et al., 1990
Mouse/(C3H × 101)F_1	Heritable translocation	control, 165, 204, 250, or 300 ppm 6 h/d, 5 d/wk for 6 wks, then 7 d/wk for 2.5 wks.	47,025 - 85,500 ppm•hrs	Positive: dose-related increase; 0.05, 2.80, 5.09, 10.84, and 25.53% translocation carriers in combined female strains	Generoso et al., 1990

[a]Defined as the number of dead implants per total implants.
[b]Defined as the average no. living embryos in experimental group/average no. for controls.
UDS = unscheduled DNA synthesis

Sega et al. (1991) also examined the effect of exposure rate on DNA alkylation of reproductive targets (sperm and testes) and hemoglobin. Male (C3H*Rl* × B/10*Rl*)F_1 mice were exposed to 75 ppm of [^{3}H]ethylene oxide for 4 h, 150 ppm for 2 h, or 300 ppm for 1 hour (300 ppm•hours); alkylation of DNA was measured 90 minutes, 1 day, 3 days, and 6 days after terminating exposure. This study showed that epididymal and vas sperm were alkylated by ethylene oxide; the level of alkylation was greater in epididymal sperm than in vas sperm, suggesting a greater susceptibility of developing sperm. Alkylation of both epididymal and vas sperm increased with exposure rate. There was no suggestion of repair, as the binding level did not decrease as a function of time after exposure. Alkylation of hemoglobin showed a exposure rate effect also, with no decrease with time after exposure.

The dominant-lethal assay is one test used to screen for mutagenicity in germ cells. Embree et al. (1977) showed that dominant lethality is induced in male Long-Evans rats exposed to 1000 ppm (1800 mg/m^3) of ethylene oxide for 4 h. The exposed males were mated with female rats each week for 10 consecutive weeks. Significant increases in postimplantation deaths were observed during the first 5 weeks of mating for ethylene oxide-exposed rats compared with controls. Postimplantation deaths are indicated by increased number of dead implants per female (weeks 2, 3, and 5) and number of dead implants/total implants (mutagenic index) (weeks 1, 2, 3, and 5). There was a significant decrease in the fertility index (number of pregnant females per number of females mated) during weeks 3 and 4 and the total number of implants per total number of pregnancies during week 2. Preimplantation losses were not affected. The increase in postimplantation deaths during the first 5 weeks suggest that ethylene oxide affected germ cells after meiosis.

Generoso et al. (1983) exposed (101 × C3H)F_1 male mice repeatedly to 255 ppm (459 mg/m^3) 6 h/day, 5 days/week for 2 or 11 weeks and evaluated dominant lethality after mating the exposed males with (C3H × C57BL)F_1 females for 3.5 days after the last exposure. Both treatments protocols produced marked increases in the number of dead implants (average = 37 and 50% after 2 and 11 weeks, respectively) and dominant lethality (average = 39 and 55% after 2 and 11 weeks, respectively). The effect was slightly greater after 11 weeks of treatment than after 2 weeks.

Generoso et al. (1986) also conducted dominant lethality test using (C3H × 101)F_1 male mice exposed to ethylene oxide at concentrations of 300, 400, or 500 ppm (540, 720, or 900 mg/m^3) for 6h/day, for 4 days; the total concentrations were 7200, 9600, or 12,000 ppm•hours. Each exposure group was accompanied by a control. The treated animals were mated each day with a different female ((SEC × C57BL)F_1) starting with the day after exposure ended and continuing for 12 days (500 ppm) or 8 days (300 and 400 ppm). The results showed that the maximum effects occurred during mating days 4.5 to 7.5 at 500 ppm; a marked decrease was observed for the number of living embryos and marked increases were observed in the number of dead implants

and the number of females with one or more dead implants. Clear, but less pronounced effects were seen at 400 ppm and only marginal effects were seen at 300 ppm. The overall dominant lethality showed a clear concentration-response relationship.

Generoso et al. (1986) also conducted a study to examine the effect of exposure rate on dominant lethality. Male mice of the same hybrid strain were exposed to ethylene oxide at concentrations of 300 ppm for 6 h/day, 600 ppm for 3 h/day, or 1200 ppm for 1.5 h/day for 4 days (total concentration = 1800 ppm•hours/day). The exposed males were mated with (SEC × C57BL)F_1 females starting 5 days after the last exposure. Each exposure group was accompanied by a control. A clear exposure-related increase in the frequency of dominant lethality was observed.

In another study, Generoso et al. (1990) evaluated the effect of inhaled ethylene oxide on dominant lethality and heritable translocations. Groups of (C3H × 101)F_1 male mice were exposed to 165, 204, 250, or 300 ppm (297, 367, 450, or 540 mg/m^3) ethylene oxide, 6 h/day, 5 days/week, for 6 weeks followed by exposure for 7 days/week for 2.5 weeks. During the last 10 days of exposure and 1 day after the last exposure the male mice were mated with T-stock or (SEC × C57BL)F_1 females. No significant dominant lethality was seen at 165 ppm in either strain as assessed by the number of living. At 204 ppm, there was a significant decrease in the number of living embryos in one strain and a significant increase in the number of females with one or more dead implants in both strains; this dose showed an overall marginal effect on dominant lethality. At 250 and 300 ppm, clear effects on dominant lethality were indicated in both strains by decreases in the number of living embryos, increases in the number of dead implants, and increases in the number of females with one or more dead implants. The frequency of dominant lethals showed concentration-related increases, but the exposure-response relationship was not linear.

In the experiment on heritable translocations, Generoso et al. (1990) evaluated the frequency of semisterile and sterile male offspring and analyzed the carriers for translocations. There was a concentration-related increase in the frequency of translocation carriers produced from each female strain; the increases achieved statistical significance ($p<0.01$ compared with controls) at all concentrations. The response curves were not linear.

3.5.2. Somatic Cells

Other genetic toxicity tests including SCE and chromosome aberration tests have been performed on peripheral lymphocytes and bone marrow cells in laboratory animals exposed to ethylene oxide by inhalation. Kligerman et al. (1983) compared the frequencies of SCEs in peripheral lymphocytes taken from male Fisher 344 rats exposed to ethylene oxide at target concentrations of 0, 50, 150, or 450 ppm for 6 h/day for 1 or 3 days. The frequency of SCEs/metaphase was significantly increased only at 450 ppm (10.4 vs 7.8 in controls) after a single

exposure, whereas the frequencies were significantly increased at all concentrations (7.5, 9.1, 10.3, and 13.6 for 0, 50, 150, and 450 ppm, respectively) after three exposures. The frequency of SCEs was similar for a single exposure to 450 ppm (2700 ppm•hours) and repeated exposures to 150 ppm (2700 ppm•hours) In addition, only repeated exposures caused increases in the number of high frequency cells with ≥ 20 SCEs/metaphase.

A study conducted by Ong et al. (1993) showed that SCEs are induced in spleen and bone marrow cells of male Fischer 344 rats exposed to ethylene oxide at concentrations of 100 ppm for 6 h/day, 300 ppm for 2 h/day, or 600 ppm for 1 h/day, 5 days/week, for 3, 6, or 9 months. The frequency of SCEs in spleen cells did not show a clear concentration-response relationship at any time point, but a cumulative response was seen as duration of exposure increased. The frequency of SCEs in bone marrow cells was highest at the lowest concentration and there was no clear increase with duration of exposure.

The frequency of SCEs was also increased in lymphocytes of New Zealand white rabbits exposed to ethylene oxide at concentrations of 200 or 400 ppm, 6 h/day or 1500 ppm for 15 minutes two times a day, 5 days/week up to a cumulative concentration of about 48,000 ppm•hours (Yager, 1987). A clear exposure-rate effect was not observed.

Vergnes and Pritts (1994) reported that male Fischer 344 rats and male B6C3F_1 mice exposed to 200 ppm ethylene oxide, 6 h/day, 5 days/week for 4 weeks had significantly elevated frequency of micronuclei in polychromatic erythrocytes in their bone marrow. The mean percent of micronucleated cells was 0.79% for rats (0.30% for controls) and 0.72% for mice (0.22% for controls).

Lynch et al. (1984b) reported that the frequency of SCEs and chromosome aberrations were significantly increased at both concentrations in lymphocytes of adult male cynomolgus monkeys exposed to 50 or 100 ppm of ethylene oxide, 7 h/day, 5 days/week for 2 years. Mitotic activity of the lymphocytes was also reduced.

3.5.3. DNA Alkylation

Ethylene oxide is a reactive epoxide that readily alkylates DNA and proteins without metabolic activation (Golberg, 1986). Ehrenberg et al. (1974) detected radioactive binding to the nucleic acid fraction of tissues from mice exposed to radioactive ethylene oxide at 29 ppm for 82 minutes and analyzed 73 minutes after exposure. The relative binding activities in the tissues were as follows: kidney≈spleen>lung>liver>testes>brain. Ehrenberg et al. (1974) further identified 7- (2-hydroxyethyl)guanine (7-HEG) as one of the DNA alkylation products formed after exposure to ethylene oxide. Potter et al. (1989) exposed male Fischer 344 rats by nose only inhalation to [^{14}C]ethylene oxide at concentrations of 1, 10, or 33 ppm for 6 hours and isolated DNA from brain, lung, liver, spleen, kidney, and testes. A linear relationship was observed for the formation of 7-HEG and concentration of ethylene oxide in air. Alkylation frequencies ranged from 0.0786-0.118, 0.777-0.964, and 3.03-3.66 nmole 7-HEG/g DNA at concentrations of 1, 10, and 33 ppm, respectively, for all tissues except testis,

which was 60% lower (0.065, 0.466, and 2.00, respectively). Bolt and Leutbecher (1993) exposed male Sprague-Dawley rats to [^{14}C]ethylene oxide in a closed system until the ethylene oxide disappeared from the atmosphere; a linear increase was again observed for the formation of 7-HEG adducts in liver and spleen (exposure concentration in ppm was not provided). The animals were sacrificed immediately after exposure.

In a time-course study, Walker et al. (1992a) exposed male B6C3F$_1$ mice to 100 ppm of ethylene oxide for 1 or 3 days or 1, 2, or 4 weeks (6 h/day, 5 days/week). Persistence of DNA adducts was assessed after exposing male mice to 100 ppm and male F344 rats to 300 ppm for 4 weeks and analyzing the tissue DNA 1, 3, and 7 days after the last exposure. DNA adducts were measured in lung, kidney, liver, spleen, testes, and brain. In control mice, 2-6 pmole of 7-HEG/mg DNA was detected. At the early time points (not further described), formation of 7-HEG adducts was not different from that of controls, but as exposure duration increased adduct formation increased attaining a steady state concentration only in lung by 4 weeks. After exposure for 4 weeks, 7-HEG adducts showed a greater persistence in rat tissues than in the mouse, except for the kidney. Half-life of disappearance was 6.9 days for mouse kidney and 1 to 2.3 days for other tissues in the mouse; for rats the half-life of disappearance ranged from about 2.9 to 5.8 days for all tissues. Two minor adducts (O^6-(2-hydroxyethyl)guanine [O-HEG] and 3-(2-hydroxyethyl)guanine [3-HEG)]) were also analyzed in the rats exposed to 300 ppm for 1 to 4 weeks. Steady-state concentrations of 1.0-1.2 pmole O-HEG/mg DNA was achieved by 2 weeks in brain, kidney, lung, and spleen, whereas steady-state was achieved after a few days for formation of 3-HEG adducts (1 pmole adduct/mg DNA). After a 4-week exposure the concentration of these adducts was 250- to 300-fold lower than the concentration of 7-HEG.

Walker et al. (1992a) also conducted another study using male mice and rats exposed to 0, 3, 10, 33, or 100 ppm ethylene oxide for 6 h/day, 5 days/week for 4 weeks. An additional group of rats was exposed similarly to 300 ppm. After a 4-week exposure to 100 ppm, formation of 7-HEG adducts was similar in all mouse tissues (21-38 pmole 7-HEG/μmol guanine, with testes containing the lesser amount. In rats exposed to 100 ppm for 4 weeks, 7-HEG formation was lower in liver, kidney, and testes (44-55 pmole 7-HEG/μmol guanine) than other tissues (81-105 pmole 7-HEG/μmol guanine); the lowest amount was found in testes. The dose-response relationship for 7-HEG formation was nonlinear for rats and mice (lung, brain and spleen).

3.6. Summary

Acute lethality data are summarized in Table 17. Mice are the most sensitive species followed by dogs and rats. Four-hour LC_{50}s range from 660 ppm for female mice to 1972 ppm for male rats, and 1-hour LC_{50} s ranged from 4439 ppm for female rats to 5748 ppm for male rats; a 1-hour study was not available for the mouse. The lowest concentration causing death in a 4-hour exposure study was 533 ppm (20% mortality) for female mice. A slightly higher concentration of 800 ppm cause 100% mortality in male mice. The lowest concentration

causing death in a 60-minutes exposure study was 3966 ppm (40% mortality) for female rats. Lethal concentrations of ethylene oxide vapor are irritating to the eyes and upper and lower respiratory tract. Death was not due to respiratory irritation, but to respiratory failure, probably involving nervous system toxicity. Lethal concentrations of ethylene oxide also cause neurological effects manifested by absence of tail and toe pinch reflex and startle reflex, ataxia, semiconsciousness, and convulsions. In addition, vomiting occurs in dogs and diarrhea occurs in rats and dogs, which also may be due to a neurological mechanism and not to a direct effect on the gastrointestinal tract. Pathological lesions develop in the liver and kidney and respiratory tract of animals exposed to lethal concentrations.

Clinical signs observed in animals that survived a single exposure to ethylene oxide vapor were not different from those observed in animals that died. Eye and respiratory tract irritation and evidence of neurological effects are the primary effects observed in animals surviving exposure to ethylene oxide. Further, the effects were usually reversible within a few days after exposure depending on the concentration of ethylene oxide vapor. Rabbits appear to be the most resistant species to effects of ethylene oxide vapor; only a mild hindlimb weakness was seen after exposure to 13,349 ppm for 30 minutes. Several studies on repeated exposures (6 h/day, 5 days/week) to ethylene oxide vapor were available; durations ranged from 6 weeks using dogs, 10 to 14 weeks using rats and mice, to 24 months using monkeys. Clinical signs observed after repeated exposures are similar to those observed after a single exposure. However, respiratory irritation progresses to secondary effects; neurological effects progress to hindlimb weakness, muscle atrophy and paralysis. Growth retardation, mild anemia, and pathologic lesions in adrenal gland, thymus, nasal cavity, kidney, and spleen also occur after repeated exposures to ethylene oxide vapor. Neurological effects including hindlimb paralysis are reversible; and may be completely resolved several months after terminating exposure.

Table 17. Summary of Lethality Data for Experimental Animals

Species/sex	LC_{50} [a]		Exposure time (minutes)	Comments	Reference
	ppm	mg/m^3			
Rat/male	1,460	2,630	240	lowest experimental concentration causing lethality was 882 ppm (20%)	Jacobson et al., 1956
Rat/male	1,972	3,550	240	lowest experimental concentration causing mortality was 2,026 ppm (80%); no mortality at 1,850 ppm	Nachreiner, 1991
Rat/female	1,537	2,767	240	lowest experimental concentration causing mortality was 1,443 ppm (20%); no mortality at 1,021 ppm	Nachreiner, 1991
Rat/male&female	1,741	3,134	240	No comments	Nachreiner, 1991
Rat/male	5,748	10,346	60	lowest experimental concentration causing mortality was 5,546 ppm (20%); no mortality at 4,827 ppm	Nachreiner, 1992
Rat/female	4,439	7,990	60	lowest experimental concentration causing mortality was 3,966 ppm (40%); no mortality at 3,609 ppm	Nachreiner, 1992
Rat/male&female	5,029	9,052	60	no comments	Nachreiner, 1992
Rat/sex not specified	ND	ND	30	1:100 (10,679 ppm) was fatal to rats; no additional information; 1:150 (7,119 ppm) was not fatal	Walker and Greeson, 1932
Mouse/female	835	1,504	240	lowest experimental concentration causing mortality was 533 ppm (20%); lowest concentration tested	Jacobson et al., 1956
Mouse/male	ND	ND	240	LC_{50} was not calculated; 100% mortality at 800 ppm; no deaths at 400 ppm	NTP, 1987
Mouse/female	660	1,188	240	lowest experimental concentration causing mortality was 800 ppm (80%); no mortality at 400 ppm	NTP, 1987

Table 17. Continued					
Species/sex	**LC_{50}[a]**		**Exposure time (minutes)**	**Comments**	Reference
	ppm	**mg/m^3**			
Mouse	ND	ND	30	1:150 (7119 ppm) was fatal to mice	Walker and Greeson, 1932
Dog/male	960	1,730	240	no deaths occurred at 710 pm	Jacobson et al., 1956
Guinea pig	ND	ND	480	1,300 ppm caused death	Waite et al., 1930
Guinea pig	ND	ND	330	3,000 ppm caused death	Waite et al., 1930
Guinea pig	ND	ND	190	3,000 ppm caused death	Waite et al., 1930
Guinea pig	ND	ND	150	7,000 ppm caused death	Waite et al., 1930
Guinea pig	ND	ND	60	25,000 ppm caused death	Waite et al., 1930
Guinea pig	ND	ND	10	63,000 ppm caused death	Waite et al, 1930

[a]LC_{50} or the percent mortality at the lowest experimental concentration causing mortality.

Several studies showed that exposure to ethylene oxide vapors cause developmental and reproductive effects in mice and rats. These studies are summarized in Table 18. There are inconsistencies in the developmental toxicity studies. The studies by Snellings et al. (1982a) and BRRC (1993) showed developmental effects at concentrations ≥ 50 ppm in rats exposed for 6 h/day (300 ppm•hours), whereas the study by Saillenfait et al. (1996) showed developmental effects only at concentrations ≥ 800 ppm in rats exposed for 0.5 hours three times per day (1200 ppm•hours). The difference is not due to strain sensitivity, because the BRRC (1993) and Saillenfait et al. (1996) studies used Sprague-Dawley rats. These studies suggest that high concentrations for a short duration are less effective than lower concentrations for longer durations. No developmental effects were induced in rabbits exposed to 150 ppm. Reproductive toxicity studies showed effects at concentrations ≥ 50 ppm in rats and ≥ 200 ppm in mice.

Ethylene oxide is a direct alkylating agent that is genotoxic in numerous in vitro and in vivo test systems. Ethylene oxide vapor is genotoxic in mammalian germ cells as evidenced by induction of dominant lethality, heritable translocations, DNA strand breaks, and UDS (see Table 16). It is genotoxic in somatic cells as indicated by induction of SCEs, chromosome aberrations, or micronuclei in peripheral lymphocytes, spleen cells, or bone marrow cells. In addition to its genotoxic activity in somatic cells, ethylene oxide is carcinogenic in mice and rats. Positive results have been obtained using the mouse lung tumor bioassay (≥ 70 ppm) and the standard 2-year bioassays in mice and rats at concentrations ≥ 100 ppm. The carcinogenicity results are summarized in Table 15. IARC (1994) considers the evidence based on animals data to be sufficient for carcinogenicity of ethylene oxide.

4. SPECIAL CONSIDERATIONS

4.1. Metabolism/Disposition/Kinetics

Ethylene oxide is metabolized primarily by two pathways. Ethylene oxide can react with water to form ethylene glycol and, after several additional steps, oxalic acid (Golberg, 1986). Martis et al. (1982) showed that ethylene oxide, administered to dogs by intravenous injection, is rapidly hydrolyzed to ethylene glycol. Ethylene oxide reacts with chloride ions to form 2-chloroethanol followed by glutathione conjugation (Glutathione-S-transferace) and the formation of *S*-hydroxyenthyl)cysteine (Goldberg, 1986; Fennell, 1986) Fennell (1996) reported that physiologic

Table 18. Developmental and Reproductive Effects of Ethylene Oxide Vapor

Species	Exposure	Effect	Reference
Rat	0, 10, 33, 100 ppm, 6 h/day, gd 6-15	33 ppm – NOEL 100 ppm – mild retarded growth of fetus	Snellings et al., 1982a
Rat	0, 50, 125, 250 ppm, 6 h/day, gd 6-15	50 ppm – slight fetal growth retardation 125 ppm – maternal effects and fetal growth retardation 250 ppm – more severe maternal effects and fetal growth retardation	BRRC, 1993
Rat	0, 150 ppm, 7 h/day, 5 d/wk, premating, gd 7-16, or 1-16	growth retardation of fetus regardless of stage of exposure	Hackett, 1982
Rat	0, 400, 800, 1200 ppm, 0.5 h/day, gd 6-15	no effects on the fetus at any concentration	Saillenfait et al., 1996
Rat	0, 200, 400, 800, 1200 ppm, 0.5 h, 3 times per day, gd 6-15	800 ppm – fetal growth retardation 1200 ppm – maternal effects and fetal growth retardation	Saillenfait et al., 1996
Mouse	0, 1200 ppm, 1½ h, gd 1	fetal deaths, hydrops, and other malformations	Rutledge and Generoso, 1989
Mouse	0, 200, 400 ppm, 6 h/day, 5, 15, or 25 exposures	200 ppm: abnormal spermatozoa 400 ppm: abnormal spermatozoa	Ribeiro et al., 1987
Rat	0, 10, 33, 100 ppm, 6 h/day, 1-gen reprod.	33 ppm – NOEL 100 ppm – reproductive and fetal effects	Snellings et al., 1982b
Rat, males	0, 50, 100, 250 ppm, 6 h/day, subchronic	50 ppm – abnormal sperm, teratic type 100 ppm – abnormal sperm, teratic type 250 ppm – abnormal sperm, testicular degeneration	Mori et al., 1991
Rabbits	0, 150 ppm, 7 h/day, gd 7-19 or 1-19	no developmental effects	Hackett et al., 1982

based pharmacokinetic (PBPK) models indicated that glutathione conjugation accounts for about 10% of ethylene metabolism in humans, with most of the remainder undergoing hydrolysis. PBPK models indicated that

glutathione conjugation accounts for 75% of ethylene oxide metabolism in mice and 50% in rats. Golberg (1986) noted that ethylene oxide is not a substrate for epoxide hydrolase, and Brown et al. (1996). Brown et al. (1996) found no difference in the production of ethylene glycol by heat inactivated and active mouse liver cytosol.

Brugnone et al. (1986) studied workers exposed to 0.2 to 22.5 mg/m^3 (0.11 to 12.3 ppm) of ethylene oxide and showed that, at steady-state, 75 to 80% of inhaled ethylene oxide is absorbed into the body. The concentration of ethylene oxide in alveolar air ranged from 0.05 to 7 mg/m^3 (0.03 to 3.8 ppm). The venous blood:alveolar air coefficients ranged from 12 to 17 and the venous blood:environmental air coefficients ranged from 2.5 to 3.3. Brugnone et al. (1986) calculated a mean absorption of 7.2 to 7.7 mg of ethylene oxide for an 8-hour exposure to 2 mg/m^3 (1.11 ppm) and an alveolar ventilation rate of 10 L/minutes. Filser et al. (1992) used the data presented by Brugnone et al. (1986) to calculate a mean half-life of 42 minutes for ethylene oxide in humans.

Maples and Dahl (1993) reported that blood uptake gradually increased during the first 15 minutes and reached a plateau at about 60 ng/g blood in male F344 rats exposed to 5 ppm of ethylene oxide vapor for 60 minutes. Ehrenberg et al. (1974) calculated a biological half-time ($t_{½}$) of 9 minutes for male CBA mice exposed to ethylene-[1,2-^{3}H]oxide at concentrations ranging from 1.15 to 33 ppm (average concentrations) for 60 to 107 minutes. These investigators inferred that 2.5 μmole/kg (approximately equal to 2.5 μmole/L) is absorbed after an exposure of 1 ppm•hour. Martis et al. (1982) reported a mean half-life of about 33 minutes for the dog administered ethylene oxide by intravenous injection. They also noted that elimination kinetics was not dose dependent in the dog.

Brown et al. (1996) reported $t_{½}$ for ethylene oxide clearance from the blood as 13.8 ± 3.0 minutes for male rats and 10.8 ± 2.4 minutes for female rats exposed to 100 ppm of ethylene oxide; similar values were obtained for 330 ppm. For mice, the $t_{½}$ was 3.12 ± 0.2 minutes and 5.4 ± 0.5 minutes for male mice and 2.4 ± 0.2 minutes and 5.6 ± 0.2 minutes for female mice exposed to 100 and 330 ppm, respectively. The authors noted that the increase in $t_{½}$ in mice exposed to 330 ppm was due to saturation of metabolism in mice probably due to glutathione depletion. They measured ethylene oxide concentrations in blood, muscle, brain and testes 2 to 10 minutes after a 4-hour exposure. Peak tissue concentrations were similar for all tissues except testes, which was 50 and 20% lower in the mouse and rat, respectively, than in other tissues. Ethylene oxide concentrations were slightly higher in the tissues of the rat (except for testes) exposed to 100 ppm than in mice, whereas at 330 ppm, the concentrations were slightly higher in the mice than in rats. The authors also noted that clearance of ethylene oxide from the tissues was similar to that of blood.

Formation of hemoglobin adducts has been used as a measure of exposure to ethylene oxide vapor. Potter et al. (1989) reported that hemoglobin adduct formation showed a linear trend and no evidence of

saturation in male F344 rats exposed to 0, 3, 10, or 33 ppm for 6 h. Adduct formation measured as nmole of N^1-HEHis/g globin was as follows: 0.136, 1.03, and 4.64 for 3, 10, and 33 ppm, respectively. Walker et al. (1992b) also showed that hemoglobin adduct formation showed a linear trend in mice and rats exposed to 3 to 33 ppm of ethylene oxide (6 h/day, 5 days/week for 4 weeks), but was nonlinear over the entire exposure range of 3 to 100 ppm for mice and 3 to 300 ppm for rats.

4.2. Mechanism of Toxicity

Ethylene oxide is a direct-acting alkylating agent; it alkylates DNA and proteins. Ethylene oxide is also a mild primary irritant and a central nervous system depressant.

Finelli et al. (1983) noted that distal axonal neuropathy is characterized by primary axonal degeneration with secondary demyelination affecting distal segments of long tract fibers without involving the neuronal bodies. They postulated that ethylene oxide affects the peripheral and central nervous systems by interference with metabolism of neuronal perikaryon or axonal transport, thus inhibiting delivery of essential metabolites to nerve terminals. Ohnishi et al. (1985) noted axonal degeneration of myelinated nerve fibers in rats receiving subchronic inhalation exposure to ethylene oxide.

The mechanisms by which ethylene oxide induces developmental and testicular toxicity (not including genetic damage to germ cells) are not known. Protein alkylation may be involved, i.e., alkylation of enzymes in testicular toxicity (Mori et al., 1989). It is likely that protein alkylation is also involved in the induction of developmental toxicity.

Some of the toxic effects of ethylene oxide are probably mediated by alkylation of DNA or proteins, which can alter the structure and functional activities of genes, chromosomes, or protein. Genetic toxicity indicated by increased frequencies of SCEs, chromosome aberrations, and micronuclei is probably caused by DNA alkylation. Carcinogenicity is probably mediated by a genetic toxicity resulting from DNA alkylation.

Generoso et al. (1986) suggested that dominant lethality involves alkylation of chromosomes. In another study, heritable translocations were confirmed by cytogenetic analysis of the offspring, thus showing structural alterations in chromosomes, which could be due to DNA alkylations. Although the mechanism by which ethylene oxide induces effects in the zygote manifested by fetal deaths, resorptions, and structural fetal defects is not known, Generoso et al. (1987) and Katoh et al. (1989) noted that the data indicated that genetic damage is a likely mechanism. However, cytogenetic analysis of zygotes and midgestational fetuses ruled out numerical and structural alterations in chromosomes (Katoh et al., 1989). Gene mutations and specific locus mutations were also ruled out (Russell et al., 1984). Therefore, Katoh et al. (1989) proposed that the effects may be mediated by a nonmutagenic process involving changes in gene expression.

5. DATA ANALYSIS AND PROPOSED AEGL-1

5.1. Human Data Relevant to AEGL-1

There are no human data directly related to AEGL-1 derivation. Humans have been exposed to ethylene oxide at a wide range of concentrations. These studies did not correlate effects with exposures concentrations.

5.2. Animal Data Relevant to AEGL-1

The same data sets described for AEGL-2 are considered for deriving AEGL-1 values.

5.3. Derivation of AEGL-1

No AEGL-1 values are proposed. The odor threshold and sensory irritation occur at ethylene oxide concentrations higher than those causing systemic effects. In addition, all AEGL-2 values are below the odor threshold. It would not be valid to propose values below the definition of AEGL-1.

6. DATA ANALYSIS AND PROPOSED AEGL-2

6.1. Human Data Relevant to AEGL-2

Nonlethal effects of ethylene oxide in humans are summarized in Table 5. The epidemiologic studies presented suggestive evidence of adverse reproductive outcomes (Hemminki et al., 1982; Rowland et al., 1996); however, these studies had a number of limitations that makes them unsuitable for quantitative evaluation of AEGL values. Other nonlethal effects were shown to be reversible upon termination of exposure; however, some effects, particularly those occurring in the nervous system, are likely to cause concern to an exposed population, . Several studies described effects in humans exposed to ethylene oxide, but only three involved single or very short-term exposures. The study by Deschamps et al. (1992) showed respiration irritation, nonimunological asthma, and peripheral neuropathy in one subject accidentally exposed to concentrations at the odor threshold ($\geq$260 ppm) for 4 h/day for 4 days. Respiratory irritation could be attributed to each daily exposure, whereas peripheral neuropathy could have been caused by a single exposure and exacerbated upon repeated exposures. Other studies have shown that peripheral neuropathy may be exacerbated by repeated exposures to ethylene oxide (Finelli et al., 1983). The study by Laurent (1988) showed respiratory tract irritation, nervous system effects, and hemolysis in five workers exposed to ethylene oxide at the odor threshold for 30 minutes. Salinas et al. (1981) described nervous system effects leading to unconsciousness, apnea, and muscle twitching in an individual exposed to a calculated concentration of 500 ppm for $\leq$5 minutes. There is considerable uncertainty regarding the exposure concentration reported by Salinas et al. (1981); therefore, this study should not be used to derive AEGL values. In the remaining studies showing nonlethal effects, duration of exposure was not reported or the subjects were exposed repeatedly for durations ranging from 2 weeks to more than a year. Genetic lesions in somatic cells were documented in individuals after single exposure. Somatic lesions are not relevant endpoints

for evaluating AEGL-2 levels, because the only disease state associated with genetic lesions in somatic cells is carcinogenicity.

6.2. Animal Data Relevant to AEGL-2

The primary animal studies that can be used to derive AEGL-2 values include a 4-hour study in rats (Embree et al., 1977); developmental toxicity studies in rats reported by Snellings et al. (1982a), BRRC (1993), and Saillenfait et al. (1996); and a reproductive toxicity study in mice (Ribeiro et al., 1987). Genetic toxicity in germ cells should be considered in the evaluation of AEGL-2 exposure levels, but genetic toxicity in somatic cells should not because of the uncertainty of associating genetic lesions in somatic cells with a disease state other than carcinogenicity. The reproduction studies (Snellings et al., 1982b) cannot be used to derive AEGL-2 levels, because it is difficult to attribute the observed effects to a single exposure to ethylene oxide. The Embree et al. (1977) study showed central nervous system toxicity, eye irritation, respiratory tract irritation, and dominant lethality in rats exposed to 1000 ppm for 4. The Snellings et al. (1982a) and BRRC (1993) developmental toxicity studies showed growth retardation in rats exposed to 100 ppm and 50 ppm, respectively, for 6 h/day during organogenesis; the effects at 50 ppm appeared to approximate the threshold for growth retardation. The Saillenfait et al. (1996) study showed growth retardation in rats exposed to 800 or 1200 ppm for 3 × 0.5 h/day during organogenesis and maternal effects at 1200 ppm, but not at concentrations ≤ 1200 ppm for 0.5 h/day or 400 ppm for 3 × 0.5 h/day. The results of the Saillenfait et al. (1996) study showed inconsistencies, and the results differ considerably from those of other developmental toxicity studies. Ribeiro et al. (1987) reported abnormal spermatozoa in mice exposed to 200 or 400 ppm for 6 h/day for 1 to 5 weeks (5-25 exposures).

6.3. Derivation of AEGL-2

The Deschamps et al. (1992) study in which an individual was exposed to ethylene oxide at the odor threshold for 4 h/day would result in an AEGL-2 value of 87 ppm for a single 4-hour exposure assuming the odor threshold is 260 ppm and applying an uncertainty factor of 3 for intraspecies sensitivity. The data from the Laurent (1988) and Deleixhe et al. (1986) studies in which five workers were exposed to the odor threshold for 30 minutes would result in an AEGL-2 value of 87 ppm for a 30-minutes exposure. An uncertainty factor of 3 would account for intraspecies sensitivity (enzyme polymorphism and heart or respiratory diseases). Both values are associated with a high degree of uncertainty because they were based on odor detection and there is a wide variation in the odor threshold, ranging from 260 ppm as reported by Hellman and Small (1974) and 700 ppm (with a 95% confidence limit of 317 to 1540 ppm) as reported by Jacobson et al. (1956). The human studies are not considered to be adequate for deriving AEGL-2 values.

The rat study by Embree et al. (1977) was used to derive AEGL-2 values. Neurotoxicity along with respiratory tract and eye irritation and dominant lethality occurred in rats exposed to 1000 ppm for 4 hours. An

uncertainty factor of 3 was applied for intraspecies variability; glutathione-S-transferase polymorphism suggest variations in the metabolism of ethylene oxide in humans. An uncertainty factor of 10 is applied for interspecies sensitivity because the experimental exposure concentration was close to the lethal concentration for rats and above the lethal concentration for mice. The total uncertainty factor is 30. Time frame extrapolation was performed according to ten Berge's equation where n = 1.2 as derived from the rat lethality data. The resulting AEGL-2 values are 190, 110, 33, and 19 ppm for 30-minute, 1-, 4-, and 8-hour exposures. These AEGL-values should be protective of potential developmental effects. AEGL-2 values are summarized below:

AEGL-2 VALUES			
30 minutes	1 hour	4 hours	8 hours
190 ppm	110 ppm	33 ppm	19 ppm

Reference: Embree et al., 1977

Comments: This was a dominant lethality study; neurotoxicity and eye and respiratory tract irritation was observed as clinical signs of toxicity.

Uncertainty factors: 3 for intraspecies variability based on potential polymorphism in the glutathione detoxification pathway for ethylene oxide and for protection of individuals with respiratory and heart diseases.

10 for interspecies sensitivity because the rat was not the most sensitive species and the experimental concentration is near the lethal concentration for rats and above lethal concentration for mice.

Human data showed that exposure to ethylene oxide at concentrations approximating the odor threshold ($\geq$ 260 ppm) caused some eye and respiratory tract irritation, hematologic effects, and nervous system effects that were reversible within 21 days. The proposed AEGL-2 value for 30 minutes is below the most conservative estimate of the odor threshold, a concentration that caused some reversible respiratory and neurologic effects.

7. DATA ANALYSIS AND PROPOSED AEGL-3

7.1. Human Data Relevant to AEGL-3

No human lethality data are available for deriving AEGL-3 values. Epidemiologic data provided limited evidence that exposure to ethylene oxide is associated with an increased risk of lymphatic and hematopoietic cancer (IARC, 1994). Quantitative assessments of human cancer data will not be attempted for data sets providing only limited evidence.

7.2. Animal Data Relevant to AEGL-3

Several lethality studies are available for deriving AEGL-3 values. One-hour inhalation studies have been conducted in male and female rats (Nachreiner, 1992) and 4-hour inhalation studies have been conducted in male and female rats (Jacobson et al., 1956; Nachreiner, 1991), male and female mice (Jacobson et al., 1956; NTP, 1987), and male dogs (Jacobson et al., 1956). The LC_{50} values varied for the three species studied: mice were slightly more sensitive than dogs, which were more sensitive than rats. All studies were well-conducted, but the Nachreiner (1991, 1992) studies were more comprehensive regarding the endpoints evaluated. There were some intraspecies variations for the rat and mouse studies. The mice and rats used by Jacobson et al (1956) were of unspecified strain, whereas the Nachreiner study (1991, 1992) used Sprague-Dawley rats and the NTP (1987) study used $B6C3F_1$ mice. Therefore, strain differences could have accounted for some of the variations in LC_{50} values. For the dog data, probit analysis was applied to a data set consisting of either 100% mortality or 100% survival, thereby producing a highly uncertain LC_{50} value that should be interpreted with caution.

Long-term exposure studies have shown that inhaled ethylene oxide is carcinogenic to the mouse (Adkins et al., 1986; NTP, 1987) and rat (Snellings et al., 1984b; Lynch et al., 1984a; Garman et al., 1985). Concentrations associated with significant increases in tumor incidences are 100 ppm for 6 h/day repeatedly for 2 years or 200 ppm for 6 months.

In addition to the lethality and carcinogenicity studies, a study in which mice were exposed to 1200 ppm for 1.5 hours on the day of mating (Rutledge and Generoso, 1989) should be considered when evaluating data pertinent to deriving AEGL-3 values. Rutledge and Generoso (1989) observed a high incidence of late fetal deaths and hydrops, a severe abnormality that may be associated with fetal deaths. Although the exposure time was critical (the first 24 hours after mating) for observing these effects, any value derived for AEGL-3 should be protective of the zygote during this developmental stage.

7.3. Derivation of AEGL-3

Lethality thresholds (LC_{01}) were derived from the mouse, rat, and dog data (Table 19) The LC_{01} for dogs exposed for 4 hours is much lower than the values obtained for the other species; however, the results from the dog data have a higher degree of uncertainty than the results for other species. The LC_{01} values derived from the mouse and rat data range from 264 to 922 ppm for a 4 hours exposure, with the mouse values being the lower than those of the rat.

The rat study by Jacobson et al. (1956) was selected to derive the AEGL-3 values. An uncertainty factor of 3 for intraspecies sensitivity was applied to the LC_{01} value (628 ppm). Glutathione-S-transferase polymorphism (Fennell, 1996) suggest variations in the detoxification of ethylene oxide; because ethylene

oxide is a respiratory irritant individuals with respiratory and heart disease may respond differently to acute intoxication. An interspecies uncertainty factor of 3 is applied, because the rat was not the most sensitive species tested for lethality. Systemic uptake of ethylene oxide is similar across species (Rhomberg et al., 1990), but mice and rats differ in various kinetic parameters (tissue uptake, metabolism kinetics, adduct formation, elimination rates, etc.) (Walker et al., 1992a,b; Brown, 1996; Fennell, 1996). Metabolism is saturated at a lower concentration in mice than in rats (Fennell, 1996). The LC_{50} values show about a twofold to threefold difference in the mortality response of mice and rats. The total uncertainty factor used to derive AEGL values is 10.

Table 19. Estimates of the Threshold for Lethality (LC_{01}) to Ethylene Oxide				
Species	**Exposure duration (h)**	**LC_{50} (ppm)**	**LC_{01} (ppm)**	**Reference**
Dog	4	960	120	Jacobson et al., 1956
Mouse	4	623[a]	264	NTP, 1987
	4	835	406	Jacobson et al., 1956
Rat	4	1460	628	Jacobson et al., 1956
	4	1741[a]	922	Nachreiner, 1991
Rat	1	5029[a]	2494	Nachreiner, 1992

[a]Combined data sets for males and females

Applying a total uncertainty factor of 10 to the 4-hour LC_{01} (628 ppm) derived from the rat data (Jacobson et al., 1956) results in an AEGL-3 value of 63 ppm. ten Berge equation ($C^n \times t = k$, where n = 1.2) was used to extrapolate to other time points. The value of n was derived empirically from the 1 and 4-hour LC_{50} values for rats. The AEGL-3 values for 30 minutes, 1 h, and 8 hours are 360, 200, and 35 ppm. These values are summarized below:

AEGL-3 VALUES			
30 minutes	1 hour	4 hours	8 hours
360 ppm	200 ppm	63 ppm	35 ppm
Reference:	Jacobson et al., 1956		
Comments:	well-conducted study using adequate numbers of animals at each exposure level; probit analysis was used to extrapolate lethality data for male rats to LC_{01} ; the rat is twofold to threefold less sensitive than the mouse		
.			
Uncertainty factors:	3 for intraspecies variability based on potential polymorphism in the glutathione detoxification pathway for ethylene oxide, the need to protect individuals with respiratory and heart diseases, and the steepness of the concentration-response relationship 3 for interspecies sensitivity because the rat and mouse differ in mortality response by twofold to threefold; systemic uptake, distribution, and modes of action are likely to be similar, but metabolism in the mouse saturates at a lower concentration than the in rat.		

Ethylene oxide has carcinogenic activity in laboratory animals. Dose-response data was used to estimate the excess lifetime risk associated with a single exposure to ethylene oxide. Calculations for AEGL-3 estimates are presented in Appendix A of this document. EPA's linearized multistage model (GLOBAL86) (Howe et al., 1986) was used to derive a slope factor for ethylene oxide from incidence data on alveolar/bronchiolar adenomas/carcinomas in female mice. This site showed the highest incidence and was, therefore, considered to be the most sensitive target. AEGL-3 values based on carcinogenicity data are 2688, 1344, 336, and 168 ppm for 30-minutes, 1-hour, 4-hour, and 8-hour exposures, respectively. These values are higher than those derived from lethality data and are not recommended for AEGL-3.

The proposed AEGL-3 value for a 30-minutes exposure is higher than the odor threshold of 260 ppm as reported by Hellman and Small (1974). Deschamps et al. (1992) reported toxic effects, but no lethality, for 4-hour exposures at the odor detection level. Laurent (1988) also reported toxic effects, but no lethality, for 30-minutes exposures at the odor detection level. The odor detection level can range from 260 to 1540 ppm. Salinas reported very serious neurological effects for a 2- to 3-minutes exposure to 500 ppm (calculated concentration).

8. SUMMARY OF PROPOSED AEGLs

8.1. Proposed AEGLs

The proposed AEGL values are presented in Table 20. No lethality data for humans were available for deriving the AEGL-3 values; the limitations in the epidemiologic studies precluded an unequivocal conclusion regarding carcinogenicity based on human data. Data from long-term animal studies were used to estimate AEGL-3 values based on carcinogenicity. The calculations showed that the AEGL-3 values based on carcinogenicity; the excess lifetime cancer risk is less than the risk of death caused by acute toxicity. Therefore, the AEGL-3 values were based on an approximation of the lethality threshold ($LC_{\theta 1}$) for rats.

The effects of ethylene oxide have been shown to be reversible except for lethality, cancer, and some of the developmental effects. Data for deriving AEGL-2 values were based on neurotoxicity and irritation to the eyes and respiratory tract. The AEGL-2 values should, however, be protective of developmental toxicity.

AEGL-1 values are not proposed because the odor threshold and sensory irritation occur at concentrations above those causing systemic effects and any proposed values would not meet the definition of AEGL-1. In addition, the proposed AEGL-2 values are below the odor threshold.

Table 20. Proposed AEGL Values For Ethylene Oxide					
Classification	**30 minutes**	**1 hour**	**4 hours**	**8 hours**	**Endpoint (Reference)**
AEGL-3	360 ppm (643 mg/m^3)	200 ppm (357 mg/m^3)	63 ppm (113 mg/m^3)	35 ppm (63 mg/m^3)	Lethality Jacobson et al., 1956
AEGL-2	190 ppm (339 mg/m^3)	110 ppm (196 mg/m^3)	33 ppm (59 mg/m^3)	19 ppm (34 mg/m^3)	Nervous system effects, eye and respiratory irritation; dominant lethality Embree et al., 1877
AEGL-1	No values derived				

8.2. Comparison of AEGLs with Other Standards and Criteria

Table 21 summarizes standards and guidelines established by various agencies and organizations. The ACGIH-TLV, NIOSH-REL, NIOSH-STEL, and OSHA-PEL are based on cancer risk associated with lifetime occupational exposures and should not be compared with the acute values derived for emergency standards. The NIOSH-IDLH is based on an LC_{50} value of 800 ppm for a 4-hour exposure. This value was adopted for a 30-minutes exposure. The ERPG-3 value is higher than the AEGL-3 value for a 1-hour exposure, and the ERPG-2 value is lower than the AEGL-2 value for 1 h. The 1-hour NAC-EEGL of 20 ppm is less than the proposed AEGL-1 of 66 ppm for a 1-hour exposure.

Table 21. Standards and Guidelines for Ethylene Oxide	
Agency/Organization	**Exposure concentration**
ACGIH – TLV-TWA (ACGIH, 1995-96)	1 ppm (A2)
OSHA – PEL (OSHA, 1993)	1 ppm, TWA 5 ppm (15-minutes "excursion")
NIOSH - IDLH (U.S. DHHS, 1994a,b)	800 ppm
NIOSH – REL (U.S. DHHS, 1994a,b)	<0.1 ppm, TWA
NIOSH – STEL (U.S. DHHS, 1994a,b)	5 ppm (10 minutes/day, ceiling)
AIHA – ERPG 1 (1-hr) (AIHA, 1994)	Not applicable
AIHA – ERPG 2 (1-hr) (AIHA, 1994)	50 ppm
AIHA – ERPG 3 (1-hr) (AIHA, 1994)	500 ppm
NRC – EEGL (1-hr) (NRC, 1986b)	20 ppm
NRC – EEGL (24-hr) (NRC, 1986b)	1 ppm

EEGL = emergency exposure guidance level; ERPG = emergency response planning guidelines; IDLH = immediately dangerous to life or health; PEL = permissible exposure limit; REL = recommended exposure limit; STEL = short-term exposure limit; TLV = threshold limit value; TWA = time-weighted average.

8.3. Confidence in AEGLs

There are a number of uncertainties associated with deriving AEGL values for ethylene oxide. No human data were available for deriving AEGL-3 values. The lack of definitive exposure data precluded using human data to derive AEGL-1 and AEGL-2 values. Exposure was based on the odor detection threshold, which ranged from 260 to 1540 ppm.

Animal data were used to derive all AEGL values, and there are inherent uncertainties when animal data are extrapolated to human exposure scenarios. However, the mechanism of action of ethylene oxide, which probably involve DNA or protein alkylation, is likely to be similar in animals and humans. There appear to be no species differences between ethylene oxide concentrations in air and hemoglobin adduct formation suggesting that uptake is similar across species. Metabolism appears to be qualitatively similar, but quantitative difference in that the two major pathways (hydrolysis and glutathione conjugation) could result in species differences in tissue concentrations and disposition.

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APPENDIX

CALCULATIONS OF AEGL VALUES

CALCULATION OF AEGL-2 VALUES

Key Study: Embree et al., 1977

Toxicity Endpoint: Nervous system effects, eye and respiratory irritation; dominant lethality at 1000 ppm for 4 hours

Time scaling: ten Berge's equation: $C^n \times t = k$, where n = 1.2 derived from rat data

Uncertainty factors: 3 for intraspecies variability

10 for interspecies sensitivity

Calculations:

4-h exposure C = 1000 ppm/30 (uncertainty factor) = 33.3333 ppm
$C^n \times t = k$; n = 1.2 C = 33.3333 ppm, t = 4 h, k = 268.8528 ppm•hours
$C = (k/t)^{1/2}$ = (268.8528 ppm•hours/4 hours)$^{1/2}$ = 33 ppm

0.5-h AEGL-2 $C = (k/t)^{1/2}$ = (268.8528 ppm•hours/0.5 hours)$^{1/2}$ = 188 ppm (rounded to 190 ppm)

1-h AEGL-2 $C = (k/t)^{1/2}$ = (268.8528 ppm•hours/1 hours)$^{1/2}$ = 105.8 ppm (rounded to 110 ppm)

8-h AEGL-2 $C = (k/t)^{1/2}$ = (268.8528 ppm•hours/8 hours)$^{1/2}$ = 19 ppm

CALCULATION OF AEGL-3 VALUES

Key Study: Jacobson et al., 1956

Toxicity Endpoint: Lethality; the LC_{50} for white male rats was 1460 ppm for a 4-h exposure. The data were extrapolated to a LC_{01} (628 ppm) to approximate the lethality threshold.

Time scaling: ten Berge's equation: $C^n \times t = k$, where n = 1.2 derived from rat data

Uncertainty factors: 3 for intraspecies sensitivity

3 for interspecies sensitivity

Calculations:

4-h AEGL-3 C = 628 ppm/10 (uncertainty factor) = 62.8 ppm
$C^n \times t = k$; c = 62.8 ppm, n= 1.2, t = 4 h, k = 574.9255 ppm•hours
$C = (k/t)^{1/2}$ = (574.9255 ppm•hours/4 hours)$^{1/2}$ = 62.8 ppm (rounded to 63 ppm)

0.5-h AEGL-3 $C = (k/t)^{1/2}$ = (574.9255 ppm•hours/0.5 hours)$^{1/2}$ = 355 ppm (rounded to 360 ppm)

1-h AEGL-3 $C = (k/t)^{1/2}$ = (574.9255 ppm•hours/1 hours)$^{1/2}$ = 199 ppm (rounded to 200 ppm)

8-h AEGL-3 $C = (k/t)^{1/2}$ = (574.9255 ppm•hours/8 hours)$^{1/2}$ = 35 ppm

PRELIMINARY CANCER ASSESSMENT OF ETHYLENE OXIDE

In 1985 EPA reported a unit risk or q_1* for inhalation exposure to ethylene of 1×10^{-4} μg/m^3 based on the combined incidences of leukemias and brain gliomas in Fischer 344 rats as reported by Snellings et al., (1981) (U.S. EPA, 1985). A study by NTP (1987) was completed after EPA conducted its risk assessment of ethylene oxide. This study was summarized in Table 14 of the text and the data for lung tumors in female mice will be used to calculate another slope factor (q_1*). The calculations of a slope factor and the AEGL values for carcinogenicity are presented below.

Data summary (NTP, 1987): Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 50, or 100 ppm for 6 h/day, 5 days/week for 102 weeks. The incidence of lung adenomas/carcinomas in females was 2/49, 5.48, 22/49 for 0, 50, or 100 ppm, respectively.

Derivation of the slope factor for ethylene oxide:

Convert exposure concentrations for 6 h/day and 5 days/week to continuous exposure:

50 ppm × 6/24 × 5/7 = 8.93 ppm × 1.8 = **16.1 mg/m^3**

100 ppm × 6 h/24 hours × 5 days/7days = 17.86 ppm × 1.8 = **32.2 mg/m^3**

The slope factor (q_1*) derived from the linearized multistage model is 8.82×10^{-3} mg/m^3)^1 or **8.82×10^{-3} μg/m^6)$^{-1}$**

The calculations for AEGL values following the method presented by NRC (1986a) are presented below.

To calculate a "virtually safe dose" (VSD of d) at a cancer risk of 10^6:

d = $10^{-6}/8.82 \times 10^{-6}$ μg/m^3 = **1.13×10^{-1} μg/m^3**

To calculate the total cumulative dose for a total lifetime exposure of 70 years, which is equivalent to 25,600 days:

total d = d × 25,600 = 1.13×10^{-1} μg/m^3 × 25,600 = **2.90×10^3 μg/m^3**

To account for the uncertainties about the stage at which a carcinogen may act, and because this method was derived for persons of young military age, a factor for the additional risk is applied to the dose.

2.90×10^3 μg/m^3/2.8 = **1.04×10^3 μg/m^3**

To calculate the dose for a lifetime risk of 10^{-4}:

1.04×10^3 μg/m^3 × $10^{-4}/10^{-6}$ = 1.04×10^5 μg/m^3 = 1.04×10^2 mg/m^3

1.04×10^5 μg/m^3/1000 μg/mg = 1.04×10^2 mg/m^3 or 104 mg/m^3

104 mg/m^3 = **57.6 ppm**

Therefore, a single exposure to ethylene oxide at 57.6 ppm for 24 hours would present a cancer risk of 10^{-4}. The total exposure to ethylene oxide for a 24-h period is 1382 ppm•hours. Haber's law can be used to scale the 24-h exposure to the time frames relevant to AEGLs.

$c \times t = k$, where c = concentration, t =time, and k is a constant.
k = 57.6 ppm × 24 hours = 1382 ppm•hours

To calculate the exposure concentration for other time frames, rearrange the equation as follows to determine the concentration of ethylene oxide.

c = k/t, where, t = 0.5, 1, 4, or 8 h

24-h	=	57.6 ppm (104 mg/m^3)
8-h	=	**173 ppm (311 mg/m^3)**
4-h	=	**346 ppm (623 mg/m^3)**
1-h	=	**1382 ppm (2488 mg/m^3)**
0.5 h	=	**2764 ppm (4975 mg/m^3)**

These values based on carcinogenicity exceed the values based on lethality data and are not proposed for AEGL-3.