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Office of Health and
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April 1984
External Review Draft

Research and Development

EPA

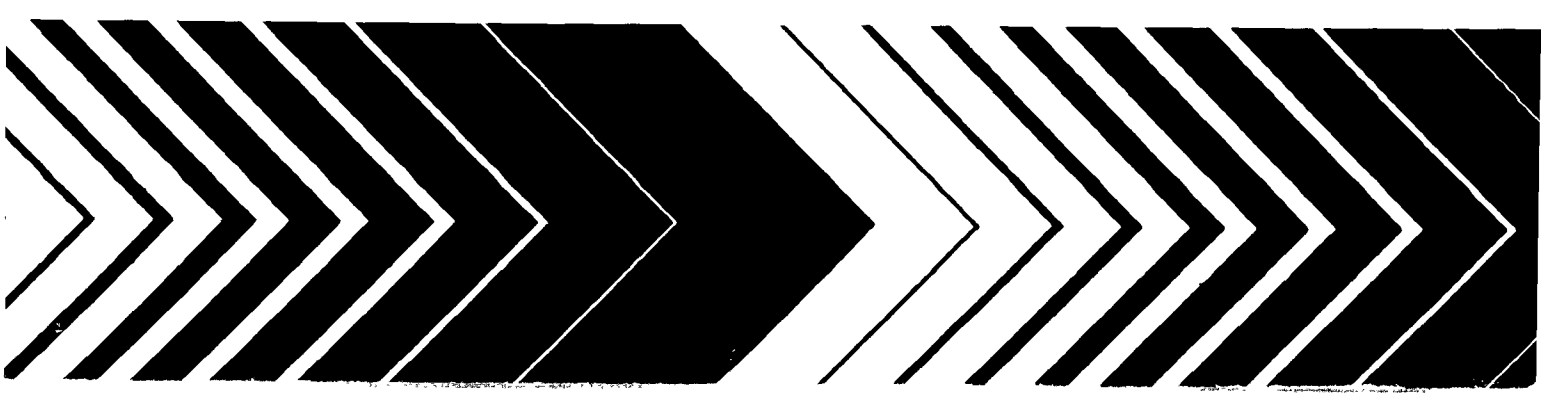
Health Assessment Document for Ethylene Oxide

Review Draft

(Do Not
Cite or Quote)

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.



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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
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DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency Policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. The health assessment document was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of ethylene oxide as a hazardous air pollutant. However, the scope of this document has since been expanded to address multimedia aspects.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses are placed in perspective with observed environmental levels.

The EPA's Office of Health and Environmental Assessment (OHEA) is responsible for the preparation of the health assessment document. The OHEA Environmental Criteria and Assessment Office (ECAO-RTP) had overall responsibility for coordination and direction of the document (Dr. Robert M. Bruce, Project Manager). The chapters addressing physical and chemical properties, sampling and analysis, air quality and biological effects in animals and man were originally written and revised by Syracuse Research Corporation with the exception of chapters or sections addressing mutagenicity, teratogenicity and reproductive effects and carcinogenicity. The air quality chapters (5, 6, 7) were reviewed by Radian Corporation under contract to the Office of Air Quality Planning and Standards and recommendations proposed.

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1. SUMMARY AND CONCLUSIONS

The largest single use of ethylene oxide is as an intermediate in the synthesis of ethylene glycol. However, small amounts of this epoxide are used as a sterilant or pesticide in commodities, pharmaceuticals, medical devices, tobacco, and other items. Although this use is only a small fraction of the total epoxide consumption, it represents a considerable potential for human exposure.

The pharmacokinetics of ethylene oxide have not been studied extensively. There were no studies found in the literature searched concerning the absorption of this chemical. However, the toxicity data suggests that absorption occurs via the respiratory and gastrointestinal tracts. Two studies (Ehrenberg et al., 1974; Appelgren et al., 1977) have shown that ethylene oxide is widely distributed in various tissues (liver, kidney, lung, testes, brain, spleen, and intestinal mucosa) following inhalation exposure and intravenous administration.

Acute exposure to ethylene oxide in humans has resulted in symptoms of headache, vomiting, dyspnea, and diarrhea. Similar symptoms have been reported by Blackwood and Erskine (1938), Cobis (1977), and Anonymous (1947). Thiess (1963) reported that high concentrations of ethylene oxide for brief periods produced bronchitis, pulmonary edema, and emphysema. Salinas et al. (1981) reported neurological symptoms (convulsive movements) in a woman who was exposed for a brief period to 500 ppm ethylene oxide.

Anaphylactic reactions have been observed in patients using ethylene oxide sterilized plastic tubing for hemodialysis (Poothullil et al., 1975) or

cardiac catheterization (Pessayre and Trevoux, 1978). Hirose et al. (1953) and Clarke et al. (1966) reported hemolysis in patients following the use of ethylene oxide sterilized plastic tubings.

The acute toxic effects of ethylene oxide in laboratory animals have been extensively reviewed. Exposure to concentrated ethylene oxide produces systemic poisoning, with symptoms of salivation, nausea, vomiting, diarrhea, convulsion, and death (Hine and Rowe, 1973). Symptoms of respiratory irritation, incoordination, and cardiac arrhythmia have also been reported (Sexton and Henson, 1949).

The subacute and chronic effects of ethylene oxide in man are not well documented. Both Jensen (1977) and Gross et al. (1979) have reported neurotoxicity in humans following inhalation exposure to ethylene oxide.

The ability of ethylene oxide to cause teratogenic or adverse reproductive effects has been examined in a number of species (mouse, rat, rabbit, monkey, and human) by two routes of administration (inhalation and intravenous). Hackett et al. (1982) reported that rats, but not rabbits, exposed to 150 ppm ethylene oxide administered by inhalation displayed signs of maternal toxicity and toxicity to the developing conceptus. Laborde and Kimmel (1980) and Kimmel (1982) reported that 150 mg/kg ethylene oxide administered intravenously to mice and rabbits caused maternal toxicity and developmental toxicity. Laborde et al. (1982) reported that ethylene chlorohydrin (ECH), a reaction product of ethylene oxide, produced adverse effects on maternal and fetal well-being in mice but not in rabbits at 120 mg/kg administered intravenously and produced adverse developmental effects without significant toxicity when administered ECH at 60 mg/kg intravenously. In a one-generational study, Snellings et al. (1982) reported that 100 ppm

ethylene oxide administered by inhalation caused severe adverse effects including a higher incidence of infertility, longer gestational periods, a decrease in the number of pups born, and a decrease in the number of implantation sites. Hollingsworth et al. (1956) observed testicular degeneration in hamsters and rats inhaling 204 to 357 ppm ethylene oxide. In a more recent study, Lynch et al. (1983) reported adverse effects on sperm concentration motility but not morphology in Cynomologuous monkeys exposed to 50 and 100 ppm ethylene oxide by inhalation. In humans, Hemminki et al. (1982) conducted an epidemiologic study of nursing personnel exposed to ethylene oxide and found an association between ethylene oxide exposure and spontaneous abortion.

In conclusion, the available information indicates that ethylene oxide produces developmental toxicity in laboratory animals when conducted at or near maternally toxic doses. Ethylene oxide produces adverse reproductive effects and testicular toxicity at levels lower than those which produce general toxicity. Finally, ethylene oxide is reported to be associated with spontaneous abortions in nursing personnel exposed to ethylene oxide in hospital occupational settings.

Ethylene oxide has been shown to induce gene mutations in bacteria, fungi, higher plants, Drosophila, and cultured mammalian cells in tests conducted without the use of exogenous hepatic metabolic activation systems. It is therefore a direct-acting mutagen. Strong positive responses were found in bacteria (10-fold to 18-fold increase over negative controls), higher plants (33-fold increase), and mammalian cells in culture (2-fold to 20-fold increases). Less strong, but clearly positive, reponses were found in Drosophila (2-fold to 3-fold increases). Based on these positive findings in

different test systems in a wide range of organisms, ethylene oxide is judged to be capable of causing gene mutations.

Ethylene oxide has also been shown to be clastogenic, in that it causes dominant lethal effects in mice and rats; chromosomal aberrations in higher plants, Drosophila, mice, and rats; and micronuclei in mice and rats. Based on these positive findings in different test systems, ethylene oxide is judged to be capable of causing chromosomal aberrations. It has also been shown to induce sister chromatid exhange (SCE) in rabbits, rats, and humans.

Tissue distribution studies have shown that ethylene oxide reaches the gonads. This result is consistent with evidence that ethylene oxide causes unscheduled DNA synthesis (UDS) in germ cells of male mice and heritable mutations in insects and rodents (i.e., sex-linked recessive lethals and heritable translocations in Drosophila, dominant lethals in rats and mice, and heritable translocations in mice). Ethylene oxide can therefore be regarded as mutagenic both in somatic cells and in germ cells.

Based on the available data, there is overwhelming evidence that ethylene oxide is a direct-acting mutagen that has the potential to cause mutations in the cells of exposed human tissue. The observations that ethylene oxide reaches and reacts with mammalian gonadal DNA, and causes heritable mutations in intact mammals, indicates that it may be capable of causing heritable mutations in man provided that the pharmacokinetics of ethylene oxide in humans also results in its distribution to the DNA of germ cells.

Three epidemiologic studies showed a significant association between ethylene oxide exposure and the occurrence of cancer. Two of the studies found an excess risk of leukemia associated with ethylene oxide exposure. While these studies have shortcomings and are not definitive, they do,

nevertheless, constitute limited, bordering on inadequate, evidence for human carcinogenicity under the International Agency for Research on Cancer (IARC) classification scheme for the evaluation of carcinogenic risk to humans.

Positive results for the carcinogencity of ethylene oxide have been obtained by subcutaneous injection in mice and by intragastric administration in rats. In addition, two long-term chronic inhalation studies in rats have shown statistically significant responses for leukemia, brain tumors and peritoneal mesothelioma. The animal evidence is sufficient under the IARC classification system for experimental animals. Considering both the animal and human evidence for carcinogenicity, especially leukemias in both humans and rats, the Carcinogen Assessment Group (CAG) classifies ethylene oxide as being probably carcinogenic to humans and, therefore, belonging in the IARC Group 2A. Because of the very few human cancers, however, this classification borders on a Group 2B classification. Assuming that ethylene oxide is carcinogenic in humans, upper-limit potency estimates have been calculated using both the animal and human data base.

Estimates of carcinogenic relative potencies of ethylene oxide in rats and humans suggest that humans may be more sensitive than animals to the carcinogenic effects of ethylene oxide. Supporting this suggestion are data indicating that humans have greater sensitivity than rats to chromosome abnormalities induced by ethylene oxide exposure. The unit risk estimate of lifetime cancer risk resulting from continuous exposure to air that contains an ethylene oxide concentration of 1 $\mu g/m^3$ for humans is 3.6×10^{-4}, while the 95% upper-limit estimate for animals based on rat studies is 1.0×10^{-4}. The

potency index for ethylene oxide, which is based on both the unit risk value and molecular weight, is in the lower part of the the third quartile of 54 suspect carcinogens evaluated by the CAG.

2. INTRODUCTION

EPA's Office of Research and Development has prepared this health assessment to serve as a "source document" for Agency use. This health assessment was originally developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulations of ethylene oxide under Section 112 of the Clean Air Act. However, based on the expressed interest of other agency offices, the scope of this document was expanded to address ethylene oxide in relation to sectors of the environment outside of air. It is fully expected that this document will serve the information needs of many government agencies and private groups that may be involved in decision-making activities related to ethylene oxide.

In the development of the assessment document, existing scientific literature has been surveyed in detail. Key studies have been evaluated and summary and conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified.

The document considers all sources of ethylene oxide in the environment, the likelihood for its exposure to humans, and the possible effect on man and lower organisms from absorption. The information found in the document is integrated into a format designed as the basis for performing risk assessments. When appropriate, the authors of the document have attempted to identify gaps in current knowledge that limit risk evaluation capabilities.

3. PHYSICAL AND CHEMICAL PROPERTIES

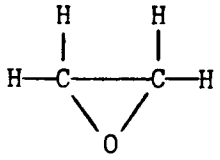
3.1. SYNONYMS AND CAS NUMBER

Synonyms: 1,2-epoxyethane
ethylene oxide
oxirane

CAS Number: 75-21-8

3.2. STRUCTURAL AND MOLECULAR FORMULAS

Structural formula:



Molecular formula:

C_2H_4O

3.3. TORTIONAL ANGLES AND BOND DISTANCES (Hirose, 1974)[a]

	Tortional Angles
<HCH	116.9
<COC	61.62
θ[b]	21.6
ϕ[c]	7.97

	Bond Distances, Å
r_m (C-C)	0.1462
r_m (C-H)	0.1086
r_m (C-O)	0.1428

[a] From microwave spectra

[b] Angle of C-C bond to H_2C plane

[c] (<OCC)/(2-θ)

3.4. PHYSICAL PROPERTIES OF PURE ETHYLENE OXIDE

3.4.1. Description. Ethylene oxide is a colorless, flammable gas which condenses at low temperatures to a colorless, clear, mobile liquid (Cawse et al., 1980; Hawley, 1981).

3.4.2. Molecular Weight.

44.05 (Weast, 1980)

3.4.3. Melting Point.

-111°C (Weast, 1980)

3.4.4. Boiling Point.

10.4°C (at 101.3 kPa = 1 atm) (Cawse et al., 1980)

3.4.5. Boiling Point Change with Pressure Change (Δbp/pressure at 100 kPa).

0.25 K/kPa (Cawse et al., 1980)

0.033 K/torr (Cawse et al., 1980)

3.4.6. Density.

d_{10}^{10}: 0.8824 (Weast, 1980)

3.4.7. Coefficient of Cubical Expansion (at 20°C, per °C).

0.00161 (Cawse et al., 1980)

3.4.8. Refractive Index (at 7°C).

1.3597 (Weast, 1980)

3.4.9. Vapor Pressure (Cawse et al., 1980).

Temperature	Vapor Pressure	
°C	kPa	Torr
-40	8.35	62.6
-30	15.05	112.9
-20	25.73	193.0
-10	42.00	315.0
0	65.82	493.7
10	99.54	746.6
20	145.8	1093
30	207.7	1558
40	288.4	2163
50	391.7	2938
60	521.2	3909
70	681.0	5108
80	875.4	6566
90	1108.7	8315
100	1385.4	10390

3.4.10. Aqueous Solubility[a] (Cawse et al., 1980).

Pressure		Temperature		
kPa	torr	5°C	10°C	20°C
20	150	45	33	20
27	202.5	60	46	29
40	300.0	105	76	49
53	397.5	162	120	74
67	502.5	240	178	101
80	600.0	NT	294	134
93	697.5	NT	NT	170
101	757.5	NT	NT	195

[a]Solubility in mℓ vapor/mℓ water, vapor volume at 0°C and 1 atm

NT = Not tested

3.4.11. Freezing Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide		Freezing Point
Weight %	Mole %	°C
0	0	0.0
2.5	1.0	-0.9
5	2.1	-1.6 (eutectic)
10	4.4	5.6
15	6.7	8.9
20	9.3	10.4
30	14.9	11.1 (max)
40	21.4	10.4
50	29.0	9.3
60	38.0	7.8
70	48.8	6.0
80	62.1	3.7
90	78.6	0.0
100	100	-112.5

3.4.12. Boiling Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide		Boiling Point
Weight %	Mole %	°C
0	0	100
2.5	1.0	70
5	2.1	58
10	4.4	42.5
15	6.7	38
20	9.3	32
30	14.9	27
40	21.4	21
50	29.0	19
60	38.0	16
70	48.8	15
80	62.1	13
90	78.6	12
100	100	10.4

3.4.13. Flash Point (tag open cup).

<-18°C (Cawse et al., 1980)

3.4.14. Flash Point of Aqueous Solutions (Cawse et al., 1980).

Ethylene Oxide Weight %	Flash Point Closed Cup (°C)
1	31
3	3
5	-2

3.4.15. Explosive Limits in Air, Volume % (Cawse et al., 1980).

Upper Limit 100%

Lower Limit 3%

3.4.16. Heat of Combustion at 25°C (Cawse et al., 1980).

5.17 kJ/mol

1.24 kCal/mol

3.4.17. Log Octanol/Water Partition Coefficient.

-0.30 (Hansch and Leo, 1979)

3.4.18. Ultraviolet Spectroscopic Data (Weast, 1980).

λ = 169 nm (gas)

log ε = 3.58

λ_2 = 171 nm (gas)

log ε = 3.57

3.5. PHYSICAL PROPERTIES AND DESCRIPTION OF COMMERCIAL ETHYLENE OXIDE

The physical properties and description of commercial ethylene oxide are described in Table 3-1.

TABLE 3-1

Manufacturers' Specifications for Ethylene Oxide[a,b]

	BASF	Celanese	Dow	Jefferson	Shell	Wyandotte
Purity, wt % min	99.95	99.95	NA	NA	NA	NA
Water, wt % max	0.005	0.02	0.03	0.03	0.03	NA
Aldehydes, as acetaldehyde, wt % max	0.005	0.01	0.005	0.025	0.010	0.003
Acidity, as acetic acid, wt % max	0.002	0.002	0.002	0.005	0.0020	0.002
CO_2, wt % max	0.005	NA	0.002	NA	NA	0.005
Total Cl as Cl^-, wt % max	0.005	NA	0.005	nil	NA	0.0005
Nonvolatile residue, g/100 mℓ, max	0.010	0.01	0.01[c]	0.01	0.010	0.01
Color, APHA, max	10	10	5	NA	10	10
Residual Odor	NA	none	NA	none	none	mild
Appearance	NA	clear	NA	clear	clear	NA
Acetylene, max	NA	NA	0.0005	nil	NA	NA

[a]Source: U.S. EPA, 1980

[b]This information was obtained from the respective manufacturer's product data sheets, available from each manufacturer on request.

[c]Presently, 0.005 g/100 mℓ in Dow ethylene oxide (Kurginski, Dow Chemical Co.)

NA = Not available; wt = weight; max = maximum; min = minimum

Commercial grade ethylene oxide has a purity of >99.9%. Specific impurities include trace quantities of water, aldehydes (specified as acetaldehyde), acid (specified as acetic acid), chloride, and an unspecified residue. Since commercial grade ethylene oxide is virtually pure, its physical properties are the same as those previously described.

3.6. CHEMICAL PROPERTIES

The majority of information contained in this section was taken from Cawse et al. (1980).

Ethylene oxide is a highly reactive epoxide. Industrially, it is used principally as an intermediate for a wide variety of compounds. Most of its reactions involve opening the epoxide ring. An exception is the formation of oxonium salts with strong anhydrous mineral acids.

3.6.1. Reduction. Catalytic hydrogenation or chemical reduction of ethylene oxide results in the formation of ethanol.

3.6.2. Clathrate Formation. Ethylene oxide and water form a stable clathrate containing 6.38 to 6.80 molecules of ethylene oxide to 46 units of water in the unit cell. The maximum observed melting point for these compounds is 11.1°C (Section 3.4.11).

3.6.3. Polymerization. Low molecular weight polymers can be formed by the reaction of ethylene oxide and water or alcohols. The average molecular weight of these polymers (polyethylene glycols) ranges from 200 to 14,000, depending upon the reaction conditions. High polymers, with molecular weights

ranging from 90,000 to 4 x 10^6, are formed by coordinate anionic polymerization. This reaction involves the coordination of a metallic compound with ethylene oxide to initiate the reaction. Numerous organometallic and alkaline earth compounds and mixtures are used as catalysts. This process is important in the formation of non-volatile residues during ethylene oxide storage (Section 3.5). The primary catalyst for this process is rust, and no inhibitor has been found.

3.6.4. Other Reactions. Table 3-2 lists a number of other reactions ethylene oxide undergoes that are representative of its chemistry.

3.6.5. Hydrolysis and Related Reactions. Epoxides degrade in water by hydrolysis and related ionic reactions and, possibly, by radical oxidations. The hydrolysis chemistry involves cleaving a carbon-oxygen bond of the cyclic ether to form ethylene glycol. Bronsted et al. (1929) noted the pathways for ethylene oxide hydrolysis in aqueous hydrochloric acid, describing hydrolysis as a combination of a noncatalytic reaction (herein referred to as the spontaneous hydrolysis) and an acid-catalyzed hydrolysis. Reaction with chloride paralleled hydrolysis; chloride and epoxide reacted without catalysis and with acid catalysis.

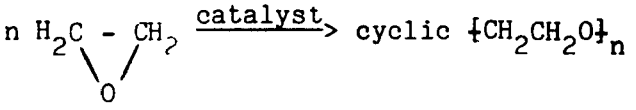
Long and Pritchard (1956) demonstrated that epoxide hydrolysis was also base catalyzed. For any epoxide, the degradation pathways are as follows for the spontaneous (I), acid-catalyzed (II), and alkali-catalyzed hydrolyses (III):

(I) $C_2H_4O + H_2O \xrightarrow{k_N} HOCH_2CH_2OH$

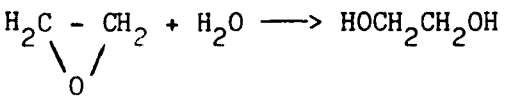
TABLE 3-2

Typical Reactions of Ethylene Oxide

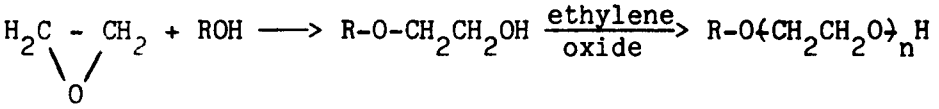
1. Crown Ethers



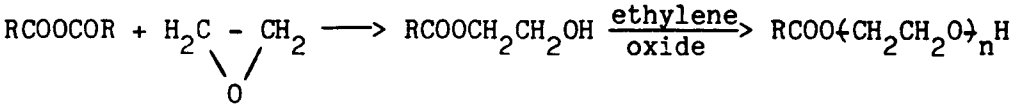
2. Hydrolysis



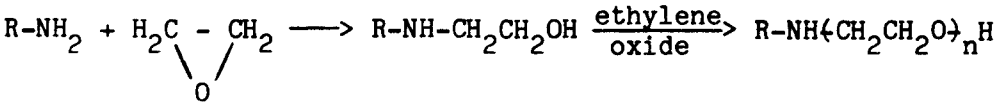
3. Reaction with Alcohols



4. Reaction with Organic Acids and Acid Anhydrides



5. Reaction with Ammonia and Primary and Secondary Amines



6. With Hydrogen Sulfide and Mercaptans (e.g., glutathione, cystine)

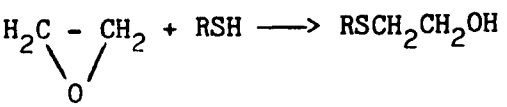
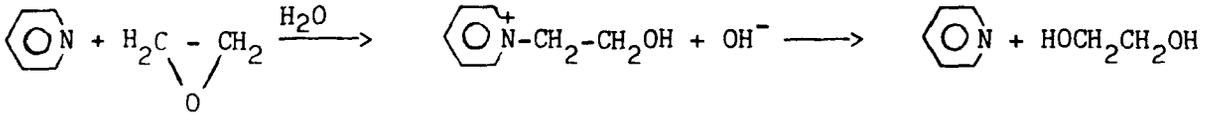
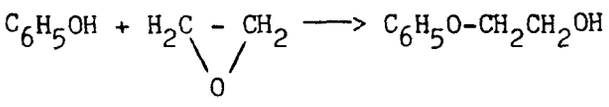


TABLE 3-2 (cont.)

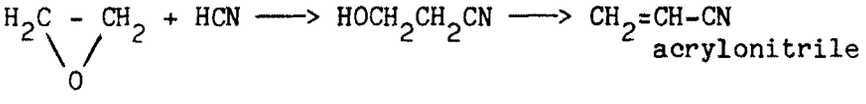
7. Reaction with Pyridine (and possibly other nitrogen heterocycles)



8. With Phenols



9. With Hydrogen Cyanide



(II) $C_2H_4O + H_2O^+ \xrightarrow[H_3O^+]{k_A} HOCH_2CH_2OH$

(III) $C_2H_4O + H_2O \xrightarrow[OH^-]{k_B} HOCH_2CH_2OH$

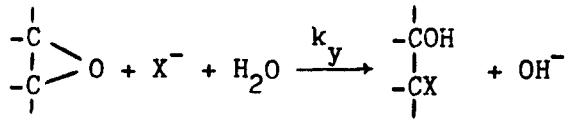
Table 3-3 summarizes hydrolysis data for ethylene oxide. The temperature coefficients for the rate constants are the following:

$\log k_A = 10.753 + \log T - 0.0255/R - 79.5/RT$ (Long et al., 1957)

$\log k_N = 7.726 - 79.5/RT$ (Lichtenstein and Twigg, 1948)

$\log k_B = 9.312 - 75.3/RT$ (Lichtenstein and Twigg, 1948)

Epoxides can also react with nucleophiles (anions or Lewis bases) by pathways which parallel hydrolysis (reaction with water or hydroxide). The chemistry, although similar to hydrolysis, is more complex. The epoxide ring can be cleaved by spontaneous reaction or by acid-catalyzed reaction:



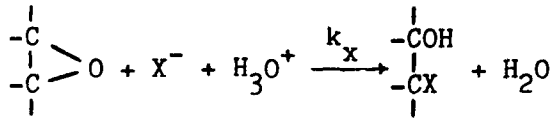


Table 3-4 summarizes specific rate constants for reactions of ethylene oxide with various anions. The consensus agrees that the spontaneous reaction is S_N2, but disagreement exists whether acid catalyzed epoxide ring opening is A1-like or A2-like (Long et al., 1957; Lamaty et al., 1975; Pritchard and

TABLE 3-3

Hydrolysis Kinetics of Ethylene Oxide

Temperature (k)	Specific Rate Constant		
	$k_A \times 10^3$ ($M^{-1}S^{-1}$)	$k_N \times 10^7$ (S^{-1})	$k_B \times 10^4$ ($M^{-1}S^{-1}$)
293	5.34[a]	3.61[a]	NR
293.2	NR	4.2[b]	0.65[b]
298	9.3[c]	6.75[c]	1.0[d]
298	NR	5.62[f,g]	NR
298	NR	6.17[f,h]	NR
298	NR	6.61[f,i]	NR
298	9	5.56[j]	1.1
298	NR	5.8[k]	NR
NR	10.0[d]	NR	NR
303.2	16.9[e]	NR	NR

[a]Bronsted et al., 1929
[b]Lichtenstein and Twigg, 1948
[c]Eastham and Latremouille, 1952
[d]Pritchard and Long, 1956
[e]Long et al., 1957
[f]Conway et al., 1983
[g]River water pH 7.4
[h]Sterile river water pH 7.4
[i]Sterile distilled water
[j]Long and Pritchard, 1956
[k]Koskikallio and Whalley, 1959
NR = Not reported

TABLE 3-4

Specific Rates of Reaction of Anions and Lewis Bases with Ethylene Oxide

Lewis Base or Anion	Temperature K	$10^6 k_y$ (ℓ/mole - sec)[a]	$10^2 k_x$ (ℓ^2/mole^2 - sec)[a]
Cl^-	293	NR	2.17 (water)[b]
	298	NR	3.67 (water)[b]
	298	0.305[e]	NR
	300	NR	8.23 (50% aqueous ethanol)[c]
Br^-	293	NR	8.67 (water)[b]
	298	NR	14.5 (water)[b]
Pyridine	291	200 (water)[d]	NR

[a] k_y = neutral reaction; k_x = acid catalyzed

[b] Bronsted et al., 1929

[c] Lamaty et al., 1975

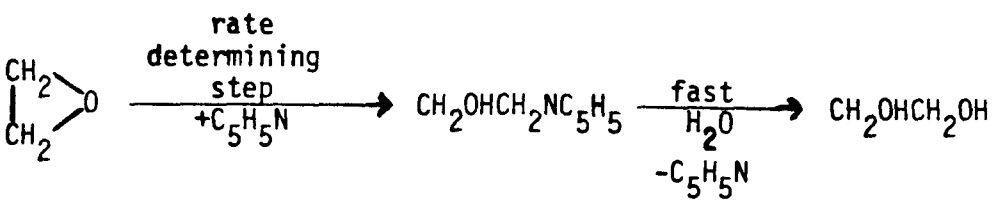
[d] Pritchard and Siddiqui, 1973

[e] Conway et al., 1983

NR = Not reported

Long, 1956; Pritchard and Siddiqui, 1973; Virtanen and Kuokkanen, 1973). A discussion of the mechanism is beyond the scope of this review.

Some products of epoxide reaction with Lewis bases or with anions are not stable. For example, tertiary amines, such as pyridine, are capable of catalyzing epoxide hydrolysis to glycol:



Aqueous chemical degradation in the environment can be estimated from the contributions of hydrolysis (Equation 1) and anion reactions (Equation 2):

$$\frac{-dC_{epox}}{dt} = (k_N + k_A C_{H_3O^+} + k_B C_{OH^-}) C_{epox} \quad (1)$$

$$\frac{-dC_{epox}}{dt} = (k_{yi} C_{Ai} + k_{xi} C_{Ai} C_{H_3O^+}) C_{epox} \quad (2)$$

where C_{Ai}, k_{yi}, and k_{xi} refer to the concentration and specific rate constants for each anion or Lewis base. The overall degradation rate is the sum of all contributions, as given in Equation 3:

$$-\frac{dC_{epox}}{dt} = [k_N + k_A C_{H_3O^+} + k_B C_{OH^-} + \Sigma(k_{yi} + k_{xi} C_{H_3O^+}) C_{Ai}] C_{epox} \quad (3)$$

The relative importance of chemical hydrolysis and reaction with chloride was assessed for ethylene oxide. Degradation half-lives and product distributions (chlorohydrin to glycol ratios) were estimated for freshwater and marine water (NaCl concentration of 3% or 0.513M). The following specific rate constants from Tables 3-3 and 3-4 were utilized:

k_N 0.661×10^{-6} s^{-1}
k_A 9×10^{-3} M^{-1} s^{-1}
k_B 1×10^{-4} M^{-1} s^{-1}
k_{yCl} 0.305×10^{-6} M^{-1} s^{-1}
k_{xCl} 3.67×10^{-2} M^{-2} s^{-2}

Estimates were calculated for pH 5, 7, and 9, which is approximately the pH range of natural waters. Half-lives for chemical degradation and the chlorohydrin/glycol ratios (for sea water reactions) are summarized below:

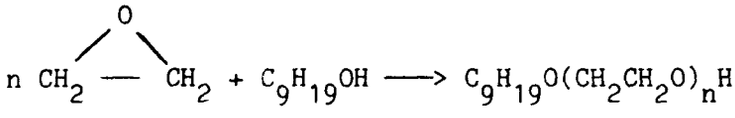
	Calculated Ethylene Oxide Half-Life at 298K (hours)		
pH	5	7	9
Freshwater	256	291	291
Saline Solution			
0.85% (physiological)		273	
1%	240	270	270
3% (marine)	212	236	236

Conway et al. (1983) used buffered (pH=7) sterile solutions of 0, 1, and 3% NaCl to hydrolyze ethylene oxide and reported half-lives of 314, 265, and 224 hours, respectively. The half-lives for river water (pH 7.4), sterile river water (pH 7.4), and sterile distilled water reported by these authors were 341, 310, and 293 hours, respectively. The chlorohydrin/glycol ratio experimentally determined by Conway et al. (1983) was 0.11 and 0.23 for 1 and 3% saline solutions.

From the data presented, some understanding of the fate of ethylene oxide in biological fluids can be determined. The hydrolysis half-life in physiological saline (0.85%) is 273 hours or 11.4 days. This long a half-life would clearly allow for other reactions to take place. As an example, the half-life for the ethylene oxide reaction with pyridine in water is 58 minutes. Other nucleophiles (e.g., RS^-, $PhNH_2$) present in biological systems, are known to be more nucleophilic than pyridine, and may react with ethylene oxide in biological systems much more rapidly than either water or chloride.

Hydrolysis or hydrolysis-type reactions are also the most significant industrial reactions of ethylene oxide. Ethylene glycol is the hydrolysis product; higher glycols (diethylene, triethylene, and polyethylene glycols) and glycol ethers are the result of the reaction of ethylene oxide with glycols and alcohols, respectively.

Glycol esters of carboxylic acids and phenols, and ethers of cellulose, starch, and other polyols are also prepared as described above. For example, reaction of ethylene oxide and nonylphenol yields nonylphenoxypolyethoxyethanol, a non-ionic, surface-active agent (Blackford, 1976a).



$$n\ CH_2\text{—}O\text{—}CH_2 + C_9H_{19}OH \longrightarrow C_9H_{19}O(CH_2CH_2O)_nH$$

Ethylene oxide reacts with amines by pathways similar to reactions with hydroxyl compounds. Reaction of ethylene oxide and ammonia yields the commercially important ethanolamines:

$$NH_3 + nH_2C\text{-}CH_2 \longrightarrow H_2N \left(CH_2CH_2O \right)_n H$$

where n is typically 1 to 4. Choline is prepared by reacting trimethylamine with ethylene oxide (Jukes, 1964):

$$(CH_3)_3N + H_2C\text{-}CH_2 \longrightarrow (CH_3)_3\overset{+}{N}\text{-}CH_2CH_2OH$$

Some ionic reactions of ethylene oxide are listed in Table 3-2.

3.6.6. Free Radical Reactions. The free-radical chemistry of ethylene oxide is of particular importance in determining its fate in the atmosphere. The most important free-radical reaction is the reaction with hydroxyl radical.

Only one reported study of the reaction of the hydroxyl radical with ethylene oxide was found in the available literature. Fritz et al. (1982) reported the results of a study utilizing a laser photolysis/resonance fluorescence (LPRF) unit designed to study the reactions of OH radicals with anthropogenic pollutants. Hydroxyl radical was generated by HNO_3 photolysis and radical concentrations were measured by the system. The authors studied the reaction over three temperatures, 297, 377, and 435K, at 10 torr (Ar). The following relations were reported:

$$k_{(297K)} = (8.0 \pm 1.6) \times 10^{-14} \text{ cm}^3\text{/molec. S.}$$

and

$$k_{(T)} = (1.1 \pm 0.4) \times 10^{-11} \exp(-1460/T) \text{ cm}^3\text{/molec. S.}$$

where the error limits are the $\geq$90% confidence limit (3σ). The mechanism they reported involves hydrogen abstraction, followed by ring opening, reaction with oxygen, NO, and finally decomposition to carbon monoxide and formaldehyde. Ring opening may take place either before O_2 addition or after NO reaction.

4. SAMPLING AND ANALYTICAL METHODS

4.1. SAMPLING

The state-of-the-art in air sampling utilizes solid sorbents. Samples can subsequently desorb by solvent or thermal means. Critical factors in the method are the capacity of the sorbent to retain the epoxide during the collection and the complete desorption of the epoxide.

Brown and Purnell (1979) evaluated Tenax GC sampling tubes for use in ambient air monitoring studies and found them to be inappropriate for ethylene oxide. Although most of the 71 compounds tested were adequately retained, ethylene oxide was not, having the third poorest retention.

Pellizzari et al. (1976) evaluated Tenax GC and other sorbents for sampling atmospheric propylene oxide (very similar to ethylene oxide). Table 4-1 compares the breakthrough volumes for several sorbents. The effect of humidity on the breakthrough volume was tested for Tenax GC. Breakthrough volume increased from 4.0 to 4.5 ℓ/g when humidity was increased from 41 to 92%. Pellizzari et al. (1976) also examined the effect of storage time on the recovery of diepoxybutane (300 ng) loaded onto Tenax GC cartridges. They desorbed it thermally and analyzed it by GC. When analysis was immediate, recovery was 100%. After the loaded cartridge was stored for 1 week, the recovery dropped to 76%. Combined transport (6 days) and storage yielded recoveries of 75 and 64% after 1 and 2 weeks, respectively. Since Brown and Purnell (1979) and Pellizzari et al. (1976) used comparable methods for determining the breakthrough volume, it appears that propylene oxide and

TABLE 4-1

Breakthrough and Safe Sampling Volumes for Propylene Oxide with Several Sorbents

Sorbent	Breakthrough Volume ℓ/g (sorbent)[a]	Safe Sampling Volume (ℓ/g)[b]
PBL Carbon	36	9
PCB Carbon	40	10
SAL9190	40	10
MI808	24	6
Tenax GC (35/60)[c]	4	1
Porapak Q (100/120)	4	1
Chromosorb 101 (60/80)	4	1
Chromosorb 102 (60/80)	8	2
Chromosorb 104 (60/80)	>36	9

[a] Pellizzari et al., 1976

[b] Brown and Purnell, 1979

[c] Mesh size

ethylene oxide behave similarly. Brown and Purnell (1979) have noted that, under the conditions of the test (5 to 600 mℓ/minute flow rate, <100 ppm vapor concentration, <20°C, and <95% relative humidity), the breakthrough volume is not <50% of the retention volume, and a safe sampling volume is 50% of the retention volume. Thus, it appears that if propylene oxide behavior is analogous to ethylene oxide, then ethylene oxide will likely be detected only rarely using solid adsorbants, even if it is present, since the great majority of monitoring studies use air samples larger than the breakthrough volume for ethylene oxide.

The National Institute for Occupational Safety and Health (NIOSH) has published standard procedures for ethylene oxide collection in air (NIOSH, 1977). The procedure calls for the sampling of 5 ℓ of air through glass tubes packed with activated coconut shell charcoal. For ethylene oxide, two tubes mounted in series are used; the front and back-up tubes contain 400 and 200 mg, respectively, of charcoal. The front and back-up sections are individually measured for epoxide. If the back-up portion contains >25% of the epoxide, the analysis is not considered valid. The method suggests desorbing the epoxide with carbon disulfide. The required solvent amount is 2.0 mℓ for ethylene oxide. Aliquots of the desorbed solutions are then analyzed by GC with flame ionization detection. NIOSH (1977) conducted tests on the analytical parameters. Ethylene oxide was examined at concentrations from 41 to 176 mg/m^3 (23 to 98 ppm); precision ($\overline{CV}_T$) was 0.103 (or standard deviation of 9.3 mg/m^3), and accuracy was 0.9% lower than the "true" value. NIOSH (1977) recommended sample concentrations of 20 to 270 mg/m^3 for this method for industrial hygiene monitoring.

Romano and Renner (1975) described the results of a six laboratory intercomparison of three methods for sampling ethylene oxide in surgical equipment. The study was administered through the Z79 Subcommittee on Ethylene Oxide Sterilization of the Association for Advancement of Medical Instrumentation. The three sampling methods were vacuum extraction with sample freezeout, headspace analysis, and acetone extraction. The vacuum-freezeout technique requires distillation of volatiles from the sample, and freezing them in a cold trap. The sample is then vaporized and an aliquot is removed with a vacuum syringe for GC analysis. Romano and Renner (1975) reported that the method requires greater time and equipment than the other techniques and is subject to errors from equipment leaks. Its advantages are that it is the most sensitive, and since the sample injected into the GC is a vapor, column life is long. Acetone extraction consists of partitioning the epoxide between the sample and the acetone solvent. Its advantage is its simplicity. Its disadvantages include its inability to quantitatively extract epoxide, problems from impurities in the solvent and extraction of other compounds from the plastics, the reduced lifetime of columns because of these impurities, and low sensitivity. In headspace analysis, the sample is placed into a vial which is equipped with a septum for gas withdrawal by syringe. The epoxide partitions between the sample and headspace gases. The advantages of this technique include its ease of performance, speed, sensitivity, and relatively long column life. Its disadvantage is that leaks in septa, vial caps, etc., can yield low measurements.

Romano et al. (1973) reported that the headspace technique has a lower limit of 0.1 ppm and that the technique can be automated. Romano and Renner (1975) evaluated results for the three methods at six laboratories by analysis

of variance. Among overall methods, there were no significant differences; however, slight differences between laboratories were detected.

Ben-Yehoshua et al. (1971) extracted fruit pulp by blending it with 50 mℓ of analytical grade acetone for 30 seconds, filtering the homogenate to clarity. The samples were then stored at -10°C in bottles with self-sealing stoppers. Measurements (by GC) of added ethylene oxide and its residues were accurate to ±5%.

Scudamore and Heuser (1971) extracted wheat flour and other commodities, including coconut, sultanas, lentils, and ground nuts with 5:1 (v/v) analytical grade acetone-water. The extraction used as little as 3 mℓ solvent/g sample. A contact time of 24 hours was sufficient to yield ethylene oxide recoveries (by GC) of ≥95%.

Pfeilsticker et al. (1975) extracted 10 g of grain (not crushed) with 5 mℓ of methanol using continuous agitation for 24 hours. Recovery of ethylene oxide (25 ppm) was 73% and standard deviation (with GC analysis) was 1.70 ppm.

Brown (1970) sampled and analyzed surgical materials (plastic and rubber) for ethylene oxide residues by means of a three column chromatography system. Brown (1970) could separate ethylene oxide and its degradation product, ethylene chlorohydrin. Samples were extracted with p-xylene (3 days contact) or co-sweep distillation. The three column system consisted of: I. Fluorisil, II. acid-celite, and III. Fluorisil. The p-xylene solution was passed through Column I; ethylene chlorohydrin remained fixed in the column and ethylene oxide passed through. The ethylene oxide solution was passed through the acid-celite column which converted it to ethylene chlorohydrin. Column III retained the ethylene chlorohydrin, which was subsequently eluted with petroleum ether. The sample was concentrated with a Kuderna-Danish

apparatus, and then analyzed by GC. Brown (1970) reported values as low as 1.8 ppm, but accuracy, precision, and minimum detection limit were not described.

4.2. ANALYSIS

Thus far, GC analysis for ethylene oxide has only used flame-ionization detection or thermal conductivity detection. Neither detection system is selective, so the epoxides must be separated from all interferences, and the choice of analytical column depends on potential interferences. Columns for epoxide analysis have included uncoated Poropak Q, QS, and R, and Chromosorb 102 (Taylor, 1977a,b; Ben-Yehoshua and Krinsky, 1968; Steinberg, 1977), and a variety of coated columns. The most common liquid phases appear to be SE-30, Carbowax 20M, and polypropylene glycol (Ben-Yehoshua and Krinsky, 1968; Casteignau and Halary, 1972; Steinberg, 1977; Hughes et al., 1959). Bertsch et al. (1974) used a 100m x 0.5mm capillary column coated with Emulphor ON 870. The GC methods in current use appear capable of epoxide analysis at the ppm level.

Other analytical methods include various wet chemical techniques. Epoxides can be analyzed by ring opening with specific reagents and subsequent analysis for the reagent or one of its products (Dobinson et al., 1969). Mishmash and Meloan (1972) reported perhaps the most recent use of this approach. Butylene oxide was hydrolyzed to its glycol, then the glycol was oxidized with periodic acid. Residual oxidant was analyzed by adding CdI_2-starch, and then measuring the starch-I_3 complex concentration at 590 nm. They claimed a detection limit in the nmole range.

5. SOURCES IN THE ENVIRONMENT

5.1. PRODUCTION

5.1.1. Quantities Produced. Production volumes and sales quantities for ethylene oxide are listed in Table 5-1 for the years 1972 to 1982.

5.1.2. Producers, Production Sites, and Distribution. The producers, production sites, and annual capacities of ethylene oxide are listed in Table 5-2. ICI Americas is building a new ethylene oxide plant in Bayport, Texas; the nameplate capacity is rated at 520 million pounds/year (Anonymous, 1981a). Dow will add 400 million pounds/year capacity onto its Plaquemine, Louisiana, facility during the fourth quarter of 1983. Union Carbide is building a 400 million pounds/year unit in Alberta, Canada, slated to be on stream in 1985. PPG Industries and DuPont are conducting a feasibility study to determine whether or not to move the former's idle Guayanilla, Puerto Rico, facility (rated at 300 million pounds/year) to Beaumont, Texas, to be operated jointly by both.

5.1.3. Production Methods and Processes.

5.1.3.1. INTRODUCTION -- The majority of information in this section was obtained from Cawse et al. (1980).

TABLE 5-1

Ethylene Oxide Production[a,b]

Year	Production	Sales[c]
1982[d]	5200 (2359)	NA
1981	4937 (2240)	NA
1980	5220 (2368)	531 (241)
1979	5665 (2570)	560 (254)
1978	5012 (2273)	525 (238)
1977	4364 (1980)	549 (249)
1976	4184 (1898)	439 (199)
1975	4467 (2026)	409 (186)
1974	3893 (1766)	457 (207)
1973	4167 (1890)	501 (227)
1972	3962 (1797)	454 (206)

[a]Source: USTC, 1974, 1975; USITC, 1976, 1977a, 1977b, 1978, 1979, 1980, 1981

[b]All quantities are expressed in millions of pounds; SI units in millions of kilograms are given in parentheses.

[c]The difference between production and sales does not enter the merchant marketplace.

[d]Projected (Source: Anonymous, 1982)

NA = Not available

TABLE 5-2

Ethylene Oxide Producers, Plant Sites, Capacities, Processes, and Technology[a]

Company	Location	Annual Capacity[b]	Process Oxidant	Technology
BASF Wyandotte, Indust. Chem. Group Basic Chems. Div.	Geismar, LA	481 (216)	oxygen	Shell
Calcasieu Chem. Corp.[c]	Lake Charles, LA	225 (101)	oxygen	Shell
Celanese Corp. Celanese Chem. Co., Inc.	Clear Lake, TX	425 (191)	oxygen	Shell
Dow Chemical U.S.A.	Freeport, TX	260[d] (117)	air	Dow
	Plaquemine, LA	450[e] (203)	air	Dow
Eastman Kodak Co. Eastman Chemical Prod., Inc. Subsid. Texas Eastman Co.	Longview, TX	195 (88)	oxygen	Shell
ICI Americas, Inc., Petrochems. Div.	Bayport, TX	520 (234)[f]	NA	NA
Inter-North, Inc. Northern Petrochem. Co., Subsid. Petrochems Div.	Joliet, IL	230 (104)	oxygen	Scientific Design
Olin Corp., Olin Chems. Group	Brandenburg, KY	110 (50)	oxygen	Shell
PPG Industries, Inc. Chems. Group, Chem. Div.-U.S.	Beaumont, TX	155 (70)	air	Scientific Design

TABLE 5-2 (cont.)

Company	Location	Annual Capacity[b]	Process Oxidant	Technology
Shell Chemical Co.	Geismar, LA	700 (315)	oxygen	Shell
Sun Olin Chemical Co.	Claymont, DE	100 (45)	oxygen	Shell
Texaco, Inc.				
Texaco Chemical Co., Div.	Port Neches, TX	700 (315)	air	Scientific Design
Union Carbide Corp.				
Chems. and Plastics Div.	Seadrift, TX	1000 (450)	air	Union Carbide
	Taft, LA	1250 (563)	air	Union Carbide
Union Carbide Carbie, Inc., Subsid.	Ponce, PR	640 (288)	air	Union Carbide

[a]Sources: Anonymous, 1981a; SRI International, 1981a,b; Cawse, 1980

[b]Capacities are expressed in millions of pounds; capacities in millions of kilograms are in parentheses.

[c]Plant is on indefinite standby as of January 31, 1981 (Anonymous, 1981a).

[d]Approximately 200 million pounds/year (90 million kg/year) additional capacity can be obtained from a chlorohydrin unit used for propylene oxide production.

[e]Expansion of 400 million pounds/year (180 million kg/year) is due in the fourth quarter of 1983.

[f]Under construction

NA = Not available

Ethylene oxide is produced almost exclusively by direct oxidation, using either air or oxygen. Other processes cannot compete with the lower operating costs of direct oxidation. Only one plant in the United States currently has chlorohydrin capacity (Dow at Freeport, Texas; see Table 5-2). The major drawback of the direct oxidation process is the loss of ≈25 to 30% of the ethylene to carbon dioxide and water.

5.1.3.2. DIRECT OXIDATION -- The overall reaction for direct oxidation can be represented as follows:

$$H_2C{=}CH_2 + \pi O_2 \xrightarrow{Ag} H_2C\!-\!CH_2 \quad (O\text{ bridge})$$

5.1.3.2.1. Air-Based Oxidation -- The schematic for air-based ethylene oxidation is presented in Figure 5-1. Little detailed information is available concerning process technology; however, the salient features of the process are presented below.

In the first section, air and ethylene are fed into the recycle gas stream (the recycle gas contains unreacted starting material from the main absorber). The recycle stream is fed into a bank of tubular main reactors, the number of reactors depending chiefly on the capacity of the plant, activity of the catalyst, and size of the reactors. In the main reactor, the ethylene is oxidized to ethylene oxide, carbon dioxide, and water, as well as minor components such as formaldehyde and acetaldehyde.

Ethylene conversion to ethylene oxide per pass in the main reactors is 20 to 50%. Oxidation inhibitors (e.g., vinyl chloride, ethylene dichloride) are added to retard carbon dioxide formation. The process stream leaving the

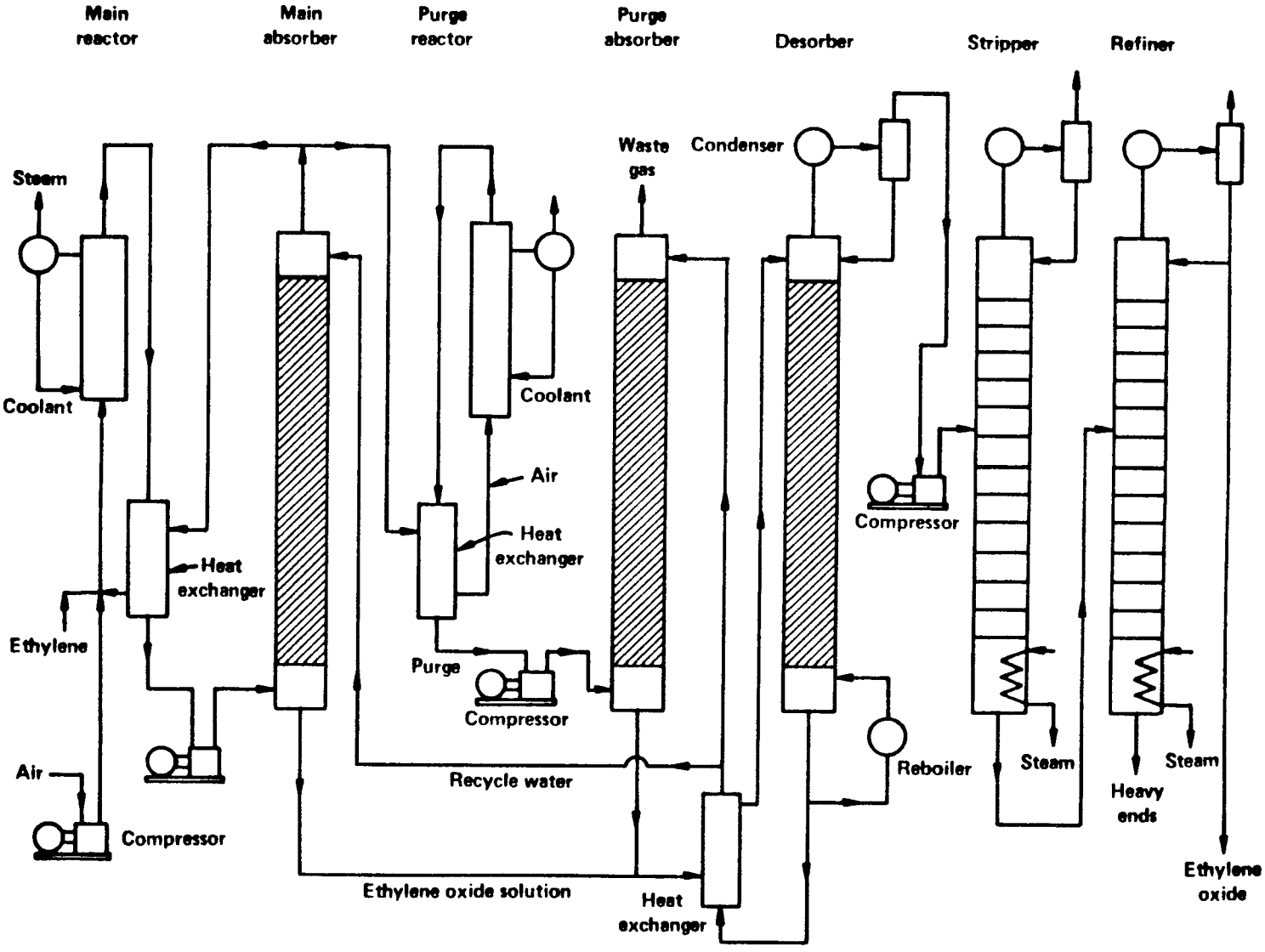


Figure 5-1

Schematic for air-based ethylene oxidation (Schultze, 1965)

reactor may contain 1 to 2 mole % ethylene oxide. This hot effluent gas is cooled to around 35 to 40°C and fed to the main absorber.

The main absorber uses cold water to dissolve the ethylene oxide, some carbon dioxide, and traces of hydrocarbons and aldehydes. The unabsorbed gas is split overhead. The largest portion is used as recycle gas, and to cool the effluent stream from the main reactor; the gas then enters the main reactor. A much smaller portion of the absorber effluent gas is fed as the main stream to the secondary or purge reactor. The effluent from the purge reactor is heat exchanged with the main stream and sent to the purge absorber which operates in the same manner as the main absorber.

The purge reactor system reacts a large portion of the ethylene present in the purge gas from the main reactor which must be vented from the main reactor so that inert gases (principally nitrogen and carbon dioxide) do not accumulate. Although Figure 5-1 shows a two stage air-based plant with a single purge reactor, some large plants have three or more stages to improve the overall yield. These plants merely place another purge reactor and absorber in series.

In some plants, the ethylene content of the vent gas is sufficiently high to make energy recovery economical. This not only produces valuable power from the vent gas, but also reduces the hydrocarbon emissions from the process.

The remainder of the process involves purification. The ethylene oxide water solution from the absorbers is heat-exchanged and sent to the desorber, where the ethylene oxide is steam stripped under reduced pressure. The

ethylene oxide is collected at the top and compressed for further purification, while the stripped water is recirculated to the main and purge absorbers.

The ethylene oxide from the desorber still contains some carbon dioxide, nitrogen, aldehydes, and traces of ethylene and ethane, and must be sent to the stripper. Here, the light gases are separated overhead and vented, while the partially purified ethylene oxide is taken from the bottom of the stripper and sent to the mid-section of a final refining column. The ethylene oxide from the refining section should have a >99.5 mole % purity.

The specific conditions used to operate ethylene oxide plants are proprietary. However, the general ranges suggested by the literature and patent reviews have been summarized by Cawse et al. (1980) and are presented in Table 5-3.

5.1.3.2.2. Oxygen-Based Oxidation -- The differences in oxygen-based and air-based oxidation processes are almost entirely the result of the change in oxidants. The main difference is that the purge reactor is absent in the oxygen-based process and a carbon dioxide removal unit and an argon vent are added. In the air-based cycle, the low per-pass conversion, the necessity of complete ethylene oxide removal in the absorber, and the accumulation of nitrogen necessitates a substantial purge system. Because of this, a staged reaction-absorption system is required. Since the oxygen-based process uses substantially pure oxygen, the recycle gas is almost entirely unconverted ethylene; hence, there is no need for a purge system. However, carbon dioxide is still produced in the oxygen system, and since this has a negative effect on catalyst selectivity, carbon dioxide must be removed. In addition to the

TABLE 5-3

Ranges of Reaction System Variables in the Direct Air-Oxidation of Ethylene Oxide[a]

Variable	Range
ethylene, mole %	2-10
oxygen, mole %	4-8
carbon dioxide, mole %	5-10
ethane, mole %	0-1.0
temperature, °C	220-277
pressure, MPa (psi)	1-3 (145-435)
space velocity[b], h^{-1}	2000-4500
pressure drop, kPa (torr)	41-152 (308-1140)
conversion, %	20-65
selectivity or yield (mole basis, %)	63-75

[a]Source: Cawse et al., 1980

[b]The space velocity is the standard volume of the reactant stream fed per unit time divided by the volume of reactor space filled with catalyst.
h = hour

carbon dioxide removal unit, an argon vent is also required. Argon is a major impurity in oxygen and can build up to the extent of 30 to 40 mole %. In spite of this additional purge, the total vent stream from an oxygen-based plant is much smaller than from an air-based plant.

As is the case with an air-based unit, the main process vent stream usually contains high hydrocarbon concentrations. In such cases, the purge stream can be used readily for energy recovery. The operating ranges for an oxygen-based process are summarized in Table 5-4.

The choice of oxygen versus air as the oxidant is based strictly on economics; in general, for small to medium capacity units (<50,000 t/year), oxygen-based plants have lower capital cost even with the necessary air separation facility. For medium to large plants (75,000 to 150,000 t/year), the air process investment is smaller unless oxygen can be purchased from a very large air separation facility. Operating costs of the facilities can differ significantly and are based on the cost of ethylene, oxygen, catalyst, and energy.

5.1.3.2.3. Chlorohydrin Processes -- The chlorohydrin process was the main method of ethylene oxide manufacture until 1957. In 1972, the Dow Chemical Company converted the remaining chlorohydrin capacity to the production of propylene oxide, and the process was not used again for ethylene oxide production until 1975. The Dow Chemical Company has built-in flexibility for using the chlorohydrin process to produce either propylene oxide or ethylene oxide. Since 1975, part of this capacity has been used for ethylene oxide. During 1975, the Dow Chemical Company made between 25 and 50 million pounds of ethylene oxide via the chlorohydrin process (Blackford, 1976b). The

TABLE 5-4

Ranges of Reaction System Variables in the Direct Oxygen-Oxidation of Ethylene Oxide[a]

Variable	Range
ethylene, mole %	15-40
oxygen, mole %	5-8.5
carbon dioxide, mole %	5-15
ethane, mole %	0-2
argon, mole %	5-15
nitrogen, mole %	2-60
methane, mole %	1-60
temperature, °C	220-275
pressure, MPa (psi)	1-2.2 (145-319)
space velocity[b], h^{-1}	2000-4000
conversion, %	7-15[c]
selectivity or yield (mole basis, %)	70-77

[a]Source: Cawse et al., 1980

[b]The space velocity is the standard volume of the reactant stream feed per unit time divided by the volume of reactor space filled with catalyst.

[c]At 30 mole % ethene

h = hour

chlorohydrin process is attractive commercially only when a good supply of captive low-cost chlorine and lime or caustic soda is available. Also, satisfactory markets or disposal facilities are needed for the by-products produced (Schultze, 1965).

The chlorohydrin process starts by conversion of ethylene to ethylene chlorohydrin with hypochlorous acid. The chlorohydrin is converted to ethylene oxide by dehydrochlorination with slaked lime. Two major by-products, 1,2-dichloroethane (≈100 to 150 pounds/1000 pounds ethylene oxide) and bis(2-chloroethyl)ether (≈70 to 90 pounds/1000 pounds ethylene oxide), are formed during the chlorohydrin formation; acetaldehyde (5 to 10 pounds/1000 pounds ethylene oxide) is produced during the dehydrochlorination.

The formation of ethylene oxide from ethylene chlorohydrin can be represented by the following equation:

$$2\ HOCH_2CH_2Cl + Ca(OH)_2 \longrightarrow 2\ \underset{\diagdown O \diagup}{CH_2CH_2} + CaCl_2 + 2H_2O$$

Ethylene chlorohydrin is formed in the lower section of a reaction tower. Gases are separated from the dilute chlorohydrin solution in the top section and the vent gases from the condensing apparatus pass in series to water and caustic scrubbers, where residual chlorine and HCl gas are removed before recycling the unreacted ethylene. The aqueous chlorohydrin solution is mixed with a 10% solution of milk of lime at the inlet to the hydrolyzer (Schultze, 1965).

The crude ethylene oxide product from the hydrolyzer contains about 77.5% ethylene oxide, 10% water, 12% chlorinated organic compounds (principally

1,2-dichloroethane and bis(2-chloroethyl)ether), and 0.5% acetaldehyde together with small amounts of hydrocarbon gases. This crude ethylene oxide is refined in two columns; the first column removes chlorinated hydrocarbons and the second column removes acetaldehyde.

5.2. USES OF ETHYLENE OXIDE

A description of the various uses of ethylene oxide is given below:

	Pounds[a]	Percent of Total
Ethylene glycol	3.2×10^6	62%
Nonionic surface-active agents	0.62×10^6	12%
Glycol ethers	0.31×10^6	6%
Ethanolamines	0.26×10^6	5%
Miscellaneous applications (higher glycols, urethane polyols, sterilant, fumigant, export)	0.78×10^6	15%

Source: Anonymous, 1981a

[a]Based on 1982 production estimates of 5200×10^6 pounds.

The major users and use sites for ethylene oxide are listed in Table 5-5. As can be seen from this table, a very large percentage of production is captively consumed by the primary manufacturers. A general description of the various uses of ethylene oxide is presented below.

5.2.1. Ethylene Glycol. By far, the largest single use of ethylene oxide is its use captively as an intermediate in the synthesis of ethylene glycol, which is currently produced by hydration of ethylene oxide. Current industry capacity to produce ethylene glycol is 5815 million pounds annually (Anonymous, 1981b). The growth in consumption of ethylene oxide has largely depended on its use as an intermediate for ethylene glycol production

TABLE 5-5

Users and Use Sites of Ethylene Oxide

Company	Location	Ethylene Glycol	Glycol Ethers	Diethylene Glycol	Ethanol-amine	Triethylene Glycol	Polyethylene Glycol
BASF Wyandotte Corp.	Geismar, LA	+	-	+	-	-	-
	Wyandotte, MI	-	-	-	-	-	+
Calcasieu Chem.	Lake Charles, LA	+	-	-	-	-	-
Celanese Chem.	Clear Lake, TX	+	-	+	-	+	-
Dow Chem.	Freeport, TX	+	-	+	+	+	+
	Plaquemine, LA	+	-	+	-	+	-
	Midland, MI	-	+	-	+	-	-
Eastman Kodak	Longview, TX	+	+	+	-	+	-
Northern Petrochem.	Morris, IL	+	-	+	-	-	-
Olin Corp.	Brandenburg, KY	+	+	+	+	+	+
PPG Ind.	Beaumont, TX	+	+	+	-	+	-
	Guayanilla, PR	+	-	+	-	+	-
Shell Chem.	Geismar, LA	+	+	+	-	+	-
Texaco Jefferson Chem.	Port Neches, TX	+	+	+	+	+	+
Union Carbide	Seadrift, TX	+	+	+	+	+	-
	Taft, LA	+	+	+	-	+	-
	Penuelas, PR	+	+	+	-	+	-
	Texas City, TX	-	-	+	-	-	-
	Institute and S. Charleston, WV	-	-	-	-	-	+
Ashland Chem.	Janesville, WI	-	-	-	-	-	+
Hoadag Chem.	Skokie, IL	-	-	-	-	-	+

[a]Source: SRI International, 1977

[b]+ indicates user of ethylene oxide, - indicates non-users of ethylene oxide

(Blackford, 1976b). Ethylene glycol is mainly used for polyester production and antifreeze formulations (Anonymous, 1981c).

5.2.2. Nonionic Surface-Active Agents. Of the nonionic surface-active agents synthesized from ethylene oxide, ≈25% are of the cyclic variety, while ≈75% are of the acyclic variety. In the cyclic group, ethylene oxide is used to make ethoxylate alkyl phenols and alkylphenol-formaldehyde condensates. Production of ethoxylated nonylphenol is probably the largest volume product of the cyclic group; another large-volume product is ethoxylated dodecylphenol. These surface-active agents are primarily used in detergents. The acyclic surface-active category includes ethylene oxide used in the synthesis of surface-active polyethylene glycol esters, ethoxylated alcohols, polyether polyols, ethoxylated fats and oils, and miscellaneous ethoxylated products, such as mercaptans, glycols, and polyols (Cogswell, 1980). Industry estimates that ethylene oxide consumption for acyclic surface-active agents is expected to increase. The manufacture of ethoxylated linear alcohols, used in heavy-duty liquid detergents, will account for most of this growth (Cogswell, 1980).

5.2.3. Di-, Tri-, and Polyethylene Glycols. Ethylene oxide and ethylene glycol react to form diethylene glycol, triethylene glycol, and polyethylene glycol. Diethylene and triethylene glycols are obtained mainly as by-products of ethylene glycol manufacture. Diethylene glycol is used to produce polyester resins, as a textile lubricant, and in solvent extraction. Triethylene glycol is used as a humectant and in natural gas dehydration, vinyl plasticizers, and polyesters. Industry capacity to make diethylene glycol is 472

million pounds/year; capacity to make triethylene glycol is ≈145 million pounds/year (SRI International, 1977).

5.2.4. Glycol Ethers. Ethylene oxide is combined with alcohols to manufacture glycol monoethers, which include ethylene glycol monomethyl, monoethyl, and monobutyl ethers; diethylene and triethylene monoethyl, monomethyl, and monobutyl ethers (Cogswell, 1980). Solvent applications dominate the many uses of glycol ethers. Industry capacity to make glycol ethers is 865 million pounds annually (SRI International, 1977).

5.2.5. Ethanolamines. Ethylene oxide reacts with ammonia to form a mixture of mono-, di-, and triethanolamines. The proportion of the three ethanolamines is dependent upon the ratio of reactants used. About 25 to 30% of all ethanolamines are used for soaps and detergents, 5 to 20% for gas conditioning, 10% by the metal industry, 8% for textiles, 5 to 15% for toilet goods, and the remainder in varied applications (Blackford, 1976b).

5.2.6. Miscellaneous Applications. Ethylene oxide is consumed in the synthesis of numerous commercial chemicals. The largest amount in the miscellaneous group goes into production of polyether polyols for flexible polyurethane foams. In 1978, about 100 million pounds (45 million kg) of ethylene oxide were consumed in these polyols (Cogswell, 1980).

Approximately 17 million pounds of ethylene oxide are used annually to make the medicinals, choline and choline chloride (Cogswell, 1980).

Approximately 10 million pounds of ethylene oxide are used annually in the manufacture of hydroxyethyl starch, which is a semi-synthetic gum used in

textile sizing and adhesives (Cogswell, 1980). Hydroxyethyl cellulose is produced by reacting cellulose with ethylene oxide. About 25 million pounds (11 million kg) of ethylene oxide are used annually to make these adhesive additives (Cogswell, 1980).

Arylethanolamines are made by reacting ethylene oxide with either aniline or aniline derivatives. It is estimated that 3 million pounds (1.4 million kg) of ethylene oxide are used annually for arylethanolamines (Cogswell, 1980). They are used as intermediates for monoazo dyestuffs.

Acetal copolymer resins are produced by catalytically copolymerizing 1,3,5-trioxane with a cyclic ether having at least two adjacent carbon atoms (e.g., ethylene oxide). Ethylene oxide consumption for these resins is believed to have amounted to ≈2 to 3 million pounds/year (0.9 to 1.4 million kg) from 1977 to 1978 (Cogswell, 1980).

Like nonionic surface-active agents, ethylene oxide is used to produce ethoxylated cationic surface-active agents. Several million pounds of ethylene oxide are used annually to produce these cationic agents, such as ethoxylated (coconut oil alkyl) amine, ethoxylated (tallow alkyl) amine, and various ethoxylated fatty acid amino amides (Blackford, 1976).

Small amounts of ethylene oxide are also consumed as a fumigant, as a food and cosmetic sterilant, and in hospital sterilization (Gilmour, 1978). In 1975, an estimated 0.1 million pounds of ethylene oxide were used for fumigant purposes (Landels, 1976). By contrast, Dow Chemical (Kurginski, 1979) has estimated that 0.2% of production (≈10 million pounds/year) of ethylene oxide is used as a fumigant; however, the exact amount is not available.

5.2.7. Discontinued Uses of Epoxides. Until 1953 (when acetylene was first used), all acrylonitrile was produced by the catalytic dehydration of ethylene cyanohydrin that was prepared from ethylene oxide and hydrogen cyanide. The reaction may be represented as follows:

$$H_2C\!-\!CH_2 \;(\text{O}) + HCN \longrightarrow HOCH_2CH_2CN \longrightarrow CH_2 = CHCN + H_2O$$

In 1956, American Cyanamid Company closed down its 35 million pounds/year plant at Warners, New Jersey, which was based on this process. From then until 1966 when it was discontinued, this process was used only by Union Carbide at Institute, West Virginia (Blackford, 1974). In 1965, Union Carbide consumed 90 million pounds of ethylene oxide to make acrylonitrile. No other significant discontinued uses of ethylene oxide are known.

5.2.8. Projected or Proposed Uses. Wood treatment is a potentially important market for epoxides (Anonymous, 1977). The USDA Forest Product Laboratory has reported that treating southern yellow pine with epoxides (including ethylene oxide, propylene oxide, and butylene oxide) improves its durability. The treatment adds 20 to 30% (by weight) of the epoxide to the wood.

5.2.9. Alternatives to Uses for Ethylene Oxide. More than 99% of the United States' production of ethylene oxide is used as a chemical intermediate in chemical syntheses of glycols and other compounds. Alternatives would require production routes from raw materials other than ethylene oxide.

Roughly 62% of the ethylene oxide production is hydrolyzed to ethylene glycol. A new process for making ethylene glycol directly from ethylene has

been developed by Halcon, Inc. (Klapproth, 1976). Ethylene is reacted with acetic acid in the presence of a catalyst to form mono- and diacetates, which are then hydrolyzed to ethylene glycol. Oxirane Corporation has constructed an 800 million pounds/year plant based upon this technology in Channelview, Texas. This capacity represents ≈25% of the total industry ethylene glycol capacity.

As far as the other compounds synthesized from ethylene oxide are concerned, no information was available on synthesis from other raw materials.

About 0.1 million pounds of ethylene oxide are used as a fumigant annually (Dow Chemical estimates that the volume of ethylene oxide used as a fumigant is <0.2% of total production, which in 1978 would equal 10 million pounds; Kurginski, 1979). Since there are many commercial fumigants available, it seems possible that many of its fumigant uses might be replaced by an alternative fumigant.

5.3. POTENTIAL FOR ENVIRONMENTAL CONTAMINATION

5.3.1. Air Emissions from Production. Air emissions from direct oxidation ethylene oxide plants of all types consist mainly of ethylene, ethylene oxide, and traces of ethane. The main process vent stream is responsible for most of the air emissions in both air- and oxygen-based units. In air units, this vent is located on the last purge reactor absorber and is principally spent air (N_2, O_2, and some inert gases), carbon dioxide, traces of ethylene oxide, and generally <2 mole % hydrocarbons. A catalytic converter is sometimes added to the main process vent in an air system.

The analogous vent stream from an oxygen-based system is about 10^2 smaller and contains a much higher hydrocarbon concentration, and is consequently used as a fuel. Table 5-6 presents approximate concentrations of typical vent stream contaminants for the main process vent and the purge gas vent.

Approximate amounts of vented reaction stream have been estimated. For unburned vent gas from an oxygen-based unit, the total hydrocarbon emissions have been estimated to be ≈12 g/kg product. If methane is used as a diluent and the purge gas incinerated, the emissions can be reduced to ≈4 g/kg product. In an air-based unit without catalytic combustion of the purge gas, hydrocarbon emissions are estimated to be >30 g/kg product. The use of a catalytic converter can reduce emissions to ≈15 g/kg product. In a study conducted for the U.S. EPA, the total ethylene oxide emissions in 1978 were estimated to be about 2×10^6 pounds (9.09×10^6 kg) (SAI, 1982).

Process waters for ethylene oxide manufacture and use appear to be minor problems with respect to waste treatment. The major aqueous waste is draw-off from separator bottoms (Liepins et al., 1977). The process water is recycled in its manufacture and its primary use as an intermediate in ethylene glycol manufacture (Sittig, 1962, 1965). The aqueous waste from direct oxidation plants will contain small amounts of glycols, aldehydes, and heavy glycols (Cawse et al., 1980). No information was available on how much of the process water eventually is treated, and no specific details were provided on treatment methods. The waste water will contain high BOD, but inorganic composition and refractory organics appear minimal problems with ethylene oxide manufacture or ethylene glycol production from ethylene oxide (Sittig, 1962, 1965; Spencer, 1971). Conventional water treatment (including filtration and

TABLE 5-6

Typical Vent Gas Composition for Both Air- and Oxygen-Based Ethylene Oxide Plants*

Stream	Range, mole %	
	Air-Based	Oxygen-Based
Main Process Vent		
nitrogen	85-93	2-35
oxygen	1.0-5	5-7
methane	0-0.9	1-35
ethane	trace-0.2	trace-0.2
ethylene	trace-2.5	13-35
ethylene oxide	0-0.01	0-0.01
carbon dioxide	5-15	5-15
argon	NP	5-15
water	0.1-1.5	0.1-0.5
CO_2 Rich Purge Gas (water-free)		
nitrogen	13-25	NP
oxygen	1-26	0.02
ethylene and hydrocarbons	2.5-8.0	0.3-0.9
ethylene oxide	0-1.0	NP
carbon dioxide	62-80	99-99.7
inert compounds	NP	0.005-0.015

*Source: Cawse et al., 1980

NP = Not present

flocculation) with a biological treatment appears sufficient (Spencer, 1971; Shenderova et al., 1972).

There is no solid waste associated with ethylene oxide manufacture.

5.3.2. Handling, Transport, and Storage. Ethylene oxide could be emitted to the atmosphere as the result of fugitive emissions or venting during its handling, transport, or storage. No specific information was available to describe these losses. Information on current practices, procedures, or environmental controls was sparse and no monitoring information was available. The following paragraphs discuss potential releases of epoxides without making any attempt to establish relative importance.

Bulk shipments of ethylene oxide are commonly made by railroad freight tanker; the sizes of the tankers are commonly 10,000 and 20,000 gallons. Shipments are also made in special 55-gallon drums and by highway truck tankers. Ethylene oxide is stored in bulk containers, as well as in smaller quantities in 55-gallon drums.

No information was available on the usual emission controls used on storage and transport containers. "Padded" containers, if used, would conserve vapors which would otherwise be vented to the atmosphere. Emissions could also occur during equipment purging in routine maintenance, gauge glass blowdown, or leaks.

Release is also possible during transfer. In normal practice, railway tankers are loaded and unloaded directly from or into storage tanks. The transfer utilizes nitrogen pressurization to ≈50 psi or pumping. Faulty equipment or over-pressurization can cause epoxide emissions. Small amounts spilled during handling could also release some ethylene oxide.

A concern in addition to normal working and handling losses is release from a storage container or transport-related accident. This could vary in scope from a relatively minor incident, such as release through a pressure safety valve or a rupture disc, to a major accident in which an entire storage container or tanker would rupture. No information was available to predict how often the minor release accidents do, in fact, occur or on the amount of ethylene oxide they annually release.

Storage, transport, and handling methods have been extensively described in literature supplied by manufacturers (BASF Wyandotte Corp., 1972; Dow Chemical Company, 1977; Jefferson Chemical Company, undated a and b; Oxirane Corporation, undated) and safety information sources (NFPA, 1975; MCA, 1971). This literature chiefly concerns safety of humans and property. Tank cars for ethylene oxide and propylene oxide are specified as ICC-105A100W and 105A100. These are equipped with pressure relief valves which vent excessive pressure into the atmosphere. The epoxides should preferrably be stored in an area detached from the plant site and storage tanks should be diked. Ethylene oxide should be equipped with cooling pipes. Tanks must be equipped with pressure relief valves, but specific instructions on emission control of excess pressure was not included. Vapor recompression systems could be applied to prevent emissions (Spencer, 1971).

5.3.3. Potential Environmental Formation. The major source of potential inadvertent production in the environment of ethylene oxide is probably the combustion of hydrocarbon fuels. Hughes et al. (1959) utilized gas-liquid partition chromatography to separate and identify oxygenated derivatives of hydrocarbons that were found in the combustion products of hydrocarbon fuels.

Among the oxygenated combustion products identified were ethylene oxide and propylene oxide. Barnard and Lee (1972) identified these compounds in the oxygenated combustion products from n-pentane combustion. Seizinger and Dimitriades (1972) identified ethylene oxide as a component of automobile exhaust. The fuels used were simple hydrocarbons, not gasoline, but all were components of gasoline; no lead was present. Stationary sources of hydrocarbon combustion may also emit large quantities of these compounds into the environment.

Ethylene oxide has been identified in tobacco smoke (Binder and Lindner, 1972; Binder, 1974). It is not uncommon for tobacco to be treated with ethylene oxide by cigarette manufacturers for its fumigant properties.

Binder and Lindner (1972) determined that the ethylene oxide concentration of unfumigated tobacco was 0.02 μg/mℓ, while fumigated tobacco had a concentration of 0.05 μg/mℓ and extensively fumigated tobacco had a concentration of 0.30 μg/mℓ. Binder determined the ethylene oxide content of smoke from unfumigated tobacco as 1 μg/g.

Epoxides are formed in the photochemical smog cycle. Olefins can be converted to the corresponding epoxides by reaction with an organic peroxide (Altshuller and Bufalini, 1965). Alkyl peroxides can decompose to yield an epoxide and oxy radical (NAS, 1976).

Water disinfection has the potential to convert olefins to epoxides. Olefin conversion during chlorination would proceed by the same route as for chlorohydrination production of the epoxide. However, this process would require conversion of ethylene to the chlorohydrin (Morris, 1975; Carlson and Caple, 1977). Since ethylene is very volatile, this process seems unlikely.

5.4. SUMMARY

This section discusses production, uses, and emissions of ethylene oxide. Ethylene oxide is produced virtually exclusively by direct oxidation using either air or oxygen. Its 1981 production volume was 4937 million pounds, down from 5220 million pounds in 1980.

The major emission sources from production facilities are the main process vent for both air and oxygen units and the purge gas vent for air units; fugitive emissions are also a major source. Total air emissions from production have been estimated to be around 2 million pounds based on 1978 production (50×10^6 pounds). Ethylene oxide also enters the atmosphere from handling, storage, and transfer operations, as well as the disposal of process wastes. There is no solid waste from ethylene oxide manufacture.

Greater than 90% of the ethylene oxide produced is used captively as a chemical intermediate, where there is some potential for environmental contamination. Up to 10 million pounds are used annually for fumigation and sterilization; when used as such, ethylene oxide emissions might be significant. Ethylene oxide may also be produced by hydrocarbon combustion (e.g., automobile exhaust).

6. ENVIRONMENTAL FATE, TRANSPORT, AND DISTRIBUTION

6.1. INTRODUCTION

Epoxides are not persistent in the environment. Available information on their chemical and biological properties characterizes them as highly reactive. The available information on transport was not sufficient to develop a definite description of transport characteristics. Interphase transport from water to air seems to be a slow process, but evaporation of ethylene oxide applied as a sterilant or a fumigant appears to be a rapid process. High water solubility and high vapor pressure result in significant mobility within water or air.

Epoxide degradation has been fairly well characterized, and indicates that ethylene oxide is reactive in all media. Available information on its ionic reactions indicates that chemical (see Section 3) and biological degradation follow parallel pathways with respect to products. Its degradation in water, soil, commodities, and manufactured products proceeds through ionic reactions. Its degradation in the atmosphere has not been well characterized with respect to processes or products. Available information indicates that it is very reactive in photochemical smog cycle reactions. No information was available on whether ionic reactions (e.g., with water vapor or water within aerosols) significantly contributed to its degradation in the atmosphere.

6.2. ETHYLENE OXIDE FATE IN WATER

Ethylene oxide will degrade in water by hydrolysis and related nucleophilic reactions; aqueous radical reactions will not be a significant process. The hydrolysis chemistry of ethylene oxide has been discussed in Section 3.6.4, and the information presented there will be used in the present discussion.

Ethylene oxide has a hydrolysis half-life of 12.2 days in pure water, 12.9 days in filtered (0.22 μm filtered) Kanawha River water, and 14.2 days in unfiltered Kanawha River water (Conway et al., 1983). The Kanawha River water had a pH of 7.4 and the initial ethylene oxide concentration was ≈70 mg/ℓ. These variations in hydrolysis rates are well within the error limits discussed by Mabey and Mill (1978) in regard to hydrolysis experiments.

It is interesting to note that the presence of a microbial population in the unfiltered river water did not materially decrease the half-life of the ethylene oxide merely from related hydrolysis reactions with the moieties present in biological systems. Although the microbial concentration was not reported, the lack of a significant change in rate may indicate that such reactions are not significant in river water. In addition, it should be noted that the half-life of 12 to 14 days allows for sufficient time for exposure of ethylene oxide to biota and possibly humans, although the latter would be much less likely given the addition of hypochlorite in water treatment plants. Conway et al. (1983) also reported that pH variations would have less of an effect on the rate of hydrolysis than temperature over a pH range of 5 to 10.

Evaporation from water also appears to be a significant process. Conway et al. (1983) reported the calculated relative desorption coefficient α_d (α_d = K_d (ethylene oxide)/$K_d(O_2)$, K_d is the desorption coefficient) to be 0.31,

0.34, and 0.36 for 10, 20, and 30°C water. Experimental values for 22°C water are 0.36 for no wind and 0.39 for a 5 m/s wind, are reasonably consistent with a calculated value of 0.34, and may be the result of increased turbulence and wind flow. These values of α_d indicate that ethylene oxide will be desorbed from a water body with a rate dependent upon the actual oxygen-transfer rate in a specific system. The rate of desorption will be less than that for volatile low solubility organics such as toluene, benzene, and chloroform, which have an α_d of around 0.65 (Rathburn and Tai, 1981).

Conway et al. (1983) also measured the biochemical oxygen demand (BOD) using 2 mℓ of domestic sewage/BOD bottle. They found the biooxidation as a percent of theoretical to be 5, 22, 40, and 52% on days 5, 10, 15, and 20, respectively. Conway et al. (1983) suggested that in a sewage treatment plant where the microbial population is much higher, biodegradation may be very fast. However, from their data it is not possible to determine whether or not the chemical actually being degraded is ethylene oxide or ethylene glycol from hydrolysis, since the hydrolysis half-life, which is ≈14 days, is similar to the BOD half-life of a little less than 20 days.

Hendry et al. (1974) reported the rate constant for the reaction of one epoxide with alkyloxy radical proceeding by α hydrogen abstraction to be 8.5×10^4 M^{-1} s^{-1}/α-hydrogen or 3.4×10^5 M^{-1} s^{-1} for ethylene oxide. Given an alkyloxy radical concentration in ambient water of 10^{-14} M, the half-life for this process is ≈6 years. Hence, for ethylene oxide, hydrolysis and evaporation appear to be the dominant fate processes, while no definitive statement regarding biodegradation can be made.

6.3. ETHYLENE OXIDE FATE IN SOIL

Pertinent data regarding chemical degradation of ethylene oxide in soil were not located in the available literature. It seems reasonable, however, that given the major components of soil, the half-life of ethylene oxide in soil would be shorter than in water.

6.4. ETHYLENE OXIDE FATE IN THE ATMOSPHERE

Epoxide degradation in the atmosphere can be inferred from information derived from their oxidation by free-radical pathways. Little direct information on epoxide behavior in the environment was available.

Atmospheric reactivity of volatile organics has been characterized by their relative reaction rates with hydroxyl radicals in the gas phase (Cupitt, 1980; Darnall et al., 1976). However, there are a number of difficulties in determining an atmospheric half-life or lifetime for ethylene oxide based on hydroxyl radical reactions. The most important is in choosing the appropriate hydroxyl radical concentration. The second difficulty is more fundamental and questions the appropriateness of chosing the OH reaction as the dominant removal mechanism. A number of different modeling and direct measurement efforts have been expended in determining the hydroxyl radical concentration in the atmosphere. These have provided a wide range of values of varying accuracy for both average and altitude specific concentrations. A reasonable compromise for an average OH concentration appears to be 1×10^6 molecules cm^{-3} based on more recent modeling efforts (Cupitt, 1983). For ground level concentrations, the values may be somewhat higher, possibly around 1.3 to 1.4 $\times 10^6$ molecules cm^{-3} during the summer (Crutzen and Fishman, 1977; Logan et al., 1981). Using these two values, a temperature of 300 K, and the Arrhenius

equation of Fritz et al. (1982) (see Section 3.6.5), the lifetimes of ethylene oxide vary between 215 days (using the lower limit of the Arrhenius equation) and 100 days (using the upper limit of the Arrhenius equation) for a hydroxyl radical concentration of 1 x 10^{-6} molecules cm^{-3}, and 159 days (using the lower limit of the Arrhenius equation) and 74 days (using the upper limit of the Arrhenius equation). Thus, given the limits, the lifetimes vary between 215 and 74 days. This lifetime is in sharp contrast with other ethers which are significantly shorter. For example, tetrahydrofuran, a five membered cyclic ether, has a lifetime of ≈1 day. Fritz et al. (1982) suggested that this is due to the distorted sp^3 bonds in ethylene oxide that give rise to a hydrogen abstraction activation energy of 5.8 kcal/mol, rather than the standard 2.8 kcal/mol.

Bogan and Hand (1978) determined the absolute rate constant of the reaction oxygen atoms [$O(^3P)$] with ethylene oxide to be (6.3 $\pm$ 0.18) x 10^{-16} cc/mcule-sec at 300 K. This rate is several orders of magnitude slower than the hydroxyl radical reaction, and yields a half-life of 1400 years, given an atmospheric $O(^3P)$ concentration of 2.5 x 10^4 molecules/cc (Graedel, 1978).

Sickles et al. (1980) used a Teflon smog chamber and the rate of ozone production to rank 19 compounds relative to propane. The chambers were outdoors and irradiated with sunlight. Purified air, an organic compound, and NO_2 were added before sunrise to multiple chambers; ethylene oxide to NO_2 ratio at the onset of each experiment was 4:0.067. Sickles et al. (1980) found ethylene oxide much less reactive than propane, with ethylene oxide being the fifth least reactive compound tested. The order of reactivities found was:

Acrylonitrile
Perchloroethylene
Ethanol
Ethylacetate
Acetone
Methanol
Acetic acid
Propane
Ethylene dichloride
Acetylene
Chloroform
Dimethyl formamide
Benzaldehyde
Methylene chloride
Pyridine
Ethylene oxide
Methyl chloroform
Phenol
Acetonitrile
Nitrobenzene

When compared to an indoor smog chamber study (Dimitriades and Joshi, 1977), the relative ordering of compounds was similar.

All of these results indicate that ethylene oxide is relatively unreactive in the atmosphere compared to other ethers. Given the shortest lifetime of 74 days for hydroxyl radical reaction, and the production volume and volatility, it should be possible to detect ethylene oxide in ambient air, yet no reports confirming its detection were found in the available literature. This lends support to the possibility that a reaction or reactions other than hydroxyl radical, possibly ionic, are dominant. This possibility is supported further by the facility with which ethylene oxide undergoes ionic reactions, yet no such reactions have been identified by atmospheric chemists. Nonetheless, the possibility that such a reaction may be dominant remains. Atmospheric constituents such as suspended particles may catalyze the decomposition of ethylene oxide as well as water vapor and other nucleophilic species. Thus, with the information currently available, no definitive state-

ments can be made regarding the atmospheric fate or lifetime of ethylene oxide.

6.5. DEGRADATION IN COMMODITIES AND MANUFACTURED PRODUCTS

Ethylene oxide is registered in the United States for use as a fumigant or sterilant on several stored food commodities and manufactured products (Goncarlovs, 1983). These include as a fumigant on furs, bulk food containers, food containers, stored grain, stored fruits, stored processed foods, garments, stored herbs, stored spices, furniture, aircraft, buses, railroad cars, laboratory animal bedding, and tobacco products. As a sterilant, it is used principally on hospital equipment and pharmaceuticals. The use of ethylene oxide as a fumigant is chiefly to protect stored products from either insect or microbial destruction. The fate of this epoxide and its residue are especially important in those materials, commodities, and products coming into close contact with humans, such as surgical equipment, pharmaceuticals, and food service and packaging materials (Wesley et al., 1965; Alguire, 1973; Holmgren et al., 1969; Gilmour, 1978).

Delineation of ethylene oxide fate in these materials has established that it will degrade to glycol and halohydrin or evaporate. The degradation could result from chemical or enzymatic activity or from some combination of the two. The halohydrin formation requires epoxide reaction with inorganic halide. The halide could be naturally present, be added, or be derived from organic halides. Bromide ion often comes from degraded methyl bromide, which is also a fumigant (Rowlands, 1971; Lindgren et al., 1968).

Scudamore and Heuser (1971) evaluated ethylene oxide fate for a variety of treated commodities. They examined degradation and apparent vaporization

of ethylene oxide and its residues. The losses of the ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin were measured over a 1-year period. Apparent first order specific rate constants, k, were calculated for epoxide dissipation. The rate constant, k combined losses from the degradation (chemical and metabolic pathways), k_D, and vaporization, k_V:

$$k = k_D + k_V$$

The glycols (ethylene and diethylene) were only determined once at either 6 months or 1 year after treatment. Effects considered included ethylene oxide treatment (dose and temperature during application), moisture content of the commodity, storage temperature, and storage in closed containers or in open trays. Ethylene oxide residues rapidly dissipated. While its estimated half-life was longest at 10°C in sealed containers, it never exceeded 2 weeks. Increasing the ethylene oxide dose had a varied effect on its loss rate. For the most part, small increases in the dose slightly decreased the loss rate, while very large increases caused larger decreases in the rate of loss and, sometimes, caused non-linear correlations. The effect of moisture content appeared varied and relatively small. Scudamore and Heuser (1971) also monitored some commercially treated products and found ethylene halohydrin residues but no ethylene oxide residues. They concluded that ethylene oxide will normally dissipate from treated commodities, but under some circumstances, small quantities could persist for several months.

Stijve et al. (1976) discussed the fate of ethylene oxide applied as a fumigant to commodities. They suggested that ethylene oxide could be retained

by physical adsorption, but that it would persist not more than a few weeks before volatilization or reaction with natural constituents of the commodity.

Ben-Yehoshua et al. (1971) examined ethylene oxide residues during the treatment of dates. They reported a small ethylene oxide loss in an empty container and ascribed this to apparent adsorption to container walls. The larger losses experienced with 2.1 kg of dates in the container resulted from ethylene oxide uptake by the fruit. The ethylene oxide loss in treated dates, which were left in open containers, was attributed to degradation to the chlorohydrin and glycol combined with volatilization.

The available information on fate of ethylene oxide applied to manufactured goods was not as extensive as that on its fate in commodities. All available information suggested that its behavior in manufactured products corresponds to the pathways of degradation and volatilization described above.

Alguire (1973) described losses of ethylene oxide from polystyrene creamer cups and cream cheese wrappers at ambient temperature and open to the environment. The ethylene oxide did not degrade on the polystyrene cups, and was lost solely through out-gassing. More than 90% vaporized in the first day, and no residual ethylene oxide remained after 5 days. Ethylene oxide loss from cream cheese wrappers primarily consisted of its conversion to ethylene glycol; no ethylene chlorohydrin was detected at any time. Ethylene oxide was completely gone by the tenth day.

Some studies have identified ethylene chlorohydrin residues in manufactured goods sterilized with ethylene oxide. These studies did not seek any information on volatilization losses. Brown (1970) identified ethylene oxide and its derivatives on treated equipment made of rubber, dacron, and polyvinylchloride, but did not detect chlorohydrin on polyethylene equipment.

Holmgren et al. (1969) measured 0 to 1500 ppm chlorohydrin on 21 ethylene oxide treated drugs.

6.6. BIOACCUMULATION IN AQUATIC ORGANISMS

Specific experimental information regarding the bioaccumulation of ethylene oxide in aquatic organisms is not available. Veith et al. (1979) have suggested, however, the use of the following equation to calculate bioconcentration factors (BCF):

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

where K_{ow} is the partition coefficient between octanol and water. Using this equation and the log K_{ow} of -0.30, reported by Hansch and Leo (1979), the BCF for whole fish was calculated to be 0.34.

6.7. SUMMARY

This section discusses the results of studies relating to the fate of ethylene oxide in the environment. In water, ethylene oxide will degrade by hydrolysis and related nucleophilic reactions with a half-life on the order of 12 to 14 days at 298 K. Lower temperatures will lengthen the half-life; pH changes will have a minimal effect. Volatilization will also be a significant process although less so than for sparingly soluble solutes (e.g., toluene, chloroform, benzene). There is no conclusive evidence indicating microbial degradation will be significant; however, the components of sewage sludge may react rapidly with ethylene oxide. The fate of ethylene oxide in soil will likely be similar to water; its half-life will probably be shorter.

The fate of ethylene oxide in the atmosphere is not clear from the information presented in the available literature. Rate constants are available for hydroxyl radical and oyxgen atom [$O(^3P)$] reactions as well as smog chamber studies. All predict a lifetime of sufficient length to allow for measurement, the shortest calculated lifetime being 74 days. Nonetheless, no confirmed reports detailing the measurement of ambient levels of ethylene oxide were found. The possibilitv of nucleophilic reactions in the gas phase may explain this.

In commodities, food containers, and manufactured goods, ethylene oxide appears to volatilize or hydrolyze to glycol or halohydrin with a half-life on the order of 2 weeks.

7. ENVIRONMENTAL LEVELS AND EXPOSURE

7.1. INTRODUCTION

The purpose of this document is to present available information relevant to human health effects that could be caused by this substance.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used to support any conclusions regarding risks to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

7.2. ENVIRONMENTAL LEVELS

Ambient monitoring has portrayed ethylene oxide as an almost non-existent contaminant of environmental or biological samples. Although ethylene oxide is rarely identified in monitoring studies, its principal degradation products (glycols and halohydrins) have been identified.

No monitoring data was available for ethylene oxide in biological tissues, except for some tissue distribution studies. Since epoxides are reactive alkylating agents, it is reasonable to expect such results (Anderson, 1971).

Only one ambient air monitoring study reporting the presence of ethylene oxide in air was found in the available literature. Bertsch et al. (1974) tentatively identified ethylene oxide in the ambient air near the University of Houston. However, the authors used Tenax as an adsorbant for trapping air contaminants and its use casts doubt on their tentative identification, since Tenax does not adequately retain ethylene oxide.

U.S. EPA (1976) listed one monitoring observation for ethylene oxide in water. It was observed in the effluent from a chemical plant in Bandenburg, Kentucky. No other epoxide observation was reported. U.S. EPA (1976) also noted observations of ethylene halohydrin, but its origin might be from industrial wastes rather than residues from epoxide.

No other reports of ethylene oxide in ambient air or water were found, yet SAI (1982) reported that the maximum exposure concentration level of ethylene oxide, based on dispersion models, was 5 $\mu g/m^3$ (2.77 ppb). One reason for this could be its reactivity, but another reason could be the lack of an adequate sampling method (see Section 4). Most sampling methods either lose significant amounts of ethylene oxide on even short term storage, or use adsorbants with a very poor affinity for ethylene oxide (e.g., Tenax GC); however, this is certainly not the case for all studies, especially those using freeze-out techniques. Since environmental samples are rarely as high as workplace samples (particularly in the case of a reactive molecule such as ethylene oxide), the well documented NIOSH (1977) method becomes inadequate.

The problem is compounded by the fact that few, if any, monitoring studies are undertaken to identify only one compound in the environment. Thus, these studies must assume some compromise between completeness and speed, making it impossible to optimize conditions for any one compound.

Several studies have examined the residues of ethylene oxide applied to commodities and manufactured goods as a fumigant and disinfectant. The information on residues in commercial products is discussed here. Another portion of this report (Section 6.5) describes investigations on the fate of this epoxide. The present section differs from the previous section in that the information here concerns residues in actual commercial products.

Scudamore and Heuser (1971) evaluated ethylene oxide and its metabolites in commercially treated products, and also did some fate studies (discussed in Section 6.5). While they never detected ethylene oxide in commercial products, they did find ethylene chlorohydrin residues ranging from 10 to 70 ppm.

Lindgren et al. (1968) reviewed studies on residues from ethylene oxide treatment, most of which were fate studies rather than ambient monitoring studies. Their review suggested that residual epoxide could be present in commercial products.

Ethylene oxide is a common sterilant for surgical equipment. Its fate in plastic and rubber surgical equipment parallels its behavior in commodities. Brown (1970) monitored residues on various hospital equipment sterilized with ethylene oxide. Ethylene oxide was observed in three samples, one of which had received treatment ≈80 days previously. Ethylene chlorohydrin was detected in 10 samples.

7.3. EXPOSURE

The available data concerning the environmental levels of ethylene oxide are insufficient to properly estimate exposure, however, a general overview is helpful. Over 5 billion pounds (>2 billion kg) of ethylene oxide are produced yearly. The vast majority is used captively as a synthetic intermediate. Possibly, 10 million pounds (4.5 million kg) are used for fumigation/sterilization, which includes food commodities, medical devices, pharmaceuticals, and cosmetics. This use constitutes the only documented potential exposure to ethylene oxide; however, the extent of this exposure needs to be determined. Ethylene oxide also appears to be a product of incomplete combustion, and has been identified in automobile and diesel exhaust and tobacco smoke. It can be formed during the photochemical smog cycle, but appears to be rapidly destroyed.

7.4. SUMMARY

This section discusses the results of monitoring studies conducted to measure the levels of pollutants, including ethylene oxide, in the environment. Very little information is available on ambient monitoring, no confirmed detection of ethylene oxide in air has been reported, and only one report exists for water. The lack of more monitoring reports may be because most, but not all, sampling methods would miss ethylene oxide even if present. Several studies have examined the persistence and fate of ethylene oxide in commodities and commercial goods including food, medical supplies, and drugs.

8. ECOLOGICAL EFFECTS

8.1. MICROORGANISMS AND INSECTS

Ethylene oxide is utilized as a fumigant for foods and spices (particularly grains), and shows major microbial, insecticidal and acaricidal activity (Sykes, 1964; Lindgren and Vincent, 1966).

Fumigation with ethylene oxide has been used to control a wide variety of bacteria, fungi, rickettsia and viruses. Sykes (1964), for example, reported that exposures to gaseous ethylene oxide at concentrations of 1 to 10% will kill Bacillus globigii, Staphylococcus aureus, Escherichia coli, Chromobacterium prodigiosum, and Mycobacterium phlei within a few hours. Roberts et al. (1943) found that 10% gaseous ethylene oxide will kill Bacillus anthracoides in 8 hours. Ethylene oxide also produced significant sporicidal activity against dry bacterial spores (Bruch and Koesterer, 1961). Exposure of Bacillus subtilis spores to 1 to 2% vapor concentrations of ethylene oxide killed $\geq$95% of the spores within 4 hours. A 5% gaseous concentration of ethylene oxide produced 90% kill of airborne B. globigii spores in <2 hours (Roberts et al., 1943). Treatment of agar slants containing yeasts and fungi with 8% gaseous ethylene oxide for 3 hours was lethal to these microorganisms (Whelton et al., 1946). Skeehan (1959) indicated that herpes simplex, vaccinia, and bovine respiratory viruses are susceptible to saturated ethylene oxide vapor treatment.

Susceptible insects common to stored products include the flour beetle, rice weevil, and grain weevil (Lindgren et al., 1954). Ethylene oxide will kill one-half the stored product insect population at a concentration range of

6 to 18 mg/ℓ (Ong, 1948). Lindgren and Vincent (1966) reported a major reduction in available tissue glutathione content of Calliphora larvae exposed to ethylene oxide. Decrease in tissue glutathione via depletion of reduced-SH groups may be the mechanism of toxicity. The insect toxicity of ethylene oxide has been ranked by Lindgren as intermediate between that of ethylene dibromide and ethylene dichloride. A bibliography of ethylene oxide insecticidal properties, citing 185 references, has been published (Young and Busbey, 1935).

8.2. PLANTS

Pertinent data regarding the effects of ambient exposure of ethylene oxide on plants were not found in the available literature. As detailed in Section 9.4, ethylene oxide is capable of inducing mutations and chromosomal aberrations in plants.

8.3. AQUATIC ORGANISMS

Limited information is available on the toxicity of ethylene oxide to aquatic organisms. The acute toxicity of ethylene oxide appears to be moderate, as indicated by LC_{50}'s in the range of 84-90 mg/ℓ for fish, a mean 48-hour LC_{50} of 212 mg/ℓ for Daphnia, and a mean 48-hour LC_{50} of 745 mg/ℓ for brine shrimp (Table 8-1). LC_{50} values for the hydrolysis product ethylene glycol were >10,000 mg/ℓ for the above species except goldfish (which were not tested) (Conway et al., 1983). If reacted to form ethylene chlorohydrin, the 96-hour LC_{50} for fathead minnows was about 90 mg/ℓ (Conway et al., 1983).

TABLE 8-1

ute Aquatic Toxicity of Ethylene Oxide[a]

Test Procedure	Test Organism	LC_{50} (95% Confidence limits), mg/ℓ 24 hr	48 hr	96 hr	Reference
range-finding[b], static, aerated	fathead minnow	274 (150-500)	NA	NA	Conway et al., 1983
range-finding[b], static, sealed under oxygen	fathead minnow	86 (50-150)	NA	NA	Conway et al., 1983
definitive static acute[c] (no aeration)	fathead minnow	90 (63-125)	89 (63-125)	84 (73-96)	Conway et al., 1983
static acute	goldfish	90	NA	NA	Bridie et al., 1979
static acute	Daphnia magna	>300 270 260	300 137 (83-179) 200 (150-243)	NA NA NA	Conway et al., 1983
static acute	brine shrimp	>500 350 570	>500 1000 490	NA NA NA	Conway et al., 1983

[a]Source: Conway et al., 1983

[b]Range-finding tests used 2 fish/test concentration

[c]Definitive tests used 10 fish/test concentration

NA = Not applicable

9. BIOLOGICAL EFFECTS IN ANIMALS AND MAN

9.1. PHARMACOKINETICS

9.1.1. Absorption. No pertinent data regarding the absorption of ethylene oxide were found in the available literature. However, acute toxicity data suggest that absorption occurs readily via the respiratory and gastrointestinal tracts (Table 9-1).

9.1.2. Distribution. Information concerning the distribution of ethylene oxide in the body is limited. Two studies have shown that it is found in many tissues following inhalation exposure and intravenous administration.

Ehrenberg et al. (1974) conducted inhalation studies with radioactively labeled [1,2-^{3}H] ethylene oxide. Following exposure of mice to 1.15 ppm of the labeled chemical in air for 75 minutes, the highest levels of radioactivity (in unidentified chemical form) were associated with proteins isolated from the lungs, kidneys, and liver. Lower levels of radioactivity were measured in the testes, brain, and spleen, but additional organs were not analyzed.

Appelgren et al. (1977) carried out whole body autoradiography on mice that were injected intravenously with radioactive [^{14}C] ethylene oxide (label position unspecified). Preliminary inhalation studies with labeled ethylene oxide showed a similar tissue distribution of the compound as that seen following intravenous injection, except for a high initial labeling of respiratory mucosa (data not shown). Two minutes after the injections, concentrations of radioactivity 2 to 3 times those seen in the blood were

TABLE 9-1

Acute Toxicity of Ethylene Oxide

Route	Species	Sex	Strain	Dose	Response	Reference
oral	rat	M	Wistar	330 mg/kg	LD_{50}	Smyth et al., 1941
oral	rat	M	NR	100 mg/kg	0/5 died	Hollingsworth et al., 1956
oral	rat	M	NR	200 mg/kg	5/5 died	Hollingsworth et al., 1956
oral	guinea pig	M,F	NR	270 mg/kg	LD_{50}	Smyth et al., 1941
oral	rabbit	M,F	NR	631 mg/kg	LD_{50}	Woodward and Woodward, 1971
ihl.	rat	M	white	1460 ppm/4 hours	LC_{50}	Jacobson et al., 1956
ihl.	rat	M,F	Sherman	4000 ppm/4 hours	LC_{50}	Carpenter et al., 1949
ihl.	guinea pig	NR	NR	7000 ppm/2.5 hours	LC_{low}	Waite et al., 1930
ihl.	mouse	F	white	835 ppm/4 hours	LC_{50}	Jacobson et al., 1956
ihl.	dog	M	beagle	960 ppm/4 hours	LC_{50}	Jacobson et al., 1956
i.v.	rabbit	M,F	NR	178 mg/kg	LD_{50}	Woodward and Woodward, 1971
i.v.	rat	M	NR	355 mg/kg	LD_{50}	Bruch, 1973
i.p.	rat	M,F	NR	178 mg/kg	LD_{50}	Bruch, 1973
i.p.	mouse	M,F	NR	178 mg/kg	LD_{50}	Bruch, 1973
i.p.	rabbit	M,F	NR	251 mg/kg	LD_{50}	Woodward and Woodward, 1971
s.c.	rabbit	M,F	NR	200 mg/kg	LD_{50}	Woodward and Woodward, 1971

Ihl. = inhalation; i.v. = intravenous; i.p. = intraperitoneal; s.c. = subcutaneous; NR = not reported

observed in the liver, kidneys, and pancreas. Tissue labeling 20 minutes to 4 hours after exposure showed high levels of radioactivity in the liver, kidneys, lungs, intestinal mucosa, epididymis, cerebellum, and testes. Twenty-four hours after injection, radioactivity was still found in the liver, intestinal mucosa, epididymis, cerebellum, bronchi, and bone marrow. Since these observations were made on autoradiographs, quantitative results were not reported. The extent of bioexchange of the radioactive label into natural body constituents also could not be determined in this study.

9.1.3. Metabolism. Comprehensive studies designed to fully characterize the metabolic fate of ethylene oxide have not been conducted.

Significant concentrations of ethylene glycol were detected in the plasma of 4 beagle dogs following the intravenous administration of 25 mg/kg or 75 mg/kg ethylene oxide on separate occasions (Martis et al., 1982). Urinary excretion data indicated that 7-24% of the administered dose was excreted in the urine within 24 hours as ethylene glycol; the mean percentages of the low and high doses that were excreted in the urine were 13.5 $\pm$ 3.5% and 14.2 $\pm$ 8.1%, respectively.

Two urinary metabolites were detected when [1,2-^{14}C] ethylene oxide was administered to Sprague-Dawley rats via single intraperitoneal injection at a dosage of 2 mg/kg (Jones and Wells, 1981). The urinary metabolites were *S*-(2-hydroxyethyl) cysteine (9% of the dose) and *N*-acetyl-*S*-(2-hydroxyethyl) cysteine (33% of the dose), which suggests that the metabolism of ethylene oxide involved conjugation with glutathione. A small percentage of the dose was exhaled as $^{14}CO_2$ and as unchanged ethylene oxide (Section 9.1.4).

In the inhalation study with mice summarized in Section 9.1.2 (Ehrenberg et al., 1974), the only urinary metabolite characterized was 7-hydroxyethylguanine, which accounted for a minor amount (0.007%) of the total urinary radioactivity. Significant alkylation of tissue proteins was found, but alkylation of DNA was confirmed by the high specific activity of tritium as 7-hydroxyethylguanine. Cumming et al. (1981) reported large differences in the patterns of initial alkylation as well as removal of total alkylation products from the DNA of various tissues (i.e., testis, liver, lung, kidney, spleen) of mice following inhalation exposure to tritium-labeled ethylene oxide. Thus, ethylene oxide distributes and reacts extensively throughout the body.

9.1.4. Elimination. In the inhalation study with mice conducted by Ehrenberg et al. (1974) using tritium labeled [1,2-^{3}H] ethylene oxide (see Section 9.1.3), it was found that 78% (mean value) of the absorbed radioactivity was excreted in the urine within 48 hours. The biological half-life in mice was reported to be ≃9 minutes, thus indicating rapid urinary elimination.

Approximately 43% of the administered radioactive dose of [1,2-^{14}C] ethylene oxide (2 mg/kg, single injection) was excreted in the urine of mice over 50 hours, most of which (≃40%) appeared within 18 hours of dosing (Jones and Wells, 1981). Two urinary metabolites, S-(2-hydroxyethyl) cysteine and N-acetyl-S-(2-hydroxyethyl) cysteine accounted for 9 and 33% of the dose, respectively. Within 6 hours, 1.5% of the dose was exhaled as $^{14}CO_2$ and 1% as unchanged ethylene oxide, but these are not maximum values (exhaled radioactivity was not sampled at later post-exposure times).

Martis et al. (1982) investigated the elimination kinetics of intravenously administered ethylene oxide in beagle dogs. Four dogs received

single 25 and 75 mg/kg injections of compound on separate occasions, and venous blood was sampled for ethylene oxide and ethylene glycol at 0, 0.08, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 7.0 and 24 hours after administration. It was found that the ethylene oxide cleared rapidly from the plasma, and that in all cases concentrations decreased to <2% of the zero-time value within 5 hours. The plasma concentration of ethylene oxide declined exponentially, and first order rate constants of 0.025 $\pm$ 0.006 min^{-1} and 0.023 $\pm$ 0.010 min^{-1} for the low and high dosages, respectively, were calculated from the plasma concentration-time data using a curve-fitting computer program. These rate constants corresponded to plasma half-lives of 29.3 $\pm$ 5.7 min and 36.5 $\pm$ 18.5 min. It was noted that the lack of significant differences in kinetic parameters (i.e., elimination rate constant, plasma half-life, apparent distribution volume, total body clearance) at the two dose levels indicates that the elimination kinetics are not dose-dependent. Ethylene glycol was formed quite rapidly following the administration of ethylene oxide, and plasma concentrations reportedly exhibited the characteristics of a metabolite in a one-compartment model; maximum plasma concentrations of ethylene gylcol were reached by 90 $\pm$ 24.5 minutes (25 mg/kg) and 120 $\pm$ 42.4 minutes (75 mg/kg) post-injection. Plasma concentration-time data for ethylene glycol following the intravenous injection of 35 and 106 mg/kg of ethylene glycol indicated half-lives of 177.1 $\pm$ 29.3 and 264.9 $\pm$ 90 minutes, respectively.

9.2. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

9.2.1. Effects in Humans.

9.2.1.1. ACUTE EXPOSURE -- Case reports indicate that headache, nausea, vomiting, dyspnea, and/or respiratory irritation are common effects of acute inhalation exposure (Greaves-Walker and Greeson, 1932; Blackwood and Erskine, 1938; von Oettingen, 1939; Anonymous, 1947; Sexton and Henson, 1949; Hollingsworth et al., 1956; Curme and Johnston, 1952; Salinas et al., 1981). Symptoms of poisoning have been reported to be delayed by several hours following exposure. Similar effects (e.g., marked nausea and profuse vomiting), as well as mild leukocytosis and blisters (discussed subsequently), developed in three chemical plant workers who were dermally drenched with 1% aqueous ethylene oxide solution (Sexton and Henson, 1949). Inhalataion exposure to high concentrations of ethylene oxide for brief periods has been associated with bronchitis, pulmonary edema, and emphysema (Theiss, 1963), as well as convulsive movements (Salinas et al., 1981). In a controlled study of the effects of ethylene oxide on human volunteers, Greaves-Walker and Greeson (1932) observed that ethylene oxide at ≈2200 ppm was slightly irritating to four subjects. At a 5-fold higher concentration, the compound had a definite effect on nasal mucosa within ≈10 seconds.

Three chemical plant workers, who were drenched with 1% aqueous ethylene oxide solution, developed marked nausea and profuse vomiting several hours following exposure (Sexton and Henson, 1949). Large vesiculated blisters developed in the areas of exposed skin, and two workers who had complete blood counts taken showed a mild leukocytosis.

Cobis (1977) reported a very low incidence of health-related effects due to exposure to ethylene oxide in Veteran's Administration medical facilities. Ethylene oxide was used for sterilization purposes in 162 hospitals and 7 outpatient clinics over an average of 8.2 years. Only 12 employees were

reported to have been involved in exposure incidents, and symptoms included watering eyes, nausea, and skin irritation. These cases are currently being followed to determine possible exposure sequelae. The average exposure concentration was not given, and it is presumed (although not stated) that the employees were exposed to ethylene oxide vapor.

The dermatological effects of ethylene oxide contact have been reviewed by Taylor (1977). Concentrated ethylene oxide evaporates rapidly from the skin and produces a freezing effect, resulting in burns ranging from first through third degree severity. Ethylene oxide gas retained in porous materials that have not been properly aired can produce skin irritation. Foot burns (Phillips and Kay, 1949) and hand burns (Royce and Moore, 1955), for example, have been observed in workers that wore ethylene oxide-sterilized rubber boots and rubber gloves, respectively. Biro et al. (1974) described a hospital incident in which 19 women were burned by surgical gowns and drapes that had been sterilized with ethylene oxide. Joyner (1964) found in a 2-year retrospective study of medical records that ethylene oxide plant workers had experienced exposure-related burns.

Sexton and Henson (1949) described the dermatological reactions that occurred in 6 men whose skin was directly exposed to a 1% water solution of ethylene oxide for periods ranging from 15 minutes to 3 hours. The men with the maximum exposures (2-3 hours) exhibited the most marked cutaneous effects (vesicular eruptions), but nausea and vomiting were the only systemic effects noted.

In a subsequent study, Sexton and Henson (1950) applied 1 to 100% solutions of ethylene oxide to the skin of 8 volunteer subjects for time intervals that ranged from 20 seconds to 95 minutes. The magnitude of skin

injury appeared to be related to the duration of contact and the concentration. The most hazardous concentrations of ethylene oxide were in the 50% range, since the manifestation arbitrarily examined in this study (minimal second degree burn demonstrated as an area of erythema with one or more superimposed vesicles) was produced in 45 seconds with this solution. The degree of skin injury was proportionately decreased at concentrations both greater and less than 50%. The lowest ethylene oxide concentration investigated (1%) produced a mild reaction (erythema) after 50 minutes of exposure. The milder skin reactions at concentrations >50% were attributed to the fact that the more concentrated solutions boil vigorously, thus preventing efficient skin penetration; the more dilute solutions lacked sufficient chemical to cause injury except after prolonged contact. Delayed skin sensitization developed in 3 of the 8 subjects.

Shupack et al. (1981) demonstrated that human skin reactions were directly related to total dose when exposures were to ethylene oxide that was retained in permeable materials. In tests with 12 unsensitized volunteers, it was found that patch materials that rapidly lose ethylene oxide (i.e., fabric or rubber) elicited few reactions, even at ethylene oxide levels as high as 3000-5000 ppm after 4 to 8 hours of contact. Patch materials that lost ethylene oxide slowly produced mild skin reactions (erythema plus edema) at material levels as low as 1700 ppm (PVC film) and 1000 ppm (PVC blocks) after similar durations of contact. Patches were removed from the subjects after 1, 2, 4 and 8 hours; it was found that most of the ethylene oxide diffused from the fabric and rubber patches within 1 hour and from the PVC film patches within 4 hours, but that the PVC block retained a substantial portion of ethylene oxide residue at 4 hours. In a subsequent experiment the same

subjects (i.e., those previously exposed in the first experiment) were exposed to patch materials that retained ethylene oxide the longest (thick PVC blocks and petrolatum applied to Webril pads). It was found that the reactions were most widespread when the ethylene oxide levels in these materials approximated 1000 ppm; erythema appeared in 10 of the 12 PVC block subjects and 10 of the 12 petrolatum subjects after 4-8 hours of contact, and cleared within 3 to 4 days. Reactions were not elicited at nominal levels of 10 or 100 ppm ethylene oxide in PVC or petrolatum, although one subject who had developed sensitivity to 1000 ppm ethylene oxide in PVC block in the first experiment showed a mild delayed reaction to 100 ppm. Little or no reaction developed to patches that contained ethylene oxide by-products that were present in the original patches (i.e., ethylene glycol and ethylene chlorohydrin), indicating that ethylene oxide was the toxic agent.

Although incidental findings in the Sexton and Henson (1950) and Shupack et al. (1981) experimental studies described above suggest that ethylene oxide can cause skin sensitization, Theiss (1963) did not observe sensitization in ethylene oxide plant workers who were challenged with a single dermal application of 1% after an average of 10.4 years of occupational exposure. Anaphylactic reactions have been observed in patients using ethylene oxide sterilized plastic tubing for hemodialysis (Poothullil et al., 1975) or cardiac catheterization (Pessayre and Trevoux, 1978). These symptoms included uticaria, breathlessness, and hypotension. In a follow-up study on a patient apparently sensitized to contact with hemodialysis tubing, Dolovich and Bell (1978) illustrated that this patient showed a positive skin test response to ethylene oxide-serum albumin conjugate, and produced <u>in vitro</u> histamine

release to this antigen. This response indicates that a specific IgE antibody to ethylene oxide had been induced in this patient.

Clinical reports of hemolysis following usage of ethylene oxide sterilized plastic tubings have also been published (Hirose et al., 1953; Clarke et al., 1966). Ethylene oxide, rather than a chemical reaction product, is implicated, since this type of effect can be prevented by extensive aeration of ethylene oxide sterilized plastic devices.

Ethylene oxide vapors in high concentrations are irritating to the eyes, but ocular contact with liquid ethylene oxide can cause severe burns. A workman exposed to ethylene oxide in an unstated manner was reported to have suffered a corneal burn, but healing was observed within 48 hours following a corneal denudement procedure (McLaughlin, 1946). Thiess (1963) described two cases of accidental eye injury with ethylene oxide. A nurse was exposed to a direct blast of ethylene oxide from a sterilizer cartridge, and developed an epithelial keratitis of the cornea within 3 hours. Within 24 hours, the eye was entirely normal. The second case involved a patient who received a squirt of liquid ethylene oxide (concentration not stated) in the eye and was treated immediately by extensive washing with water; this resulted only in irritation of the conjunctivae that persisted for about 1 day.

9.2.1.2. SUBCHRONIC AND CHRONIC EXPOSURE -- Limited information is available on toxic effects of subchronic or chronic ethylene oxide exposure in humans. The information is largely derived from clinical case reports from retrospective mortality studies.

Gross et al. (1979) reported on four cases of apparent ethylene oxide-induced neurotoxicity. This occurred in a plant in which a sterilizer was

found to have leaked for 2 months of operation. The exact levels of ethylene oxide were unknown, but the four individuals involved reported that they could intermittently smell the ethylene oxide gas, roughly indicating a level of >700 ppm. The length of exposure to ethylene oxide from the leaking sterilizer was 3 weeks for cases 1 and 2, 2 weeks for case 3, and 2 months for case 4. Three of the four cases had worked as sterilizer operators for more than 2 years and were exposed to ethylene oxide from the leaking sterilizer for 2, 3 or 8 weeks; the fourth had been an operator for only 3 weeks and was exposed for the duration.

The individual who had been exposed to ethylene oxide for 3 weeks had noted conjunctival and mucosal irritation and transient blunting of the senses of smell and taste, and developed headache, nausea, vomiting and lethargy that was followed by acute encephalopathy (recurrent major motor seizures at 20-30 minute intervals). Two of the other three operators were symptomatic (i.e., headaches, numbness and weakness in the extremities, fatiguability, one case of memory/thinking disturbances) and had abnormal neurological examination results that were consistent with sensorimotor neuorpathy. Nerve conduction studies were abnormal in the three operators, including the asymptomatic patient, and were compatible with the diagnosis of sensorimotor neuropathy. Removal from exposure resulted in relief of symptoms within 2 weeks. Two of the individuals returned to work under normal conditions of lower ethylene oxide exposure, but improvement in nerve conduction was not observed; significant improvement was noted, however, in the third individual who returned to work in a position without ethylene oxide exposure.

Jensen (1977) reported that three workers using ethylene oxide sterilizers were hospitalized for neuropathy of the lower limbs. Follow-up indicated that these effects were reversible.

Jay et al. (1982) found that the four sterilizer operators described above (Gross et al., 1979), who were exposed to excessive levels of ethylene oxide from a leaking sterilizer and developed neurologic abnormalities, subsequently developed cataracts. One of the operators was exposed to the leaking sterilizer for 2 months and developed bilateral cataracts during the following 2 1/2 years; cataracts were diagnosed in the other three operators upon examination ≈3 1/2 years after exposure to the leaking sterilizer. Eight other men whose work involved exposure to ethylene oxide sterilizers (6 of the 8 were sterilizer operators), but who were asymptomatic, were subjected to complete ocular examinations, but cataracts were not found. Four of the 12 men, two of whom had not worked on the leaking sterilizer, had increased central corneal thickness with normal endothelial cell counts when compared with a control group of 12 subjects of higher average age (41 vs. 33 years). None of the patients were examined before exposure to ethylene oxide, but the authors believed it unlikely that cataracts would occur by chance in persons in this age range, particularly because none of the patients had any systemic or ocular disease that might be associated with cataract formation.

Hemoglobin values and lymphocyte counts were reported to be significantly lower and higher, respectively, in a group of Swedish ethylene oxide production workers when compared with control subjects (Ehrenberg and Hallstrom, 1967). The design and results of this study are more completely described in Section 13.5, but it should be noted that the production workers

were reported to have been exposed for 2-20 years (average of 15 years) to an unknown level of compound.

Joyner (1964) conducted a retrospective morbidity study of 37 male ethylene oxide production plant workers. These workers varied in age from 29-56 years and were exposed to typical concentrations of 5-10 ppm (range 0-55 ppm) for 5-16 years (mean 10.7 years). Age-matched controls consisted of 41 operators (mean length of service, 11.7 years) assigned to other production units, who had past exposure to many different petrochemical industry agents, but had never exhibited clinical effects attributable to systemic chemical toxicity. As detailed in Sections 9.4 and 9.5, no significant increase in health problems relative to controls was found. This evaluation should have been sufficient to identify major toxic effects of extended low-level ethylene oxide exposure, although limitations in the design of the study, as well as an insufficient period of observation, preclude evaluation of more subtle toxic or carcinogenic responses.

An excess of deaths from specific causes (including all circulatory causes) other than certain malignancies (Section 9.5) was not observed in a group of 767 male ethylene oxide workers from the Texaco Chemical Company Plant in Port Neches, Texas (Morgan et al., 1981). These cohort members had been employed for at least 5 years between January 1955 and December 31, 1977, and an industrial hygiene survey of the plant performed in July, 1977, showed that the 8-hour time-weighted average exposure to ethylene oxide was well below 50 ppm.

Hogstedt et al. (1979) conducted a cohort study of mortality among 89 full-time ethylene oxide production workers, 86 intermittently exposed maintenance workers, and a group of 66 unexposed control workers during the

years 1961-1977. As described in Section 9.5, exposure patterns were quite complex; in addition to ethylene oxide (concentrations were generallay <50 mg/m^3), workers were exposed at different times to ethylene dichloride, ethylene chlorohydrin, ethylene, low concentrations of bis(2-chloroethyl)ether, as well as traces of other chemicals. It was found that the full-time exposed cohort showed a considerable excess mortality when compared with the expected number based on national statistics. The excess mortality arises mainly from increased mortality due to stomach cancer and leukemia (Section 9.5), but also from diseases of the circulatory system. When at least 1 year of exposure and 10 years or more of induction-latency time were required for inclusion, there were 12 observed deaths attributed to the circulatory system (9 due to coronary heart disease and 3 due to cerebrovascular disease), with an expected incidence of 6.3; this difference was statistically significant ($P<0.05$). The excess mortality is of the same magnitude in a restricted cohort of those with 10 or more years of employment in ethylene oxide production and 20 years of induction-latency time (7 observed, 2.2 expected).

9.2.2. Effects in Animals.

9.2.2.1. ACUTE EXPOSURE -- The acute toxicity of ethylene oxide is summarized in Table 9-1. Exposure of mice, rats, guinea pigs, rabbits, and dogs to lethal levels of ethylene oxide has produced symptoms of mucous membrane irritation and central nervous system depression, including lacrimation, nasal discharge, salivation, nausea, vomiting, diarrhea, respiratory irritation, incoordination, and convulsions (Sexton and Henson,

1949; Hollingsworth et al., 1956; Hine et al., 1981). Animals that survived the initial exposures showed subsequent bronchitis, pneumonia, and loss of appetite, with delayed symptoms of apathy, dyspnea, vomiting, paralysis (particularly of the hindquarters), periodic convulsions and death (Waite et al., 1930; Hollingsworth et al., 1956). Prompt deaths are usually due to lung edema; delayed deaths frequently result from secondary infections in the lungs, although general systemic intoxication may also be a factor (Hine et al., 1981).

Pathological findings following lethal exposure to ethylene oxide in mice, rats, and guinea pigs showed congestion of the lungs, hyperemia of the liver and kidneys, and gray discoloration of the liver (Waite et al., 1930). Pathological findings after dealyed death caused by ethylene oxide included emphysema of the lungs, fatty degeneration of the liver, cloudy swelling of the kidney tubules, and congestion of the spleen and brain (Hollingsworth et al., 1956). Intravenously-administered ethylene oxide caused congestion in all organs of the rabbit (Greaves-Walker and Greeson, 1932). Zamlauski and Cohen (1976) have reported that infusion of ethylene oxide in the rat at blood levels of 0.45 to 4.5 mg/mℓ produced a significant decrease (≈30%) in glomerular filtration rate, which indicates effects of ethylene oxide on kidney function.

Ethylene oxide in 10% and 50% aqueous solutions produced hyperemia and edema in shaved rabbit skin when applied through cotton pads for 1 to 60 minutes (Hollingsworth et al., 1956). Bruch (1973) studied the dermal irritation properties of 2 to 10% aqueous ethylene oxide solutions in guinea pigs and rabbits. Subcutaneous injection in the guinea pig resulted in ecchymoses and skin thickening, while intradermal injection and topical

application in the rabbit resulted in mild irritation. Topical or intradermal administration of 1% ethylene oxide (0.5 mℓ), thrice weekly for 3 weeks, did not result in sensitization in guinea pigs (Woodward and Woodward, 1971).

McDonald et al. (1977) studied the ocular effects of varied concentrations of ethylene oxide in saline applied repeatedly over a 6-hour period to the eyes of rabbits. They observed a dose-dependent increase in congestion, swelling, discharge, iritis, and corneal cloudiness, indicating the irritating effect of ethylene oxide on mucous membranes and corneal epithelium. The maximum nondamaging concentration for this time period was 0.1% ethylene oxide. In another study of ocular irritation in rabbit eyes, Woodward and Woodward (1971) found slight irritation following a single application of 10% aqueous ethylene oxide (duration of exposure unknown), and a no-effect concentration of 2.1% ethylene oxide was determined. The higher values determined in this study are probably the results of a different mode of application and, therefore, different duration of exposure.

9.2.2.2. SUBCHRONIC AND CHRONIC EXPOSURE -- The subchronic toxicity of inhaled ethylene oxide has been investigated in a variety of different animal species by different routes of exposure (Hollingsworth et al., 1956; Jacobson et al., 1956). As summarized in Table 9-2, symptoms of poisoning and pathologic changes are similar to those observed in acute studies with lung, kidney, and liver damage occurring, and with neuropathy of the hindquarter and testicular tubule degeneration occurring in some species.

Hollingsworth et al. (1956) observed neurotoxic effects in animals following inhalation exposure to 357 ppm ethylene oxide vapor for several weeks (the exposure for each species is presented in Table 9-2). Rats,

TABLE 9-2

Subchronic Toxicity of Ethylene Oxide

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	20 rats (10/sex) 16 guinea pigs (8/sex) 5 mice (female) 2 rabbits (1/sex) 1 monkey (female)	841 ppm	up to 8 in 10 days (7 h/d; 5 d/wk)	Death in all animals. Pathologic changes in lungs, liver and kidneys similar to those in acute poisoning.	Hollingsworth et al., 1956
inhalation	30 mice (female, white) 20 rats (male, white)	400 ppm	30 (6 h/d; 5 d/wk)	Weight loss, reddish nasal discharge, diarrhea, labored breathing, weakness of the hind legs, and some deaths (13/20 exposed and 0/20 control rats, and 24/30 exposed and 3/30 control mice). Fifteen additional rats or mice were examined pathologically; changes were limited to a few cases of hemosiderosis in the spleen that occurred late in the exposure period.	Jacobson et al., 1956
inhalation	20 rats (10/sex) 10 mice (female)	357 ppm	33-38 (7 h/d; 5 d/wk)	Death in 10/10 mice (33 exposures) and 18/20 rats (38 exposures) caused by secondary respiratory infections. Impairment of sensory and motor function in rats prior to death, resulting in reversible hind leg muscle paralysis and atrophy.	Hollingsworth et al., 1956
inhalation	16 guinea pigs (8/sex)	357 ppm	123 in 176 days (7 h/d; 5 d/wk)	Growth depression, degeneration of the testicular tubules with replacement fibrosis (males), slight fatty degeneration of the adrenal cortex (females). No nervous system effects or mortality.	Hollingsworth et al., 1956

TABLE 9-2 (cont.)

Route	Species	Concentration	Number of Exposures	Effects	Reference
inhalation	2 monkeys (1/sex) 2 monkeys (males)	357 ppm 357 ppm	38-41 in 60 days 94 in 140 days (both schedules 7 h/d; 5 d/wk)	Growth depression and characteristic neurological impairment (e.g., hind limb paralysis and muscular atrophy, poor or nonexistent knee reflex, extensor reflex and hindquarter/genitalia pain perception). No histopathologic effects of exposure.	Hollingsworth et al., 1956
inhalation	3 dogs (male, Beagle)	290 ppm	30 (6 h/d; 5 d/wk)	Two of 3 exposed dogs showed toxic signs that included vomiting, slight tremors, transient weakness of the hind legs and decreases in red blood cells, hemoglobin, and hematocrit. Hematologic parameters normal in control dogs. Lungs showed congestion and alveolar collapse and fatty changes in the hindquarters were consistent with muscular atrophy.	Jacobson et al., 1956
inhalation	20 rats	204 ppm	127-133 in 185-193 days (7 h/d; 5 d/wk)	Weight loss, some deaths with effects on lungs (congestion, hemorrhage, emphysema, atelectasis) kidneys and testes (slight degeneration of some tubules) (slight cloudy swelling of tubules)	Hollingsworth et al., 1956
inhalation	8 guinea pig 4 rabbits (2/sex) 2 monkeys (female)	204 ppm	127-157 in 176-226 days (7 h/d; 5 d/wk)	No effect on growth or mortality. Evidence of paralysis/muscular atrophy in the rabbits and monkeys. Slight edema and congestion noted in rabbits' lungs.	Hollingsworth et al., 1956

ABLE 9-2 (cont.)

Route	Species	ncentration	Number of Exposures	Effects	Reference
inhalation	20 rats 8 guinea pigs 4 rabbits (2/sex) 2 monkeys (females)	113 ppm	122-157 in 176-226 days (7 h/d; 5 d/wk)	Growth depression and a moderate increase in lung weights in rats were the only adverse treatment-related effects noted.	Hollingsworth et al., 1956
inhalation	30 mice (females, White) 20 rats (male, White)	100 ppm	130 (6 h/d, 5 d/wk)	No clinical signs of toxicity or treatment related mortality (3/20 exposed and 3/20 control rats, and 8/30 exposed and 4/30 control mice died). No significant pathologic changes in additional groups of 60 rats or mice.	Jacobson et al., 1956
inhalation	3 dogs (male, Beagle)	100 ppm	130 (6 h/d, 5 d/wk)	Normochronic anemia (decreased RGC, Hb and hematocrit) indicated in 1 and suggested in 1 of 3 dogs. No changes in the 3rd exposed dog, or in control dogs.	Jacobson et al.,
inhalation	20 rats 8 guinea pigs 4 rabbits (2/sex) 10 mice (female)	49 ppm h/d, 5 d/wk)	127-131 in 180-184 days (7 h/d, 5 d/wk)	No adverse effects as judged by general appearance, behavior, mortality, growth, final body and organ weights, and gross or microscopic pathologic examination.	Hollingsworth et al., 1956
oral (intubation)	5 rats (female)	100 mg/kg	15 doses in 21 days (5 d/wk)	Weight loss, gastric irritation and slight liver damage, but no mortality.	Hollingsworth et al., 1956
oral (intubation)	rats (female)	, 10 or 3 mg/kg	22 doses in 30 days (5 d/wk)	No evidence of adverse effect as indicated by growth, hematology, blood urea nitrogen determinations, organ weights or gross microscopic pathology.	Hollingsworth et al., 1956

TABLE 9-2 (cont.)

Route	Species	Concentration	Number of Exposures	Effects	Reference
s.c.	rats	54 mg/kg	30	Weight loss, injection site hemorrhage and inflammation.	Hollingsworth et al., 1956
s.c.	rats	18 mg/kg	30	No observed effect.	Hollingsworth et al., 1956
s.c.	dogs	36 mg/kg	30	Anemia, hyperplastic bone marrow, and ectopic hematopoiesis.	Woodward and Woodward, 1971
i.v.	dogs	36 mg/kg	21	No observed anemia, other observations not mentioned.	Balazs, 1976

d = day; h = hour; wk = week

rabbits, and monkeys showed paralysis and atrophy of the muscles of the hind limbs. These effects were reversible after discontinuation of exposure for 100 to 132 days. Special studies on monkeys were carried out with repeated (38-94) exposures to this level of ethylene oxide. Knee jerk reflexes became very weak, pain perception in the hind quarters decreased, the cremasteric reflex was elicited, and the extensor reflex of the palms of the hind feet was abolished. Impairment of both sensory and motor function at the lumbar and sacral level of the spinal cord was indicated. Exposure of monkeys to a lower level of ethylene oxide (204 ppm for 176-226 days) produced partial paralysis and some muscular atrophy of the hind legs with moderate suppression of the leg reflexes. The Babinski reflex was present after this lower level exposure to ethylene oxide.

Preliminary results of a chronic inhalation study conducted by NIOSH have been reported (Lynch et al., 1982). Male F344 rats (80 per treatment group) and male cynomolgus monkeys (12 per treatment group) were exposed to either 50 ppm or 100 ppm ethylene oxide for 7 hours/day, 5 days/week for 24 months. Additional details of the epxerimental design are presented in Section 9.5 of this study, but it should be noted that the rats were included primarily for carcinogenicity evaluation, and that the monkeys were used to determine target organ toxicity. A number of indices were evaluated including body weights, hematology, clinical chemistry, urinalysis, opthalmology, pulmonary function, neurophysiology, neuropathology, gross and histopathology, sister chromatid exchange rates, and chromosomal aberrations in peripheral cymphocytes. The results that are currently available are summarized below.

As detailed in Section 9.3, weight gain throughout most of the study and survival were significantly depressed in the rats at both exposure levels

(Lynch et al., 1982). Weight gain was significantly depressed in the treated monkeys beginning at week 25. The livers and spleen of the rats were the only organs in which histopathological evaluations have been completed, but the preliminary terminal sacrifice spleen data indicate a dose-related induction of leukemia (Section 9.3). Hematologic anlayses showed no statistically significant change in red blood cell count in the treated rats, but white blood cell counts were highly variable and reflected the presence of the leukemia. There were no differences in the red or white blood cell counts in either of the monkey groups, although increased frequencies of chromosomal aberration and sister chromated exchanges were observed in the peripheral lymphocytes of these animals.

Significant hematological effects (i.e., anemia) have also been observed in ethylene oxide-exposed dogs. Jacobson et al. (1956) found decreased red blood cell counts, hemoglobin, and hematocrit in 2 of 3 beagle dogs that were exposed to 292 ppm ethylene oxide vapor for 6 hours/day, 5 days/week for 6 weeks. Definite (1 dog) and suggestive (1 dog) hematologic effects of the same type were also observed in 2 of 3 dogs that were similarly exposed to 100 ppm ethylene oxide for 6 months (Jacobson et al., 1956). Woodward and Woodward (1971) demonstrated a dose-related increase in anemia in dogs that were administered 6-36 mg/kg ethylene oxide in 30 daily subcutaneous injections. Pathologic examination showed hyperplastic bone marrow and ectopic hematopoiesis. Balazs (1976) was unable to repeat these findings in beagle dogs, however, with an ethylene oxide-glucose solution administered intravenously over the same concentration range in a 21-day study.

An oral feeding study using 10% ethylene oxide in olive oil was performed on rats (Hollingsworth et al., 1956). Rats fed 100 mg/kg ethylene oxide in 15

doses over 21 days showed marked weight loss, gastric irritation, and slight liver damage. Feeding of 30 mg/kg in 22 doses produced no observable adverse effects.

9.2.3. Summary of Toxicity. The primary effects of acute inhalation exposure to high concentrations of ethylene oxide gas are respiratory tract irritation and central nervous system depression. Headache, vomiting, dyspnea and diarrhea are common systemic effects of vapor exposures in humans, and excessive exposures have produced bronchitis, pulmonary edema, and convulsive movements. Similar effects have been observed in a variety of animal species, but paralysis (particularly of the hindquarters) and periodic convulsions frequently preceded death. Death in ethylene oxide-exposed laboratory animals is usually due to lung edema or secondary infections in the lungs, and postmortem pathologic findings in other organs include widespread hyperemia and congestion (e.g., liver, kidneys, spleen) and fatty degeneration (liver).

Dermatological effects of ethylene oxide following skin contact in humans following accidental or experimental exposure include edema, erythema, and vesiculation with possible bleb formation. These changes typically progress in the above sequence, vesicle formation is usually delayed (e.g., 6-12 hours), the magnitude of skin injury appears to be related to concentration and duration of contact, and the effects are reversible. Concentrated ethylene oxide evaporates from the skin resulting in a freezing effect, but more dilute solutions penetrate the skin more effectively, resulting in chemical burning; weak solutions lack sufficient chemical strength to cause injury except after prolonged contact. Skin burns have also been caused by residual ethylene oxide in clothing or footwear that was treated or

accidentally contaminated with the compound. Sensitization has also been associated with repeated dermal exposure to ethylene oxide at the sites of contact. Similar dermal irritative effects have been observed in experimentally exposed rabbits and guinea pigs, but sensitization was not demonstrated by topical or intradermal administration in guinea pigs. High concentrations of ethylene oxide vapors are irritating to the eyes of humans and animals, and direct ocular contact with liquid ethylene oxide can produce corneal injury.

Case reports indicate that neurological effects (e.g., headache/vomiting, sensorimotor neuropathy, seizures) and ocular effects (e.g., cataracts) may be primary effects of limited repeated exposure to high levels of ethylene oxide, and hematological effects (reduced hemoglobin and elevated lymphocytes) have been noted in chronically exposed ethylene oxide production plant workers. Retrospective morbidity and mortality studies of ethylene oxide production workers do not suggest, however, chemical related non-neoplastic toxicity. Subchronic exposure of different species of animals to ethylene oxide by different routes of exposure produced effects similar to those seen in acute studies; symptoms of poisoning primarily reflect neurotoxic action (e.g, hindquarter neuropathy) and pathologic changes generally occur in the lungs, kidney, and liver (e.g., congestion and degenerative changes), although testicular effects (e.g., tubule degeneration) and hematologic effects (e.g., anemia) have been observed.

9.3. TERATOGENICITY AND REPRODUCTIVE TOXICITY

Batelle Pacific Northeast Laboratories (Hackett et al., 1982) conducted teratology and reproductive studies for the National Institute for

Occupational Safety and Health investigating the effects of ethylene oxide (EtO) produced by inhalation exposure. Rabbits and rats were exposed to a single dose of EtO 150 ppm, (Union Carbide, Linda Lot No. 01901, 99.7% pure) both prior to, and during the time of organogenesis. Thirty New Zealand white rabbits per group were exposed in three different regimes (filtered air alone, EtO exposure on days 7-19 of gestation, and EtO exposure on days 1-19 of gestation). Forty-one Sprague-Dawley CD rats per group were exposed according to four different schedules (filtered air alone, EtO exposure on days 7-16 of gestation, EtO exposure on days 1-16 of gestation, and EtO exposure three weeks prior to mating and through days 1-16 of gestation).

In the rabbits, no toxic effects were observed in the mothers (i.e., changes in body weight, organ weight, histopathological changes in the organs). In addition, there were no decreases in the percentage of pregnant animals nor was there any indication of adverse effect on the fetus (i.e., decreases in fetal body weight, crown rump length, sex ratios or morphologic alterations).

In the rats, maternal toxicity was observed with sporadic decreases in food consumption, decreases in body weight, increases in kidney and spleen weights with increases in spleen weights roughly proportional to the duration of exposure. Adverse effects were also observed in the developing conceptus. There was an increase in resorptions in animals exposed both pre- and post-gestationally with a trend for early midgestational resorptions. In addition, fetal body weight, decreases in crown-rump length and increases in incomplete skeletal ossification were observed in all EtO exposed offspring, and this was especially pronounced in animals exposed both pre- and postgestationally. It was concluded from this study that exposures of 150 ppm in rats caused significant adverse effects in both the mother and developing fetus. However,

since only one dose was used in this study, it is not known whether these developmental effects would occur in the absence of maternal toxicity.

Because of concerns over adverse reproductive effects which could occur as a result of exposure to EtO or EtO reaction products left on improperly degassed surgical supplies, LaBorde and Kimmel (1980) conducted studies on the effects of EtO administered intravenously. CD-1 mice in four replicates of three treatment groups (10 animals per group) were treated with 0, 75, 150 mg/kg EtO (Eastman Organic Chemicals Co. purity not stated, EtO was injected in 5% dextrose solution). The animals were exposed in the following treatment periods of gestation; days 4-6 (period I), days 6-8 (period II), days 8-10 (period III) and 10-12 (period IV).

Clinical signs of maternal toxicity (weakness, labored breathing, tremors and death) were observed in animals injected with 150 mg/kg EtO on gestational days 4-6 (Period I), days 8-10 (Period III), and days 10-12 (Period IV) but not on days 6-8 (Period II). Decreases in mean maternal body weight gain were observed in animals in period I, period III, and period IV and was accompanied by decreases in the mean number of live fetuses in periods III and IV. Embryotoxicity, as manifested by significant reductions in mean fetal weight was observed in all four periods at the 150 mg/kg dose. There was no significant change in the mean number of implants per litter, but there was reduction in the mean number of live fetuses per litter (and also an increase in the number of dead and resorbed offspring) in periods III and IV at the 150 mg/kg level. An increase in the percent of malformed fetuses/litter were noted in periods II, III and IV at 150 mg/kg level, but in period III the incidence did not achieve statistical significance. It was concluded that the EtO exposure, under these conditions, was selectively affecting the

development of the conceptus (skeletal malformations and embrotoxicity) since EtO exposure in period III (days 6-8 of gestation) produced malformations and embryonic death while not affecting the mother (no clinical signs of toxicity). However, this conclusion was tempered somewhat because maternal deaths were observed in group III before and after days 6-8 of gestation. Although there was no dose-response relationship in the severity of adverse effects in either the mother or fetus, the types of malformations in periods II and III appeared to follow a developmental pattern. The authors reported that, in animals treated on days 6-8, cervical and upper thoracic vertebrae malformation were observed. Animals treated on days 8-10 had defects primarily in the lower thoracic region.

Another study by the same investigators (Kimmel et al., 1982) evaluated the reproductive effects of intravenous injections of EtO in rabbits. This study was reported briefly in a poster session presented at the 1982 Society of Toxicology meeting. New Zealand white rabbits were intravenously injected in two treatment regimes; 0, 9, 18 or 36 mg/kg EtO (source and purity not reported) on days 6-14 of gestation, or 0, 18 or 36 mg/kg on days 6-9 of gestation. Seventeen to twenty-one animals were examined in the group exposed on days 6-9, eighteen to twenty-four animals examined in the group exposed on days 6-14.

Maternal toxicity was observed in both exposure groups, with more severe effects observed in the groups treated on days 6-14 than on days 6-9 of gestation. Significant decreases in maternal weight gains were observed during the entire treatment at the 18 and 36 mg/kg level. These decreases included both decreases in pregnancy weight gains and decreases in absolute weight gains (weight gained during pregnancy minus uterine weight). No

embryotoxic effects were observed in the day 6-9 treatment groups, however in the 6-14 day treatment group a significant dose-related trend for decreased numbers of live fetuses/litter and resorptions/litter were observed. At the 36 mg/kg level, the incidence of resorptions/litter was statistically significantly different from control levels. Therefore, the authors concluded that intravenous administration of EtO in rabbits produced embrotoxicity, however only at doses which also produce significant maternal toxicity.

LaBorde et al. (1982) presented data at the 1982 Society of Toxicology meeting regarding the teratogenic effects of ethylene chlorhydrin (ECH), a reaction product of EtO in mice and rabbits. Since ECH is produced by the interaction of EtO and chloride ions, it is a residue of EtO that could be left on medical devices after improper degassing of EtO during sterilization. Forty-one to sixty-five CD-1 mice were intravenously injected with 60 mg/kg or 120 mg/kg ECH (source not reported, ECH was injected in 5% sterile dextrose) on days 4-6, 6-8, 8-10, or 10-12 of gestation. Seventeen to twenty-two New Zealand white rabbits were intravenously injected with 9, 18, or 36 mg/kg ECH on days 6-14 of gestation.

In this study, no adverse effect was observed in either the mother or the fetus of the New Zealand white rabbits. However, in CD-1 mice, clinical signs of toxicity (weight loss of 1 gram or more in 24 hours) were observed in the mothers in all treatment periods at the 120 mg/kg dose. Maternal weight gain during the entire treatment period and during pregnancy were significantly reduced at the 120 mg/kg level on days 4-6, 6-8 and 10-12. There was also a trend for increased resorptions/litter in animals exposed on days 4-6 and 10-12 at the 120 mg/kg level. At the 120 mg/kg dose for all treatment periods, there was a significant decrease in mean fetal weight/litter. At the 60 mg/kg

level, in animals exposed on days 8-10, there was a significant reduction in fetal weight in the absence of maternal toxicity. The authors reported a trend for an increase in the number of malformed fetuses treated on days 8-10 however, the incidence of this effect did not achieve statistical significance.

The conclusion reached by Laborde et al. (1982) was that ECH administered intravenously in mice produced embryo/fetal toxicity and possibly a slight increase in malformations at maternally toxic doses. However, at the 60 mg/kg level, in animals treated on days 8-10, fetal weight reductions occurred without maternal toxicity. Therefore, it was concluded that ECH may pose a hazard specific to the developing conceptus.

Verret (1974) investigated the toxic and teratogenic effects of ethylene chlorohydrin (ECH) in the developing chick embryo. ECH (source and purity not reported) was administered via the air cell during a pre-incubation period (0 hour) and after 96 hours of incubation at levels equivalent to 10, 25, 50, 100, and 200 mg/kg. The control groups were treated with a water vehicle or left untreated. One hundred eggs were used per group.

Ethylene chlorohydrin was found to be toxic in this system with significantly increased mortality (no hatch) at levels $\geq$25 mg/kg at the 0 hour exposure, and at levels $\geq$12.5 mg/kg at the 96 hour exposure. Statistically significant increases in structural anomalies were observed at two dose levels (50 and 100 mg/kg) at the 0 hour exposure, and at four dose levels (12.5, 25, 50, and 100 mg/kg) at the 96 hour exposure. The significance of these observations in terms of mammalian effects however, is not known since teratogenic effect in chick embryos may not be predictive of mammalian effects.

REPRODUCTIVE EFFECTS

The Carnegie-Mellon Research Institute (Snellings et al., 1982) conducted a one-generation study evaluating the effects of EtO exposure due to inhalation. Thirty male and female Fischer-344 rats were continually exposed to 10, 33 and 100 ppm ethylene oxide with the control animals exposed to filtered air. Prior to cohabitation, all groups were initially exposed to EtO for 6 hours/day, 5 days/week for 12 weeks. After one week of cohabitation females with vaginal plugs were removed, and the other animals were rotated with a different male to allow for mating for another week. At the end of two weeks all male and female animals were separated. The males were then exposed to EtO for 6 hours/day, 7 days/week for an additional three weeks. The females were exposed for 6 hours/day, 7 days/week from day one through day nineteen of gestation. On the twentieth day of exposure, females not pregnant were sacrificed. The pregnant females were allowed to deliver and five days after parturition were again exposed to EtO for 6 hours/day, 7 days/week until day 21 postpartum.

The following criteria were used to establish fertility. If a female produced a litter, or if gross examination revealed implantation sites after staining, then she was considered fertile. Any female, not becoming pregnant after two different matings was considered infertile. If the male impregnated a female after the first mating, then he was considered fertile. Any male failing to impregnate a female in two different mating periods was considered infertile. By this criteria, females exposed to 100 ppm had a higher incidence of infertility after mating with a male of proven fertility. However, this incidence did not achieve statistical significance. In the males there was no increase in infertility. In the 100 ppm group,

significantly more females had lengthened gestational period (time of vaginal plug to litter) than the control, 10 or 33 ppm groups. The control, 10 and 33 ppm groups had gestational periods of 22 days, while the 100 ppm group had gestations ranging from 22 to 31 days (7/14 rats had 22 day gestation, 4/14 rats had 23 day gestation, 3/14 rats had greater than 25 day gestation). However, since most of the animals did not have extensively long gestational delays, it is not clear whether this lengthening of gestation represented a true adverse biological effect.

In this study (Snellings et al., 1982), the number of pups was significantly reduced with a decrease in the number of implantation sites at the 100 ppm level. However, of the surviving pups, there was no effect on survival after parturition. In the parental generation, there was no adverse effect on bodyweight or organ histology (testes, epididymides, accessory sex glands, cervix, uterus, ovaries, oviducts, mammary tissues). In the F_1A generation, ≈25% of the animals suffered from sialoacryoadenitis virus infection but this infection appeared to be unrelated to the EtO exposure.

It was concluded from this study (Snelling et al., 1982) that EtO administered to rats has the potential to disrupt reproduction by causing an increased incidence of embryolethality. However, this embryotoxic effect was only observed when the animals were exposed to the highest dose (100 ppm) and not at the lower doses (10,33 ppm) of EtO.

TESTICULAR EFFECTS

Hollingsworth et al. (1956) investigated the acute and chronic toxicity of EtO in a variety of animal species. Positive responses related specifically to the male reproductive system were observed in hamsters and

rats. Eight guinea pigs were exposed to 357 ppm ethylene oxide (commercial grade EtO, 97-98.6% pure by weight) and received 123 seven hour exposures over a 176 day period. There was only moderate growth reduction in the males; however, appreciable degeneration of testicular histology was noted. In another phase of this experiment, both rats and hamsters were exposed to 204 ppm EtO, 7 hours/day, in 122 to 157 exposures given over an experimental period of 176 to 226 days. Only slight but not statistically significant decreases in testis weight of rats and guinea pigs were observed. However in rats, there was histological evidence for a degeneration of testicular tubules.

A recent study sponsored by the NIOSH described the effects of inhaled EtO on semen production in Cynomologus (Macaca fasicularis) monkeys (Lynch et al., 1983). The monkeys were exposed by inhalation to 50 and 100 ppm EtO, (Union Carbide, 99.7%) 7 hours/day, 5 days/week for 2 years. In the preliminary range-finding study, only two animals per group were used. Testicular weight was diminished in animals exposed to 100 ppm EtO but were only marginally decreased in those exposed at the 50 ppm level. Similar decreases in epididymal weights were also reported. Sperm motility was significantly reduced at the 50 and 100 ppm level, both in terms of the percent motile sperm and the ability of the sperm to travel a given distance in a given time (drive range). In the preliminary study, the sperm concentration was decreased at the 50 and 100 ppm level. In a subsequent study with larger numbers of monkeys per group (8 or 9), the same types of adverse testicular effects were observed. In this study, there was a 30% decrease in sperm concentration, 30% reduction in motile sperm, and a 3-4 fold

increase in drive range when the animals were exposed to 50 and 100 ppm EtO. However, there was no effect on sperm head morphology (Lynch et al., 1983).

In another study relevant to the effects of EtO on the testis, radiolabeled EtO was detected in autoradiograms of mice gonads (epididymis and testis) 20 minutes after intravenous injection (Appelgren et al., 1977). Radioactivity was found in the epididymis up to 24 hours after injection. The results of the dominant lethal mutagenicity test were negative although inadequacies in this study prevent a firm conclusion from being made (see a discussion of this study in Mutagenicity Section). This study is relevant to testicular effects because it establishes that EtO has access to the gonads.

ADVERSE REPRODUCTIVE OUTCOME IN HUMANS

There is little information relating to the effects of EtO on the reproductive system in humans. In one study a comparison was made between the health of 37 male employees involved in EtO production with 41 men who worked in other production units (Joyner, 1964). This study evaluated many health endpoints including genitourinary problems. The mean exposure period was 10.7 years with a general level of exposure on the order of 5 to 10 ppm. The range of exposure levels varied from 0-55 ppm. The health survey of the workers considered the following information: 1) the number of sick days taken in a 10 year period with information on the etiology and duration of the illness, 2) any medical diagnosis entered into the medical records and confirmed by an outside physician, 3) any visits to the Medical Division related to respiratory, gastrointestinal or genitourinary problems. In this study there was a higher incidence of chest abnormalities and a higher incidence of absenteeism attributable to gastrointestinal and genitourinary cause.

However, the higher incidence of absenteeism was attributable to a single individual in each category. Therefore, it was concluded that long term exposure to EtO had no adverse health effect on the men involved in EtO production. However, since this study did not deal specifically with reproductive health problems, it is of limited value in determining the potential of EtO to cause adverse reproductive effects.

A study by a Russian investigator (Yakubova, 1976) reported that female workers involved in EtO production experienced a number of gynecological and obstetrical problems. These problems included diseases of the cervix, inflammation of the uterus, obstetric anamnesis, (this word, as well as others may have been incorrectly translated) hypertonic disease, anemia, toxicosis, and shortened pregnancies. In this study, the observations were reported in an anecdotal manner with no presentation of actual data or description of methodologies. Therefore, it is of little value in the scientific review of adverse reproductive effects.

Holmberg (1979) and Holmberg and Nurminen (1980) reported case studies of a mother exposed to a variety of organic solvents. These studies describe an adverse reproductive outcome of a woman exposed to alkylphenol and dyes as well as EtO. The same woman may have been described in both these reports, however, this was not made clear in the articles. Both reports describe an infant born with hydrocephalus and Holmberg (1979) described a child with additional malformations (cleft palate, double uterus, polydactyly). These reports are not useful in establishing causal relationship between EtO exposure and congenital malformations because EtO was not the only chemical involved. A larger population size would have to be evaluated before such an association can be established.

An epidemiology study has been conducted concerning the effects of EtO exposure on pregnancy outcomes in nursing personnel. This report is the only one which adequately evaluates the possible causal association between EtO exposure and adverse human reproductive effects, and has been reviewed in depth by an Environmental Protection Agency epidemiologist (Margosches, 1983). In cooperation with the Finnish investigators the data has been critically analyzed and reviewed. The following is the text of this evaluation: "In November, 1982, K. Hemminki et al. published a study of "Spontaneous abortions in hospital staff engaged in sterilizing instruments with chemical agents" in the British Medical Journal. This study, encompassing all Finnish sterilizing staff at that time, claimed adjusted spontaneous abortion (s.a.) rates of 16.7% for "exposed" and 5.6% for nonexposed pregnancies among these staff. The report singled out ethylene oxide, glutaraldehyde, and formaldehyde use and suggested concentrations as low as 0.1-0.5 ppm EtO might have been associated with adverse outcomes. In particular, among hospital-discharge-corroborated pregnancies, the ethylene-oxide-exposed s.a. rate (22.6) significantly exceeded the control s.a. rate (9.2). Also rates among all pregnancies exposed to EtO or to glutaraldehyde differed significantly from rates among pregnancies not exposed.

This study encompassed staff employed in 1979 at hospitals throughout Finland (including tuberculosis sanitoria and mental hospitals). It was a cohort study looking at past events; determination of exposure status was based on the responses to two questionnaires. The unit for most statistical tabulations and analyses was the pregnancy; while not uncommon in the literature, such a basis cannot take into consideration the relatedness of sibling births or repeated miscarriages of a single woman.

The cohorts, sterilizing staff and controls (hereafter also called group (1) and (2), were identified by the head nurses at the study hospitals. The former were named in response to a first questionnaire that also queried chemical sterilizing agent use history at each hospital. The latter were obtained as members of cluster samples from auxiliary nurses in departments (not including chemical sterilization, x-ray, or surgery) at the time of distribution of a second questionnaire that focussed on pregnancy and employment history. The investigators obtained a very high return rate (92% among sterilizing staff, 91% among auxiliary nurse controls) and studied the 645 (63%) ever pregnant women among sterilizing staff and the 574 (55%) ever pregnant women among controls. The 17 male sterilizing staff were not studied.

While the study population was selected on the basis of hospital employment as sterilizing staff (1) or non-sterilizing-staff auxiliary nurses (2), Dr. Hemminki classified pregnancies of each group (1) member according to likelihood of exposure and the agent(s) present in order to make finer comparisons. He considered all pregnancies occurring after the first use of EtO at a hospital to be exposed to EtO unless an individual did not work at the hospital during a particular pregnancy; similarly for glutaraldehyde and formaldehyde. This was a fairly conservative classification. This study design precluded the examination of the questions whether spontaneous abortions were related to an individual's ever having been exposed to an agent.

Another limiting factor of the study design was the characterization of individual exposures in purely qualitative terms. Dr. Hemminki believes that typical exposures have averaged <1 ppm (measured by gas-tight syringes). He

bases this belief on papers published by colleagues at the Institute of Occupational Health covering a 3-year period overlapping the close of the study period, on the unchanged instrumentation of EtO use over its 20 or so years in Finland, and on the measurement method's 1 ppm detection limit. He did not, however, make any unwarranted inferences regarding possible dose-response relationships. Nevertheless, he did find sizeable differences in adjusted spontaneous abortion rates in both nurses and sterilizing professionals (these are 2 education levels of sterilizing staff) between ethylene-oxide-exposed and non exposed age-adjusting ($\leq$30) the rates among discharge-registry-identified pregnancies, the EtO-exposed s.a. rate (16.1) also exceeded the rate (9.4) in control pregnancies but no longer significantly. (Certain of the pregnancies occurring during 1973 to 1979 could be cross-identified through a national hospital discharge register and parallel analyses were carried out on this set and the total questionnaire-obtained set).

On the whole, this study and its report paid close attention to the possibilities and consequences of such typical epidemiologic afflictions as reporting and recall bias. Additionally, the methodology for statistical analysis, based on rates adjusted for such concommitant variables as age, parity, and decade of pregnancy by logistic regression, is sound (although there may be good reasons to investigate a finer categorization of age). While a "per woman" analysis, the analytic methods for incorporating an individual's pregnancy history have not yet been perfected or standarized.

Unfortunately, the investigators introduced a possible source of bias through telling the supervisory nurses (who identified group (1) and selected group (2)) the purpose of the study, including the names of the agents of

interest. Another shortcoming is the impreciseness with which hospital exposure history was determined. Finally, although the authors planned a priori to investigate relationships between EtO and spontaneous abortions and, possibly, other adverse pregnancy outcomes, the underlying relatedness of multiple pregnancies and of certain of the analyses (e.g., regroupings of the same pregnancies to look at different exposures) dilute the strength of any associations perceived in this study. Notwithstanding these limitations, this work is sufficiently suggestive to support further study of the possible associations between EtO exposure and adverse pregnancy outcomes or other reproduction effects." (Margosches, 1983).

SUMMARY OF TERATOGENICITY AND REPRODUCTIVE TOXICITY

The potential of ethylene oxide (EtO) to cause teratogenic or adverse reproductive effects has been examined in four animal species (mouse, rat, rabbit, monkey) by two routes of administration (inhalation and intravenous) (Table 9-3).

In a teratology study, Hackett et al., 1982 reported that rats exposed to a single 150 ppm dose of EtO displayed both maternal toxicity (decreases in food consumption, decreases in body weight, increases in kidney and spleen weights) and toxicity to the developing conceptus (increases in resorptions, decreases in fetal weight, decreases in crown-rump length, and increases in incomplete skeletal ossification). However, similar effects were not produced in rabbits exposed to 150 ppm EtO in this study.

LaBorde and Kimmel, 1980, administered 75 and 150 mg/kg EtO to CD-1 mice for several gestational intervals. The animals displayed signs of maternal and fetal toxicity at the highest dose level. There were maternal deaths with

TABLE 9-3

Summary of Studies

Type of Study	Route of Administration	Species	Dose of Level and Time of Exposure	Findings	References	Comments
Teratology	iv	CD-1 mouse	0, 75, 150 mg/kg day 4-6, 6-8, 8-10 or 10-12 of gestation	1. Developmental toxicity at or near dose level which produced maternal toxicity (150 mg/kg)	LaBorde and Kimmel, 1980	
Teratology	iv	New Zealand white rabbit	0, 18, 36 mg/kg day 6-9 of gestation; 0, 9, 18, 36 mg/kg day 6-14 of gestation	1. Developmental toxicity only at levels which were maternally toxic (36 mg/kg, day 6-14)	Kimmel et al., 1982	
Teratology/ Reproduction	Inhalation	Sprague-Dawley CD rat	150 ppm, 7 hr/day: day 7-16 gestation, day 1-16 gestation, 3 weeks pregestation plus day 1-16 gestations	Teratology: 1. Retarded fetal development Reproduction: 1. Maternal toxicity 2. Increase intrauterine mortality	Battelle Pacific Northwest Laboratories (NIOSH 210-80-0013) Hackett et al., 1982	Teratology: 1. Inadequacies a) no maternal toxic doses b) dose response not determined Reproduction: 1. Inadequacies a) dose-response not determined
Teratology/ Reproduction	Inhalation	New Zealand white rabbits	150 ppm, 7 hr/day: day 7-19 gestation, day 1-19 gestation	1. No teratogenic or reproductive effects	Battelle Pacific Northwest Laboratories (NIOSH contract No. 210-80-0013) Hackett et al., 1982	Teratology: 1. Inadequacies a) no maternally toxic doses b) no developmental toxic doses c) dose-response not determined Reproduction 1. Inadequacies a) dose-response not determined

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose of Level and Time of Exposure	Findings	References	Comments
*One generation reproduction	Inhalation	Fisher 344 rats	0, 10, 33, 100 ppm 12 wks prior to mating, 6 hr/day, 5 day/wk. During gestation - days 0 through day 19. During lactation - days 5 through 21.	1. No difference in F_0 fertility. No F_0 toxicity. 2. No adverse effects on F_{1a} survival, growth rate, or lactation. 3. Adverse reproductive effects at highest dose, 100 ppm. a) increased gestational length b) decreased litter size c) decreased implantation sites (i.e., decreased fecundity) d) decreased fetuses/ implantation sites (embryo lethal)	Carnegie-Mellon Research Institute, 1979 (Snelling et al., 1982)	
Chronic toxicity (male reproduction)	Inhalation	Guinea pigs	357 ppm, 123 7-hr exposure in 176 days	1. Tubular degeneration of tests with replacement fibrosis	Hollingsworth et al., 1956	
			204 ppm, 122 to 157 7-hr exposures in 176 to 226 days	1. Slight decrease in testes weight, not statistically significant.		
		Rats	204 ppm, 122 to 157 7-hr exposures in 176 to 226 days	1. Slight decrease in testes weight, not statistically significant. 2. Testes: small, slight degeneration of tubules.		
Testicular toxicity	Inhalation	Cynomologous monkeys	50, 100 ppm, 7 hrs/day for 2 years	1. Decreased testicular weight. 2. Decreased sperm concentration. 3. Decreased sperm motility. 4. No change in sperm morphology.	Lynch et al., 1983	

*A variety of experimental protocols were utilized, only those which provided positive information on reproduction effects are noted here.

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose of Level and Time of Exposure	Findings	References	Comments
Medical survey of workers	Occupational exposure	37 male workers	Mean exposure time: 10.7 years. General levels: 5-10 ppm	1. No observed increase in male reproductive disorders.	Joyner, 1964	1. Small sample size 2. Study did not evaluate fertility or testicular function.
Medical survey of workers	Occupational exposure	282 female production workers 259 female management coworkers 100 females controls	<0.2-0.3 mg/m^3	1. Gynecological disorders, spontaneous abortions, toxicosis, decrease birth weights.	Yakubova, 1976	1. Difficulties in translated material 2. Little information provided on experimental design. 3. Multiple exposures to noise and high temperatures
Case study	Occupational exposure	Pregnant female		1. Infant with hydrocephalus	Holmberg, 1979, Holmberg and Nurminen, 1980	1. Mother exposed to multiple chemicals. 2. Only one infant studied.
Epidemiology study	Occupational exposure	Pregnant female	<1 ppm	1. EtO exposure associated with an increase in spontaneous abortion	Hemminki et al., 1982	1. Possible bias introduced by supervisors who categorized participants in this study. 2. Limited exposure data
Teratology	Intravenous	CD-1 mouse	0, 60, 120 mg/kg day 4-6, 6-8, 8-10, 10-12 of gestation	1. Maternal toxicity at 120 mg/kg for all treatment periods 2. Embryotoxicity at 120 mg/kg for all treatment periods and at 60 mg/kg on days 8-10 (fetal weight reduction).	LaBorde and Kimmel, 1980	

TABLE 9-3 (cont.)

Type of Study	Route of Administration	Species	Dose of Level and Time of Exposure	Findings	References	Comments
Teratology	Intravenous	New Zealand white rabbits	0, 9, 18, 36 mg/kg day 6-14 of gestation	1. No effect on mother or fetus	LaBorde et al., 1982	1. Inadequacies a) no maternally toxic doses b) no developmentally toxic doses
Teratology and Toxicity	Air cell injection	Chick embryo	0, 10, 25, 50, 100 200, mg/kg at 0 hour incubation; 0 5, 12.5, 25, 50, 100 mg/kg at 96 hours incubation	1. Ovo-toxic at levels $\geq$25 mg/kg at 0 hour, and $\geq$12.5 mg/kg at 96 hours. 2. Teratogenic to chick embryo	Verrett, 1974	Uncertainties in extrapolating avian developmental effects to those of mammals

*Original study performed by Carnegie-Mellon Research Institute (Bayes, 1979); later published as Snellings et al., 1982

decreases in the number of implants per litter and an increase in the percentage of malformed fetuses/litter. The malformations appeared to follow a developmental pattern and in at least one gestational interval (days 8-10 of gestation) occurred in the absence of significant maternal toxicity.

Similar studies were conducted by Kimmel et al., 1982, on the effects of 18 and 36 mg/kg EtO administered intravenously to New Zealand rabbits. Significant maternal toxicity (decreases in weight gain) were observed in addition to embryotoxicity observed in the offspring (decreases in the number of live fetus/litter, increases in the number of resorptions/litter). No embryotoxicity was observed in the absence of maternal toxicity.

Laborde investigated the teratogenic effect of intravenously administered ethylene chlorohydrin (ECH), a reaction product of EtO, in CD-1 mice and New Zealand rabbits. No adverse maternal or embryotoxic effects were produced in the rabbits. However in the mice, at the highest dose (120 mg/kg) severe maternal weight loss with increases in resorptions/litter and decreases in fetal weight were observed. At the 60 mg/kg level, on gestational days 8-10, there was significant fetal weight loss in the absence of maternal toxicity. Therefore, the authors concluded that ECH may be a specific hazard to the developing conceptus at this dose level. ECH was also reported to produce adverse effects in developing chick embryos (Verrett, 1974). Structural abnormalities were produced by 12.5 to 100 mg/kg of ECH when the egg was incubated with the chemical for up to 96 hours.

In a one generational study, (Snellings et al., 1982) female rats exposed by inhalation to 100 ppm EtO had higher incidence of infertility with indications of a longer gestational period. There was a decrease in the number of pups produced by mothers exposed to 100 ppm EtO, as well as a

decrease in the number of implantation sites. However, there were no significant signs of toxicity in the mothers (no decreases in body weight or changes in organ histology).

Adverse effects on the testis resulting from EtO exposure have been reported for the hamster and rat (Hollingsworth et al., 1956) and Cynomologous monkey (Lynch et al., 1983). Hollingsworth reported testicular degeneration occuring in hamsters and rats exposed to EtO by inhalation (204 to 357 ppm). Lynch et al. (1983) reported adverse effects on sperm concentration, motility, but not morphology in Cynomologous monkeys. The monkeys in this study were exposed over two years to 50 and 100 ppm EtO by inhalation. In mice radio-labeled EtO has been found to persist in the epididymis up to 24 hours after a single injection (Appelgren et al., 1977).

Very little information exists on the adverse reproductive effects of EtO in the human. Medical surveys have described either no adverse reproductive outcome (Joyner, 1964) or a variety of adverse outcome (Yakabova, 1976). The study by Joyner, 1964 is inadequate because it does not deal specifically with adverse reproductive outcomes. The report by Yakabova, 1976 was presented in an anecdotal manner and therefore is of little scientific value. A case report described by Holmberg (1979) and Holmberg and Nurminen (1980) indicated that one women exposed to a variety of substances including EtO produced an infant with multiple defects and hydrocephalus. However, because of the multiple chemical exposures involved, this study is of little value in establishing the potential of EtO to cause adverse effects.

A recent epidemiology study has been conducted evaluating the pregnancy outcome of nursing personnel exposed to EtO (Hemminki et al., 1982). Although there were problems in the study design and collection of data, the data is

sufficient to suggest an association between EtO exposure and spontaneous abortion and warrants further examination of adverse pregnancy outcomes. Additional epidemiology studies would be helpful to more firmly establish the potential of EtO to cause adverse reproductive effects in humans.

In conclusion, EtO appears to be capable of producing developmental toxicity, i.e., structural defects, in utero death, growth retardation, and infertility in laboratory animals. The levels needed to produce these effects approach or equal the levels needed to produce toxicity in the dams. EtO has been shown to produce adverse testicular effects (testicular degeneration, poor semen quality) and was found to accumulate in the epidiymus. The effects of EtO on human reproduction have not been studied in depth, although one study indicates that EtO may be associated with spontaneous abortion (Hemminki et al., 1982). Future studies are needed to establish this effect in humans.

9.4. MUTAGENICITY

Ethylene oxide (EtO) has been evaluated for mutagenicity in several different systems including tests in bacteria, fungi, higher plants, Drosophila, mammalian cells in vitro, and rodents. Effects in humans are also reported. The available data concerning the mutagenicity of EtO are discussed below and summarized in Tables 9-4 to 9-16. The reader may also wish to refer to other reviews of the mutagenic potential of EtO (e.g., Fishbein, 1976, Wolman 1979, Ehrenberg and Hussain, 1981, and NIOSH, 1981).

GENE MUTATION STUDIES

Prokaryotic Test Systems (Bacteria)

Several investigators have shown that ethylene oxide (EtO) causes point mutations in bacteria (Table 9-4). EtO is a very effective sterilant for products that would be damaged by other sterilization methods. Bacillus subtilis var. niger is commonly used to monitor the effectiveness of EtO sterilization. Jones and Adams (1981) found that treatment of spores of these bacteria with Pennges (12:88 EtO-Freon mixture by weight for 5 minutes increased the number of colony variants by five fold over the spontaneous level. Forty aberrant isolates (out of 125 found) were plated five times in succession of these 11 reverted to typical appearance, 12 changed to other atypical appearances, and 17 remained stable. Although the changed were not well-defined genotypically these data suggest that EtO induced mutations in the surviving spores.

In a study by Rannug et al. (1976), EtO was chosen as a positive control chemical in tests of other chemical substances in the Salmonella/microsome assay. In this study, strain TA1535 was exposed to concentrations of EtO (purity not reported) ranging from 0 to 95.5 mM in a suspension test without addition of an exogenous mammalian metabolic activiation system (Table 9-4). A statistically significant dose-related response was observed (Figure 9-1) where the maximum killing was ≈20%.

In another Salmonella assay, Pfeiffer and Dunkelberg (1980) exposed strains TA98, TA100, TA1535, and TA1537 to concentrations of EtO (99.7% pure diluted in cold acetone) ranging from 0 to 200 μM (0 to 8.8 mg/plate) (Table 9-4). Between 6 and 10 trials were performed and each was conducted in duplicate. A clear dose-dependent response was observed for the base-pair substitution detecting strains TA100 and TA1535 but not for the frameshift

TABLE 9-4

Summary of Mutagenicity Testing of EtO: Gene Mutations in Bacteria

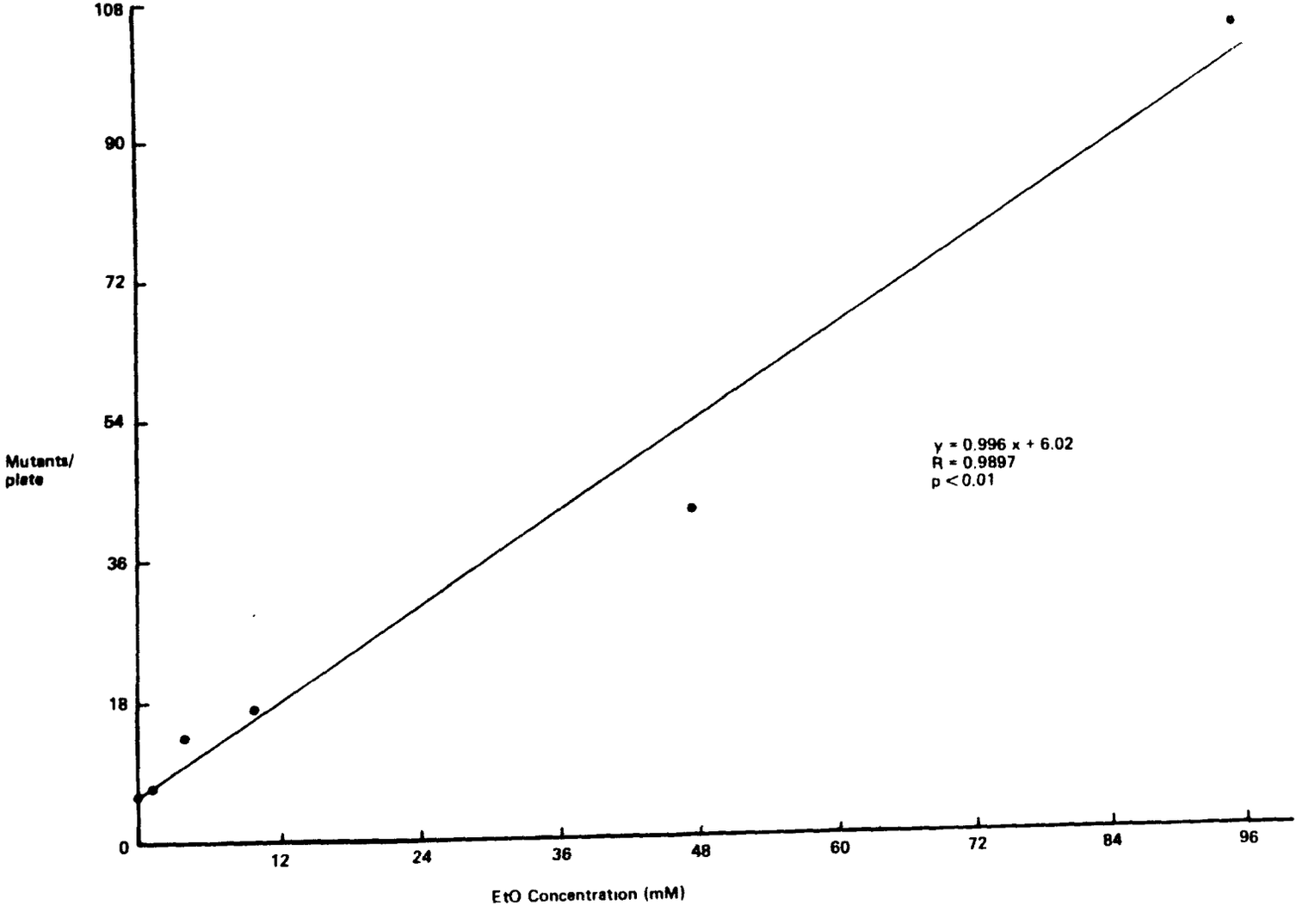
Reference	Test System	Strains	Activation System	Chemical Information	Results	Comments
Rannug et al. 1976	Salmonella/ microsome assay (suspension/ assay)	TA1535	None	Concentration tested: 0 to 95.5 mM Source: Fluka Purity: Not given Solvent: Cold ethanol	Strong positive response	1. Eto used as a positive control. 2. Dose-dependent response. 15-fold increase in revertants noted at highest dose compared to negative controls. 3. Five plates used per dose.
Pfeiffer and Dunkelberg, 1980	Salmonella/ microsome assay (plate test)	TA98 TA100 TA1535 TA1537	None	Concentration tested: 0 to 200 µmol/plate (0 to 8.8 mg/plate) Source: J.T. Baker Chemicals BV Deventer, The Netherlands Purity: 99.7% Solvent: Cold acetone	Positive	1. Dose-dependent response for TA1535 and TA100. 2. Concurrent negative control values not given. 3. Compared to lowest dose (20 µmol/ plate), revertant count at highest dose (200 µmole) was elevated 18-fold for TA1535 and 2.25-fold for TA100. 4. Between 6 and 10 independent runs were done in duplicate for each experiment.

TABLE 9-4 (cont.)

Reference	Test System	Strains	Activation System	Chemical Information	Results	Comments
Tanooka, 1979	Bacillus subtilis spores (reversion to his^+ prototrophy)	HA 101 (his met leu) TKJ 5211 (his met uvrA10) TKJ 8201 (his met polA151)	None	Concentration tested: 27.3% atmosphere of EtO gas for times ranging from 5 to 50 minutes. Source: Daicide LS gas Daido Oxygen Co. Tokyo, Japan Purity: 27.3% EtO 72.7% Freon	Positive response	1. Tests conducted in a polyethylene bag; 4×10^8 spores placed on sterile filter inside bag. 2. Negative control values not provided. 3. Revertant values expressed as mutation frequency (6×10^{-5} after 5 minutes exposure and 8×10^{-3} after 50 minutes exposure of HA 101 and TKJ 5211). 4. Lethal and mutagenic effects were enhanced in the polA strain; TKJ 8201 was 10x more sensitive than HA 101 and TKJ 5211.

FIGURE 9-1

MUTAGENIC RESPONSE OF Salmonella typhimurium STRAIN TA 1535 EXPOSED TO ETHYLENE OXIDE



Rannug et al. (1976)

detecting strains TA98 and TA1537. This result is consistent with responses observed for other alkylating agents.

Tanooka (1979) exposed spores from three different his$^-$ Bacillus subtilis strains to an EtO gas mixture (Daicide LS comprised of 27.3% EtO and 72.7% freon gas) in a plastic bag (Table 9-4). Histidine-independent revertants were selected after treatment; a repair-competent strain and a uvrA repair-deficient strain were treated for times ranging from 5 to 50 minutes. Exposure-related revertant frequencies were observed for both strains (ranging from 3×10^{-6} after 5 minutes exposure to 2×10^{-4} after 50 minutes exposure). In a similar experiment conducted with a polA strain a significantly higher dose-related revertant rate was reported compared to the results with the repair competent and uvrA strains. The revertant frequencies corresponding to 5 and 40 minutes of exposure were about 8×10^{-5}, and 3×10^{-3}, respectively. A similarly elevated sensitivity of the polA strain was observed for EtO-induced toxicity. No data were given for negative controls for any of the strains. The his$^+$ revertants produced in the repair-competent strain exposed to EtO gas for 30 minutes were characterized, and 85% of them were found to contain suppressor mutations; 15% were true revertants as measured by cotransformation of hisB$^+$ with the neighboring trpC$^+$ marker using DNA extracted from each his$^+$ colony. Although this study was not conducted using a "standard" assay system, it does indicate that EtO is mutagenic in B. subtilis.

The positive responses in these tests show that EtO causes genetic damage as evidenced by induction of mutations in bacteria. The studies described below show that EtO causes genetic damage in higher organisms too.

Eukaryotic Test Systems

Plants--

Yeast--Kolmark and Kilbey (1968) studied the induction of ad^+ revertants in Neurospora crassa strain K3/17 (macroconidia) after treatment with EtO (source and purity not given). Five doses ranging from 0.0015 to 0.15M were employed, but the corresponding mutation frequencies were not reported (Table 9-5). The purpose of the work was to study kinetics of mutation induction. In this study, ethylene oxide was found to be 15-21 times more effective as a mutagen than diepoxybutane.

Migliore et al. (1982) tested a series of aliphatic epoxides for their ability to induce forward mutations in Schizosaccharomyces pombe. EtO treatment in liquid suspension at concentrations from 0.5 to 15 mM resulted in dose-related increases in mutation frequency; survival was reduced about 60% at the high dose. One hundred fold increases in mutation frequency were noted at the high dose levels compared to the corresponding negative controls both with and without metabolic activation by phenoballitone-induced mouse liver S9 mix (50.28 $\pm$ 1.76 vs. 0.59 $\pm$ 0.22 and 66.21 $\pm$ 29.44 vs. 0.66 $\pm$ 0.59 mutations/10^4 survivors, respectively). The ranking of the chemical substances tested with respect to their relative specific activity was epichlorohydrin > EtO > glycidol > 1,2-epoxybutane > 1,1,1-trichloropropylene oxide > propylene oxide > 2,3-epoxybutane.

Angiosperms--EtO is known to be a very effective mutagen of higher plants. Many tests have been performed in which EtO has been shown to be mutagenic. The results of these studies will not be analyzed in depth. Most were directed mutagenesis tests conducted to generate desirable traits in food crops. The results of two tests, in which plants were treated with EtO, will

TABLE 9-5

Summary of Mutagenicity Testing of EtO: Gene Mutation Tests in Lower Plants (Yeast)

Reference	Test System	Chemical Information	Results	Comments
Kolmark and Kilbey, 1968	ad-3A revertants in Neurospora crassa	Concentration tested: ranged from 0 to 0.1 M (0 to 6.2 g/ℓ) EtO. Source: Imperial Chemical Industries Ltd. Purity: Not given Solvent: Distilled water	Dose-related positive response	1. Objective of work was to study kinetics of mutation. 2. Revertant values given in Figure of paper as mutation frequencies (i.e. $ad^+/10^6$ survivors).
Migliore et al. 1982	Forward mutations at the ade locus in Schizocaccharomyces pombe	Source: Montedison (Italy) Purity: 99.70% Solvent: Water and DMSO	Dose-related positive response	

Dose (mM)	Without S9 Survival	Without S9 Mutation Freq. $\times 10^{-4}$	With S9 Survival	With S9 Mutation Freq. $\times 10^{-4}$
0	100	0.66 ± 0.59	100	0.59 ± 0.22
0.5	74.78	1.89 ± 1.00	100	3.32 ± 0.96
1.5	99.19	4.17 ± 0.75	76.64	7.15 ± 0.24
5	80.3	18.77 ± 0.72	100	14.33 ± 7.62
15	35.14	66.21 ± 29.44	42.87	50.28 ± 1.76

be discussed for illustrative purposes (Ehrenberg et al., 1956, and Jana and Roy, 1975). Ehrenberg et al. (1956) administered several chemical substances, including EtO (purity not given), to dry and presoaked barley seeds and screened for sterility (dependent on chromosomal aberrations) and chlorophyll mutations (caused by gene mutations, either chromosomal or extrachomrosomal) in the developing plants (Table 9-6). The seeds were exposed to EtO either as a gas (dry seeds receiving 80% EtO for 6 days) or in solution. For the solution exposure experiments, the seeds were presoaked in 0.12 and 0.03% (0.27 and 0.07 M) solutions for 2 hours. EtO induced mutations in a dose-dependent manner as can be seen in Table 9-6. A fivefold increase in lethal mutations and a 33-fold increase in chlorophyll mutations were observed.

Jana and Roy (1975) treated dry seeds of two varieties of rice, IR8 and Dular, with EtO (purity not given) solutions from 0.1 to 0.6% (0.02 to 0.14 M) at 10°C for 8 hours at pH 7.0. The seeds were sown and the plants were grown and harvested. Seeds from single plants were collected and thoroughly mixed to obtain a random sample of seeds. These were then grown to get at least 100 plants from treated original seed for the next generation. These plants were scored for gene mutations affecting chlorophyll expression, and a dose-related mutation frequency was observed (Table 9-6). Although negative controls were not reported, and the spontaneous mutation frequency was not provided, about three times as many mutants were reported in offspring from plants receiving the highest dose compared to those receiving the lowest dose.

The positive responses observed in plants is consistent with the bacterial results and shows EtO is mutagenic in plants.

TABLE 9-6

Summary of Mutagenicity Testing of EtO: Mutation Tests in Higher Plants

Reference	Test System	Chemical Information	Results	Comments
Ehrenberg et al. 1956	Lethal (chromosomal) and chlorophyll (gene) mutations in barley.	When tested as a gas, resting seeds exposed to 80% EtO for 6 days. When tested in solution, partly presoaked seeds exposed to 0.03% and 0.12% (0.27 and 0.07 M) EtO for 2 h at 20°C.	Postive response	1. Third generation progeny not available for analysis when report written; positive response may be due to extra chromosomal mutations. 2. Mutagenic response observed after both types of treatment. 3. Half-life of EtO in water solution is around 100h at 20°C.

% EtO	% Sterility	% 2 nd generation chlorophyll gene mutations	No. spikes analyzed	treatment condition
0	4	0.054	15,861	None
0.03	5.7	0.20	2,510	Solution
0.12	9.5	0.75	1,872	Solution
80	22.1	1.8	989	Gas

TABLE 9-6 (cont.)

Reference	Test System	Chemical Information	Results	Comments
Jana and Roy, 1975	Chlorophyll gene mutations in rice (IR8 and Dular)	Concentration tested: ranged from 0 to 0.6% EtO. Seeds treated for 8 hours at 10°C and pH 7.0 Source: Eastman Organic Chemicals Purity: Not given Solvent: Not given	Dose-related positive response	1. Objective of study was to study kinetics of mutation. 2. Revertant values given in Figure in text as mutation frequencies.

% 2nd Generation Chlorophyll Gene Mutations		
%EtO	Dular	IR8
0	---	---
0.1	5.0 ± 0.36	5.9 ± 0.43
0.3	7.0 ± 0.37	7.0 ± 0.30
0.5	12.3 ± 0.32	12.0 ± 0.19
0.6	14.6 ± 0.13	13.1 ± 0.16

Animals--

Insects--EtO has also been shown to cause both gene and chromosomal mutations in animals. Bird (1952) injected adult male Drosophila melanogaster (Oregon K) with 0.5 and 0.8% (0.11 and 0.18 M) EtO to test its ability to induce sex-linked recessive lethal mutations (Table 9-7). The highest dose level approximated the LD_{50}. The exact amount administered and the purity of the sample were not reported. There were no sex-linked recessive lethals in 494 offspring of untreated flies. Ten lethals out of 713 offspring (1.4%) and 9 lethals out of 198 offspring (4.5%) were detected after treatment with 0.5% and 0.8% EtO, respectively. The dose-related positive response reported indicates EtO is mutagenic in Drosophila.

Watson (1966) fed EtO to male Oregon K Drosophila melanogaster to compare the induction of sex-linked recessive mutations with the induction of heritable translocations. A second objective of this study was to compare the effect on mutation yield of storing sperm in seminal receptacles after treatment with alkylating agents. A positive dose-related increase in both endpoints resulted from EtO treatment (Table 9-7). For the sex-linked recessive lethal test, about 3% lethals were detected at the low dose (0.4% EtO) compared to 7% at the high dose (0.7% EtO). For translocations these values were ≈0.28% and 0.7%, respectively. Negative control values were not given. Storage of EtO-treated sperm in the seminal receptacles for 6 days had no effect on the frequencies of the two types of genetic damage.

Lee (unpublished) conducted parallel experiments with unlabeled and 3H-labeled EtO to determine:

1. The relation of epxosure to level of alkylation of germ cell DNA.

TABLE 9-7

Summary of Mutagenicity Testing of EtO: Gene Mutation Tests in Insects

Reference	Test System	Strain	Chemical Information	Results	Comments
Bird, 1952	Drosophila melanogaster sex-linked recessive lethal test	Orgeon K: adult males	EtO administered by feeding, inhalation or injection. (Data not presented for first two routes of administration.) For injection experiments 0.5% to 5% solutions administered to 20 males. Dosages >0.8% lethal. 0.8% EtO killed 50% of treated flies while 0.5% EtO did not affect viability Source: Not given Purity: Not given Solvent: 0.4% saline	Dose-related positive response	1. Objective of experiment was to find most effective method of administration for routine testing. 2. Cannot determine germ cell stage specificity.
Watson, 1966	Drosophila melanogaster sex-linked recessive lethal test and heritable translocation test	Oregon K: adult males	Concentration tested: 0, 0.04, or 0.7% (0, 0.09, or 0.16 M) EtO Source: Not given Purity: Not given Solvent: Not given	Positive dose-related response	1. Objective of experiment was to determine effect of sperm storage in female seminal receptacle on mutation frequency after treatment with *monofunctional* and bifunctional alkylating agents. 2. Did not observe storage effect for EtO with respect to either endpoint. 3. Cannot determine germ cell stage specificity.

% EtO	No. Chromosomes	No. Lethals	% Lethals
0	494	0	0
0.5	713	10	1.4
0.8	198	9	4.5

	% EtO	%SLRL	% Trans.	% Trans. / % Lethal
Pre-stored	0.4	3.3	0.29	0.08
		3.6	0.39	0.1
	0.7	7.1	0.69	0.1
Post-stored	0.4	3.3	0.79	0.24
		3.1	0.37	0.12
	0.7	6.8	0.60	0.09

TABLE 9-7 (cont.)

Reference	Test System	Strain	Chemical Information	Results	Comments
Lee, unpublished	Drosophila melanogaster sex-linked recessive lethal test and gonadal		Source: Not given for unlabeled EtO ^{3}H-EtO from New England Nuclear sp. act. = 2.8 ci/mmote Purity: Not given		1. Objective of experiment was to determine the relation of exposure to level of alkylation of germ cell DNA alkylation to mutational response.

Exposure (μmole/25 ml vial)	(Dose) Alkylation/ Nucleotide x 10^{-3}	% SLRL
0	5.58	0.12
0.086	22.3	0.35 ± 0.07
0.43		0.92 ± 0.2

2. The relation of germ cell DNA alkylation to mutational responses in Drosophila melonegaster males.

For both the dosimetry and genetic test treatments ethylene oxide was given to the flies by adding 0.7 mℓ of cold water solutions to glass fiber paper in 25 mℓ scintillation vials (0.086 or 0.43 μmole/vial). Immediately afterwards 50 males were added to the vials which were sealed and treatment was continued for 24 hours at 25°C. ^{14}C-Thymidine was also given to males in the dosimetry experiment and alkylations per nucleotide of DNA were calculated based on the $^{3}H/^{14}C$ ratios in purified sperm DNA (to determine the number of alkyl groups present) and the ^{14}C/sperm cell ratio (to determine the amount of sperm cell DNA in the extraction product). The genetic data showed EtO to be an effective mutagen as dose-related increases in sex-linked recessive lethals were observed (see Table 9-7). Using the exposure-dose relation determined from the dosimetry experiments and the genetic data a doubling dose of 2.3 x 10^{-3} alkylations/nucleotide was calculated.

These studies show that EtO is distributed to the gonads of a higher eukaryote (Drosophila) and causes heritable genetic damage.

Mammalian Cells in Culture -- Three tests have been conducted to ascertain the ability of EtO to cause gene mutations in mammalian cells in culture. Brown et al. (1979) reported in an abstract that polymethacrylate (PMMA) plastic sheets and polypropylene (PP) plastic sheets and meshes sterilized by EtO gas adsorbed EtO molecules which could be released later to exert a mutagenic effect. They placed the EtO treated plastic, of unspecified size, in culture flasks containing L5178Y $TK^{+/-}$ mouse lymphoma cells for three days. This was followed by dilution in EtO-free media for 3 days prior to selection using BUdR. PMMA sheets treated for 18 hours with pure EtO were

estimated to release 8 to 40 μg EtO (as measured by gas chromatography) into the flasks, while similarly treated PP sheets and meshes released 5 to 100 μg EtO. Although the spontaneous negative control mutation frequencies were not given, the released EtO was reported to result in a 2- to 20-fold increase in induced mutation frequency relative to the controls (see Table 9-8). It was not possible to evaluate this report critically, because it was presented in abstract form.

Tan et al. (1981) administered EtO (Matheson Co., 99.7% pure, Dr. R. Cumming, personal communication) to Chinese hamster ovary cells at concentrations ranging upwards to 10 mM in the medium. Mutations at the HGPRT locus were selected after 5 hour EtO treatments both with and without an exogenous metabolic activation system (S9 mix derived from Aroclor 1254-induced rat livers) followed by a 16-18 hour recovery period and subculturing for one week. A dose-dependent positive response was obtained at concentrations causing between 10% and 90% cell killing (Figure 9-2) both with and without metabolic activation. The mutation frequency at the highest dose not resulting in excess toxicity (<80% cell killing) was roughly 10 times greater than the reported spontaneous frequency (see Table 9-8).

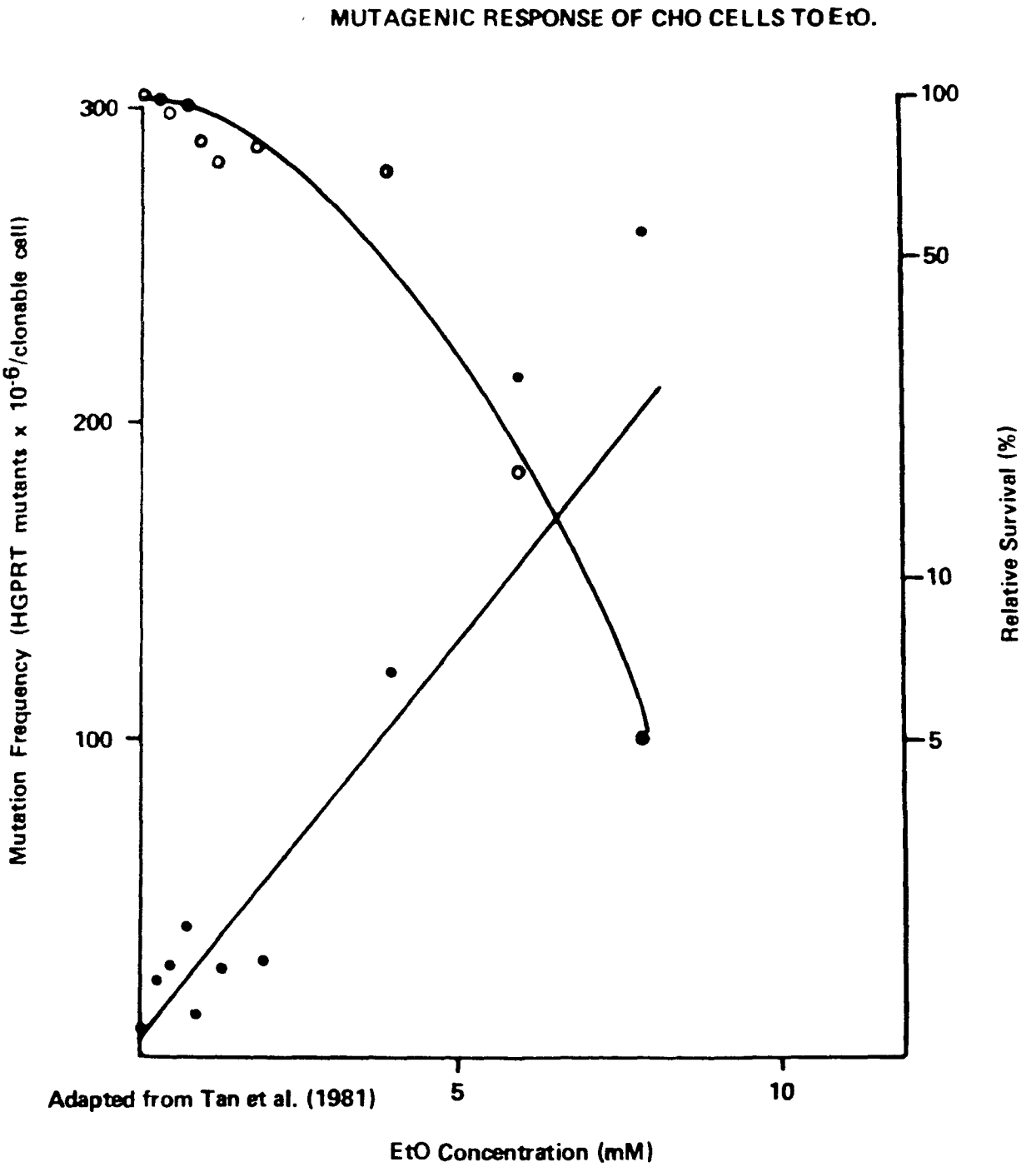
Hatch et al. (1982) and Dr. Stephen Nesnow personal communication (1983) exposed Chinese hamster V-79 cells to EtO gas at concentrations up to 7500 ppm and selected for ouabain - and 6-thioguanine resistant mutants. Significant numbers of mutants were produced for both genetic markers. There was a dose-related increase in mutation frequency. The response for the highest dose was 20 times greater than negative out rats at reported to be repeatable but this could not be verified because the work was reported in an abstract.

TABLE 9-8

Summary of Mutagenicity Testing of EtO: Mammalian Cells in Culture

Reference	Test System	Activation System	Chemical Information	Results	Comments
Brown et al., 1979	L5178Y TK+/- mouse lymphoma gene mutation assay	None	Polymethacrylate (PMMA) plastic sheets and polypropylene (PP) plastic sheets and meshes sterilized for 18 h in pure gaseous EtO. PMMA retained EtO and established concentrations of 8-40 µg/20 mℓ cultured medium (1-5 x 10^{-5}M EtO). PP retained EtO and established concentrations of 5-100 µg/20 mℓ in cultured medium. Source: Not given Purity: Not given Solvent: None	2 to 20-fold induced mutation frequency observed	1. Presented in abstract. 2. Chemical concentrations measured by gas chromatography. 3. Two EtO metabolites also tested. At the low, but unspecified, level tested, ethylene glycol residues did not produce an effect. Chlorohydrin produced residues of 15-30 µg/piece of PP. Direct addition of this compound to the medium resulted in a 2-3 x induced mutation frequency.
Tan et al., 1981	CHO-K_1-BH_4 HGPRT Chinese Hamster Ovary cell gene mutation assay	Liver S9 mix from Aroclor 1254-induced Sprague-Dawley rats	Concentrations tested 0 to 10 mM	Dose-related positive response with and without activation	1. Concentrations and induced mutants extrapolated from Figure 9-1 of text. 2. 250-300 mutants/10^6 cells at high dose both with and without activation compared to 0-10 mutants/10^6 cells in negative controls. 3. Direct acting mutagen. 4. EtO both cytotoxic and mutagenic.

FIGURE 9-2


MUTAGENIC RESPONSE OF CHO CELLS TO EtO.
Mutation Frequency (HGPRT mutants x 10^{-6}/clonable cell)
300
200
100
100
50
10
5
Relative Survival (%)
5
10
EtO Concentration (mM)
Adapted from Tan et al. (1981)

The studies by Brown et al. (1979), Tan et al. (1981) and Hatch et al. (1982) indicate that EtO causes gene mutations in cultured mammalian cells.

CHROMOSOME ABERRATION STUDIES

Many studies have shown that heritable chromosome aberrations are induced in plants after EtO exposure (e.g., Moutschen et al. [1968] in barley and Mackey [1968] in wheat). These studies will not be discussed in this report. Most were directed mutagenesis studies designed to obtain desirable variants. The ability of EtO to cause such mutations shows it to be an effective clastogen in plants.

Dominant Lethal Tests

EtO causes chromosome damage both in mammalian germ cells and somatic cells (Tables 9-9 to 9-13). EtO has been tested in dominant lethal tests in both rats and mice and has yielded a positive response in each (Table 9-9). The precise nature of the damage causing dominant lethal effects is not known, but there is a good correlation between chromosome breakage in germ cells and dominant lethal effects (Matter and Jaeger, 1975). When dominant lethal effects are observed in the offspring of treated males, it can be concluded that the test agent reached the gonads and likely caused genetic damage. Embree et al. (1977) conducted a dominant lethal test with Long Evans rats. Twelve-week-old males inhaled 1000 ppm EtO for 4 hours (Matheson Gas Products, Newark, California, purity not given). The LC_{50} is reported to be 1462 ppm per 4 hours. Embree et al. (1977) reported signs of toxicity after treatment but no deaths. Immediately following treatment, each male was mated to two virgin females per week for 10 weeks. The females were sacrificed 17 days

TABLE 9-9

Summary of Mutagenicity Testing of EtO: Dominant Lethal Tests

Reference	Test System	Mating and Sacrifice	Chemical Information	Results	Comments
Embree et al., 1977	Dominant lethal assay in Long Evans rats	Each male placed with 2 virgin females per week for 10 weeks. Females sacrificed on the 17th day after first exposure to male.	12 week old male animals exposed to 1000 ppm EtO via inhalation for 4 hours Source: Not given Purity: Not given	Positive response. Significant increase in postimplantational fetal deaths during first 5 weeks of the experiment % Dead Implants Week: 1 — EtO: 12* — Control: 2 2 — 30* — 10 3 — 30* — 4 4 — 9 — 8 5 — 10* — 4 10 — 9 — 11 *P<0.05	1. Animals exhibited toxicity but no deaths resulted. 2. Pattern of positive response indicates postmeiotic effect.
Generoso et al., 1980	Dominant lethal assay: male mice T stock (Experiment I) and (101 x C3H)F_1 (Experiment II)	Experiment I: Mated to 2 virgin (SEC x C57B1)F_1 females about 12 weeks old. Females replaced when vaginal plug observed. Sacrificed 12-15 days later. Experiment II: Mated to 2 virgins from one of the following stocks T, (SEC x C57BL)F_1, (101 x C3H)F_1, or (C3H x C57BL)F_1. Sacrifice 12-15 days after observation of vaginal plug.	Single i.p. injection of 150 mg/kg. Maximum volume of 1 mℓ Source: Eastman Kodak Co. Purity: Not given Solvent: Double-distilled water	Positive response observed for days 2.5-11.5. Corresponds to treated spermatozoa and late spermatids. During this period 12 to 31% dead implants in treated group compared to 3 to 5% dead implants in negative control group. Little or no difference in the yield of dominant lethal mutations in male postmeiotic germ cells when mated to females from different stocks.	1. i.p. route of administration chosen to mimic implanation of medical device.

TABLE 9-9 (cont.)

Reference	Test System	Mating and Sacrifice	Chemical Information	Results	Comments
Appelgren et al., 1977	Dominant lethal assay: mice	Males mated to 3 virgin females per week. Females sacrificed on 17th day after first exposure to a male.	Single injection of either 0, 0.025, 0.05, or 0.1 g/kg of EtO given i.v. Source: Not given Purity: Not given Solvent: Saline	Negative response	1. Reported data of dominant lethal test from work by Bateman. 2. Positive controls showed a significant dose-related positive response. 3. Highest dose is 1/3 that used by Generoso et al., 1980; route of administration different from those used by Generoso et al. and Embree et al. (1977). 4. Conducted whole body autoradiography study. Determined EtO distributed to various tissues in the body, including gonads, after either inhalation or injection.

TABLE 9-10

Summary of Mutagenicity Testing of EtO: Heritable Translocation Test

Reference	Test System	Strains	Chemical Information	Results	Comments
Generoso et al., 1980	Heritable translocation	T stock males treated and mated to (SEC x C57BL)F_1 females	Single daily intraperitoneal injection of 0, 30, or 60 mg/kg of EtO weekdays for 5 weeks	Dose-related positive response	1. Shape of response curve consistent with dose-squared kinetics. 2. Demonstrates capability of EtO to cause heritable genetic damage in mice <u>in vivo</u>.

Dose (mg/kg)	Translocation Heterozygotes Frequency	%
0	0/822	0
30	6/456	1.32
60	38/406	9.36
60	6/72	8.33

TABLE 9-11

Summary of Mutagenicity Testing of EtO: Chromosome Aberration Tests

Reference	Test System	Chemical Information	Results	Comments
Fomenko and Strekalova, 1973	Chromosomal aberrations in bone marrow from rats	Concentration tested: 0.001-0.003 and 0.030-0.060 mg/liter for 2, 4, 8, and 30 days by inhalation Source: Not given Purity: Not given Solvent: Not given	Time-dependent positive response at highest dose	1. Method of preparing cells for analysis not given. 2. Criteria for scoring aberrations not given. 3. Definition of terms not given. 4. Insufficient information for adequate evaluation of results.
Strekalova, 1971	Chromosome aberrations in bone marrow from random bred white rats	Concentration tested: 9 mg/kg per os	Positive response reported	1. Animals killed 24 and 48 hours after treatment. 2. Chromosome preparations made from bone marrow squashes. 3. Criteria for classification of aberrations not defined. 4. Insufficient information for adequate evaluation of results.
Poirier and Papadopulo, 1982	Chromosomal aberrations in the human amniotic cell line FL.	Source: Matheson Gas products Purity: Commercial Grade	Dose-related positive response	1. 1 hour vapor exposure. 2. Selected data presented only for cells harvested 72 hours after exposure.

EtO Dose (mM)	% Abnormal Metaphases	Chromatid aberrations/100 cells: Breaks	Exchanges	% Survival
0	10.8	3.0	5.4	100
5	21.7	15.0	5.0	58
7.5	59.7	37.6	45.5	25
10	77.8	79.2	115.1	9.2

TABLE 9-12

Summary of Mutagenicity Testing of EtO: Micronucleus Tests

Reference	Test System	Chemical Information	Results	Comments
Appelgren et al., 1978	Micronucleus test: NMRI mice and Sprague-Dawley rats	Concentration tested: 0 to 0.3 g/kg (mice) or 0 to 0.2 g/kg (rats) via intravenous injection 30 and 6 hours before the animals are killed. Source: Not given Purity: Not given Solvent: Cold water	Dose-dependent response in mice. Increased incidence in rats, but severe bone marrow depression prevented further characterization.	1. The animals given the highest doses died after the first or second injection. 2. 1000 polychromatic erythrocytes screened for micronuclei per animal.
Conan et al., 1979	Micronucleus test: Swiss mice	Concentration tested: Two injections. Doses ranged from 0-200 mg/kg for i.p. injection, or 0-5 mg adsorbed to implanted plastic devices. Source: Not given Purity: Not given Solvent: Water	Dose-dependent positive response after i.p. injection.	
Jenssen and Ramel, 1980	Micronucleus test: CBA mice (males)	Concentration tested 0-175 mg/kg Source: Fluka AG, Switzerland Purity: Not given Solvent: Not given	Positive response	1. Two-fold increase noted in micronucleus formation (0.33 $\pm$ 0.10 in controls compared to 0.93 $\pm$ 0.31 at 150 mg/kg.

TABLE 9-13

Summary of Mutagenicity Testing of EtO: Chromosome Mutations in Human Populations

Reference	Test System	Chemical Information	Results	Comments
Theiss et al., 1981	Chromsome aberrations: peripheral blood of occupationally exposed workers	Exposure: 1. Long-term (>20 years) 2. <20 years 3. Long-term plus accident 4. Accident 5. Control	Mutagenic effect indicated Aberrations excluding gaps: 1. a. 3.5 b. 2.7 2. 2.3 3. 2.2 4. 1.4 5. a. 1.4 b. 1	1. Workers were exposed to other alkylene oxides besides EtO. Cannot assign damage to one agent.
Pero et al., 1981	Chromosome aberrations: peripheral blood lymphocytes from EtO exposed workers	Exposure levels: 0.5 to 1.0 ppm in air	Suggestive positive response for aberrations excluding gaps. Noted only in comparison	1. Both exposed groups has significantly higher levels of total aberrations (breaks and gaps) compared to the control group

after caging with a treated male. Statistically significant ($P<0.05$) increases in postimplantation deaths were observed on weeks 1, 2, 3, and 5 after treatment, but not other weeks, indicating EtO exerts its effects on postmeiotic cells. It should be noted that the statistical significance of increases observed for weeks 1 and 5 may have been due to low negative control values for the corresponding weeks.

Generoso et al. (1980) also observed an increased incidence in postimplantation deaths in mice during the first two weeks after administration of 150 mg/kg EtO (Eastman Kodak, purity not given) by a single intraperitoneal injection. One dose of 200 mg/kg EtO was shown to kill 10 out of 12 mice. The testing for dominant lethal effects in this study was done two ways. In the first experiment, T stock males treated with EtO were mated to two virgin (SEC x C57BL)F_1 females. When females were impregnated, as evidenced by the observation of a vaginal plug, they were replaced with other females. These females were also replaced after the observation of a vaginal plug and so forth for three weeks post-treatment. The females were sacrificed 12 to 15 days after the observation of the vaginal plug and were dissected to determine the frequency of dominant lethal effects. A significant increase in postimplantation deaths was observed in females that were bred with treated males between days 2.5 and 11.5 post-treatment (from 12 to 31% dead implants in treated group compared to 3 to 5% dead implants in negative control group). This indicates that late spermatids and spermatozoa are sensitive to the test compound. In the second experiment (101 x C3H)F_1 males were injected with EtO and divided equally into four groups. Four days post-treatment they were mated either to T stock, (SEC x C57BL)F_1, (101 x C3H)F_1, or (C3H x C57BL)F_1 females. The females were checked for vaginal plugs each morning until the

8th day post-treatment and were killed for uterine analysis 12 to 15 days after the observation of a vaginal plug. The purpose of this experiment was to determine whether the different stocks of mice differed with respect to the ability of oocytes to repair genetic damage induced in the treated male genome. The results of this experiment were consistent with those of the first experiment in showing an increased incidence of postimplantation deaths. However, no significant difference was observed when (101 x C3H)F_1-treated males were mated to females of different stocks.

Appelgren et al. (1977) studied the whole-body distribution of radiolabeled EtO in mice and reported the results of a dominant lethal test. Male mice were treated with [^{14}C] ethylene oxide (sp. act. not given) by inhalation or intravenous (i.v.) injection. The animals were later sacrificed and autoradiograms of midsagittal sections were prepared. The autoradiograms from mice that inhaled EtO differed qualitatively from those that received the material intravenously in only one respect: the mucosal membranes of the respiratory tract of animals that inhaled the compound accumulated EtO. In experiments conducted using the i.v. route of administration, EtO was present in the gonads (epididymis and testicle) 20 minutes after administration. Radioactivity was still present in the epididymis 24 hours after injection. These observations that EtO reaches the gonads are consistent with the positive dominant lethal responses reported by Embree et al. (1977) and Generoso et al. (1980). However, the results of the dominant lethal test cited by Appelgren et al. (1977) were negative, in that there was no increase in the incidence of dominant lethal mutations. The highest dose used in this study was 100 mg/kg, as compared to the 150 mg/kg used by Generoso et al. (1980). Since the chemical was administered by i.v. injection in the study by

Appelgren et al. (1977) and intraperitoneally by Generoso et al. (1980), it is not clear whether the apparently negative response in the study of Appelgren (1977) is attributed to the difference in the dose or to other factors.

The positive dominant lethal tests reported by Embree et al. (1977) and Generoso et al. (1980) indicate that EtO reaches the germinal tissue in intact mammals and causes genetic damage. Although these tests do not unambiguously demonstrate heritable effects caused by EtO, the positive heritable translocation test reported by Generoso et al. (1980) does. Mouse-specific locus tests, which measures heritable gene mutations, are now underway at Oak Ridge National Laboratory and Research Triangle Institute, and the results should provide additional insight into the ability of EtO to cause heritable mutations in intact mammals.

Heritable Translocation Test

In conjunction with their study of dominant lethal effects, Generoso et al. (1980) tested EtO for its ability to cause heritable translocations in mice (Table 9-10). T stock male mice were given 0, 30, or 60 mg EtO per kg once daily, weekdays, for 5 weeks. Immediately after the last injection each male was caged with three (SEC x C57BL)F_1 females. After one week the treated males were removed, and the females were separated from each other. In the control group, each male was left with one of the three females for ≈5 months after the first litters were born in order to produce additional progeny. The incidence of heritable translocations was as follows: negative control, 0%; 30 mg/kg, 1.32%; and 60 mg/kg, 9.36%. These positive results demonstrate that EtO causes heritable chromosomal mutations in whole mammals.

Chromosome Aberration Tests

The ability of EtO to cause well-defined chromosomal aberrations (breaks, rings, inversion, translocations, etc.) has been studied by several investigators. Some of these studies have been discussed previously. These include the positive heritable translocation tests (Watson, 1966 and Generoso et al. 1980), and work conducted with plants (e.g., Jana and Roy, 1975). Two additional experimental studies were evaluated (Table 9-11). One was by Fomenko and Strekalova (1973) who administered from 0.001 to 0.003 mg/liter or from 0.030 to 0.060 mg/liter EtO (purity not given) by inhalation for 2, 4, 8, or 30 days to white rats (strain unspecified). A time-related increase in total aberrations in bone marrow cells was noted in the high dose group (7.1 to 11.6%) compared to the negative controls (3.0%). The significance of these results cannot be determined, however, because of deficiencies in reporting how the chromosomes were prepared and in defining criteria for scoring aberrations.

Similarly, Strekalova (1971) reported that administration of one 9 mg/kg dose of EtO *per os* in aqueous solution resulted in an increased incidence in total aberrations in bone marrow cells scored 21 and, to a lesser extent, 48 hours later; the vague manner in which the study is reported, however, precludes an independent evaluation of the results. The most notable problem is that the terms and the criteria for scoring aberrations are not defined. Furthermore, bone marrow squashes were used to prepare metaphase chromosomes for analysis. This technique is not suitable, because it does not yield high quality chromosome spreads compared to chromosome preparations made by the air-drying technique.

Poirier and Papadopoulo (1982) exposed F1 cells (derived from human amnios) to EtO (commercially available from Matheson Gas Products) at 5, 7.5, and 10 mM for 1 hour. The corresponding cell survivors was 58, 25, and 9.2%, respectively. Three separate experiments were performed. After harvesting (at 48, 72, 0196h) and slide preparation, 150 metaphases were scored for each dose and fixation time (50 from each experiment). Dose-related increases in chromatid aberration were found. For example at 48 hours after treatment the frequency of exchanges (triradials, 'dicentric' and 'centric' rings) per 100 cells was 5.9, 10.6, 56.7, and 127.3 for the corresponding treatments of 0, 5, 7.5, and 10 mM EtO/1 hour exposure (Table 9-11).

Ethylene oxide at 50 and 100 ppm 7 hours/day, 5 days/week for 104 weeks also significantly increased the frequency of chromatid/chromosomal aberrations in peripheral lymphocytes of male Cynomolgus monkeys (Lynch et al., 1982; Dr. D. Lynch, personal communication 1983). The response was dose-related; roughly four-fold increases in cells with one or more chromatid and/or chromosome aberrations were noted in the high dose animals compared to the negative controls.

Micronucleus Formation

Three studies addressed the ability of EtO to induce micronuclei (Table 9-12). Appelgren et al. (1978) treated NMRI mice by i.v. injection with two doses of EtO ranging from 50 to 300 mg/kg, 30 and 6 hours before sacrifice and Sprague-Dawley rats according to the same regimen with doses up to 200 mg/kg EtO. Mice given 300 mg/kg died after the first injection. Rats given 200 mg/kg died after the second injection. In mice, EtO caused a highly significant dose-related increase in micronuclei. At the highest dose there

were 2.48% polychromatic erythrocytes (PCE) with micronuclei compared to 0.52% PCE with micronuclei in the negative control animals ($P<0.001$). Rats also exhibited a statistically significant increase in micronuclei, but it was not shown to be dose-related. Toxicity of the bone marrow confounded the results. The mid-dose level caused 1.08% PCE with micronuclei compared to 0.49% PCE with micronuclei in the negative controls ($P<0.05$).

Using male Swiss mice, Conan et al. (1979) conducted three different types of experiments to assess the ability of EtO, or its metabolites ethylene glycol and 2-chloroethanol, to cause micronuclei. Ethylene glycol and 2-chloroethanol were given to the experimental animals via oral administration or i.p. injection. EtO was administered by i.p. injection, i.v. injection or i.p. implantation of gas sterilized medical devices. Implantation of the EtO gas sterilized medical devices did not induce elevated numbers of polychromatic erythrocytes with micronuclei. Similarly, when EtO was injected i.v. (two injections of 100 mg/kg 24 hours apart) and the animals were killed 6 hours after the second injection, no statistically significant increase in micronucleus formation was observed after treatment. However, when EtO was given i.p. a suggestive positive response was observed. In order of increasing doses of EtO (from 0 to 4000 mg/kg i.p.), the percentage of PCE with micronuclei ranged from 0.23 to 0.47.

Jenssen and Ramel (1980) used CBA male mice in their assessment of the ability of EtO to cause micronuclei. EtO was administered i.p. at dosages up to 175 mg/kg, and micronuclei was scored in polychromatic erythrocytes 24 hours later. The response was not clearly dose-related, but a two-fold increase in micronuclei was observed in the animals at the two highest doses

(150 and 175 mg/kg) compared to negative control animals (0.93 ± 0.31% and 0.66 ± 0.19% compared to 0.38 ± 0.10%, respectively).

The positive responses obtained in the micronucleus tests of Appelgren et al. (1978) and of Jenssen and Ramel (1980) indicate that EtO reaches bone marrow and exerts a chromosome damaging (breakage and/or nondisjunction) effect on hematopoietic cells of mammals.

CHROMOSOME MUTATIONS IN HUMAN POPULATIONS

Three studies have been conducted in which workers exposed to EtO have been monitored for the induction of chromosome damage in peripheral blood lymphocytes.

Ehrenberg and Hallstrom (1967) monitored eight workers for the presence of chromosome aberrations in peripheral lymphocytes eighteen months after an acute exposure to high, but unspecified, concentrations of EtO. Ten unexposed persons were selected as controls. The two groups were not characterized in the report and it is not known how well the control group matched the exposed group. No analyzable cells were obtained from one person in the exposed group. All samples were coded and an average of 20 metaphase plates was analyzed per remaining persons (range = 6 to 26). Gross chromosome aberrations (i.e., chromosome and chromatid breaks and exchanges, supernumerary chromosomes and one case of endoreduplication) were elevated in the exposed subjects (17.5%) compared to the unexposed control subjects (4.3%). Chromosomal effects such as this are potentially heritable and represent clear evidence of genetic damage. The addition of chromosome gaps to these values increased the respective incidences to 30.2% and 16.5%. Because of the small size of the study population and the low number of metaphase spreads analyzed,

the discriminating power of the study is not great and, thus, the elevated levels of chromosome damage observed in the exposed population is judged not to be a significant positive effect.

Theiss et al. (1981) monitored 43 humans exposed to EtO and to a lesser extent other alkylene oxides for the presence of chromosomal aberrations (Table 9-13). The workers ranged from 27 to 63 years ($\bar{x}$ = 47.1 years). Exposed individuals were categorized into four groups based on the type and extent of EtO exposure they had received:

1. Long-term exposure (more than 20 years), 11 men.
2. Less than 20 years exposure, 6 men.
3. Long-term exposure plus accident, 21 men.
4. Accident (i.e., short-term high exposure to EtO), 5 men.

Subjects in the first three groups worked in plants where EtO was manufactured or processed. Personnel in the fire department or maintenance workers comprised the fourth group. The negative control group included male office and staff workers, none of whom had been exposed to radiation at the time of testing. The age of individuals in the control group ranged from 24 to 58 years ($\bar{x}$ = 38.6). The work place was monitored for EtO by means of spot samples for up to 2-hour periods and for propylene oxide by personal dosimeters for up to 10 hours over 12-hour shifts. Ethylene oxide exposures were normally <5 ppm but were found to rise to 1900 ppm for several minutes during a plant breakdown. Levels of propylene oxide were usually far below the maximum allowable concentration of 100 ppm, but higher concentrations were measured for brief periods. The percentage of aberrant metaphases, excluding gaps, in cells cultured from 70-72 hours at 37°C in two control groups was 1.4

and 1. Based on Fisher exact test analysis of the data, with Yates correction, significantly increased incidences of chromosomal aberrations were observed in Group I individuals (>20 years exposure) compared to the control group upon examination in October 1978 (3.5%, $P<0.005$). An increased incidence of aberrant metaphases was also noted when these individuals were subsequently examined in August 1979 (2.7%, $P<0.05$). No statistically significant increase was observed for the other groups. The significantly increased rate of chromosome aberrations (excluding gaps) in workers exposed to EtO for more than 20 years suggests a mutagenic effect. However, the results do not conclusively indict EtO as the causative agent, because the workers were exposed to other substances (e.g., ethylene chlorohydrin, ethyleneimine, propylene oxide, etc.) which may have caused or contributed to the effect. Furthermore, it should be noted that the authors may not have used an appropriate statistical test in their evaluation of the data.

In performing the Fisher exact test one must assume that one aberration is independent of another aberration. Within individuals this may not be the case. If a person has one aberration he may be more likely to have a second aberration particularly if the damage was induced in a stem cell. If this were the case in the study by Theiss et al. (1981) one of the basic assumptions of the Fisher-Yates test, that of independence of the observations, would not be met. A more appropriate statistical test, and one which the authors claimed to have used (but have not reported) in their analysis, is the Mann-Whitney test. Use of the Mann-Whitney test to compare Group 1 and the control group shows an increased (and perhaps biologically significant) but not <u>statistical</u> difference between the two groups in regard to aberrations.

Pero et al. (1981) also found increased incidences of chromosome aberrations in factory workers exposed to EtO (Table 9-13). The workers were divided into three groups. One was an unexposed control group and two were exposure groups (i.e., sterilizers and packers) exposed to 50% EtO and 50% methyl formate gas (0.5 to 1.0 ppm EtO) via inhalation. Chromosome breaks and gaps were scored in the peripheral blood lymphocytes from these individuals. Cells were cultured for 72 hours and 200 metaphases were scored per individual. A statistically significant increase in chromosome gaps plus chromosome breaks was observed in cells from the sterilizer EtO-exposed group (5 workers) compared to the control group (9 workers), 11-14% in exposed groups compared to 8.5% in controls, ($P<0.05$). However, with respect to breaks alone, a nonsignificant (or at best only a marginally significant) increase was noted in the comparison between sterilizers and control groups (8.2 $\pm$ 1.0% compared to 5.8 $\pm$ 1.0%, respectively, $P<0.15$). The comparison between the packer (12 individuals); 6.2 $\pm$ 0.9% and control groups was not significant.

The increased incidences of chromosome aberrations in peripheral lympocytes noted in three studies of workers exposed to EtO are consistent with one another and with the experimental animal data showing EtO to be clastogenic. They indicate that similar effects are caused in humans as well.

OTHER STUDIES INDICATIVE OF MUTAGENIC DAMAGE

Additional studies have been conducted bearing on the genotoxicity of EtO (Tables 9-14 to 9-16). These studies do not measure mutagenic events per se in that they do not demonstrate the induction of heritable genetic alterations, but positive results in these test systems do show that DNA has

TABLE 9-14

Summary of Mutagenicity Testing of EtO: SCE Formation in Human Populations

Reference	Test System	Chemical Information	Results	Comments
Johnson and Johnson, 1982	Sister chromatid exchange induction and chromosome aberrations: Industrial workers	Inhalation exposures estimated to be: Low relative exposure (1 ppm), moderate relative exposure (1-10 ppm), high relative exposure (5-200 ppm).	Dose-response association suggested	1. Levels of SCE remained elevated after termination of exposure. 2. Environmental exposure to EtO causes increased SCE formation. 3. Report based on preliminary data from relatively small sample population.
Garry et al., 1979	Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers	Maximum exposures estimated to be 36 ppm (from average measurements over one 8 hour period). Workers divided into groups based on known exposures to EtO and symptoms indicative of exposure.	Statistically significant increases in level of SCE observed in exposed individuals compared to controls (unexposed laboratory personnel).	1. Air dried fluorescence plus Giemsa chromosome preps. 2. 20 metphases scored/individual.

	SCE Months after Exposure 0	SCE Months after Exposure 6	Chromosome Aberrations Months after Exposure 0	Chromosome Aberrations Months after Exposure 6
High Potential Exposure	33	35	1.5	2
Low Potential Exposure	14	15	1.1	0.9
Inside Controls	12	12	0.6	0.78
Outside Controls	--	8	--	0.5

TABLE 9-14 (cont.)

Reference	Test System	Chemical Information	Results	Comments
Yager, 1982 and Yager et al., 1983	Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers	Exposures determined by individually monitoring workers. High exposure group received a cumulative dose >100 mg while cumulative dose for low exposure group was <100 mg.	Group / Mean Exposure (mg) / SCEs/cell Control / / 7.56 ± 1.01 Low exposure / 13 / 7.76 ± 1.05 High exposure / 501 / 10.69 ± 1.92	1. Control group carefully matched to the exposed group for age, sex and personal habits. 2. Exposure estimates based on breathing zone measurements and task frequency estimates.
Laurent et al., 1982	Sister chromatid exchange induction: peripheral blood lymphocytes collected from hospital workers.	No exposure estimates	Exposed group had statistically significant increase in SCEs compared to control group was range of SCEs for the exposed group was 9.61 - 17.57 compared to a range of 7.04 - 8.52 for the control group.	1. Control group may not have been matched for age, sex, and personal habits to the exposed group.

TABLE 9-15

Summary of Mutagenicity Testing of EtO: SCE Formation in Experimental Studies

Reference	Test System	Chemical Information	Results	Comments
Star, 1980	Sister chromatid exchanges: Cultured human fibroblasts	Concentrations tested: 0 to 3600 ppm and residues from plastic children's endotracheal tubes treated with 1400 mg/cm^3 of pure EtO for 90 minutes followed by aeration from 24 to 96 hours after sterilization. Source: STERI-Gas cartriges 3M Germany GmbH, Neuss Purity: Not given Solvent: Dulbecco's Modified Eagle's Medium	Toxic as well as mutagenic. Significant increases in SCE induction at 36 ppm. Cytotoxicity at 180 ppm and higher	1. Cultures from skin biopsies used between fifth and tenth subculture. 2. Insufficient data presented to evaluate conclusions.
Yager and Benz, 1982	Sister chromatid exchange induction: New Zealand white rabbits	Concentrations tested: 0, 10, 50, and 250 ppm by inhalation Source: Matheson Dayton, OH	Positive response at 50 and 250 ppm exposures	1. Increased SCE levels decreased after exposure ended but still remained above baseline levels 15 weeks after exposure.
Kligerman et al., 1983	Sister chromatid exchange induction: CDF rats	Concentrations tested: 0, 50, 150 and 450 ppm for 1 or 3 days by inhalation Source: Matheson Gas Product Purity: 99.7%	Dose and time dependent positive response Concentration / SCEs/Metaphase: 0 — 7.5 ± 0.5 50 ± 7 — 9.1 ± 1.3* 140 ± 17 — 10.3 ± 1.3* 144 ± 33 — 13.6 ± 1.3*	1. Significant increases at 50 ppm show effects induced at levels to which workers have been exposed. Until recently TWA was 50 ppm. 2. Data for 3 days exposure groups shown.

*Significantly different from controls by one-tailed Dunnett's test

TABLE 9-16

Summary of Mutagenicity Testing of EtO: Unscheduled DNA Synthesis

Reference	Test System	Chemical Information	Results	Comments
Cumming et al. (in press)	Unscheduled DNA synthesis: testicular DNA of (101 x C3H)F_1 mice	Concentration tested:		
		a. 600 and 800 ppm for 2, 4, 6, or 8 hours. [^{3}H] dThd administered intratesticularly immediately after administration	a. Dose-dependent increase in UDS over lower range of doses tested (e.g., 70 dpm/10^6 cells, 48 dpm/10^6 cells, and 8 dpm/10^6 cells for 800 ppm, 600 ppm, and negative controls at 4 hours)	
		b. Same as above except [^{3}H] dThd administered at different times after termination of exposure	b. UDS peaks 2 hours after end of exposure period at day 5 for 300 ppm; at day 1 for 500 ppm	
		c. 300 and 500 ppm 8 h/day for 5 days. Aliquots of animals sacrificed daily	c. Response peaked at day 5 for 300 ppm; at day 1 for 500 ppm	
		d. 500 ppm for 2, 4, 6, and 8 h. 6 animals given 80 mg/kg 3-methyl chloranthrene, 6 animals drank water with 1 mg/mℓ sodium phenobarbital for 1 week prior to exposure, 6 animals uninduced controls	d. UDS response dramatically reduced in animals receiving mixed-function oxidase inducers	
		Source: Matheson Co., East Rutherford, NJ		
		Purity: 99.7%		
Pero et al., 1981	Unscheduled DNA synthesis: Human lymphocyte cultures	Exposure levels: 0.5 to 1.0 ppm in air	Positive response	1. UDS induced by exposure to N-acetoxy acetyl aminofluorene (NA-AAF). 2. Decreases in NA-AAF-induced UDS measured biochemically and by autoradiography in lymphocytes from EtO-exposed workers. UDS peaked at 2 mM exposure NA-AAF.

been damaged. Such test systems provide supporting evidence useful for qualitatively assessing genetic risk.

SCE Formation in Human Populations

Three studies have been reviewed concerning the induction of SCEs in humans (Table 9-14). Lambert and Lindblad (1980) studied peripheral lymphocytes from five female workers in a German sterilization plant to determine if EtO exposure causes genotoxic effects in vivo as measured by SCE formation. A description of the exposure these workers received was not reported. The frequency of SCE formation in exposed individuals was increased (19.1%) compared to the unexposed control group (14.6%). Although the small sample size and uncharacterized exposure these workers received preclude a definitive assessment of the ability of EtO to cause SCEs in humans, the results are considered to indicate genetic toxicity in somatic cells of the exposed workers.

In a preliminary, unpublished report Johnson and Johnson (1982) described how they monitored workers at three sterilant facilities for the presence of SCEs and chromosome aberrations in peripheral blood lymphocytes. Based on environmental sampling the workers were assigned to one of the following categories depending upon the plant site at which they worked: high relative exposure (5-200 ppm), moderate relative exposure (1-10 ppm), and low relative exposure (1 ppm). The numerical exposure values represent the estimated range of an 8-hour time weighted average inhalation exposure. Employees at each plant were further categorized as to high or low potential for EtO exposure based on their job description and other factors. During the course of the study it was noted that the SCE levels in the control group of presumably

unexposed workers at Plant III were higher than those of other control groups available for comparison at the time (12/metaphase compared to 7/metaphase). The study was therefore expanded to include an additional control group, which was taken from the local community and matched by sex and age to potentially exposed Plant III employees.

The preliminary analysis of data indicates a consistent dose-response trend at Plant III for SCE induction both at an original monitoring and later after 6 months of no further EtO exposure (mean values of 12, 14, and 33 SCEs/metaphase for internal controls, low potential exposure and high potential exposure groups, respectively, compared to 8 SCEs/metaphase for the external control groups). A much less pronounced trend was noted at Plant II, and the SCE data for Plant I showed no significant difference between potentially exposed and control groups. Analysis of the chromosome aberration data suggests a dose-related increase in damage, but the magnitude of differences between groups is not great. Thus, it appears that a dose-response association exists between exposure to EtO and SCEs in humans and that the increased levels of SCEs appears to be stable, perhaps suggesting long-lived adverse effects caused by human exposure to EtO. However, it is important to bear in mind that these conclusions are based on preliminary data from a relatively small study population.

In a study of 12 EtO exposed workers from the instruments and materials sterilization areas of a hospital, Garry et al. (1979) reported increased SCE levels in the peripheral blood lymphocytes. The maximum exposure sampled 15 feet from the sterilizer was estimated to be 36 ppm based on an infrared spectroscopy measurement over one 8-hour period during the course of the study. Individuals reporting upper respiratory irritation had statistically

significant increases in the incidence of SCEs compared to the control population of 12 unexposed persons working in the adjacent operating room (10.3 $\pm$ 1.8 vs. 6.4 $\pm$ 0.47, $P<0.01$).

Yager (1982 and Yager et al. 1983) also monitored hospital workers (14) exposed to EtO. Thirteen persons not exposed to EtO served as matched controls. Cumulative exposure doses during the 6 months prior to blood sampling were estimated by monitoring air concentrations during defined tasks using a Wilkes-Miran^{TM} 1A Gas Analyzer and multiplying this value by the number of sterilizer loads processed. Based on these estimates, the workers were assigned to low exposure dose group (13 $\pm$ 18 mg EtO) or the high exposure dose group (501 $\pm$ 245 mg EtO). An increased incidence of SCEs/cell was observed in the high dose group (10.7 $\pm$ 1.92) compared to the low dose (7.8 $\pm$ 1.05) and unexposed control (7.56 $\pm$ 1.01) groups.

Laurent et al. (1982) also collected peripheral blood from hospital workers exposed to EtO. Ten persons in good health and not exposed to any known toxicants were selected as the negative control group. It was not reported whether the controls were matched for sex, smoking habits, etc. They do not appear to have been matched for age because the age of the control group ranged between 20 and 35 years while that of the EtO exposed workers ranged between 23 and 51 years. No estimate was made of the exposure received by the sterilizers but they had a significantly elevated level of SCE compared to the controls (13.02 $\pm$ 2.294 vs. 7.86 $\pm$ 0.479).

The increased incidences of SCEs observed in five groups of workers exposed to EtO do not demonstrate mutations but do indicate that EtO can cause genotoxic effects in somatic tissue of humans in vivo.

SCE Formation in Experimental Studies

Human cells in culture also exhibited increased SCE levels after exposure to EtO (Table 9-15). Star (1980) exposed skin fibroblast cells from normal healthy human tissue biopsies from 0 to 3600 ppm EtO or to plastic children's endotracheal tubes sterilized with 1400 mg/cm^3 EtO at 55°C for 90 minutes followed by aeration in room air for varying times from 24 to 96 hours. The cell lines were kept frozen in liquid nitrogen and used between their 5th and 10th subculture. The placement of the plastic tubes in the culture medium resulted in EtO concentrations ranging from 12 to 800 ppm as estimated by gas chromatography of head space material. Excessive cell killing precluded scoring SCEs above 600 ppm for the experiment. No statistically significant increase in SCEs was noted in the experiment using the endotracheal tubes, but a consistent apparently dose-related rise in SCEs was noted in this part of the study at doses >217 ppm. In the other set of experiments a statistically significant increase in SCE induction was reported at 36 ppm. However, insufficient data are presented to permit an adequate evaluation of the results.

A membrane dosimetry system was developed by Garry et al. (1982) to enable the measurement and determination of dose-response relationships for in vitro exposure to toxic gases. Elevated SCEs were observed in peripheral lymphocytes cultured from healthy humans at as little as 10 μg/mℓ (in the media) during a 20-minute exposure period. A dose-related increase was noted up to EtO concentrations of 35 μg/mℓ (the highest dose tested). At this dose there were about 20 SCEs/cell compared to control levels of roughly 5 SCEs/cell.

Yager (1982) and Yager and Benz (1982) administered from 10 to 250 ppm EtO gas to four-month-old male New Zealand white rabbits via inhalation. Eight animals were placed in each exposure chamber and exposed 6 hours/day, 5 days/week, for 12 weeks. Blood samples were obtained from the marginal ear vein at 1, 7, and 12 weeks of exposure and 2, 7, and 15 weeks after exposure. Three animals per chamber were used for serial blood sampling for SCE and hematological assays (i.e., red cell count [total and differential], white cell count, hematocrit, and hemoglobin concentration). One animal was held in reserve and four animals were sacrificed immediately at the end of the 12-week exposure period for analysis of reduced glutathione (GSH) in liver and blood. Positive and negative controls were performed using intraperitioneal (i.p.) injections of mitomycin C and Hanks balanced salt solution, respectively, at each time point. Exposure to 10 ppm did not cause a detectable increase in the incidence of SCEs; however, exposure to 50 and 250 ppm did cause an increase in SCEs (9.47 $\pm$ 0.26 and 13.17 $\pm$ 0.32, respectively) that decreased after exposure ended, but still remained above baseline levels (7.8 $\pm$ 0.23) 15 weeks after exposure (8.45 $\pm$ 0.30). Hematological and GSH measurements from the animals did not differ from controls.

After exposures to EtO of 0, 50, 150 or 450 ppm for 6 hours/day for 1 or 3 days blood was removed from male CDF rats by cardiac puncture, cultured in the presence of 5-bromodoxyuridine and scored for SCEs and chromosome breakage (Kilgerman et al., 1983). No significant dose-dependent increase in chromosome breakage was observed but there was a concentration dependent increase in SCEs. Animals in the highest dose group exposed for 3 days had 13.6 $\pm$ 1.3 SCEs/cell compared to the control value of 7.8 $\pm$ 0.5 SCEs/cell. SCE induction was also significantly elevated after 3 days to 50 ppm (9.1 $\pm$

1.3) showing effects at levels to which workers have been exposed. There was no significant reduction in mitotic activity or slowing of cell kinetics.

Unscheduled DNA Synthesis

Cumming et al. (in press) tested EtO for its ability to cause UDS in germ cells of male mice after inhalation exposures. Four experiments were performed in which hybrid mice (101 x C3H)F_1 were treated with 99.7% pure EtO (Matheson Co.). In the first experiment, the effect of differential time exposures on UDS induction was assessed. Animals were treated with 600 and 800 ppm EtO from 2 to 8 hours, after which exposed animals were anaesthetized with metofane and injected intratesticularly with [^{3}H]thymidylic acid (dThd). A dose-dependent increase in UDS was found over the lower end of the dose range for the first 4 hours of exposure in that a higher response was seen at 800 ppm than at 600 ppm (e.g., 70 dpm/10^6 cells for 4-hour exposure at 800 ppm compared to 48 dpm/10^6 for 4-hour exposure at 600 ppm; controls incorporated 8 dpm/10^6 cells). Due to the toxicity of EtO at 800 ppm it was only possible to measure up to 6 hours exposure for this concentration. In a second experiment, EtO administration was the same as above, but [^{3}H]dThd was administered to the animals at different times after removal from EtO exposure to characterize the UDS response at different times after treatment. UDS was found to increase with time to a peak 2 hours after the end of the exposure period and to fall afterwards. Two additional sets of experiments were performed. The first was a work week exposure regimen of (300 and 500 ppm for 5 hours/day for 5 days), and the second involved pretreatment of the animals with mixed-function oxidase inducers (either a single i.p. injection of 80 mg/kg 3-methylcholanthrene or administration of drinking water containing 1

mg/mℓ phenobarbital for 1 week prior to EtO treatment). Concerning the work week exposures, little effect was noted after the first two exposure periods at 300 ppm. An effect was subsequently noted which rose to a maximum after the 5th exposure period. At 500 ppm the maximum effect was seen after the first exposure period. Apparently, increased levels of DNA damage occurred throughout the week, but after the third exposure period the capacity to respond to this damage appeared to be limited.

Pero et al. (1981, 1982) treated peripheral lymphocytes taken from EtO exposed workers with 10 mM N-acetoxy-2-acetylaminofluorene (NA-AFF) for 1 hour and subsequently measured the incorporation of [^{3}H] thymidylic acid into DNA to detect unscheduled DNA synthesis (UDS) (Table 9-16). NA-AAF-induced UDS was found to be inversely related to the duration of worker exposure to EtO and to the number of chromosome breaks observed. This suggests an inhibition of the cellular DNA-repair capacity by EtO. Biochemical and autoradiography studies were consistent with this response. When NA-AFF-treated lymphocytes were exposed to EtO, it was found that concentrations above 2 mM resulted in inhibition of UDS.

As was the case for the studies of sister chromatid exchange induction these results do not show that EtO is mutagenic but do indicate it causes damage to DNA and are consistent with the results showing the EtO causes mutations.

SUMMARY AND CONCLUSION ON THE MUTAGENICITY OF ETHYLENE OXIDE

Ethylene oxide (EtO) has been shown to induce gene mutations in bacteria, fungi, higher plants, Drosophila, and cultured mammalian cells in tests conducted without the use of exogenous hepatic metabolic activation systems.

It is therefore a direct-acting mutagen. Strong positive responses were found in bacteria (10 to 18-fold increase over negative controls), higher plants (33-fold increase), and mammalian cells in culture (2 to 20-fold increases). Less strong, but clearly positive, responses were found in Drosophila (two-fold to three-fold increases). Based on these positive findings in different test systems in a wide range of organisms, EtO is judged to be capable of causing gene mutations.

EtO has also been shown to be clastogenic, in that it causes dominant lethal effects in mice and rats; chromosomal aberrations in higher plants, Drosophila, mice, and rats; and micronuclei in mice and rats. Based on these positive findings in different test systems, EtO is judged to be capable of causing chromosomal aberrations. It has also been shown to induce sister chromatid exchange (SCE) in rabbits, rats and humans.

Tissue distribution studies have shown that EtO reaches the gonads. This result is consistent with evidence that EtO causes unscheduled DNA synthesis (UDS) in germ cells of male mice and heritable mutations in insects and rodents (i.e., sex-linked recessive lethals and heritable translocations in Drosophila, dominant lethals in rats and mice and heritable translocations in mice). EtO can therefore be regarded as mutagenic both in somatic cells and in germ cells.

Based on the available data, there is overwhelming evidence that EtO is a direct-acting mutagen that has the potential to cause mutations in the cells of exposed human tissue. The observations that EtO reaches and reacts with mammalian gonadal DNA, and causes heritable mutations in intact mammals, indicates that it may be capable of causing heritable mutations in man provided that the pharmacokinetics of EtO in humans also results in its

distribution to the DNA of germ cells. Thus, EtO should be considered to be a potential human mutagen.

9.5 CARCINOGENICITY

The purpose of this section is to evaluate the likelihood that ethylene oxide (ETO) is a human carcinogen and, on the assumption that it is a human carcinogen, to provide a basis for estimating its public health impact and evaluating its potency in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior, have an important bearing on both the qualitative and the quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. The carcinogenicity of ETO has also been evaluated by the International Agency for Research on Cancer (1976). This section presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and, finally, a summary and conclusions dealing with all of the relevant aspects of the carcinogenicity of ETO.

9.5.1 Animal Studies

Only a few studies have been conducted to assess the carcinogenicity of ETO. Most of the reported studies have dealt with subcutaneous administration and skin painting of the compound in mice, and intragastric administration in rats. These studies are discussed briefly herein. Two lifetime inhalation studies in rats have been performed (Snellings et al. 1981 and Lynch et al. 1982), and they will be described in detail.

9.5.1.1 Mice--Reyniers et al. (1964) conducted a study of female germ-free mice that developed tumors (63/83) after being accidentally exposed to ETO-treated ground-corncob bedding for 150 days, and were moved to untreated bedding for the rest of their lifespans. These animals developed ovarian, lymphoid, and pulmonary tumors. Colony mates maintained on untreated bedding did not develop

tumors. All males exposed to ETO-treated bedding died, with necropsy showing massive hemorrhage. The causative agent was not identified, since chemical analysis of the bedding was not done. The high number of tumors could have been due to other chemicals (such as ethylene glycol or 2-chloroethanol, both derived from ethylene oxide) or to a viral agent, although the author believed that a viral agent was unlikely. High toxicity is indicated by these findings in male mice. Because germ-free mice are T-lymphocyte deficient, they may be more susceptible than normal animals to tumor development, or the tumor development may be due to immune suppression. At present, however, there is no evidence to support these hypotheses.

Dunkelberg (1979) studied the oncogenic activity of ETO dissolved in tricaprylin and administered subcutaneously to the interscapular area of groups of 100 female NMRI mice in weekly dosages of 0.1, 0.3, and 1.0 mg. The incidence of spontaneous subcutaneous tumors in these mice was between 0 and 2%. Preliminary results up to the 91st week of treatment showed that 6, 8, and 12 local tumors (sarcomas) occurred in mice receiving total ETO doses of 9.1, 27.3, and 91.0 mg, respectively. No local tumors occurred in mice receiving no treatment or tricaprylin alone. The number of tumors at sites distant from the injection area was not significantly greater in the group treated with ETO than in the two control groups. The final report of this study (Dunkelberg 1981) covers the period from the start of the study to 106 weeks, at which time all of the animals were sacrified. No increase in tumors at remote sites was observed.

Lifetime skin painting studies with 10% ETO in acetone (three times weekly) were performed on 30 female mice by Van Duuren et al. (1965). Application of 0.1 mL of ETO solution to the clipped dorsal skin produced no tumors. Median survival time for the mice was 493 days. The investigators indicated that rapid evaporation of the compound from the skin was responsible for the negative results observed.

9.5.1.2 Rats--Walpole (1958) injected 12 rats subcutaneously with a maximum total ETO dose of 1 g/kg (dissolved in arachis oil) over 94 days (dosing schedule not specified). Rats were observed for their lifetimes following treatment, and no tumors were observed. Since the total amount of ETO administered and the frequency of injection were not specified, it is difficult to evaluate this negative result.

Dunkelberg (1982) administered ETO intragastrically by gavage at two dosages, 30 and 7.5 mg/kg body weight, to two groups of 50 female Sprague-Dawley rats with empty stomachs twice weekly for a period of nearly 3 years, using salad oil as the solvent. One group was treated with the solvent alone, and the other group was left untreated. A positive control group was treated with β-propiolactone. The test substances were dissolved in 1 mL of oil immediately before treatment. The design of the experiment is summarized in Table 9-17 and the results are summarized in Table 9-18. ETO induced local tumors, mainly squamous cell carcinomas of the forestomach. The first tumor occurred in the 79th week. The tumor rates were 62% in the 30 mg/kg group and 16% in the 7.5 mg/kg group. In addition, carcinomas in situ, papillomas, and reactive changes of the squamous epithelium of the forestomach were observed in other animals. An unspecified number of tumors occurred in the glandular stomach. ETO did not induce tumors at sites away from the point of administration. Survival decreased in the positive control group.

Two other studies designed to test for chronic toxicity of ETO reported no tumors; however, the exposure and observation periods were too short to adequately test the carcinogenicity of ETO in rats, mice, monkeys, guinea pigs, and rabbits (Hollingsworth et al. 1956, Jacobson et al. 1956).

9.5.1.2.1 Snellings et al. (1981) Inhalation Study. A 2-year inhalation study (unpublished) was performed by Bushy Run Research Center, Pittsburgh,

TABLE 9-17. DESIGN SUMMARY FOR CARCINOGENICITY TESTING OF ETO BY INTRAGASTRIC ADMINISTRATION TO SPRAGUE-DAWLEY RATS
(adapted from Dunkelberg 1982)

Group	Single dose (mg/kg body wt) (2x weekly)	Average total dose (mg/kg body wt)	Number of animals
Ethylene oxide I	30.0	5112	50
Ethylene oxide II	7.5	1186	50
Oil (vehicle)	1.0 mL	-	50
Untreated	-	-	50
β-Propiolactone	30.0	2868	50

TABLE 9-18. TUMOR INDUCTION BY INTRAGASTRIC ADMINISTRATION OF ETO IN FEMALE SPRAGUE-DAWLEY RATS
(adapted from Dunkelberg 1982)

Dose	Number of rats with stomach lesions			
	Reactive changes[a]	Carcinoma in situ	Fibrosarcoma	Squamous cell carcinoma
7.5	9	4	0	8
30.0[b]	11	4	2	29

No stomach tumors were seen in either vehicle-controls or untreated controls.
[a]Reactive changes of the squamous epithelium of the stomach comprised hyperkeratosis, hyperplasia, and papillomas.
[b]Fifteen animals from the ethylene oxide I group developed stomach tumors, of which 10 exhibited metastasis and invasive growth into neighboring organs.

Pennsylvania (Snellings et al. 1981). Fischer 344 rats were exposed to 100, 33, and 10 ppm of ETO vapor by the inhalation route, 6 hours/day, 5 days/week, for approximately 2 years. Two groups were exposed to untreated air under similar conditions. Whole-body exposures were conducted in a dynamic exposure system in which the vapor concentration levels were determined by gas chromatography.

Initially, 120 rats per sex per group were exposed, with interim sacrifices of 10 animals each at 6 and 12 months and 20 animals at 18 months to determine possible treatment-related effects. Interim and terminal evaluation included hematology, serum clinical chemistry, urinalysis, body weight, organ weight, bone marrow cytogenetic studies, and gross and histologic examinations.

In the cytogenetic studies, no statistically significant differences were noted for the "percentage of abnormal cells," the "average number of chromosomal aberrations per cell," or the "total number of chromosomal aberrations (per rat)" for either males or females exposed to ETO at 100 ppm when compared with values obtained for the air-control groups. However, statistically significant chromosomal aberrations have been found in other ETO studies (see section on mutagenicity).

Histopathologic examination was performed on all tissues of each air-control group and the 100 ppm group at 6 months and at 12- and 18-month necropsy intervals. At 6, 12, and 18 months, for the two lower groups (10 and 33 ppm), this histopathologic examination was performed only when the tissue had gross lesions. At the 24-month necropsy interval, the histopathologic examination was performed on all tissues of rats in the 100 ppm group and both control groups, and on potential target tissues, selected tissues, and tissues with gross lesions in the two lower-dose groups (10 and 33 ppm).

During the 15th exposure month, all rats became infected with sialodacryoadenitis (SDA) virus infection. Clinical signs of infection were noted during the 62nd and 63rd exposure weeks. After the 64th exposure week, the exposures were temporarily terminated to permit recovery from the viral infection. Very low mortality had been observed prior to the infection of the initial 120 rats per sex per exposure group; no more than five in any group of one sex had died or were sacrificed because of a moribund condition. During the 64th and 65th

exposure weeks, a total of 24 rats died. There was a higher rate of mortality among female rats in the 100 ppm exposure group than in any other group. Gross and microscopic examination of tissues of the animals that died during this infection period revealed no pathologic findings sufficient to explain the cause of death. Most of the clinical signs associated with the infection subsided after 2 weeks of no exposure, as the mortality rate and body weights returned to preinfection values. As a result, the exposure was restarted. No increase in mortality in association with this disease had been reported in the literature.

According to Snellings et al. (1981), the total numbers of rats that died or were sacrificed in a moribund condition were 49, 39, 28, 31, and 29 for the males and 53, 31, 25, 19, and 20 for the females in the 100 ppm, 33 ppm, 10 ppm, Air Control I, and Air Control II groups, respectively. One additional male in the 33 ppm group and one female in Air Control Group I were accidentally killed.

The cumulative mortality data and statistical significances for male and female rats are shown in Tables 9-19 and 9-20, respectively. The cumulative percentage dying in the 100 ppm group for both sexes was significantly higher than that of controls for at least the last four exposure months of the study. Very few significant differences were observed in males of the 33 ppm group.

During the 15th exposure month, the mortality rate of females in the 100 ppm group increased significantly. This increase was also noted for males in the 100 ppm group and females in the 33 ppm group, but to a lesser degree. Since the SDA virus may have contributed significantly to this mortality, the data were re-evaluated by Snellings et al. (1981), using the number of rats alive at the beginning of month 17 as the starting point. This re-evaluation eliminated the immediate effects of the SDA virus infection. The results of these calculations, presented in Tables 9-21 and 9-22, indicate a significant

TABLE 9-19. CUMULATIVE PERCENTAGES OF MALE FISCHER 344 RATS THAT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER EXPOSURE TO ETO VAPOR[a] (adapted from Snellings et al. 1981)

Exposure month	Exposure concentration					
	100 ppm[b]	33 ppm[b]	10 ppm	Air Control I	Air Control II	Combined controls
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.8	0.0	0.4
6	0.0	0.0	0.0	0.8	0.0	0.4
7	0.0	0.0	0.0	0.8	0.0	0.4
8	0.0	0.0	0.0	0.8	0.0	0.4
9	0.0	0.9	0.9	0.8	0.0	0.4
10	0.0	1.8	0.9	0.8	0.0	0.4
11	0.0	2.8	0.9	0.8	0.0	0.4
12	1.0	2.8	0.9	0.8	0.0	0.4
13	1.0	4.8	0.9	0.8	0.0	0.4
14	3.0	4.8	0.9	1.8	0.0	0.9
15	7.0	6.8	0.9	1.8	2.0	1.9
16	7.0	8.8	1.9	1.8	1.0	1.9
17	7.0	9.8	2.9	2.9	4.1	3.5
18	10.4	9.8	2.9	5.1	5.2	5.2
19	11.7	12.5	2.9	5.1	5.2	5.2
20	18.2	15.1	8.0	9.0	6.5	7.8
21	24.7(-,-,a)	20.3	10.6	11.5	10.4	11.0
22	27.3(-,-,-)	29.4(-,a,a)	14.4	17.9	11.7	14.8
23	44.2(a,c,c)	36.0(-,b,b)	18.3	21.8	13.0	17.4
24	50.7(a,c,c)	39.9(-,a,-)	25.9	12.9	20.8	25.2
24.5	55.9(a,b,c)	42.5	31.0	34.6	28.6	31.6
25.0	65.2(a,-,b)	54.2	38.3	41.9	42.6	42.3

[a]Life table analysis, adjusted for scheduled interim sacrifices.

[b]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

TABLE 9-20. CUMULATIVE PERCENTAGES OF FEMALE FISCHER 344 RATS THAT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER EXPOSURE TO ETO VAPOR[a] (adapted from Snellings et al. 1981)

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm	10 ppm	Air Control I	Air Control II	Combined controls
1	0.0	0.0	0.0	0.8	0.0	0.4
2	0.0	0.0	0.0	0.8	0.0	0.0
3	0.0	0.0	0.0	0.8	0.0	0.0
4	0.0	0.8	0.0	0.8	0.0	0.0
5	0.0	0.8	0.0	0.8	0.0	0.4
6	0.0	0.8	0.0	0.8	0.0	0.4
7	0.0	0.8	0.0	0.8	0.0	0.4
8	0.0	0.8	0.0	0.8	0.0	0.4
9	0.0	0.8	0.0	0.8	0.0	0.4
10	1.8	0.8	0.0	0.8	0.0	0.4
11	1.8	0.8	0.0	0.8	0.0	0.4
12	1.8	0.8	0.0	0.8	0.0	0.4
13	2.8	1.8	0.0	0.8	0.0	0.4
14	3.9	1.8	0.0	0.8	0.0	3.0
15	16.0(b,b,b)	5.9	2.0	2.8	3.1	3.5
16	18.0(b,b,b)	5.9	3.0	3.8	3.1	3.5
17	21.1(c,c,c)	6.9	5.0	3.8	3.1	3.5
18	22.2(b,c,c)	10.3	6.2	6.1	4.3	5.2
19	25.0(a,c,c)	15.5	11.3	8.6	5.6	7.1
20	30.4(b,a,b)	16.8	11.3	9.9	12.3	11.0
21	34.4(b,a,b)	22.0	12.6	9.9	16.3	13.0
22	41.3(c,b,c)	24.6	13.9	9.9	18.9	14.3
23	49.5(c,b,c)	32.4	24.2	18.8	22.9	20.8
24	63.3(c,c,c)	35.2	28.5	22.9	25.8	24.3
24.5	70.0(c,c,c)	41.1	34.7	25.9	25.8	25.9

[a]Life table analysis, adjusted for scheduled interim sacrifices.

[b]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$

TABLE 9-21. CUMULATIVE PERCENTAGES OF MALE FISCHER 344 RATS THAT WERE ALIVE AT THE BEGINNING OF MONTH 17, BUT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER SUBSEQUENT EXPOSURE TO ETO VAPOR[a]
(adapted from Snellings et al. 1981)

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm	10 ppm	Air Control I	Air Control II	Combined controls
17	0.0	1.1	1.0	1.0	2.1	1.6
18	3.7	1.1	1.0	3.3	3.2	3.3
19	5.0	4.0	1.0	3.3	3.2	3.3
20	12.0	6.8	6.2	7.2	4.6	5.9
21	19.0	12.6	8.8	9.9	8.6	9.2
22	21.8	22.6	12.8	16.4	9.9	13.2
23	$40.0^{(a,c,c)}$	$29.8^{(-,a,-)}$	16.7	20.3	11.2	15.8
24	$46.9^{(-,c,b)}$	34.1	24.5	28.2	19.2	23.7
24.5	$52.5^{(-,b,b)}$	36.9	29.7	33.4	27.1	30.3
25.0	$62.5^{(-,-,a)}$	49.8	37.1	40.8	41.4	41.2

[a]Life table analysis, adjusted for scheduled interim sacrifices.
[b]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

TABLE 9-22. CUMULATIVE PERCENTAGES OF FEMALE FISCHER 344 RATS THAT WERE ALIVE AT THE BEGINNING OF MONTH 17, BUT DIED OR WERE SACRIFICED IN A MORIBUND CONDITION AFTER SUBSEQUENT EXPOSURE TO ETO VAPOR[a] (adapted from Snellings et al. 1981)

	Exposure concentration					
Exposure month	100 ppm[b]	33 ppm	10 ppm	Air Control I	Air Control II	Combined controls
17	3.7	1.1	2.1	0.0	0.0	0.0
18	5.1	4.7	3.2	2.3	1.2	1.8
19	8.4	10.2	8.6	5.0	2.6	3.8
20	15.1	11.6	8.6	6.3	9.4	7.8
21	20.1	17.1	9.9	6.3	13.6	9.8
22	28.4(b,-,a)	19.9	11.2	6.3[c]	16.3[c]	11.2
23	38.4(a,-,b)	28.2	21.8	15.5	20.4	17.9
24	55.2(c,c,c)	31.2	26.2	19.8	23.4	21.6
24.5	63.4(c,c,c)	37.4	32.6	22.9	23.4	23.2

[a]Life table analysis, adjusted for scheduled interim sacrifices.

[b]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

[c]Control I group differed significantly from Control II group at the $P < 0.05$ level only for the 22-month mortality count.

increase in mortality in the 100 ppm group versus the controls for both males and females, but the increased mortality was not significant until month 23 for the males and month 22 for the females. In no time interval was the cumulative percentage mortality value for either sex in the 33 ppm group significantly different from that of combined controls. However, from the 21st month on, the values for both sexes in the 33 ppm group were higher than those for both control groups. At no time were significant increases in mortality observed in the 10 ppm exposure group of either sex.

Of the many tumor types occurring in the Snellings et al. (1981) study, five types, which may be treatment related, are reviewed here: subcutaneous fibroma, peritoneal mesothelioma, pancreatic adenoma, pituitary adenoma, brain neoplasm, and mononuclear cell leukemia. The authors presented no evidence that the SDA viral infection increased the tumor incidence in the experimental groups. The time to first tumor for some neoplasms (but not for mononuclear cell leukemias) was decreased in the high-dose group as compared to controls, as shown in Table 9-23. Median time-to-tumor was not reduced.

Histopathologic examinations were performed on tissues of all the rats in the 100 ppm group and both control groups. In the 33 and 10 ppm groups, only those tissues that had gross lesions were examined. Therefore, some small tumors in these two groups may have been missed, yielding an erroneously low estimate of tumors.

In male rats sacrificed at 24 months, a statistically significant increase in subcutaneous fibromas (10/28, 35.7%) was observed in the group exposed to 100 ppm ETO as compared with combined controls (3/91, 3.3%) (Table 9-24). An increased prevalence of these tumors was also observed in the 10 ppm group (8/48, 17%); however, this increase was not significant. No increase in subcutaneous fibromas was observed in the 33 ppm group. The authors concluded

TABLE 9-23. SUMMARY OF SELECTED TUMOR INCIDENCE COMPARISONS FOR MALE AND FEMALE FISCHER 344 RATS EXPOSED TO ETO FOR TWO YEARS
(adapted from Snellings et al. 1981)

Ethylene oxide concentration ppm	Total number of rats With tissues examined	With tumor[a]	Time in months to: First tumor	Median tumor[b]
	Mononuclear cell leukemia - Males			
100	119	26	19	24
33[c]	81	25	13	25
10[c]	79	21	20	25
0-I	116	20	18	23
0-II	118	18	21	25
	Mononuclear cell leukemia - Females			
100	113	28(c,b,c)	18	24
33[c]	79	24(c,c,c)	18	24
10[c]	77	14	19	25
0-I	118	9	19	24
0-II	117	13	18	23
	Peritoneal mesothelioma - Males			
100	119	22(c,c,c)	15	23
33[c]	91	7(a,a,a)	18	25
10[c]	89	3	20	--
0-I	114	2	18	--
0-II	116	2	20	--
	Pituitary adenoma - Males			
100	117	27	15	25
33[c]	79	16	15	25
10[c]	80	27	18	25
0-I	117	28	17	25
0-II	117	22	18	25
	Pituitary adenoma - Females			
100	117	32	10	24
33[c]	90	38	17	25
10[c]	90	39	16	24
0-I	119	38	15	25
0-II	116	38	18	25

[a]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

[b]Medians were not presented if the total number of a particular tumor was three or less.

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

[c]Only organs with gross lesions were histologically examined from this exposure level at the 6-, 12-, and 18-month sacrifice intervals.

TABLE 9-24 . ETO 2-YEAR VAPOR INHALATION STUDY: 24-MONTH FINAL SACRIFICE FREQUENCY OF EXPOSURE-RELATED NEOPLASMS FOR 110- TO 116-WEEK-OLD FISCHER 344 RATS
(adapted from Snellings et al. 1981)

	ppm of Ethylene Oxide				
Organs/Findings/Sex	100[a]	33[a]	10[a]	Control I	Control II
Total number examined grossly					
Male	30	39	51	48	49
Female	26	48	54	60	56
Pituitary					
Adenomas					
Male	12/29[b]	13/39	15/51	16/48	13/49
Pancreas[c]					
Adenomas					
Male	5/30	1/2	2/3	2/48	5/49
Subcutis[d]					
Fibromas					
Male	10/28(c,c,c)	1/34	8/48(a,a,b)	1/44	2/47
Peritoneum					
Mesotheliomas					
Male	4/30	4/39	2/51	1/48	1/49
Spleen					
Mononuclear cell leukemias					
Male	8/30	10/39	9/51	5/48	8/49
Female	15/26(c,c,c)	14/48(b,b,b)	11/54(-,-,a)	5/60	6/55

[a]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

[b]Numerator equals number of rats with specified finding. Denominator equals number of rats for which specified tissues were examined.

[c]Tissues from 33- and 10-ppm groups examined only if gross lesions were present. Since tissues were not examined from all rats, data from the 33- and 10-ppm groups were not statistically compared with data from other groups.

[d]Examined only if gross lesions were present (except flank region skin and subcutis, which was routinely examined microscopically).

that the increased prevalence of subcutaneous fibromas in the 100 ppm group represented an effect of treatment. It should be noted, however, that histologic examinations were performed only on skin sections that showed gross lesions; therefore, many tumors too small for gross detection were probably missed. When the incidences of this tumor type were added to those for animals that died spontaneously or were euthanized when moribund, the totals were even higher in both the 100 and 10 ppm groups than in the controls (Table 9-25).

An increase in the frequency of peritoneal mesothelioma was observed in all of the male treatment groups sacrificed at 24 months (4/30 at 100 ppm, 4/39 at 33 ppm, 2/51 at 10 ppm vs. 1/48 for the Control I group and 2/84 for the Control II group) (Table 9-24). Although the increase was not significant at any dose level, this enhanced prevalence in the 100 and 33 ppm groups is considered a treatment-related effect. This tumor was also found in a large number of treated animals that died spontaneously or were euthanized when moribund. When the tumor incidence in this latter group was added to that for animals sacrificed at 24 months, the numbers were much higher than controls and were statistically significant for the high-dose group versus controls (21/80 at 100 ppm, 6/80 at 33 ppm, 3/80 at 10 ppm vs. 1/80 for the Control I group and 2/80 for the Control II group) (Table 9-25).

Pancreatic adenomas were statistically significant for the male high-dose group sacrificed at 24 months and the animals that died spontaneously or were euthanized when moribund (11/80 at 100 ppm, 1/43 at 33 ppm, 2/32 at 10 ppm vs. 2/80 in the Control I group and 5/80 in the Control II group) (Table 9-25). Tissues from the 33 and 10 ppm groups were examined only if gross lesions were present in the 24-month sacrifice group, which may explain the paucity of tumors in these groups (Table 9-24). The denominator in Table 9-25, the number

TABLE 9-25. ETO 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF EXPOSURE-RELATED NEOPLASMS AT 24-MONTH FINAL SACRIFICE AND IN FISCHER 344 RATS DYING SPONTANEOUSLY OR EUTHANIZED WHEN MORIBUND[a]
(adapted from Snellings et al. 1981)

	ppm of Ethylene Oxide				
Organs/Findings/Sex	100[b]	33[b]	10[b]	Control I	Control II
Pituitary					
Adenomas					
Male	24/79[c]	16/79	26/79	24/79	19/78
Pancreas[d]					
Adenomas					
Male	11/80(b,-,a)	1/43	2/32	2/80	5/80
Subcutis[e]					
Fibromas					
Male	15/78(c,b,c)	3/75	10/77(b,a,b)	1/76	3/78
Peritoneum					
Mesotheliomas					
Male	21/80(c,c,c)	6/80(-,-,a)	3/80	1/80	2/80
Spleen					
Mononuclear cell leukemias					
Male	25/80	23/80	21/80	20/80	18/80
Female	27/80(c,a,c)	24/80(b,a,b)	14/80	9/80	13/76

[a]Concerning the animals that died spontaneously or were euthanized when moribund, it was not specified whether tissues were examined microscopically only when gross lesions were present, or if all tissues were reviewed in this way. It is therefore assumed that all of the tissues from these animals were studied histologically, whether or not gross lesions were observed. Not to have performed such studies would have yielded erroneously low frequencies of exposure-related neoplasms.

[b]Superscripts in parentheses denote values significantly higher than those of control groups. First letter denotes degree of significance vs. Control I group; second letter denotes degree of significance vs. Control II group; third letter denotes degree of significance vs. combined controls (C-I plus C-II).

a = $0.05 > P > 0.01$ b = $0.01 > P > 0.001$ c = $P < 0.001$ - = not significant

[c]Numerator equals number of rats with specified finding. Denominator equals number of rats for which specified tissues were examined.

[d]Tissues from 33- and 10-ppm groups were examined only if gross lesions were present. Since tissues were not examined from all rats, data from the 33- and 10-ppm groups were not statistically compared with data from other groups.

[e]Examined only if gross lesions were present (except flank region skin and subcutis, which was routinely examined microscopically).

of rats for which the specified tissue was examined, may be erroneously high for the data combining the 24-month sacrifice with the animals that died spontaneously or were euthanized when moribund.

While Tables 9-23 and 9-24 show no significant increase in the frequency of pituitary adenomas in the groups of treated males, Table 9-23 shows some indication of a decreased time-to-tumor. In males, the first pituitary adenomas appeared at 15 months in the 100 and 33 ppm groups, and in the 17th or 18th month in all other groups; in females, the corresponding times were 10 months for the 100 ppm group versus at least 15 months for all other groups. The time-to-tumor decreased significantly with increasing dose ($P < 0.01$ for males, $P < 0.0001$ for females), suggesting that the normal incidence of pituitary adenomas was accelerated by exposure to ETO.

An increased frequency of mononuclear cell leukemia was observed in the ETO-treated animals at the 24-month sacrifice interval (Table 9-24). Statistical significance was observed in females in both the 100 and 33 ppm groups versus combined controls ($P < 0.01$). The responses for the 24-month sacrifice were 15/26 (58%), 14/48 (29%), and 11/115 (10%) for the 100, 33, and 10 ppm groups and combined controls, respectively. The frequencies for male rats were not significantly increased in the treated versus the control groups.

In females, the results for animals dying spontaneously or euthanized when moribund and for those sacrificed at 24 months remained statistically significant for the two higher-dose groups versus combined controls. The frequencies for females (Table 9-25) were 27/80 (34%), 24/80 (30%), 14/80 (18%), and 22/156 (14%) for the 100, 33, and 10 ppm groups and combined controls, respectively, with statistically significant differences in the two higher-dose groups versus combined controls ($P < 0.01$) and a significantly positive linear dose-response

trend ($P < 0.01$). The trend became even stronger ($P < 0.00001$) when the proportions were adjusted for early mortality. These data suggest that exposure to ETO not only increased the total incidence of leukemia but also accelerated its rate of development (Figure 9-3). The authors also reported that the number of female rats with three or more tumors was significantly ($P < 0.001$) increased in the 100 ppm group as compared to the controls.

A letter to the U.S. Environmental Protection Agency (Browning 1982) stated that a recent histologic examination of all brain tissue from the Snellings et al. (1981) study revealed the presence of primary brain neoplasms (Tables 9-26 , 9-27 , and 9-32). These tumors were shown to be statistically significant by the Fisher Exact Test in both males and females.

In summary, ETO has produced significant increases of several tumor types in rats. A dose-related increase in mononuclear cell leukemia occurred in female rats. The occurrence of pituitary adenoma appeared to be accelerated in female rats exposed to 100 ppm, although there was no statistically increased incidence of these tumors. The frequency of peritoneal mesothelioma was treatment-related in the male rats exposed to 100 and 33 ppm. Further, a significant increase occurred in subcutaneous fibromas in male rats. Increases in brain neoplasms were also observed in both sexes.

9.5.1.2.2 National Institute for Occupational Safety and Health Inhalation Study (Lynch et al. 1982). Another chronic inhalation study (unpublished draft) on ETO and propylene oxide (PO) was performed by the National Institute for Occupational Safety and Health (NIOSH) (Lynch et al. 1982). In the present report, only the preliminary findings of the ETO section of the study will be discussed. Male Fischer 344 rats (80 in each group) and 12 male cynomolgous monkeys were exposed to ETO at either 50 or 100 ppm for 7 hours/day, 5 days/week, for 24 months. Each treatment group consisted of 80 rats and 12 monkeys

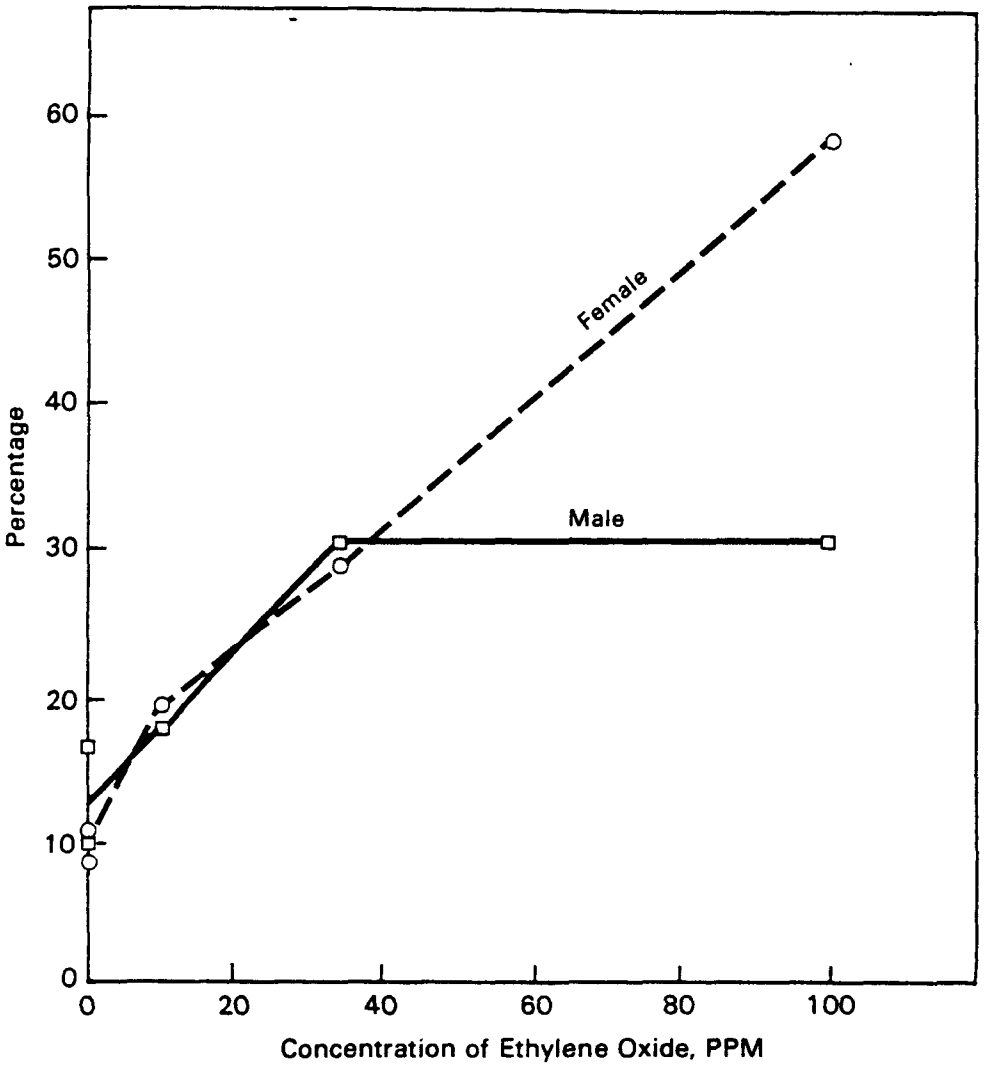


Figure 9-3 . Percentages of male and female Fischer 344 rats with histologically confirmed mononuclear cell leukemia at 24-month sacrifice. (Snellings et al. 1981)

TABLE 9-26. ETO 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF PRIMARY BRAIN NEOPLASMS IN FISCHER 344 RATS
(adapted from Snellings et al. 1981)

	Exposure level (ppm)				
Sex	100	33	10	0 (CI)	0 (CII)
		18-month sacrifice[a]			
Male	0/20	1/20	0/20	0/20	0/20
Female	1/20	0/20	0/20	1/20	0/20
		24-month sacrifice[a]			
Male	3/30	1/39	0/51	1/48	0/49
Female	2/26	2/48	0/51	0/60	0/56
		Dead/euthanized moribund[a]			
Male	4/49	3/39	1/28	0/30	0/29
Female	1/53	1/31	1/24	0/18	0/20
	18- and 24-month sacrifices and dead/euthanized moribund[a] (Combined from above)				
Male	7/99	5/98	1/99	1/98	0/98
Female	4/99	3/99	1/95	1/98	0/96
		Two-year study[b]			
(Combined 6-, 12-, 18-, and 24-month sacrifices and dead/euthanized moribund animals)					
Male	7/119[b] P=0.002[c]	5/118 P=0.017[c]	1/119	1/118	0/118
Female	4/119 P=0.045[c]	3/119	1/115	1/118	0/116

[a]Numerator equals the number of brains with primary neoplasms. Denominator equals total number of brains examined microscopically.
[b]Numerator equals the number of brains with neoplasms. Denominator equals total number of brains examined microscopically. Although animals sacrificed at 6 and 12 months are included, no brain neoplasms were discovered in these groups. The 6- and 12-month animals can be eliminated by subtracting 20 from each denominator.
[c]Fisher Exact Test.

at the start of the study. Rats and monkeys were housed together in the same chambers during the 7-hour exposure period. Food and water were available ad libitum except during the exposure periods. In analyzing for carcinogenicity, only limited data were available for monkeys because of their longer lifespans; however, the authors reported that there was no evidence of leukemia in any of the exposed monkeys.

TABLE 9-27 . ETO 2-YEAR VAPOR INHALATION STUDY: FREQUENCY OF PRIMARY BRAIN NEOPLASM TYPES IN FISCHER 344 RATS
(Combined data for 6-, 12-, 18-, and 24-month sacrifices, and dead/euthanized moribund animals)
(adapted from Snellings et al. 1981)

	Exposure level (ppm)				
Neoplasm type	100	33	10	0 (CI)	0 (CII)
	Males[a]				
Granular cell tumor	1/119	1/118	1/119	0/118	0/118
Astrocytoma/oligodendroglioma/mixed glioma	5/119	2/118	0/119	1/118	0/118
Malignant reticulosis-microglioma	1/119	2/118	0/119	0/118	0/118
	Females[a]				
Granular cell tumor	1/119	1/119	0/118	1/118	0/116
Astrocytoma/oligodendroglioma/mixed glioma	2/119	2/119	1/118	0/118	0/116
Malignant reticulosis-microglioma	1/119	0/119	0/118	0/118	0/116

[a]Numerator equals the number of brains with primary neoplasms. Denominator equals total number of brains examined microscopically. Although animals sacrificed at 6 and 12 months are included, no brain neoplasms were discovered in these groups. The 6- and 12-month animals can be eliminated by subtracting 20 from each denominator.

An overall statistically significant depression in weight gain was noted for ETO-exposed rats. This development, which appeared to begin at about week 7 for the 100 ppm group and at week 15 for the 50 ppm group, continued throughout the study. Survival was also adversely affected by exposure to ETO, with estimated mean survival times of greater than 720 days for the controls, 690 days for the 50 ppm group, and 653 days for the 100 ppm group. An outbreak of mycoplasma infection also caused an abrupt decline in survival at about 480 days into the study.

With respect to pathology, the authors report that the livers and spleens of the ETO-exposed rats were the only organs for which histopathologic evaluations were completed. While the results are preliminary (Table 9_{-28}), the data obtained at terminal sacrifice indicate that the incidence of leukemia followed a dose-response pattern ranging from 33.3% in controls to 64.3% in the 100 ppm group ($P = 0.07$, Table 9_{-28}). The one-tailed test for linear trend at terminal sacrifice was significant at the $P < 0.05$ level. Using a two-tailed test, the significance level was $P = 0.08$. These preliminary data, therefore, do provide some evidence of ETO-induced leukemia. The data from moribund sacrifice and deaths (Table 9_{-28}) merely accentuate both the early toxicity and the mortality in the 100 ppm group as compared with the other groups, and the relatively high leukemia rates in these rats. Neither these rats nor the total was significantly higher than controls.

Lynch et al. (1982) also reported that exposure to ETO significantly increased the incidence of peritoneal mesotheliomas. These tumors were present on the tunica vaginalis surrounding the testes and epididymis, and occasionally spread to the peritoneal cavity. A non-significant increase in pheochromocytomas was observed in exposed groups (Table 9_{-29}).

TABLE 9-28. LEUKEMIA INCIDENCE IN MALE FISCHER 344 RATS[a] EXPOSED TO ETO FOR 2 YEARS
(Lynch et al. 1982)

Treatment group	Leukemia incidence		
	Terminal sacrifice only (%)	Moribund sacrifice and death (%)	Terminal sacrifice plus moribund sacrifice and death (total)
Control	7/21 (33.3%)	5/18 (27.9%)	12/39 (30.8%)
Ethylene oxide, 50 ppm	12/27 (44.4%)	26/52 (50.0%)	38/79 (48.1%)
Ethylene oxide, 100 ppm	9/14 (64.3%)[b]	21/62 (33.9%)	30/76 (39.5%)

[a]Based on histopathologic evaluation of spleens.
[b]P = 0.07 based on the one-tailed Fisher Exact Test.

TABLE 9-29. INCIDENCE OF NEOPLASTIC LESIONS IN MALE FISCHER 344 RATS EXPOSED TO ETO FOR 2 YEARS[a]
(Lynch et al. 1982)

Organs/Findings	Exposure level (ppm)		
	Control	50	100
Adrenal			
Pheochromocytomas	8/78	14/77	13/78
Brain			
Gliomas (mixed cell)	0/76	2/77	5/79 (P = 0.032)[b]
Body cavity			
Peritoneal mesotheliomas	3/78	9/79	21/79 (P = 4.94×10^{-5})[b]
Spleen			
Mononuclear cell leukemia	24/77	38/79 (P = 0.22)	30/76

[a]Each group consisted of 80 male rats. Denominators of less than 80 reflect tissues accidentally lost or tissues that could not be examined histologically due to autolysis.
[b]Fisher Exact Test.

Lynch et al. (1982) reported the following incidences of mixed-cell gliomas in male rats: 0/76 in controls, 2/77 in the 50 ppm group, and 5/79 in the 100 ppm group. The term "glioma" was used because the tumors contained both astrocyte and oligodendroglia cells within the tumor. These findings are significant because the above-described tumors are unusual in Fischer 344 rats. Additional data collected from this study are currently being evaluated, and a final comprehensive report is scheduled to be published within a year.

9.5.1.3 Summary of Animal Studies--The Snellings et al. (1981) study, which showed an increase in leukemia in Fischer 344 rats, is also supported by a preliminary NIOSH study (Lynch et al. 1982, Table 9-28) in which an increase in leukemia appeared in rats of the same strain but of a different sex and with mycoplasma instead of SDA viral infections. Increases in peritoneal mesotheliomas were observed in both studies (Snellings et al. 1981 and Lynch et al. 1982), and significant increases in subcutaneous fibromas in the males were observed in the Snellings study. Snellings et al. (1981) also concluded that the frequencies among female rats with more than two neoplasms were significantly greater for all three groups when compared to combined controls.

Further, both studies found significant increases in brain neoplasms, a development that requires further review in terms of its possible value for risk evaluation. Like the finding of gliomas in male rats reported previously, these studies are significant because brain neoplasms are unusual in the Fischer 344 strain of rats.

In 1980, the National Toxicology Program (NTP) began a cancer bioassay in B6C3F1 mice (inhalation exposure). Exposure to ETO at 0, 5, and 100 ppm for 6 hours per day, 5 days per week began in August 1981. The final report is expected in mid-1984.

9.5.2 Epidemiologic Studies

9.5.2.1 Joyner (1964)--Joyner (1964) conducted a health evaluation of employees at an ETO plant in Texas. The evaluation included a physical examination of 37 male ETO operators, aged 29 to 56, and a similar number of age-matched controls. The operators were reported to have been exposed to ETO at approximately 5-10 ppm for the durations of their service. The controls, who were chosen from operators assigned to other production units, had been exposed to many different agents encountered in the petrochemical industry. The author stated that the mean length of service for the control group was 11 2/3 years, as compared with 10 2/3 years for the exposed group. The author used company medical records for the period 1952-1963 to compare the exposed group and controls with respect to days lost for illness, specific diagnoses, and initial visits for respiratory, gastrointestinal, or genitourinary complaints. The author found that the ETO operators who were currently employed exhibited less absenteeism, fewer symptoms, and fewer diagnosed illnesses (including malignant neoplasms) than the controls.

The author also reviewed the medical records of nine operators who had experienced accidental exposures in the previous 10 years, and seven workers other than operators who had experienced accidental exposures in the previous 8 years. Twelve of the accidental exposures were reported to be dermal exposures, while three were reported to be inhalation exposures; one exposure was reportedly to "vapor". Most of the dermal exposures produced burns. The vapor exposure produced conjunctivitis. Two of the persons with inhalation exposure suffered no symptoms; the third developed nausea and vomiting, which lasted several hours. The authors reported that the persons identified as having had accidental exposures did not exhibit any recurring medical problems. The one person who had suffered symptoms from the inhalation exposure was no longer with the company and was reportedly not available for follow-up.

Additionally, the author reviewed the medical records of eight persons who had previously worked as ETO operators for 100 months or more but who had since been transferred to another division. Among persons formerly employed as ETO operators for 100 months or more, no significant differences were found in the incidence of illness, symptoms, complaints, or absenteeism when compared to the study cohort or to controls; very little data was presented in this regard, however.

This study is inadequate for use in evaluating the carcinogenicity of ETO for several reasons. First, it is primarily a cross-sectional study of ETO operators who were employed as such at the time of the study. Workers who had developed cancer would probably no longer have been employed at the plant. Secondly, the period of observation, which in this study is the same as the duration of exposure for the current operators, may have been too short to allow adequate assessment of a carcinogenic effect. Cancer latency may be as long as 20 to 30 years; the longest observation period amoung current operators in this study was 16 1/3 years. The mean exposure for current operators was 10 2/3 years. For those with accidental exposures, the longest follow-up was 10 years. For the eight workers with over 100 months (8 1/3 years) of exposure, the length of follow-up was not indicated. Third, the sample sizes studied were so small that only an extremely large carcinogenic effect could be detected.

9.5.2.2 Ehrenberg and Hallstrom (1967)--Ehrenberg and Hallstrom (1967) conducted a hematologic investigation of workers at a factory that manufactured and used ETO. A preliminary investigation in 1960 revealed certain hematologic differences between 28 exposed persons who worked in an area of the factory "where leakage of ethylene oxide from tube joints, pumps, etc. was possible (and at least occasionally occurred)," and 26 controls in other departments not working in contact with ETO. The sex of the study subjects was not reported. The ages of persons in the exposed group were reported to be about the same as those in the

control group. The exposed persons were reported to have been active in the ETO department for 2 to 20 years, with an average of 15 years. One case of leukemia (chronic lymphatic type) was observed in the exposed group; the expected number of leukemia cases in the exposed group was not reported. No cases of leukemia were found in the controls. Three cases of anisocytosis were found in the exposed group and none in the controls, a finding which the authors suggested may indicate a disturbed bone marrow function. Hemoglobin values were reported to be significantly ($P < 0.05$) lower in the exposed group than in the controls, and lymphocytes per mm^3 were reported to be significantly ($P < 0.01$) higher in the 27 exposed healthy persons than in the 20 healthy controls (the presence of disease may affect the white blood cell count; thus, only "healthy" persons were considered in the latter comparison). It should be noted that three persons who were reported to have been accidentally exposed to high levels of ETO were added to the exposed group for the lymphocyte/mm^3 comparison (for a total of 31 persons in the exposed group). The authors did not state where these three persons worked or even whether they worked in the factory.

Because of these differences relating to hemoglobin and lymphocytes, and because ventilation was improved in the plant, the authors did a second study of the factory workers in 1961. The second study was expanded to include all of the workers in the plant. Workers were divided into four categories: "66 persons not working with ethylene oxide (including the 1960 control group); 86 persons intermittently working in ethylene oxide premises; 54 persons who had once been working in contact with ethylene oxide for some period of time; and 37 persons permanently working in the ethylene oxide area (including the 1960 exposed group)." The only hematologic analysis in the second study was for lymphocytes. The authors found an elevated lymphocyte count in the exposed group as compared with controls, but this difference was not significant ($P >$

0.05) for either healthy individuals or the total group. The authors suggested that this lack of a significant difference could possibly be attributed to improved ventilation and safety control in the factory, the small number (17) of healthy persons in the group permanently exposed (vs. 27 healthy exposed individuals in the 1960 investigation), and/or the average age difference between the exposed and control groups. The average age of the enlarged control group was reported to be "significantly" lower than that of the exposed group, and in general, a decrease in lymphocyte count with age was found. A significant age difference between the exposed group and the controls was not present in the 1960 examination. It should be noted that for those persons examined in the 1960 investigation a significant difference in average lymphocyte count between the exposed group and the controls occurred again when the two groups were examined in 1961.

The authors also compared the number of chromosome aberrations in eight persons accidentally exposed to ETO with that in a control group of 10 persons, and found that chromosome aberrations were significantly elevated in the exposed group. Details of the statistical analysis were not given.

In conclusion, Ehrenberg and Hallstrom (1967) found one leukemia case among 28 workers exposed to ETO. The authors indicated that the probability of such an occurrence was small, but its statistical significance was not calculated. The result of the study also suggested that ETO may elevate lymphocyte counts and reduce hemoglobin values.

9.5.2.3 Hogstedt et al. (1979a)--A follow-up study of these same workers with regard to mortality and cancer incidence was done by Hogstedt et al. (1979a). The follow-up period included the years from 1961 to 1977. The authors reported that the workers in this factory were exposed to various chemicals. During the period from 1941 to 1947, it was estimated that the air concentrations were

5 mg/m^3 ethylene chlorohydrin, 100 mg/m^3 ethylene dichloride, 0.05 mg/m ^{3} bis(2-chloroethyl)ether, and 600 mg/m^3 ethylene. The authors also cited the possibility that concentrations up to 1000 times greater than those reported may have occurred for short periods of time. For ETO, the exposure was reported to be probably < 25 mg/m^3, although there were occasional exposures to the chemical at 1300 mg/m^3 (odor threshold). During the 1950s and until 1963, the authors reported that the average air concentration of ETO in the factory was probably 10 to 50 mg/m^3, although peaks above the odor threshold still occurred. Random samples in the 1970s showed a range of 1 to 10 mg/m^3 for ETO and 10 to 25 mg/m^3 for propylene oxide, with the latter concentrations occasionally being as high as 120 to 150 mg/m^3.

The study included three subcohorts composed of 66 men who had never taken part in work involving exposure to ETO, 86 intermittently exposed men (maintenance workers), and 89 men whose work involved full-time exposure. In the full-time exposed group, a total of 9 cancer deaths were observed while only 3.4 were expected ($P < 0.01$). There were no statistically significant differences between the observed and expected number of cancer deaths in the other two exposure groups. Five of the nine cancer deaths seen in the full-time exposed cohort were either from cancer of the stomach (three deaths) or from leukemia (two deaths). Deaths from both causes were significantly ($P < 0.01$) elevated in comparison with the numbers expected (3 observed versus 0.4 expected for stomach cancer and 2 observed versus 0.14 expected for leukemia deaths). One of the leukemia deaths was from chronic lymphatic leukemia, and the other was from acute myeloid leukemia. The death from chronic lymphatic leukemia may well have been the same case that was reported in the Ehrenberg and Hallstrom (1967) study. Although the maintenance group showed no overall excess cancer mortality, the cancer deaths that occurred in this group were restricted to cancers of the

esophagus, stomach, and lymphatic system. The lymphatic system cancer death was from chronic lymphatic leukemia.

Cases of cancer in surviving subjects were identified by the Swedish Cancer Registry. By this method, two cases were identified among full-time exposed workers (testis cancer and urinary bladder cancer), two cases among maintenance workers (glottis cancer and prostate cancer), and one case (thyroid cancer) among unexposed workers. This raised the total number of cancer cases (both living and dead) identified during the follow-up period among full-time exposed workers to 11, with an expected number of 5.9 ($P < 0.05$). The expected number of cases by tumor site was not indicated. Among maintenance workers and unexposed workers, the total numbers of observed cases were raised to three and two, respectively. The expected number of total cancer cases for these two latter groups was not reported.

In summary, deaths from cancer of all sites, deaths from stomach cancer, and deaths from leukemia were each significantly ($P < 0.01$) elevated among the full-time exposed cohort. The total number of malignancies was also significantly ($P < 0.05$) elevated in this group. Workers in the full-time exposed cohort were exposed to several chemical agents, however, and the excess cancer incidence and mortality in this cohort cannot necessarily be ascribed to the ETO exposure.

9.5.2.4 Hogstedt et al. (1979b)--Hogstedt et al. (1979b) reported three cases of leukemia among workers in a small factory in Sweden between 1972 and 1977, in a study of a different population than the one studied by Ehrenberg and Halstrom (1967) and Hogstedt et al. (1979a). The factory had used 50% ETO and 50% methyl formate since 1968 for sterilizing hospital equipment. The number of persons who worked with the actual sterilization procedure was few, but the

treated boxes were stored in a hall where 30 women worked. Because of leakage from the treated boxes, "the average exposure in the storage hall was actually higher than in the sterilization room." Exposure measurements made in 1977 showed storage hall concentrations of 2 to 70 ppm, with 8-hour time-weighted average concentrations being calculated at 20 $\pm$ 10 ppm. The concentration was 1500 ppm inside newly sterilized boxes and 150 ppm on the floor outside the boxes. During the period from 1968 to 1977, 70 persons had been employed at some time in the storage hall, and another 160 had been employed in the neighboring rooms or as sterilizing operators. The expected number of leukemia cases in this group for the above period would have been 0.2. This was calculated by multiplying the person-years of observation by the sex- and age-specific national leukemia incidence for 1972.

The first of the three reported leukemia cases was that of a woman who had begun working in the storage hall in 1966. In 1972, at the age of 51, she was diagnosed as having chronic myeloid leukemia, and died in 1977. The second case was that of a woman who had begun working in the storage hall in 1968, and in early 1977, at the age of 37, was diagnosed as having acute myelogenetic leukemia. As of July 1978, her leukemia was in complete remission. The third reported case involved a man who had been the local manager of the plant since 1965. It was estimated that his exposure to ETO was 3 hours per week. In 1974, at the age of 56, he was diagnosed as having primary macroglobulinemia, and died in 1976. The authors stated that the two women had not been exposed to radiation, benzene, or other leukemia-inducing agents, but that the man had had occasional contact with benzene.

It should be noted that primary or Waldenstrom's macroglobulinemia, as was diagnosed in the plant manager, is not considered a leukemia under the International Classification of Diseases (ICD), Eighth Revision. As a result, the

Carcinogen Assessment Group (CAG) requested clarification from the primary author of the study on the classification of the case of macroglobulinemia as a leukemia case. In correspondence to the CAG (Hogstedt 1983), Hogstedt stated that Waldenstrom's macroglobulinemia was recognized by most experts in Sweden in the late 1970s to be a type of leukemia, but was considered by early 1983 to be a type of non-Hodgkin's lymphoma. In the same letter to the CAG, Hogstedt indicated, as he had in an earlier letter to Dr. Peter Infante of the U.S. Occupational Safety and Health Administration (Hogstedt 1981), that he and his fellow authors, since publication of their 1976 study, had calculated an expected number of leukemia cases based on Swedish incidence data for 1968-77. They found that the the expected number was 0.1, as opposed to the 0.2 that had been calculated from 1972 Swedish incidence data and reported in the Hogstedt et al. (1979b) article. The probability of the occurrence of two cases of leukemia (excluding the Waldenstrom's macroglobulinemia case), given the expectation of 0.1, is less than 0.01. (Had the expected number of leukemuia cases been 0.2, the probability would have been less than 0.02.) The probability that two cases would occur in the group working in the storage hall (where the two leukemia cases worked) is even lower, however, because the expected number of cases, 0.1, was calculated for the entire population of the factory.

Hogstedt et al. also suggested that the combination of ETO and methyl formate may produce a special carcinogenic risk, since methyl formate, the authors indicated, exhibits its antibacterial effect by affecting DNA structure. No literature reference was cited by the authors as to this point, however. A literature search conducted for the Carcinogen Assessment Group by the Environmental Mutagen Information Center at the Oak Ridge National Laboratory (Stafford 1983) failed to find any literature citations for mutagenicity studies of methyl formate.

9.5.2.5 Morgan et al. (1981)--Morgan et al. (1981) conducted a retrospective study of 767 workers potentially exposed to ETO who had worked for at least 5 years at the Texaco Chemical Company plant in Port Neches, Texas, between January 1955 and December 31, 1977. The authors provided no analysis of the cohort with respect to length of follow-up. An industrial survey of the plant (performed in July 1977) showed that the 8-hour time-weighted average exposure to ETO was "well below" 50 ppm, except in the area around the tank car loading operations, where readings were as high as 6000 ppm. Among the 767 male workers potentially exposed to ETO in the study cohort, there were 11 deaths from malignant neoplasms, where 15.24 would have been expected on the basis of U.S. vital statistics.

There were more deaths than expected from pancreatic cancer (SMR* = 377, 3 observed versus 0.8 expected), bladder cancer (SMR = 322, 1 observed versus 0.31 expected), brain and central nervous system cancer (SMR = 285, 2 observed versus 0.7 expected), and Hodgkin's disease (SMR = 570, 2 observed versus 0.35 expected). Although the 95% lower confidence limits for these SMRs were all less than 100, the number of deaths from pancreatic cancer and the number of deaths from Hodgkin's disease were each significantly ($P < 0.05$) more than expected by hypothesis testing using the Poisson test. Excess mortality from leukemia was not found. Because their study cohort was small and because excess cases of leukemia following exposure to ETO were found in the studies by Hogstedt et al. (1979a, b), the authors calculated the magnitude of the relative risk of mortality from leukemia, given the sample size of the cohort, that could be detected at the 95% confidence level with a power of 80%. This relative risk was calculated to be 10.5 (an SMR of 1050). In conclusion, it should be stated that the observed mortalities from pancreatic cancer and from

*Standardized mortality rate.

Hodgkin's disease were each significantly elevated among the study cohort, and that the study cohort may have been too small for an adequate evaluation of the risk of mortality from leukemia or other cancer types. Furthermore, there was no indication by the authors that sufficient allowance had been made for a cancer latency period.

9.5.2.6 Theiss et al. (1982)--Theiss et al. (1982) conducted a cohort mortality study of 602 persons who had been employed for six months or longer in the alkylene oxide (ethylene oxide/propylene oxide) production or processing areas of nine BASF Aktiengesellschaft, Ludwigshafen plants in West Germany during the period from 1928 to 1980. Vital status was ascertained for 523 of the 536 German employees in the cohort, while that of only 30 of the 66 non-German employees could be determined. Thus, the percentage of overall follow-up in this study was 92% (553 of 602). In addition to alkylene oxides, the workers were reported to have been exposed to a variety of other compounds.

The expected mortality for the total cohort and for those within the cohort who were observed for a minimum of 10 years was calculated using mortality data for Ludwigshafen, Rhinehessia-Palatinate, and the Federal Republic of Germany. The observed and expected numbers of cancer deaths for those persons observed for at least 10 years are reported in Table 9-30 . The observed number of deaths from cancer of any site was not significantly ($P < 0.05$) higher than that expected based on mortality data for Ludwigshafen, Rhinehessia-Palatinate, or the Federal Republic of Germany. Deaths from cancer of the brain among alkylene oxide workers followed for at least 10 years did, however, approach statistical significance ($P < 0.07$) in comparison with those expected based on Ludwigshafen or Rhinehessia-Palatinate mortality data.

The authors also compared the observed number of cancer deaths with that expected, using an internal cohort of 1,662 styrene workers. The minimum obser-

TABLE 9-30. COMPARISON OF OBSERVED NUMBERS OF CANCER DEATHS IN BASF-AKTIENGESELLSCHAFT, LUDWIGSHAFEN PLANTS 1928-80 FOR PERSONS HAVING 10 YEARS OF OBSERVATION FOLLOWING EXPOSURE TO ALKYLENE OXIDE WITH THAT EXPECTED BASED ON MORTALITY STATISTICS FOR RHINEHESSIA-PALATINATE 1970-75, LUDWIGSHAFEN 1970-75, AND THE FEDERAL REPUBLIC OF GERMANY 1971-74, BY ICD CODE AND CAUSE OF DEATH
(adapted from Theiss et al. 1982)

ICD No.[a]	Cause of death	Observed deaths	Rhinehessia-Palatinate 1970-75		Ludwigshafen 1970-75		Federal Republic of Germany 1971-74	
			No.	P-value	No.	P-value	No.	P-valu
140-199[c]	Malignant tumors	10	--	--	--	--	11.816	--[b]
151	Malignant tumor of the stomach	2	1.852	0.552	1.765	0.527	2.033	--[b]
156	Malignant tumor of the gall bladder	1	0.201	0.182	0.243	0.216	--[c]	--[c]
162	Malignant tumor of the bronchii	4	3.769	0.520	3.956	0.568	--[c]	--[c]
188	Malignant tumor of the urinary bladder	1	0.469	0.374	0.532	0.413	--[c]	--[c]
191	Malignant tumor of the brain	1	0.071	0.068	0.066	0.064	--[c]	--[c]
193-199	Squamous cell carcinoma of unknown primary site	1	0.743	0.525	1.047	--[c]	--[c]	--[c]
205	Myeloid leukemia	1	0.148	0.138	0.145	0.135	0.756	0.531
230-239	Tumor of unknown character	1	0.454	0.365	0.426	0.347	--[c]	--[c]

[a]International Classification of Diseases Code, Eighth Revision.
[b]The probability of observed deaths occurring by chance was not provided by the authors because the observed deaths were fewer than expected.
[c]The authors did not report the number of deaths that would be expected in the cohort based on Federal Republic of Germany mortality rates for individual tumor sites other than stomach and myeloid leukemia.

vation period of 10 years required for the comparison in Table 9-14 was not used for this analysis. Thus, in Table 9-31 , there were 14 total observed cancer deaths, as opposed to 12 observed deaths in Table 9-30 . These results are reported in Table 9-31 . The relative risk of death from cancer of all sites in the alkylene oxide cohort in comparison to what would be expected based on cancer mortality in the styrene cohort was 1.48. Assuming that the numbers of observed and expected deaths (14 and 9.44, respectively) are both Poisson variables, the difference between the two is not statistically significant ($P < 0.05$). In the 65-74-year-old age group, the relative risk was 2.78. If it is assumed that both the observed and expected deaths are Poisson variables, the difference between the two is statistically significant at $P < 0.05$. It should be noted that although the authors reported in tabular form that 10 cancer deaths had occurred in the 65-74-year-old age group, the text indicated that 11 had occurred--a difference that obviously would function to lower the probability of cancer deaths. A major problem in evaluating this result, however, is that the workers in the alkylene oxide cohort were exposed to a variety of chemicals in addition to ethylene oxide, some of which are known or suspected carcinogens. The authors did not compare the alkylene oxide and styrene cohorts with regard to the number of deaths by individual tumor site.

The authors also analyzed the cancer deaths by length of exposure, and did not find a dose-response. However, they gave no indication that the mortality analysis by length of employment had been adjusted for length of follow-up.

In summary, this study is inconclusive as to whether persons exposed to ETO are at an excess risk of death from cancer. There was a significant excess number of cancer deaths in the age group 65-74 in the alkylene oxide cohort, as compared to that expected based on the mortality data for a group of styrene workers. A fact that may have confounded this result is that the alky-

TABLE 9-31. RELATIVE RISKS OF DEATH FROM CANCER IN THE ALKYLENE OXIDE COHORT AS COMPARED WITH THE STYRENE COHORT, BY AGE (adapted from Theiss et al. 1982)[a]

Age group	Observed deaths	Expected deaths	Relative risk
15-24	--	--	--
25-34	--	0.35	--
35-44	--	0.47	--
45-54	--	1.61	--
55-64	4	3.41	1.17
65-74	10	3.60	2.78
75-84	--	--	--
Total	14	9.44	1.48

[a]In this analysis, a minimum observation period of 10 years was not made a requirement.

lene oxide workers were exposed to a variety of chemicals in addition to ETO, some of which are known or suspected carcinogens. Deaths from cancer of any particular site were not found to be significantly ($P < 0.05$) in excess when the expected numbers of deaths for those sites were derived using mortality data for Ludwigshafen or Rhinehessia-Palatinate. Two of the problems with this study are the small sample size and the fact that only a little more than half of the cohort was observed for 10 years or more. It should be noted that in regard to leukemia, for which Hogstedt (1979a, b) had found an association with ETO exposure, the authors found that for those persons who had had more than 10 years of exposure, one case of myeloid leukemia occurred where only about 0.15 would have been expected based on local mortality data, but this difference was not statistically significant at $P < 0.05$.

9.5.2.7 Schnorr (1982)--A proportionate mortality study by Schnorr (1982) of decedents who had been members of District 1199 of the National Hospital and Health Care Workers Union found that the proportionate mortality ratio (PMR) for neoplasms of lymphatic and hematopoietic tissue (ICD code 200-209, 8th Revision), as well as for other types of tumors, was significantly elevated for certain job categories (e.g., "service" and "nursing") that included job titles of personnel exposed to ETO (e.g., hospital central service employees, registered nurses, licensed practical nurses, and nurse's aides). Such job categories were relatively broad in their inclusion of job titles, however, and the results of the study with regard to a possible association of cancer risk with ETO exposure must therefore be judged inconclusive.

9.5.2.8 Studies in Progress--Several cohort or case-control studies testing the association of ETO exposure and the risk of cancer are currently in progress or about to begin. A cohort mortality study of approximately 1000 ETO production workers in the Kanawha Valley, West Virginia, is currently being conducted by NIOSH and the Union Carbide Corporation. The results of this study will not be available until at least mid-1984. NIOSH and the Health Industry Manufacturing Association are currently discussing plans for a cohort mortality study of medical equipment manufacturing personnel who use ETO as a sterilant. If the study is initiated, the results will not be available until at least 1985.

The U.S. Environmental Protection Agency is currently funding a case-control study of cases of cancer of the lymphatic and hematopoietic tissue among District 1199 of the National Hospital and Health Care Workers Union to determine if an association exists between such cancers and occupational exposure to ETO and/or other substances. The study, which includes 63 cases and 126 controls, is being conducted by Dr. Jeanne Stellman of Columbia University. The results are expected to be available in late 1984.

9.5.2.9 Summary of Epidemiologic Studies--In summary, three epidemiologic studies of persons occupationally exposed to ETO found a significant association between ETO exposure and either cancer incidence or mortality. The study by Hogstedt et al. (1979a) found significantly ($P < 0.01$) increased mortality for stomach cancer and leukemia among ETO production workers. Hogstedt et al. (1979b) found a significantly ($P < 0.05$) increased leukemia incidence among workers exposed to ETO used as a sterilant. The study by Morgan et al. (1981) found significantly ($P < 0.05$) increased mortality from pancreatic cancer and Hodgkin's disease.

Excess mortality from leukemia in the Hogstedt et al. (1979a) study and excess incidences of leukemia in the Hogstedt et al. (1979b) study were not limited to any particular types of leukemia. Excess deaths from leukemia in the Hogstedt et al. (1979a) study included one case of acute myeloid leukemia and two cases of chronic lymphatic leukemia. Excess cases of leukemia in the Hogstedt et al. (1979b) study included one case of acute myeloid leukemia and one case of chronic myeloid leukemia. The expected numbers of deaths or cases by type of leukemia were not calculated in either study.

It should be noted that in all three of the above-referenced epidemiologic studies, exposure of the cohort to other chemicals besides ETO was reported to have occurred or probably occurred. In the Hogstedt et al. (1979a) study, reports were made of exposure to several chemicals, of which two, ethylene dichloride and bis(2-chloroethyl)ether, are recognized carcinogens. In the Hogstedt et al. (1979b) study, ETO-exposed workers experienced concurrent exposure to methyl formate. In the Morgan et al. (1981) study, there was no mention of exposure to chemicals other than ETO, but the fact that the study was conducted at a chemical plant would suggest that exposure to other chemicals did occur.

9.5.3 Quantitative Estimation

This quantitative section deals with the unit risk for ETO in air, and the potency of ETO relative to other carcinogens that the CAG has evaluated. The unit risk estimate for an air pollutant is defined as the lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ug/m^3 of the agent in the air they breathe. These calculations are done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potencies of several agents with each other, and 2) to give a crude indication of the population risk that would be associated with air or water exposure to these agents, if the actual exposures were known.

In the sections that follow, the general assessment procedures used by the CAG are discussed. These include animal-to-human extrapolation modeling, data selection, calculation of human equivalent doses, extrapolation modeling from human epidemiologic studies, and interpretation of the resulting estimates. Following this discussion, the CAG's unit risk calculations and relative potency estimates are presented.

9.5.3.1 Procedures for the Determination of Unit Risk from Animal Data--In developing quantitative estimates of carcinogenic risks, one or both of two types of data are utilized: 1) lifetime animal studies, and 2) human studies where excess cancer risk has been associated with exposure to the agent. In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses, at incidences determined by an extrapolation model.

There is, however, no solid scientific basis for any mathematical extrapo-

lation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies. We must, therefore, depend on our current understanding of the mechanisms of carcinogenesis for guidance as to which risk model to use. At the present time, the dominant view of the carcinogenic process involves the concept that most cancer-causing agents also cause irreversible damage to DNA. This position is reflected by the fact that a very large proportion of agents that cause cancer are also mutagenic. There is reason to expect that the quantal type of biological response, which is characteristic of mutagenesis, is associated with a linear non-threshold dose-reponse relationship. Indeed, there is substantial evidence from mutagenicity studies with both ionizing radiation and a wide variety of chemicals that this type of dose-response model is the appropriate one to use. This is particularly true at the lower end of the dose-response curve; at higher doses, there can be an upward curvature, probably reflecting the effects of multistage processes on the mutagenic response. The linear non-threshold dose-response relationship is also consistent with the relatively few epidemiologic studies of cancer responses to specific agents that contain enough information to make the evaluation possible (e.g., radiation-induced leukemia, breast and thyroid cancer, skin cancer induced by arsenic in drinking water, liver cancer induced by aflatoxins in the diet). There is also some evidence from animal experiments that is consistent with the linear non-threshold model (e.g., liver tumors induced in mice by 2-acetylaminofluorene in the large-scale ED_{01} study at the National Center for Toxicological Research, and the initiation stage of the two-stage carcinogenesis model in rat liver and mouse skin).

Because its scientific basis, although limited, is the best of any of the

current mathematical extrapolation models, the linear non-threshold model has been adopted as the primary basis for risk extrapolation in the low-dose region of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk; i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear non-threshold dose-response relationship at low doses is the linearized multistage model. The multistage model employs enough arbitary constants to be able to fit almost any monotonically increasing dose-response data, and it incorporates a procedure for estimating the largest possible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

9.5.3.1.1 Description of the low-dose animal extrapolation model. Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients q_i, i = 0, 1, 2, ..., k, and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL79, developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1^* \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk, and R/q_1^* is a 95% lower confidence limit on the dose producing an extra risk of R. Let L_0 be the maximum value of the log-likelihood function. The upper limit, q_1^*, is calculated by increasing q_1 to a value q_1^* such that when the log-likelihood is remaximized subject to this fixed value q_1^* for the linear coefficient, the resulting maximum value of the log-likelihood L_1 satisfies the equation

$$2 (L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk, $P_t(d)$, is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear non-threshold concept discussed earlier. The slope, q_1^*, is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), where h is the number of dose groups in the experiment,

including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose are deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square statistic

$$X^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1-P_i)}$$

is calculated where N_i is the number of animals in the i^{th} dose group, X_i is the number of animals in the i^{th} dose group with a tumor response, P_i is the probability of a response in the i^{th} dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever X^2 is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of non-zero multistage coefficients.

9.5.3.1.2 Selection of data. For some chemicals, a number of studies in different animal species, strains, and sexes, each run at varying doses and routes of exposure, are available. In such cases, choices must be made as to which of several data sets are appropriate for use with the chosen model. The following are the procedures used by the CAG in evaluating these data for the purpose of risk estimation:

1. The data on tumor incidence are separated according to organ sites or tumor types. The dose and tumor incidence data set used in the model is the set in which tumor incidence is statistically significantly higher than in controls for at least one test dose level, and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The

data set that gives the highest estimate of the lifetime carcinogenic risk, q_1^*, is selected in most cases. However, efforts are made to exclude data sets that produce spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the data set having the larger sample size is selected for calculating carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex, and tumor sites, the geometric mean of q_1^*, estimates from each of these data sets, is used for risk assessment. The geometric mean of numbers A_1, A_2, ..., A_m is defined as

$$(A_1 \times A_2 \times \ldots \times A_m)^{1/m}$$

3. If two or more significant tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model.

9.5.3.1.3 Calculation of human equivalent dosages. In calculating human equivalent dosages, it is necessary to correct for differences in metabolism among species and for the variations in absorption factors involved in different routes of administration.

Following the suggestion of Mantel and Schneiderman (1977), it is assumed that mg/surface area/day is an equivalent dose between species. Since, to a close approximation, the surface area is proportional to the 2/3 power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per 2/3 power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner:

Let

L_e = duration of experiment

l_e = duration of exposure

m = average dose per day in mg during administration of the agent (i.e., during l_e) and

W = average weight of the experimental animal

The lifetime average exposure is then

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

When exposure is given in terms of mg/kg/day = m/Wr = s, the conversion is simply

$$\frac{m}{rw^{2/3}} = s \times W^{1/3}$$

where r is the absorption rate for ETO (assumed to be 1).

When exposure is via inhalation, as with ETO, dose calculations at experimental exposures of up to 100 ppm are performed under the assumption that the compound is a completely water-soluble gas absorbed proportionally to the amount of air breathed in. While the CAG has previously used an existing methodology to determine dose equivalency in such cases, for ETO the total body dose resulting from exposure of male Fischer 344 rats to air concentrations of 100 ppm for 6 hours has been measured as 20.24 mg/kg (Tyler and McKelvey, 1980). At 10 ppm exposures under similar conditions, the measured dose was 2.7 mg/kg. Since daily exposures in the Snellings et al. (1981) study included 10 ppm and 100 ppm, the human equivalent dosage for the above exposure is estimated as

$$d_h = 20.24 \times 5/7 \div (70/0.42)^{1/3} = 2.63 \text{ mg/kg/day for 100 ppm}$$

and

$$d_h = 2.7 \times 5/7 \div (70/0.42)^{1/3} = 0.35 \text{ mg/kg/day for 10 ppm}$$

where 0.42 kg is the average weight of the male rat in the Snellings et al. (1981) study, 70 kg is the average weight of the adult human, and 5/7 is the fraction of days exposed. By interpolation, the 33 ppm exposure is estimated as 0.94 mg/kg/day in human equivalent doses.

9.5.3.1.4 Calculation of the unit risk from animal studies. The risk associated with d $mg/kg^{2/3}/day$ is obtained from GLOBAL79, and for most cases of interest to risk assessment, can be adequately approximated by $P(d) = 1 - \exp(-q_1^* d)$. A "unit risk" in units X is simply the risk corresponding to an exposure of X = 1. To estimate this value, it is simply necessary to find the number of $mg/kg^{2/3}/day$ that corresponds to one unit of X, and substitute this number into the above relationship. For ETO, human equivalent doses will first be calculated and then fitted, together with the observed responses, to the linearized multistage model. An equivalent method of calculating unit risk would be to use mg/kg/day for the animal exposures and then to increase the j^{th} polynomial coefficient by an amount

$$(W_h/W_a)^{j/3} \qquad j = 1, 2, \ldots, k$$

and use the mg/kg/day equivalents for the unit risk values. In the section of this document that presents unit risk calculations from animal data, the final q_1^* will always represent the upper-limit potency estimate for humans.

9.5.3.1.5 Interpretation of quantitative estimates. Unit risk estimates based on animal bioassays are only approximate indications of absolute risk in populations exposed to known carcinogen concentrations. This is true for several reasons. First, there are important species differences in uptake, metabolism, and organ distribution of carcinogens, a well as in target site susceptibility, immunological responses, hormone function, dietary factors, and disease. Second,

the concept of equivalent doses for humans as compared to animals based on the relationship of weight to surface area is virtually without experimental verification as regards carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

Unit risk estimates can give rough indications of the relative potencies of given agents as compared with other carcinogens. Such comparisons are, of course, most reliable when based on studies in which the test species, strain, sex, and route of exposure are the same.

The quantitative aspects of assessing carcinogenic risks are discussed here because of the possible usefulness of this information in the regulatory decision-making process, e.g., in setting regulatory priorities, evaluating the adequacy of technology-based controls, etc. However, the uncertainty of present estimations of cancer risks to humans at low levels of exposure should be recognized. The CAG feels that, given the limited data available from animal bioassays, especially at the high dosage levels required for testing, almost nothing can be known about the true shape of the dose-response curve at low environmental levels. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk is appreciably higher than the estimated risk, but it could very well be considerably lower. The risk estimates presented in this document should not, therefore, be regarded as accurate representations of the true cancer risks even when the exposures are accurately defined. These estimates may, however, be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

9.5.3.1.6 Alternative methodological approaches. The methods used by the CAG for quantitative assessment are consistently conservative in that they tend to

result in high estimates of risk. This conservatism is primarily due to the CAG's use of the linear non-threshold extrapolation model in preference to any one of a variety of other extrapolation models that would give lower risk estimates. For purposes of comparison, descriptions of these alternative models (the one-hit, the probit, and the Weibull models) are presented in Appendix B.

Another method of risk estimation employed by the CAG involves the use of animal bioassay data as the basis for extrapolation. At present, the CAG's approach is to utilize data corresponding to the most sensitive animal responses in these studies. An alternative approach would be to use the average responses of all adequately tested bioassay animals.

Extrapolations from animals to humans can also be made on the basis of either relative weight or surface area. The latter approach, which is used by the CAG, has more of a basis in human pharmacological responses; however, at the present time there is some question as to which of the two approaches is more appropriate for use with carcinogens. Given this uncertainty, the CAG has chosen the most generally employed method, which is also the more conservative of the two. In the case of ETO inhalation studies, the use of extrapolation based on surface area rather than weight increases the unit risk estimates by a factor of 5.5 for the males and 6.8 for the females.

9.5.3.2 Humans--Model for Estimation of Unit Risk Based on Human Data--Whenever possible, the CAG utilizes data from human epidemiologic studies in preference to animal bioassay data. If sufficiently valid exposure information is available for a given compound, this information is always used by the CAG in its assessment. If the results of such studies show carcinogenic effects, the data are analyzed to give estimates of the linear dependence of cancer rates on lifetime average doses (equivalent to the factor B_H in the equation below). If human

epidemiologic studies show no carcinogenic effects when positive animal evidence is available, then it is assumed that a risk does exist, but that the risk is smaller than could have been observed in an epidemiologic study. In such cases it is assumed that the true incidence is just below the level of detection in the cohort studied, and calculations are then made to estimate an upper limit of cancer incidence, as determined largely by the size of the cohort.

Very little information exists that can support extrapolation from high-exposure occupational studies to situations in which contamination is at low environmental levels. However, if a number of simplifying assumptions are made, it is possible to construct a crude dose-response model whose parameters can be estimated using vital statistics, epidemiologic studies, and estimates of worker exposures.

In human studies, responses are measured in terms of the relative risk of an exposed cohort as compared to a control group. The mathematical model employed by the CAG assumes that for low exposures the lifetime probability of death from lung cancer (or any cancer), P_0, may be represented by the linear equation

$$P_0 = A + B_H X$$

where A is the lifetime probability of death from cancer in the absence of the agent, and X is the average lifetime exposure to environmental levels in units such as ppm. The factor, B_H, is the increased probability of cancer associated with each unit increase of the agent in air.

If it is assumed that R, the relative risk of lung cancer for exposed workers as compared to the general population, is independent of the length or age of exposure and depends only on average lifetime exposure, it follows that

$$R = \frac{P}{P_0} = \frac{A + B_H (X_1 + X_2)}{A + B_H \times X_1}$$

or

$$RP_0 = A + B_H (X_1 + X_2)$$

where X_1 = lifetime average daily exposure to the agent for the general population, X_2 = lifetime average daily exposure to the agent in the occupational setting, and P_0 = lifetime probability of dying of cancer with no or negligible ETO exposure. Substituting $P_0 = A + B_H X_1$ and rearranging gives

$$B_H = P_0 (R - 1)/X_2$$

To use the above model, estimates of R and X_2 must be obtained from appropriate epidemiologic studies. The value of P_0 is derived by means of life-table methodology from 1976 U.S. vital statistics records of age- and cause-specific death rates for males. For leukemia, the estimate of P_0 is 0.0091. This methodology is utilized by the CAG in the present document, in the section on unit risk based on human studies.

9.5.3.3 ETO Unit Risk Estimates

9.5.3.3.1 Unit risk estimate based on animal studies. The two long-term animal inhalation studies presented in the qualitative carcinogenicity section of this document showed similar results, both qualitatively and quantitatively, for the males. Both studies had significantly increased dose-related incidences of peritoneal mesotheliomas and gliomas, and some increase in mononuclear cell leukemias. These studies will be analyzed separately and then compared.

9.5.3.3.1.1 Snellings et al. (1981) (Bushy Run). This study exposed 120 Fischer 344 rats of each sex to three different doses (100 ppm, 33 ppm, and 10 ppm) of ETO vapor via inhalation for 6 hours/day, 5 days/week, for approximately

2 years. Comparable untreated (air) control groups were also used. Interim sacrifices were conducted to evaluate the time development of treatment-related effects.

The results of the study show statistically significant increases in brain gliomas (highest dose group) and in mononuclear cell leukemias in females in the two highest dose groups, and peritoneal mesotheliomas and brain gliomas in males in the two highest dose groups, all of which exhibited dose-response trends. Table 9-32 summarizes the pertinent data from this study that the CAG has used in calculating potency estimates for ETO. In connection with these data, it should be noted that the brain gliomas were not examined histopathologically until after the results of the NIOSH study (Lynch et al. 1982) had alerted the Bushy Run researchers to the possibility of the occurrence of brain neoplasias. For this reason, only 18-month, 24-month, and dead/euthanized moribund denominator figures were available for gliomas. For the male peritoneal mesotheliomas and the female mononuclear cell leukemias, the denominators in Table 9-32 correspond to the number of animals alive when the first tumor of that type was found. In the males, the first peritoneal mesothelioma was found at 15 months; in the females, the first mononuclear cell leukemia was found at 18 months (see also Table 9-23).

As reported earlier, a dose of 20.24 mg/kg of body weight has been measured for male Fischer 344 rats exposed to ETO at 100 ppm under conditions similar to those of the Snellings et al. (1981) study. For this document, dose is assumed to be equivalent between species on the basis of mg/surface area, or mg/body weight$^{2/3}$. This means that a dose of 2.63 mg/kg body weight given to a 70 kg human is assumed to produce an equivalent response to that produced by 20.24 mg/kg in the male rat. As discussed above and as shown in Table 9-32 , this

TABLE 9-32 BUSHY RUN ETO INHALATION STUDY IN FISCHER 344 RATS.
INCIDENCE OF PERITONEAL MESOTHELIOMA AND BRAIN GLIOMA IN MALES, AND MONONUCLEAR CELL LEUKEMIA AND BRAIN GLIOMA[a] IN FEMALES BY DOSE AMONG SURVIVORS TO FIRST TUMOR
(Snellings et al. 1981)

	Exposure in air (ppm)				
Group	0 (combined)	10	33	100	q_1^{*}[b] $(mg/kg/day)^{-1}$
Males					
Peritoneal meso./No. examined (%)[c]	4/187(2)	3/88(3)	7/82(8)	22/96(22)	1.1×10
P-values[d]	<0.00001		=0.02	<0.0001	
Brain gliomas/No. examined (%)[g]	1/196(0.5)	1/99(1)	5/98(5)	7/99(7)	5.0×10^{-2}
P-values	=0.0003		=0.02	=0.002	
Total	5/187(3)	4/88(5)	12/82(15)	29/96(30)	1.7×10^{-1}
P-values	<0.00001		=0.0005	<0.0001	
Human equivalent dose (mg/kg/day)[e]	0	0.35	0.94	2.63	
Females					
Mon. leukem/No. examined (%)[f]	22/186(12)	14/71(20)	24/72(33)	28/73(38)	2.9×10^{-1}
P-values	<0.00001	=0.08	0.0001	<.0001	
Brain gliomas/No. examined (%)[g]	1/194(0.5)	1/95(1)	3/99(3)	4/99(4)	4.0×10^{-2}
P-values	=0.014			=0.05	
Total	23/186(12)	15/71(21)	27/72(38)	32/73(44)	3.5×10^{-1}
P-values	<0.00001		<.0001	<.0001	
Human equivalent dose (mg/kg/day)[e]	0	0.28	0.75	2.11	

[a] See Table .
[b] 95% upper-limit unit risk estimate.
[c] Number alive at 15 months.
[d] Fisher Exact Test vs. combined controls (one tailed). P-value under controls is a one-sided Cochran-Armitage test for a dose-response trend.
[e] Based on measured doses in males of 20.24 and 2.7 mg/kg b.w. following 6 hours' exposure to ETO at 100 ppm and 10 ppm respectively. The animal-to-human dose equivalences are based on a dose per surface area factor of $(70/W_a)^{1/3}$, which increases unit risk estimates by factors of 5.5 for the males and 6.8 for the females over dose per body weight equivalences.
[f] Number alive at 18 months.
[g] Total number examined less 6- and 12-month sacrifices.

method of determining dose equivalence increases the unit risk estimates by factors of 5.5 for females and 6.8 for males over estimates obtained on the basis of mg/kg of body weight.

Table 9-32 , in presenting the total number of significant tumors by sex, sums the total number of significant tumors over the smallest denominator. This is done because time-to-tumor data on the gliomas are unavailable. Compared with the usual CAG procedure of counting the total number of animals with significant tumors, the addition of total significant tumors, as is done here, increases the risk estimate very slightly.

Calculations of the 95% upper-limit unit risk estimate, based on the linearized multistage model fitted to the data in Table 9-32 , yield a high value of $q^*_h = 3.5 \times 10^{-1}$ $(mg/kg/day)^{-1}$, based on total mononuclear cell leukemias and brain gliomas in the female rats. The responses of the males, based on total peritoneal mesotheliomas and brain gliomas, yield a value of 50% less, $q^*_h = 1.7 \times 10^{-1} (mg/kg/day)^{-1}$.

To convert the above estimate to units of ug/m^3 for humans, the following formula is used:

$$1 \text{ mg/kg/day} = 1 \text{ mg/kg/day} \times 70 \text{ kg} \times 1000 \text{ ug/mg} \times \text{day}/20 \text{ m}^3 = 3.5 \times 10^3 \text{ ug/m}^3$$

or

$$1 \text{ ug/m}^3 = 2.86 \times 10^{-4} \text{ mg/kg/day.}$$

The 95% upper-limit slope estimate in terms of ug/m^3 is thus calculated as

$$q^*_h = 3.5 \times 10^{-1} (mg/kg/day)^{-1} \times 2.86 \times 10^{-4} \frac{(mg/kg/day)}{ug/m^3} = 1.0 \times 10^{-4} (ug/m^3)^{-1}$$

To convert from ug/m^3 to ppm, the formula is

$$1 \text{ ppm} = \frac{1.2 \text{ g}}{10^{-3}\text{m}^3} \times \frac{44.1 \text{ m.w. ETO}}{28.2 \text{ m.w. air}} \times \frac{10^6 \text{ ug}}{\text{g}} \times 10^{-6}$$

$$= 1.9 \times 10^3 \text{ ug/m}^3$$

The lifetime probability of cancer from continuously breathing 1 ppm ETO in air is thus calculated as follows:

$$P = 1.0 \times 10^{-4}(\text{ug/m}^3)^{-1} \times \frac{1.9 \times 10^3 \text{ ug/m}^3}{\text{ppm}} = 1.9 \times 10^{-1}(\text{ppm})^{-1}$$

9.5.3.3.1.2 Lynch et al. (1982) (NIOSH). The NIOSH study (Lynch et al. 1982) in which male Fischer 344 rats were exposed to ETO at 50 ppm and 100 ppm 7 hours/day, 5 days/week for 2 years, produced results very similar to those of the Bushy Run study (Snellings et al. 1981). The results, shown in Table 9-33, show statistically significant increases and dose-response trends in brain gliomas and peritoneal mesotheliomas; there is a significant increase in mononuclear cell leukemias only at the lower dose, and no significant dose-response trend. Since incidence of this leukemia in controls was over 30% in this study, and since the Snellings et al. (1981) study did not show a significant increase in these leukemias, only peritoneal mesotheliomas and brain gliomas were used for risk assessment. The results of the potency calculations, shown in Table 9-33, are quantitatively nearly identical to those in Table 9-32. Based on the above analyses, the maximum animal slope potency value is still $q_1^* = 3.5 \times 10^{-1}$ $(\text{mg/kg/day})^{-1}$ based on the total mononuclear cell leukemias and brain gliomas in female rats in the Snellings et al. study.

9.5.3.3.1.3 Effects of results on different dose equivalence assumptions - OSHA vs. EPA assessments. The results of the above assessments depend to some extent on the dose equivalence assumptions. Dose equivalence in the following

TABLE 9-33. NIOSH ETO INHALATION STUDY IN MALE FISCHER 344 RATS. INCIDENCE OF PERITONEAL MESOTHELIOMA AND BRAIN GLIOMA BY DOSE, AMONG TOTAL EXAMINED. ESTIMATES OF 95% UPPER-LIMIT RISK BASED ON HUMAN EQUIVALENT DOSE (mg/kg/day) (Lynch et al. 1982)

	Exposure in air (ppm)			
	0[e]	50	100	q_1^* $(mg/kg/day)^{-1}$
Peritoneal mesothelioma/No. examined	3/78[d]	9/79	21/79[d]	1.0×10^{-1}
Brain glioma/No. examined	0/76[d]	2/77	5/79[b]	3.4×10^{-2}
Total	3/78[d]	11/79[b]	26/79[d]	1.3×10^{-1}
Human equivalent dose (mg/kg/day)[f]	0	1.59	3.06	--

[a]See Table 9-13.
[b]$P < 0.05$.
[c]$P < 0.01$.
[d]$P < 0.001$.
[e]P-values beside control incidences represent values associated with a one-sided Cochran-Armitage test for a dose-response trend.
[f]Human equivalent dose based on transforming ppm to mg/kg/day as in Table except for an adjustment for 7 hours' exposure.

discussion means the dose which will cause an equivalent response, quantitatively, in both species. The CAG has assumed that doses are equivalent on the basis of mg per surface area, an assumption for which there is some experimental evidence when first-order kinetics apply; for ETO, first-order kinetics appear to apply at exposures up to 100 ppm (Tyler and McKelvey 1980). As explained in an earlier section, use of the surface area correction increases the 95% upper-limit unit risk estimate by factors of 5.5 for the males and 6.8 for the females over estimates obtained on the basis of mg/kg/body weight.* OSHA, which assumes equivalence on an mg/kg body weight basis, calculated exposures of 19.30 mg/kg/day for males and 23.94 mg/kg/day for females exposed to ETO at 100 ppm in the Snellings et al. (1981) study, using EPA methodology (Federal Register 48[78]: 172-193) for a completely soluble gas. While the results for the males, 19.30 mg/kg/day, are within 5% of the dose measured by Tyler and McKelvey (1980) (20.24 mg/kg/day), EPA used the more accurate measured dose in this case. EPA then used the surface area correction factors for animal-to-man equivalence. Thus, on the basis of the difference in assumptions of equivalent dose alone, the EPA risk numbers are larger than OSHA's by a factor of about 6.

One other difference between the OSHA and EPA assessments (Snellings et al. 1981), is that the EPA added total significant tumors (mononuclear cell leukemias and brain gliomas for the females), while OSHA used the total number of malignant tumor-bearing animals. For EPA, this led to factors higher by 50% for the males and 20% for the females. The result, based on animal data, is that the EPA 95% upper-limit unit risk factor is larger than that of OSHA by a factor of about 8.

*Equivalence could also have been calculated directly on a ppm basis; this would have yielded a 95% upper-limit estimate approximately 1.8 times as high as that obtained on the basis of mg/kg/body weight. EPA uses direct ppm equivalence for partially soluble gases and particulates. ETO can be considered a completely soluble gas.

OSHA did not use human studies in its risk analysis. As seen in the following section, EPA's use of human studies increases this risk factor by an additional factor of 3.6, so that EPA's final value is larger than OSHA's by a factor of about 30. Finally, the estimate based on human data predicts only leukemia mortality due to ETO exposure. According to Calleman et al. (1978), alkylating compounds such as ETO could induce a spectrum of cancers, of which leukemia, because of its shorter latent periods, would be the first to appear. Thus, since OSHA's analysis based on animals predicts risks for all cancers, and EPA's upper-limit based on humans predicts risks only for leukemias, the possibility exists that even EPA's values are not protective enough.

9.5.3.3.2 Unit risk estimate based on human studies. In estimating the carcinogenic potency of ETO on the basis of human data, researchers have focused their attention on dose-response data for leukemia. In two studies (Hogstedt 1979a and Hogstedt 1979b), increased leukemias were evident. However, one of these studies (Hogstedt 1979a), described increased leukemias among production and maintenance workers who had been exposed to multiple carcinogens, including ethylene dichloride and ethylene chlorohydrin. For this reason, only the second study (Hogstedt 1979b) is used in the present analysis.

The risk assessment done on the basis of the Hogstedt et al. (1979b) study probably underestimates the carcinogenic potency of ETO because of two factors: 1) In this study, exposure started in 1968 and ended in 1977--giving a maximum latency period of only 9 years, whereas cancer usually involves a relatively long latency period. (However, the author states that leukemia incidence in Hiroshima and Nagasaki due to the atomic bomb irradiation showed a rapid increase that began shortly after exposure and reached a peak after 6 years.) 2) Since the study did not report the number of person-years of exposure, it is assumed for present purposes that all of the 230 workers were exposed for the full 9

years, an assumption which tends to underestimate the risk. Another problem with this study is that the gas used for sterilization was 50% ETO and 50% methyl formate. Little is known about the biological effects of methyl formate or of the combination of methyl formate with ETO. However, methyl formate is known to metabolize to formic acid, which is a normal body metabolite. It is assumed for present purposes that ETO was the only leukemogen in this study, although one of the cases (the man) had reported some contact with benzene in laboratory work.

Hogstedt (1979b) states, in connection with exposures in the factory studied, that infrared spectrophotometry and gas chromatography measurements in 1977 showed values ranging from 2 to 70 ppm in the factory's storage hall area. The study also reports that the calculated 8-hour time weighted average ETO concentration in the breathing zone was 20 $\pm$ 10 ppm, and that the concentration in the storage hall was higher than in the sterilization room. The accompanying table described the 70 storage hall employees as having had 8-hour exposures, while all but seven of the remaining employees were described as "occasionally exposed."

Of the two leukemia cases (acute myeloid and chronic myeloid), both people worked in the storage hall area, and neither had reported exposure to benzene. Because the two cases worked in the storage hall, the CAG has chosen to estimate the expected number of leukemia cases for the persons who worked only in that area rather than in the entire factory. Based on the reported expected leukemia incidence of 0.1 cases for the 230 exposed employees, we can estimate approximately (70/230) x 0.1 = 0.03 cases for the group exposed in the storage hall. Compared with the two observed cases, this yields a ratio of observed to expected cases of (2/0.03) = 65.7.

The estimated average exposure to ETO over the lifetime of the workers is calculated as follows:

$$20 \text{ ppm} \times 8/24 \text{ hrs} \times 240/365 \text{ days} \times 9/45.6 \text{ yrs}$$

$$\text{exposure} = 0.865 \text{ ppm}$$

where 45.6 years is the mean age of the 70 storage hall employees at the end of the study period.

The slope b_H of the lifetime probability of dying from leukemia due to a lifetime of breathing ETO at 1 ppm is given by

$$b_H = \frac{P_0(R - 1)\, X_1}{X_2}$$

where P_0 is the lifetime probability in the U.S. of dying* from leukemia in the absence of ETO exposure, R is the relative risk, X_1 is the exposure of 1 ppm, and X_2 is the exposure experienced by the factory workers. The relative risk R estimated above is 65.7; the exposure X_2 is given as 0.865 ppm. The lifetime probability of death from leukemia in the U.S. population is 0.0091. Substituting these values in the above equation gives

$$b_H = \frac{0.0091\ (65.7 - 1) \times 1 \text{ ppm}}{0.865 \text{ ppm}} = 0.68\ (\text{ppm})^{-1}$$

The probability associated with breathing ETO at 1 ppm for a lifetime is

$$P = 1 - e^{-b_H\ (1 \text{ ppm})} = 0.49$$

To convert ppm to ug/m^3, the formula is

$$1 \text{ ppm} = \frac{1.2 \text{ gm}}{10^{-3}\ m^3} \times \frac{44.1 \text{ m.w. chemical}}{28.2 \text{ m.w. air}} \times \frac{10^6 ug}{gm} \times 10^{-6}$$

$$= 1.9 \times 10^3\ ug/m^3$$

*P_0 employs both leukemia incidence cases and leukemia mortality rates. While leukemia mortality in the younger ages (<55) can be closely equated with incidence, in the older age groups chronic forms predominate in incidence, with death often occurring from other causes. Nevertheless, for this assessment it is assumed that although ETO would cause all types of leukemias, death will result from each case. In this study, the leukemias in the two women were of the acute form.

Thus the unit risk estimate in terms of ug/m^3 is

$$b_H = 0.68\ (ppm)^{-1} \times \frac{1\ ppm}{1.9 \times 10^3\ ug/m^3} = 3.6 \times 10^{-4}\ (ug/m^3)^{-1}$$

This compares with an upper-limit estimate of $1.0 \times 10^{-4}\ (ug/m^3)^{-1}$ based on the Bushy Run animal study (Snellings et al. 1981). The estimate based on the human study (Hogstedt 1979b) is 3.6 times as high.

Because this estimate is based on only two human leukemias, it raises questions about the suitability of the human response for risk assessment purposes. Furthermore, OSHA in its analysis relied only on the Snellings et al. (1981) study for its risk assessment. The reasons the CAG has chosen to use the Hogstedt (1979b) data are as follows:

1. Human data extrapolations are nearly always preferable to animal data extrapolations because of species and specific target organ.
2. Exposure in this study was actually measured.
3. The storage hall employees represented a fairly homogeneous group.
4. Both leukemia cases were in young women whose exposures and latent periods were less than 9 years.
5. Human cancer data are fully supported by animal cancer data showing strong dose-response relationships between ETO and leukemia and between ETO and other cancers. Since the human study related only 9 years of exposure and follow-up histories, it is quite probable that further follow-up will show excesses in other cancers.
6. The higher potency estimates in the human cancer study are further supported by human data showing a strong dose-response relationship between exposure to ETO and the frequency of chromosome abnormalities and sister chromatid exchange. Humans appear to be 100 times as sensitive as rats with respect to ETO-caused chromosome abnormalities.

Although the qualitative evidence supports ETO as a human leukemogen, the estimation of potency from only two cases presents enough uncertainty from a quantitative standpoint that such an estimate can be considered only roughly approximate at best. As such, the CAG considers it to be at the upper end of the range, with the lower end being the highest estimates based on animal data. This range is:

$$1.0 \times 10^{-4} \ (ug/m^3)^{-1} - 3.6 \times 10^{-4} \ (ug/m^3)^{-1}$$

9.5.3.4 Relative Potency--One of the uses of the concept of unit risk is to compare the relative potencies of carcinogens. For the purposes of the present analysis, potency is defined as the linear portion of the dose-response curve, and is used to calculate the required unit risk factors. To estimate relative potency on a per-mole basis, the unit risk slope factor is multiplied by the molecular weight of the compound, and the resulting number, expressed in terms of $(mMol/kg/day)^{-1}$, Is called the "relative potency index."

Figure 9-4 is a histogram representing the frequency distribution of relative potency indices for 54 chemicals that have been evaluated by the CAG as suspect carcinogens. The data summarized by the histogram are presented in Table 9-34. Where human data have been available for a compound, such data have been used to calculate these indices. Where no human data have been available, data from animal oral studies have been used rather than data from animal inhalation studies, since animal oral studies have been conducted for most of these compounds, and their use allows potency comparisons by route.

On the basis of leukemias in two women exposed to ETO for up to 9 years (Hogstedt 1979b), the relative potency index for ETO has been calculated as $5.6 \times 10^{+1}$. This number was derived by multiplying the slope in units of $(mg/kg/day)^{-1}$ by the molecular weight of ETO, which is 44.1. Based on the

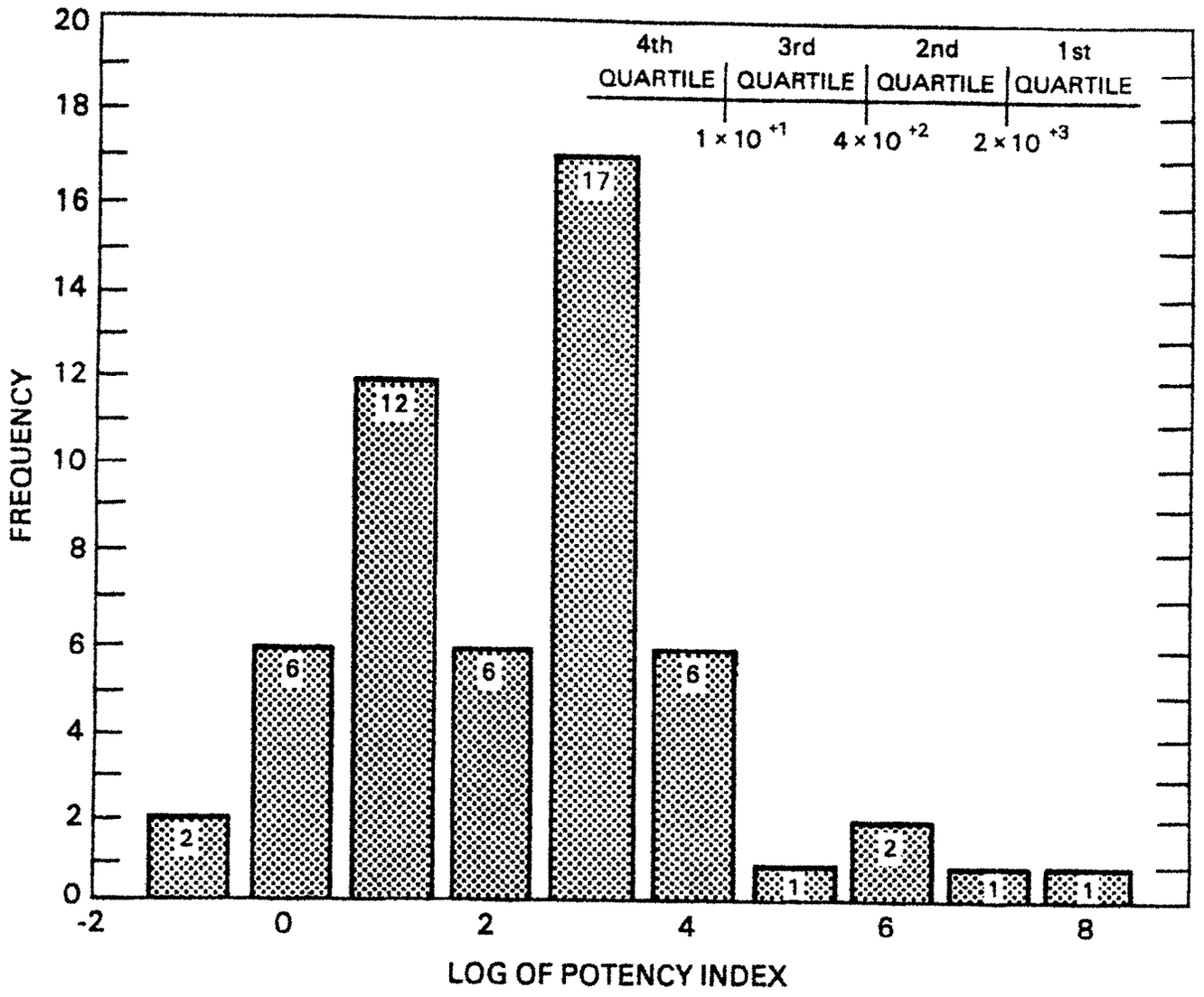


Figure 9-4 . Histogram representing the frequency distribution of the potency indices of 54 suspect carcinogens evaluated by the Carcinogen Assessment Group.

TABLE 9-34 . RELATIVE CARCINOGENIC POTENCIES AMONG 54 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS[1,2,3]

Compound	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Acrylonitrile	0.24(W)	53.1	$1x10^{+1}$	+1
Aflatoxin B_1	2924	312.3	$9x10^{+5}$	+6
Aldrin	11.4	369.4	$4x10^{+3}$	+4
Allyl chloride	$1.19x10^{-2}$	76.5	$9x10^{-1}$	0
Arsenic	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	11.5	252.3	$3x10^{+3}$	+3
Benzene	$5.2x10^{-2}$(W)	78	$4x10^{0}$	+1
Benzidene	234(W)	184.2	$4x10^{+4}$	+5
Beryllium	1.40	9	$1x10^{+1}$	+1
Cadmium	6.65(W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
Chlordane	1.61	409.8	$7x10^{+2}$	+3
Chlorinated ethanes				
1,2-dichloroethane	$6.9x10^{-2}$	98.9	$7x10^{0}$	+1
hexachloroethane	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,2,2-tetrachloroethane	0.20	167.9	$3x10^{+1}$	+1
1,1,1-trichloroethane	$1.6x10^{-3}$	133.4	$2x10^{-1}$	-1
1,1,2-trichloroethane	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
Chloroform	$7x10^{-2}$	119.4	$8x10^{0}$	+1
Chromium	41(W)	100	$4x10^{+3}$	+4
DDT	8.42	354.5	$3x10^{+3}$	+3
Dichlorobenzidine	1.69	253.1	$4x10^{+2}$	+3
1,1-dichloroethylene	$1.47x10^{-1}$(I)	97	$1x10^{+1}$	+1
Dieldrin	30.4	380.9	$1x10^{+4}$	+4

(continued on the following page)

TABLE 9-34 . (continued)

Compound	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Dinitrotoluene	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	1.14	143	$2x10^{+2}$	+2
Bis(chloromethyl)ether	9300(I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	8.51	187.9	$2x10^{+3}$	+3
Ethylene oxide	1.26(I)	44.1	$6x10^{+1}$	+2
Heptachlor	3.37	373.3	$1x10^{+3}$	+3
Hexachlorobenzene	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane				
technical grade	4.75	290.9	$1x10^{+3}$	+3
alpha isomer	11.12	290.9	$3x10^{+3}$	+3
beta isomer	1.84	290.9	$5x10^{+2}$	+3
gamma isomer	1.33	290.9	$4x10^{+2}$	+3
Hexachlorodibenzodioxin	$1.1x10^{+4}$	391	$4x10^{+6}$	+7
Methylene chloride	$6.3x10^{-4}$	84.9	$5x10^{-2}$	-1
Nickel	1.15(W)	58.7	$7x10^{+1}$	+2
Nitrosamines				
Dimethylnitrosamine	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Diethylnitrosamine	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	5.43	158.2	$9x10^{+2}$	+3
N-nitrosopyrrolidine	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	32.9	117.1	$4x10^{+3}$	+4
N-nitroso-N-methylurea	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	$4.92x10^{-3}$	198	$1x10^{0}$	0
PCBs	4.34	324	$1x10^{+3}$	+3

(continued on the following page)

TABLE 9-34 . (continued)

Compound	Slope $(mg/kg/day)^{-1}$	Molecular weight	Potency index	Order of magnitude (log_{10} index)
Phenols				
2,4,6-trichlorophenol	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Tetrachlorodibenzo-p-dioxin	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
Tetrachloroethylene	$3.5x10^{-2}$	165.8	$6x10^{0}$	+1
Toxaphene	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	$1.9x10^{-2}$	131.4	$2.5x10^{0}$	0
Vinyl chloride	$1.75x10^{-2}$(I)	62.5	$1x10^{0}$	0

Remarks:

1. Animal slopes are 95% upper-limit slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear non-threshold model.

2. The potency index is a rounded-off slope in $(mMol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

3. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available.

Hogstedt (1979b) study, this slope is 3.6×10^{-4} $(ug/m^3)^{-1}$. The transformation from ug/m^3 to mg/kg/day is performed as follows:

$$\frac{1 \text{ ug}}{m^3} \times \frac{20 \text{ } m^3}{\text{day}} \times \frac{1 \text{ mg}}{1000} \times \frac{1}{70 \text{ kg}} = 2.86 \times 10^{-4} \text{ mg/kg/day}$$

The unit risk slope can then be converted as given below:

$$b = 3.6 \times 10^{-4} \text{ } (ug/m^3)^{-1} \times \frac{1 \text{ ug}/m^3}{2.86 \times 10^{-4} \text{ (mg/kg/day)}}$$

$$= 1.26 \text{ } (mg/kg/day)^{-1}$$

The potency index for ETO is thus $1.26 \times 44.1 = 5.6 \times 10^{+1}$, putting it in the third quartile of the 54 chemicals which the CAG has evaluated as suspect carcinogens. If the lower part of the range is used, the potency index for ETO would be $3.5 \times 10^{-1} \times 44.1 = 1.5 \times 10^{+1}$, which would also rank it in the third quartile. It should be noted that the ranking of these relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals on the basis of varying routes of exposure in different species, using studies whose quality varies widely. Furthermore, all of these indices are based on estimates of low-dose risk that have been calculated by means of linear extrapolation from the observational range. The indices are therefore not valid for the comparison of potencies in the experimental or observational range if linearity does not exist there.

9.5.4 Summary -- Positive results for the carcinogenicity of ETO have been obtained by subcutaneous injection in mice and intragastric administration in rats. Two long-term chronic animal studies were performed that adequately tested the carcinogenic potential of ETO by inhalation: the Bushy Run study (Snellings et al. 1981) and that of NIOSH (Lynch et al. 1982). Snellings et al. (1981) indicated that ETO exposure resulted in an increased incidence of mononuclear

cell leukemia in females in the two highest dose groups; this increase was dose-related. The test for a linear trend was highly significant ($P < 0.0001$). There was a significant ($P = 0.045$) increase in gliomas at the highest dose, and the test for linear trend was highly significant ($P < 0.014$). In males, incidences of primary brain neoplasm, peritoneal mesothelioma, and subcutaneous fibroma were significantly elevated in at least two exposed groups. The trend analysis was significant for both mesotheliomas ($P < 0.00001$) and gliomas ($P = 0.003$) in males. In the NIOSH (Lynch et al. 1982) study, which involved only male rats, leukemia incidence was significantly increased at low doses only, while gliomas (mixed-cell) and peritoneal mesotheliomas were increased significantly in high-dose exposed groups. For these latter two sites, the dose-response trend tests were also statistically significant ($P < .01$).

Three epidemiologic studies of workers exposed to ETO demonstrated significant ($P < 0.05$) association between ETO exposure and the occurrence of cancer. Two of the studies (Hogstedt et al. 1979a and Hogstedt et al. 1979b) found an association between ETO exposure and incidence of or death from leukemia. ETO was not found to be specific for any particular type of leukemia, however. Other sites or types of cancer found to be significantly ($P < 0.05$) associated with ETO exposure include pancreatic cancer and Hodgkin's disease in the Morgan et al. (1981) study and stomach cancer in the Hogstedt et al. (1979a) study.

A range of unit risk estimates for ETO has been calculated from both animal and human data. The lower end of the range was a 95% upper-limit estimate based on total mononuclear cell leukemias and brain gliomas in female Fischer 344 rats in the Bushy Run study. The higher end was based on human leukemias in the Hogstedt (1979b) study. The unit risk estimates from the animal data were calculated from a linearized multistage model, while the human data estimate was calculated using a relative risk model. The unit risk estimate based on human

data is 3.6 times as high as that based on animal data. Extrapolation from the human leukemia data results in a highly uncertain estimate due to the small number of leukemia cases recorded. Of interest is the fact that humans have quantitatively greater sensitivity to ETO than do rats, as evidenced by the data for chromosome abnormalities.

Using the above-referenced extrapolation procedures, the range of estimates of lifetime cancer risk resulting from continuous exposure to air that contains an ETO concentration of 1 ug/m^3 is calculated to be $1.0 \times 10^{-4} - 3.6 \times 10^{-4}$. The plausibility of these estimates is enhanced when clear evidence of mutagenicity exists, as is the case with ETO.

9.5.5 Conclusions

ETO is a direct-acting alkylating agent. It reacts with mammalian DNA primarily at the N-7 position of guanine. It induces base-pair substitutions in the Ames test, and gene mutations in plants and animals. It also breaks chromosomes of plants, animals, and humans, and causes DNA damage in the spermatids of mice. The weight of the available evidence indicates that ETO is a direct-acting mutagen.

Using the criteria of the International Agency for Research on Cancer (IARC) for assessing the evidence of carcinogenicity from studies in humans (Appendix A), the CAG considers that the human data for ETO constitutes limited, bordering on inadequate, evidence that ETO is a human carcinogen. The CAG finds the animal evidence of the carcinogenicity of ETO to be sufficient. On the basis of its analysis of the human, animal, and mutagenic data cited herein, the CAG classifies ETO as being probably carcinogenic to humans and therefore as belonging in IARC Group 2A. The CAG would qualify this classification as bordering on Group 2B, however, because of limitations in the human evidence. (See Appendix A for a description of the IARC categories.)

Estimates of the relative potencies of ETO in animals and humans, made on the basis of leukemias and brain gliomas in animals and leukemias in human studies, suggest that humans may be more susceptible than animals to the carcinogenic effects of ETO. The unit risk estimate for ETO in humans is 3.6×10^{-4} $(ug/m^3)^{-1}$, while the estimate for animals, based on studies in Fischer 344 rats, is 1.0×10^{-4} $(ug/m^3)^{-1}$.

The potency index of a chemical, as calculated by the CAG, is based on both its unit risk and its molecular weight. For ETO, which has a molecular weight of 99, the potency index based on human inhalation is $5.6 \times 10^{+1}$. The potency index based on animal data is $1.5 \times 10^{+1}$. These indices rank ETO either in the third or the fourth quartile, respectively, of the 54 suspect carcinogens evaluated by the CAG.

APPENDIX 9-A

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) CRITERIA FOR EVALUATION OF THE CARCINOGENICITY OF CHEMICALS*

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN HUMANS

Evidence of carcinogenicity from human studies comes from three main sources:

1. Case reports of individual cancer patients who were exposed to the chemical or process.
2. Descriptive epidemiological studies in which the incidence of cancer in human populations was found to vary in space or time with exposure to the agents.
3. Analytical epidemiological (case-control and cohort) studies in which individual exposure to the chemical or group of chemicals was found to be associated with an increased risk of cancer.

Three criteria must be met before a causal association can be inferred between exposure and cancer in humans:

1. There is no identified bias which could explain the association.
2. The possibility of confounding has been considered and ruled out as explaining the association.
3. The association is unlikely to be due to chance.

In general, although a single study may be indicative of a cause-effect relationship, confidence in inferring a causal association is increased when several independent studies are concordant in showing the association, when the association is strong, when there is a dose-response relationship, or when a reduction in exposure is followed by a reduction in the incidence of cancer.

*International Agency for Research on Cancer. 1982. IARC Monographs: Evaluation of the Carcinogenic Risk of Chemicals to Humans, Supplement 4. Lyon, France.

The degrees of evidence for carcinogenicity from studies in humans were categorized as:

i. Sufficient evidence of carcinogenicity, which indicates that there is a causal relationship between the agent and human cancer.

ii. Limited evidence of carcinogenicity, which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding, could not adequately be excluded.

iii. Inadequate evidence, which indicates that one of three condidtions prevailed: (a) there were few pertinent data; (b) the available studies, while showing evidence of association, did not exclude chance, bias or confounding; (c) studies were available which do not show evidence of carcinogenicity.

ASSESSMENT OF EVIDENCE FOR CARCINOGENICITY FROM STUDIES IN EXPERIMENTAL ANIMALS

These assessments were classified into four groups:

i. Sufficient evidence of carcinogenicity, which indicates that there is an increased incidence of malignant tumors: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.

ii. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) the studies involve a single species, strain, or experiment; or (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting; or (c) the neoplasms produced often occur spontaneously and, in the past, have been difficult

to classify as malignant by histological criteria alone (e.g., lung and liver tumors in mice).

iii. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect; or that within the limits of the tests used, the chemical is not carcinogenic. The number of negative studies is small, since, in general, studies that show no effect are less likely to be published than those suggesting carcinogenicity.

iv. No data indicates that data were not available to the Working Group.

The categories sufficient evidence and limited evidence refer only to the strength of the experimental evidence that these chemicals are carcinogenic and not to the extent of their carcinogenic activity nor to the mechanism involved. The classification of any chemical may change as new information becomes available.

EVALUATION OF CARCINOGENIC RISK TO HUMANS

At present, no objective criteria exist to interpret data from studies in experimental animals or from short-term tests directly in terms of human risk. Thus, in the absence of sufficient evidence from human studies, evaluation of the carcinogenic risk to humans was based on consideration of both the epidemiological and experimental evidence. The breadth of the categories of evidence defined above allows substantial variation within each. The decisions reached by the Group regarding overall risk incorporated these differences, even though they could not always be reflected adequately in the placement of an exposure into a particular category.

The chemical, groups of chemicals, industrial processes or occupational exposures were thus put into one of three groups:

Group 1

The chemical, group of chemicals, industrial process or occupational exposure is carcinogenic to humans. This category was used only when there was sufficient evidence from epidemiological studies to support a causal association between the exposure and cancer.

Group 2

The chemical, group of chemicals, industrial process or occupational exposure is probably carcinogenic to humans. This category includes exposures for which, at one extreme, the evidence of human carcinogenicity is almost "sufficient", as well as exposures for which, at the other extreme, it is inadequate. To reflect this range, the category was divided into higher (Group A) and lower (Group B) degrees of evidence. Usually, category 2A was reserved for exposures for which there was at least limited evidence of carcinogenicity to humans. The data from studies in experimental animals played an important role in assigning studies to category 2, and particularly those in Group B; thus, the combination of sufficient evidence in animals and inadequate data in humans usually resulted in classification of 2B.

In some cases, the Working Group considered that the known chemical properties of a compound and the results from short-term tests allowed its transfer from Group 3 to 2B or from Group 2B to 2A.

Group 3

The chemical, group of chemicals, industrial process or occupational exposure cannot be classified as to its carcinogenicity to humans.

APPENDIX 9-B

COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimate of unit risk from animals presented in the body of this document was calculated by use of the linearized multistage model. This non-threshold model is part of a methodology for estimating a conservative linear slope at low extrapolation doses that is usually consistent with the data at all dose levels in an experiment. The model holds that the most plausible upper limits of risk are those predicted by linear extrapolations to low levels of the dose-response relationship.

Other non-threshold models that have been used for risk extrapolation are the one-hit, the log-Probit, and the Weibull models. The one-hit model is characterized by a continuous downward curvature, but is linear at low doses. Because of its functional form, the one-hit model can be considered the linear form or first stage of the multistage model. This fact, together with the downward curvature of the one-hit model, means that the model will always yield low-level risk estimates that are at least as large as those obtained with the multistage model. In addition, whenever the data can be fitted adequately to the one-hit model, estimates based on the one-hit model and the multistage model will be comparable.

The log-Probit and the Weibull models, because of their general "S" curvature, are often used for the interpretation of toxicological data in the observable range. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-Probit model was originally used in biological assay problems such as potency assessments of toxicants and drugs, and is most often used to estimate such values as percentile lethal dose or percentile effective dose. The log-

Probit model developed along strictly empirical lines, in studies where it was observed that several log dose-response relationships followed the cumulative normal probability distribution function, Φ . In fitting the log-Probit model to cancer bioassay data, assuming an independent background, this relationship becomes

$$P(D;a,b,c) = c + (1-c)\,\Phi\,(a+b\log_{10} D) \quad a,b > 0 \leq C < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individual tolerances, and b is an estimate of the log-Probit dose-response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a quantifiable probability of transforming a single normal cell into a cancer cell. In this model, the probability distribution function is

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates (a = background or zero dose rate, and b = linear component or slope of the dose-response model). In considering the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model, known as the Weibull model, is of the form

$$P(D;b,k) = 1-\exp-(bd^k) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, the model yields low-dose estimates of risks that are

usually significantly lower than either the multistage or one-hit models, which are linear at low doses. All three of these models--the multistage, the one-hit, and the Weibull--usually project risk estimates that are significantly higher at low exposure levels than those projected by the log-Probit model.

The results of both the male and female rat data sets from the Bushy Run (Snellings et al. 1981) study are presented in Table B-1. Surprisingly, for the female rats, both the Weibull and log-Probit models yielded larger estimates of risk than the multistage model, which in this case produced results identical to those produced by the one-hit model. For the males, the one-hit model produced the highest estimates and the log-Probit model produced the lowest; in this case, the multistage, one-hit, and Weibull all produced similar results.

TABLE B-1. ESTIMATES OF HUMAN LOW-DOSE RISK BASED ON DATA FROM MALE AND FEMALE FISCHER 344 RATS IN THE BUSHY RUN ETO INHALATION STUDY, AS DERIVED FROM FOUR DIFFERENT MODELS. ALL ESTIMATES INCORPORATE ABBOTT'S CORRECTION FOR INDEPENDENT BACKGROUND RATE

Continuous human exposure ppm	Maximum likelihood estimates of additional risks				95% upper confidence limit of additional risks			
	Multistage model	One-hit model	Weibull model	Log-Probit model	Multistage model	One-hit model	Weibull model	Log-Probit model
Males								
.001	3.1×10^{-4}	1.3×10^{-4}	2.5×10^{-5}	3.1×10^{-1}	9.2×10^{-5}	1.6×10^{-4}	1.3×10^{-4}	5.0×10^{-9}
0.01	3.1×10^{-3}	1.3×10^{-3}	3.3×10^{-4}	1.6×10^{-6}	9.2×10^{-4}	1.6×10^{-3}	1.4×10^{-3}	1.5×10^{-5}
0.1	3.1×10^{-2}	1.3×10^{-2}	4.3×10^{-3}	8.6×10^{-4}	9.2×10^{-3}	1.6×10^{-2}	1.3×10^{-2}	3.9×10^{-3}
1	8.4×10^{-2}	1.2×10^{-1}	5.5×10^{-2}	5.4×10^{-2}	8.8×10^{-2}	1.6×10^{-1}	9.7×10^{-2}	9.7×10^{-2}
Females								
.001	1.4×10^{-5}		3.6×10^{-3}	1.2×10^{-4}	1.9×10^{-4}		1.5×10^{-2}	1.0×10^{-3}
0.01	1.4×10^{-3}		1.4×10^{-2}	3.0×10^{-3}	1.9×10^{-3}		4.3×10^{-2}	1.5×10^{-2}
0.1	1.4×10^{-2}		5.0×10^{-2}	3.3×10^{-2}	1.9×10^{-2}		1.1×10^{-1}	9.2×10^{-2}
1	1.3×10^{-1}		1.7×10^{-1}	1.7×10^{-1}	1.7×10^{-1}		2.5×10^{-1}	2.5×10^{-1}

Animal exposure 0, 33 ppm, 100 ppm 6 hours/day, 5 days/week.

DATA

	Human eq. dose - mg/kg/day					Human eq. dose - mg/kg/day			
Males	0	0.35	0.94	2.63	**Females**	0	0.28	0.75	2.11
No. tumors/No. examined	5/187	4/88	12/82	29/96		23/186	15/71	27/72	32/73

Conversions for low doses: Humans 1 mg/kg/day = 1.84 ppm in air

or 1 ppm air = .543 mg/kg/day

Multistage and one-hit models gave identical results in females.

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