NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for ETHYLENE OXIDE

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NTP Report on Carcinogens Listing for Ethylene Oxide

Carcinogenicity

Ethylene oxide is *known to be a human carcinogen* based on evidence from studies in humans and experimental animals and from supporting mechanistic data. Ethylene oxide is a direct-acting alkylating agent that has been used as an industrial intermediate and as a disinfectant/sterilant. The DNA damaging activity of ethylene oxide provides its effectiveness as a sterilant, and it is this same property that accounts for its potential carcinogenic risk to humans. An IARC (1994) reevaluation of data on ethylene oxide resulted in upgrading its classification from "probably carcinogenic to humans" (Group 2A) to "carcinogenic to humans" (Group 1), though the epidemiology studies alone provided only "limited evidence" for the carcinogenicity of ethylene oxide in humans. In making their overall evaluation of this direct-acting alkylating agent, the IARC panel utilized supporting mechanistic data (see below).

Several epidemiological studies evaluated by IARC (1994) reported an association between exposure to ethylene oxide and increased leukemia and stomach cancer risk (Hogstedt et al., 1979, 1986; Hogstedt, 1988); however, other studies found no significant excesses in cancer risk (Morgan et al., 1981; Kiesselbach et al., 1990; Teta et al., 1993; Steenland et al., 1991; Hagmar et al., 1991; Bisanti et al., 1993). In most studies, exposure information was limited. The most frequently reported association in exposed workers has been for lymphatic and hematopoietic cancer. A meta-analysis of 10 distinct cohort studies of workers exposed to ethylene oxide found no association between exposure to ethylene oxide and increased risk of pancreatic or brain cancers. There was a suggestive risk for non-Hodgkin's lymphoma and for stomach cancer (Shore et al., 1993).

The largest study of U.S. workers exposed to ethylene oxide at plants producing sterilized medical supplies and spices (Steenland et al., 1991) found no increase in mortality from any cause of death; however, an increase in mortality from all hematopoietic neoplasms, concentrated in the subcategories lymphosarcoma, reticulosarcoma, and non-Hodgkin's lymphoma, was observed among males. An analysis of the exposure-response data from the study by Steenland et al. (1991) found a positive trend in risk with increasing cumulative exposure to ethylene oxide and mortality from lymphatic and hematopoietic neoplasms. This trend was strengthened when analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukemia and non-Hodgkin's lymphoma combined). The relationship between cumulative exposure to ethylene oxide and leukemia was positive, but nonsignificant (Stayner et al., 1993).

In the study by Teta et al. (1993), leukemia risk was increased in workers exposed for more than 10 years to ethylene oxide. A more recent study found an increased incidence of breast cancer in a cohort of workers who used ethylene oxide as a sterilant (Norman et al., 1995). The occupational groups most studied are workers who use ethylene oxide as a sterilant and those who work in the production of ethylene oxide and its derivatives. The likelihood of confounding occupational exposures to other chemicals is generally lower in sterilization workers than in chemical workers.

Experimental studies in laboratory animals demonstrated that ethylene oxide is carcinogenic at multiple organ sites in rats and mice. Sites of tumor induction in mice included the hematopoietic system, lung, Harderian gland, mammary gland, and uterus (NTP 326, 1987). Sites of tumor induction in rats included the hematopoietic system, brain, and mesothelium (Snellings et al., 1984; Garman et al., 1985; Lynch et al., 1984). The IARC (1994) evaluation

noted that ethylene oxide is associated with malignancies of the lymphatic and hematopoietic system in both humans and experimental animals. No additional cancer studies of ethylene oxide in experimental animals have been reported since the IARC (1994) review.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Ethylene oxide is a direct-acting alkylating agent that forms adducts with biological macromolecules including hemoglobin and DNA. Measurements of hemoglobin adducts (hydroxyethyl histidine and hydroxyethyl valine) have been used to monitor occupational exposure to ethylene oxide. IARC (1994) noted that ethylene oxide induces a dose-related increase in the frequency of hemoglobin adducts in exposed humans and rodents.

The major DNA adduct of ethylene oxide is N7-(2-hydroxyethyl)guanine. Dose-related increases in this adduct, as well as smaller amounts of O6-(2-hydroxyethyl)guanine and N3-(2-hydroxyethyl)adenine, have been measured in rodents exposed to ethylene oxide. Background levels of hemoglobin and DNA adducts of ethylene oxide in humans and experimental animals have been suggested to arise from endogenous production of ethene (ethylene) by gut flora or metabolism of unsaturated dietary lipids (Törnqvist, 1996).

Ethylene oxide is genotoxic at all phylogenetic levels, including prokaryotic and lower eukaryotic organisms, as well as *in vitro* and *in vivo* mammalian systems. Ethylene oxide induces gene mutations and heritable translocations in germ cells of exposed rodents. Significant dose-related increases in the frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes (Galloway et al., 1986; Lerda and Rizzi, 1992; Tates et al., 1991; Yager et al., 1983; Sarto et al., 1984; Stolley et al., 1984; Mayer et al., 1991; Schulte et al., 1992, 1995; Major et al., 1996), of micronuclei in erythrocytes (Tates et al., 1991; Högstedt et al., 1983; Schulte et al., 1995), of DNA single-strand breaks in peripheral mononuclear blood cells (Fuchs et al., 1994; Oesch et al., 1995), and of *hprt* mutations in peripheral lymphocytes (Tates et al., 1991) have been observed in workers occupationally exposed to ethylene oxide. Similar genotoxic effects have been observed in rodents exposed to ethylene oxide. For direct-acting mutagenic chemicals, increases in chromosome aberration frequency appear to be a good predictor of increased human cancer risk. Thus, all measurable genotoxic endpoints that are considered to be indicators of chemical carcinogenesis have been observed in both humans and experimental animals exposed to ethylene oxide.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded; or

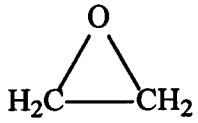
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 CHEMICAL PROPERTIES

Ethylene Oxide
[75-21-8]



1.1 Chemical Identification

Ethylene oxide (C_2H_4O, mol. wt. = 44.06) is also called:

Amprolene	Merpol
Anprolene	NCI-C50088
Anproline	Oxacyclopropane
Dihydrooxirene	Oxane (VAN)
Dimethylene oxide	Oxidoethane
ENT-26263	α,β-Oxidoethane
EO	Oxirane
1,2-Epoxyethane	Oxirane (9CI)
Ethane, 1,2-epoxy-	Oxirene, dihydro-
Ethene oxide	Oxyfume
Ethox	Oxyfume 12
ETO	Sterilizing Gas Ethylene Oxide 100%
ETOX	t-Gas
FEMA no. 2433	T-Gas

Ethylene oxide has a UN shipping number of 1040. The RCRA waste number is U115.

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless	Budavari (1996)
Physical State	Gas	Budavari (1996)
Melting Point, °C	-111	Budavari (1996)
Boiling Point, °C	13.2 @ 746 mm Hg	IARC (1994)
	10.8 @ 760 mm Hg	IARC (1994)
Density		
Liquid	0.882 @ 10 °C/10 °C	IARC (1994)
Odor	sweet etheric odor	Hoechst Celanese Polyester Intermediates et al. (1995)
Solubility	soluble in water, acetone, benzene, ethanol, diethyl ether	IARC (1994)
Partition Coefficients:		
Log octanol/water (log P)	-0.30	IARC (1994)
Vapor pressure	145.6 kPa @ 20 °C	IARC (1994)

Ethylene oxide is a colorless gas at room temperature and normal pressure, but is a liquid at or below 12 °C (Budavari, 1996). The liquid has a characteristic ether-like odor (Hoechst Celanese Polyester Intermediates et al., 1995). It reacts readily with acids resulting in ring opening. Vapors may be flammable or explosive if there is inadequate heat dissipation (IARC, 1994).

Ethylene oxide is available commercially in the United States as a high-purity chemical that contains a maximum of 0.03% water, 0.003% aldehydes as acetaldehyde, and 0.002% acidity as acetic acid (HSDB, 1998). It has been sold as a mixture with either carbon dioxide or fluorocarbon 12 to reduce its fire hazard.

2.0 HUMAN EXPOSURE

2.1 Use

The primary use of ethylene oxide is as an intermediate in the production of several industrial chemicals, most notably ethylene glycol. In 1986, 59% of the ethylene oxide produced was used to manufacture ethylene glycol and polyester. By 1995, the demand for ethylene oxide in ethylene glycol and polyester production was approximately half and half (Chem. Mark. Rep., 1995). Ethylene glycol is used primarily in automotive antifreeze and polyester is used in fibers, films, and bottles. Ethylene oxide was also used to produce nonionic surfactants (14%) in household and industrial detergents, ethanolamines (8%), glycol ethers (6%) used as solvents, intermediates, and for other purposes, diethylene glycol (6%), and triethylene glycol (2%) (Chem. Mark. Rep., 1987b; cited by NTP, 1998). Less than 1 to 2% of the industrial production of ethylene oxide is used as a fumigant and sterilizing agent for a variety of purposes and materials, including hospital equipment and foods (NIOSH, 1976; ATSDR, 1990). By the mid-1990s, ethylene oxide use for sterilization in hospitals was being replaced by other systems (Biomed. Mark. Newlett., 1995). The estimated 8 to 9 million lb used for sterilization and fumigation in 1996 represented about 0.1% of the total demand for ethylene oxide (SRIc, 1997).

At one time, it was used in the production of acrylonitrile, but the process ended in 1966 (ATSDR, 1990). Ethylene oxide has also been used to accelerate the maturing of tobacco leaves.

It has been investigated for use as an agent to improve wood durability (CHIP, 1982b; IARC V.11, 1976; both cited by NTP, 1998).

Other uses include ethoxylation products of long-chain alcohols and amines, alkyl phenols, cellulose, starch, poly(propylene glycol), and ethylene carbonate. Used directly in the gaseous form or in nonexplosive gaseous mixtures with nitrogen, carbon dioxide, or dichlorofluoromethane, ethylene oxide can serve as a disinfectant, fumigant, sterilizing agent, and insecticide. As a fumigant, ethylene oxide kills pests and microorganisms in spices and seasonings, furs, furniture, nuts, tobacco, books, drugs, leather, motor oil, paper, soil, animal bedding, clothing, and transport vehicles. As a sterilizing agent, it purifies cocoa, flour, dried egg powder, coconut, fruits, dehydrated vegetables, cosmetics, and dental, medical, and scientific supplies (IARC, 1994).

2.2 Production

Ethylene oxide has been ranked among the top 50 largest volume chemicals produced in the United States for the past several years by *Chemical and Engineering News*. The worldwide production capacity exceeds 8 million tons per year (Hoechst Celanese Polyester Intermediates et al., 1995). The 1997 *Directory of Chemical Producers* identified 11 companies producing ethylene oxide at 13 plants (SRIa, 1997).

Year	Production (billions of pounds)
1997	8.2
1996	7.2
1995	7.6
1994	7.2
1993	5.3
1992	5.8
1991	5.2
1990	5.4
1989	5.8
1988	6.0
1987	4.8
1986	5.4
1985	5.4
1984	5.7
1983	> 5.5
1982	~ 5.0
1981	4.9
1980	5.2
1979	5.7

Sources: Data were taken from Chem. Eng. News, 1996-1998 and USITC, 1980-1985 (cited by NTP, 1998).

Year	Imports	Exports
	(millions of pounds)	
1989	33.6	12.1
1988	32	18
1987	> 28	35
1986	3	~ 28
1985	23	> 62.3
1984	12.4	24.7
1983	-	-
1982	9.48	3.3

Sources: Import and export data were taken from ATSDR, 1990-9R068; USDOC Imports, 1990; USDOC Exports, 1986 and 1990; cited by NTP, 1998; and Chem. Prod., 1988.

The current process for production of ethylene oxide is the direct vapor phase oxidation process (Hoechst Celanese Polyester Intermediates et al., 1995). The process oxidizes ethylene with air or oxygen in the presence of a silver catalyst at 10-30 atm (1-3 MPa) and 200-300 °C to give ethylene oxide (IARC, 1994).

The chlorohydrin process used to be the primary process for ethylene oxide production. In this process, ethylene chlorohydrin is prepared by treating ethylene with hypochlorous acid (chlorine in water), which is then converted to ethylene oxide by reaction with calcium oxide. The chlorohydrin process has been phased out since 1931 and is not used on an industrial scale in the United States because of its inefficiency (IARC, 1994).

2.3 Exposure

The primary routes of potential human exposure to ethylene oxide are inhalation, ingestion, and dermal contact. A risk of potential occupational exposure exists for workers involved in ethylene oxide production, in the manufacture of its end products, or in the use of these compounds in occupational settings (ATSDR, 1990). Because ethylene oxide is highly explosive and reactive, the process equipment containing it generally consists of tightly closed and highly automated systems, which decreases the risk of occupational exposure (NCI DCE, 1985h; cited by NTP, 1998). Workers in the synthetic organic chemicals manufacturing industry using ethylene oxide are required to wear respirators when air concentrations exceed the PEL. Personnel in workplaces up to 50 ppm ethylene oxide in the air should wear full facepiece respirators with an ethylene oxide-approved canister (Ludwig, 1994).

2.3.1 Measurement of Exposure

Ethylene oxide forms DNA and hemoglobin adducts. These adducts have been used to monitor human exposure to ethylene oxide (see Section 6).

2.3.2 Commercial Facility Emission Estimates

Ethylene oxide emissions from commercial sterilization facilities in the United States were estimated from data in a 1985 survey of medical equipment suppliers, information provided to EPA (1986, 1988, 1989), and engineering judgment (U.S. EPA, 1993). Emissions ranged from 520 to 20,000 kg per year per unit, depending upon chamber volume, number of facilities, and amount of ethylene oxide used (Table 2-1). Emissions expected from mobile beehive fumigator units were not included in the estimation.

Table 2-1. Average Ethylene Oxide Emissions from Three Sizes of Commercial Sterilization Facilities (U.S. EPA, 1993)

Chamber vol., m^3 (no.)	Mean EO use, kg/yr	EF[a]	Mean EO emissions, kg/yr
< 11 (87)	580	0.90	520
11-56 (71)	6,500	0.65	4,200
> 56 (38)	37,000	0.54	20,000

[a]emission factor (kilograms emission/kilograms used)

2.3.3 Occupational Exposure

NIOSH collected data on potential exposure to specific substances in the National Occupational Hazard Survey (NOHS) from 1972 to 1974 (NIOSH, 1976) and in the National Occupational Exposure Survey (NOES) from 1981 to 1983 (NIOSH, 1990). The industries most likely to use ethylene oxide for sterilization or chemical synthesis are listed in Tables 2-2 and 2-3.

Industries that may use only a small portion of the total ethylene oxide produced are responsible for high occupational exposures to many workers. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 107,450 workers in 74 job categories were potentially exposed to ethylene oxide in the workplace. This estimate was based on observations of the actual use of the compound and tradename products known to contain the compound (NIOSH, 1976). NIOSH estimated that approximately 75,000 health care workers employed in sterilization areas in the period 1972-1974 were potentially exposed to ethylene oxide, and that an additional 25,000 health care workers may have been exposed due to improper engineering and administrative controls (NIOSH 35, 1981; cited by NTP, 1998). NIOSH conducted a limited field survey of hospitals and found that ethylene oxide concentrations near malfunctioning or improperly designed equipment may reach transitory levels of hundreds or even a few thousand parts per million, but time-weighted average (TWA) ambient and breathing zone concentrations were generally below the OSHA standard of 50 ppm (CHIP, 1982b; cited by NTP, 1998).

In a separate survey, OSHA estimated that in 1983, 80,000 U.S. health care workers were directly exposed to ethylene oxide, and 144,000 medical device and related industry workers were incidentally exposed (NCI DCE, 1985h; cited by NTP, 1998; IARC V.36, 1985). More recently, OSHA estimated that as many as 100,000 health care technicians may be exposed to ethylene oxide in the workplace. Health care technicians are typically exposed to quick, concentrated bursts of the gas when the door of a sterilizing machine is opened (Science, 1986). The National Occupational Exposure Survey (1981-1983) estimated that 50,132 workers, including 28,942 women, potentially were exposed to ethylene oxide (NIOSH, 1984). [Presumably, the data have been reevaluated since 1984. Current estimates based on the NOES are given in Table 2-3.] This estimate was derived from observations of the actual use of the compound (98% of total observations) and the use of the tradename products known to contain the compound (2% of total observations). A small population of workers may potentially be exposed to ethylene oxide during the fumigation of spices. OSHA estimated that 160 workers were directly exposed to the gas during spice manufacture (NCI DCE, 1985h; cited by NTP, 1998).

TABLE 2-2. POTENTIAL ETHYLENE OXIDE EXPOSURE IN SELECTED INDUSTRIES REPORTED IN THE 1972-1974 NATIONAL OCCUPATIONAL HAZARD SURVEY (NOHS) (NIOSH, 1976)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
0742	VETERINARY SERVICES, SPECIALTIES	230	1,838	1,608
2013	SAUSAGES AND OTHER PREPARED MEATS	17	748	35
2051	BREAD, CAKE, AND RELATED PRODUCTS	47	773	
2082	MALT BEVERAGES	65	1,170	
2261	FINISHING PLANTS, COTTON	10	435	300
2647	SANITARY PAPER PRODUCTS	34	2,041	
2821	PLASTICS MATERIALS AND RESINS	4	413	
2824	ORGANIC FIBERS, NONCELLULOSIC	7	8,524	1,324
2833	MEDICINALS AND BOTANICALS	116	5,718	729
2834	PHARMACEUTICAL PREPARATIONS	112	6,087	5,637
2841	SOAP AND OTHER DETERGENTS	11	2,019	106
2842	POLISHES AND SANITATION GOODS	25	49	
2843	SURFACE ACTIVE AGENTS	45	1,565	
2869	INDUSTRIAL ORGANIC CHEMICALS, NEC	79	4,017	236
2893	PRINTING INK	45	5,124	356
2899	CHEMICAL PREPARATIONS, NEC	94	1,976	
2911	PETROLEUM REFINING	90	2,048	
3011	TIRES AND INNER TUBES	7	2,850	
3021	RUBBER AND PLASTICS FOOTWEAR	72	2,581	
3069	FABRICATED RUBBER PRODUCTS, NEC	211	5,025	2,694
3079	MISCELLANEOUS PLASTICS PRODUCTS	44	173	
3841	SURGICAL AND MEDICAL INSTRUMENTS	28	743	307
7211	POWER LAUNDRIES, FAMILY & COMMERCIAL	48	2,449	1,921
7216	DRY CLEANING PLANTS, EXCEPT RUG	1,821	9,793	6,190
7219	LAUNDRY AND GARMENT SERVICES, NEC	62	246	
7391	RESEARCH & DEVELOPMENT LABORATORIES	43	1,217	309
8062	GENERAL MEDICAL & SURGICAL HOSPITALS	2,594	96,468	81,992
8072	DENTAL LABORATORIES	316	2,529	316
SUBTOTAL, LISTED INDUSTRIES		**6,277**	**168,619**	**104,060**
SUBTOTAL, OTHER INDUSTRIES		4,380	102,064	16,026
TOTAL		10,657	270,683	120,086

TABLE 2-3. POTENTIAL ETHYLENE OXIDE EXPOSURE IN SELECTED INDUSTRIES REPORTED IN THE 1981-1983 NATIONAL OCCUPATIONAL EXPOSURE SURVEY (NOES) (NIOSH, 1990)

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLOYEES	FEMALE EMPLOYEES
0722	VETERINARIANS AND ANIMAL HOSPITALS	78	783	
2033	CANNED FRUITS AND VEGETABLES	41	372	
2121	CIGARS	6	6	
2262	FINISHING PLANTS, SYNTHETICS	18	683	
2818	INDUSTRIAL ORGANIC CHEMICALS, NEC	41	1,036	
2819	INDUSTRIAL INORGANIC CHEMICALS, NEC	21	518	
2831	BIOLOGICAL PRODUCTS	33	660	
2834	PHARMACEUTICAL PREPARATIONS	14	280	
2842	POLISHES AND SANITATION GOODS	48	3,625	
2843	SURFACE ACTIVE AGENTS	21	1,078	
2851	PAINTS AND ALLIED PRODUCTS	158	1,059	
2879	AGRICULTURAL CHEMICALS, NEC	43	691	
3069	FABRICATED RUBBER PRODUCTS, NEC	11	33	
3079	MISCELLANEOUS PLASTICS PRODUCTS	98	4,914	
3585	REFRIGERATION MACHINERY	17	545	
3841	SURGICAL AND MEDICAL INSTRUMENTS	14	35	
3842	SURGICAL APPLIANCES AND SUPPLIES	8	17	
5014	TIRES AND TUBES	680	2,720	
7211	POWER LAUNDRIES, FAMILY & COMMERCIAL	352	352	
7391	RESEARCH & DEVELOPMENT LABORATORIES	5	93	
8061	HOSPITALS	1,035	16,328	
8092	SANATORIA, CONVALESCENT & REST HOMES	56	1,064	
SUBTOTAL, LISTED INDUSTRIES		**2,798**	**36,892**	
SUBTOTAL, OTHER INDUSTRIES		4,613	70,558	
TOTAL		7,411	107,450	

Ethylene oxide was used as a reaction chemical to modify starch in the starch processing area of an industrial U.S. wastewater treatment plant. Exposures (personal breathing zone concentrations) for full shift operators ranged from undetectable to 0.43 mg/m^3 (0.24 ppm) and from undetectable to 2.5 mg/m^3 (1.4 ppm) for full shift mechanics.

Production of ethylene oxide

IARC (1994) reviewed a number of studies of exposure at production facilities. Exposure data were collected in 1987 from 11 ethylene oxide production units in the United States. The highest mean 8-hr time-weighted average (TWA) was 2.9 mg/m^3 (1.6 ppm) with a range of 0.36 to 6.8 mg/m^3 (0.20 to 3.8 ppm); short-term mean exposure levels for maintenance workers were as high as 19.6 mg/m^3 (10.9 ppm). Respirators were used in operations where engineering controls were not feasible. The manufacture of ethylene oxide usually entails exposure to a variety of other chemicals, e.g., unsaturated aliphatic hydrocarbons, other epoxides, and chlorinated aliphatic hydrocarbons (IARC, 1994).

Use of ethylene oxide as a chemical intermediate

Workers employed in a Brazilian industry using ethylene oxide as an intermediate were biologically monitored for exposure to ethylene oxide (Ribeiro et al., 1994). Ambient air measurements in the general area, made during a 3-month sampling period, indicated that workers were exposed to 2-5 ppm TWA for an 8-hr working day. Blood samples were taken from 75 workers and 22 controls (no occupational exposure to ethylene oxide) matched for sex, age, and smoking habits. Cytogenetic methods and analyses showed significant increases in chromosomal aberrations, micronuclei in binucleated lymphocytes, and hemoglobin adducts (HOEtVal) in the exposed group. However, the frequencies of micronucleated cells in buccal mucosa were not significantly different between the exposed and control groups.

Use of ethylene oxide for industrial sterilization

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of products, such as medical equipment and products (surgical products, single-use medical devices, etc.), disposable health care products, pharmaceutical and veterinary products, spices, and animal feed. Although much smaller amounts of ethylene oxide are used in sterilizing medical instruments and supplies in hospitals and for the fumigation of spices, it is during these uses that the highest occupational exposure levels have been measured (IARC, 1994). Measurements of worker exposure levels in U.S. hospitals, summarized below, showed a range of exposure concentrations (0-794 ppm), depending on operation, conditions, and duration of sampling.

Use of ethylene oxide in hospitals

In hospitals, ethylene oxide is used as a gaseous sterilant for heat-sensitive medical items, surgical instruments, and other objects and fluids coming in contact with biological tissues. Large sterilizers can be found in central supply areas of most hospitals and small sterilizers are used in clinics, operating rooms, tissue banks, and research facilities. Worker exposure may occur during the following operations and conditions: changing pressurized ethylene oxide gas cylinders; leaking valves, fittings, and piping; leaking sterilizer door gaskets; opening of the sterilizer door at the end of a cycle; improper ventilation at the sterilizer door; improperly or unventilated air gap between the discharge line and the sewer drain; removal of items from the

sterilizer and transfer of the sterilized load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; and passing near sterilizers and aerators during operation (IARC, 1994).

Exposure mostly results from peak emissions during operations such as opening the door of the sterilizer and unloading and transferring sterilized material. Short-term (2-30 min) exposure concentrations from below the level of detection to 186 mg/m^3 (103 ppm) were measured in personal samples from hospital sterilizer operators in studies conducted by NIOSH during 1977-1990. With the proper use of engineering controls and work practices, exposure levels can be very low (full shift exposure, < 0.1 ppm; short-term exposure, < 2 ppm). However, the use of personal protective equipment in U.S. hospitals was generally limited to wearing gloves, with no use of respirators, when workers were transferring sterilized items (IARC, 1994).

A recent study of hazardous materials incidents in Massachusetts found that most accidental releases at hospitals involved ethylene oxide (Kales et al., 1997). Detailed exposure data, including personal and area monitoring, were obtained for employees of Massachusetts hospitals during 1990-1992 (LaMontagne and Kelsey, 1997). During this period, 23% of hospitals exceeded the OSHA action level (0.5 ppm) at least once, 24% exceeded the short-term exposure limit (STEL = 5 ppm), and 33% reported accidental exposures to ethylene oxide in the absence of personal monitoring.

A study in a large tertiary-care hospital demonstrated that standard industrial hygiene practices can result in nearly "zero exposure" without personal protective equipment or prohibitive costs (Elias et al., 1993). Instantaneous measurements showed a reduction of peak levels from 500 ppm to 0-2.8 ppm from use of engineering and administrative controls.

2.3.4 Emissions in Air

In 1985, U.S. emissions of ethylene oxide in air were approximately 5,000 Mg (metric tones) per year. The following lists percentages of total air emissions by use: sterilization and fumigation sites, 57%; production and captive use, 31%; medical facilities, 8%; and ethoxylation, 4% (IARC, 1994).

One entry route into the environment for ethylene oxide is as fugitive emissions lost during production, or as vented gases (ATSDR, 1990). Fugitive emissions amounted to some 1.28 million lb in 1978. No information was available to indicate loss with solid waste. There is an estimated emission of 142,600 lb during storage. All ethylene oxide used as a fumigant (up to 10 million lb) is released into the environment. The EOIC estimated that about 3 million lb of ethylene oxide are released into the air each year. Additional sources of ethylene oxide in the environment include inadvertent production from combustion of hydrocarbon fuels (estimated to be millions of pounds annually), cigarette smoke (from ethylene oxide-fumigated tobacco), ethylene oxide degradation products of certain bacteria, photochemical smog, and water disinfection (the latter source only minimal). It has been estimated that about 3 million lb per year were lost to the air and that about 800,000 lb per year were lost to water, representing 0.07% of the 1980 production. Most producers reported that water containing ethylene oxide is treated at a biopond before being discharged from the plant. Several producers stated that steps are underway to reduce the water-ethylene oxide discharges from the ethylene oxide plants to the waste treatment areas, so this number should decrease significantly in the near future. Those producers who have monitored ethylene oxide at the fence line reported nondetectable amounts

in the water analyzed. Five ethoxylation companies reported that a total of 4,000 lb per year was lost to the air, while none was lost to water (CHIP, 1982b; cited by NTP, 1998).

Significant gaseous releases of ethylene oxide to the environment are the result of uncontrolled industrial emissions (ATSDR, 1990). These occur during the loading or unloading of transport tanks, product sampling procedures, and equipment maintenance and repair (CHIP, 1982b; cited by NTP, 1998). The Toxic Chemical Release Inventory (EPA) listed 197 industrial facilities that produced, processed, or otherwise used ethylene oxide in 1988 (TRI88, 1990). In compliance with the Community Right-to-Know Program, the facilities reported releases of ethylene oxide to the environment which were estimated to total 4.7 million lb. By 1995, the total release to air was lower, 839,229 lb (157 facilities releasing at least 10 lb) (TRI95, 1997). The U.S. EPA (1994) estimated that its final air toxics rule for controlling ethylene oxide emissions from commercial sterilization and fumigation operations would reduce ethylene oxide atmospheric emissions by 2 million lb annually from an estimated 114 sources.

2.3.5 Other Occurrences

The risk of potential consumer exposure to ethylene oxide occurs mainly through the use of products which have been sterilized with the compound. These include medical products; articles in libraries, museums, and research laboratories; beekeeping equipment; certain foods and dairy products; cosmetics; transportation vehicles; and articles of clothing (NIOSH 35, 1981; cited by NTP, 1998). EPA reported that small amounts of ethylene oxide, used as a fumigant, were found in some food commodities, such as cocoa, flour, dried fruits and vegetables, and fish. Other sources, however, list ethylene oxide as a fumigant for only three foods: spices, black walnuts, and copra. Residual ethylene oxide may also be found in foods temporarily following fumigation. It may react with water and inorganic halides (Cl^- and Br^-) from foods, producing glycols and halohydrins. Researchers concluded that the persistence or disappearance of ethylene oxide and its by-products in fumigated commodities depends on the grain size, type of food aeration procedures, temperature, and storage and cooking conditions. Most fumigated commodities had levels of ethylene oxide below 1 ppm after 14 days in normal storage conditions (ATSDR, 1990). Ethylene oxide residues were detected in the following food products sampled from Danish retail shops: herbs and spices (14-580 mg/kg), dairy (0.06-4.2 mg/kg), pickled fish (0.08-2.0 mg/kg), meat products (0.05-20 mg/kg), cocoa products (0.06-0.98 mg/kg), and black and herb teas (3-5 mg/kg; one sample contained 1,800 mg/kg). No ethylene oxide residue was detected in a follow-up study of 59 honey samples (IARC, 1994).

Used as a pesticide, some of the ethylene oxide producers and ethoxylators have measured ethylene oxide residuals in ethoxylated products at their plants. The amount varied, depending on the material, and ranged from nondetectable to a few instances of 200 ppm in an ethoxylated surfactant. Surfactants, however, represent a small percentage of the end uses of ethylene oxide. Spot analyses of several ethoxylates by one producer showed the average ethylene oxide concentration was 20 ppm. Most products made from ethylene oxide are distilled or processed further so that no unreacted ethylene oxide is present. The ethoxylators who reported ethylene oxide residuals stated that the concentrations were found to be < 5 ppm. Unreacted levels in these products should reduce with time due to reaction, storage, further pumping, and other processing (CHIP, 1982b; cited by NTP, 1998).

Exposure of the general population to ethylene oxide may occur via inhalation and food ingestion, although there was no information indicating that ethylene oxide is a common

contaminant in food. Most of the ingested ethylene oxide comes from the use of the food additive polyethylene glycol. Also, the use of ethylene oxide polymer is permitted in beer; however, FDA indicates that the compound is not presently used in this capacity. There are also no data to indicate that ethylene oxide is a common constituent of air or water sources of any type in any geographic location within the United States. The limited data available indicate the presence or absence of ethylene oxide in water (drinking water supplies, groundwater, etc.) on a national scale. In water, the compound reacts to form glycols (ATSDR, 1990). The majority of ethylene oxide used as a fumigant or sterilant evaporates and is hydrolyzed by water vapor and oxidized by hydroxy free radicals (CHIP, 1982b; cited by NTP, 1998). From its chemical and physical properties, it can be inferred that ethylene oxide in soil will either volatilize as water evaporates, will leach down into the soil, or will be removed by runoff if water-saturated conditions persist. It is, therefore, unlikely that ethylene oxide will accumulate in organic sediments. No data on the accumulation and/or fate of ethylene oxide in the soil environment are available; however, due to its high level of reactivity, the formation of glycols may occur (ATSDR, 1990). Other sources of exposure to ethylene oxide for the general population may be the by-products of gasoline combustion and cigarette and tobacco smoke (ATSDR, 1990; IARC, 1994).

2.4 Regulations

EPA regulates ethylene oxide under the Clean Air Act (CAA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Resource Conservation and Recovery Act (RCRA), Superfund Amendments and Reauthorization Act (SARA), and Toxic Substances Control Act (TSCA). Under CAA, ethylene oxide has been designated a hazardous air pollutant and potential human health hazard. Under CERCLA, a reportable quantity (RQ) of 10 lb has been established. It is regulated as a hazardous constituent of waste under RCRA. EPA subjects the compound to reporting requirements under SARA and TSCA. A Rebuttable Presumption Against Registration (RPAR) for ethylene oxide has been issued under FIFRA. EPA has changed labeling requirements for pesticide products containing ethylene oxide that are used for sterilization purposes. These changes will require modifications in workplace design and practice in hospitals and health care facilities.

Emission standards for ethylene oxide from commercial sterilizers/fumigators were implemented in 1994 (U.S. EPA, 1994). Existing and new sources that use one to 10 tons must achieve a 99% emission reduction in the sterilization chamber vent, but no controls are required for the aeration room vent or chamber exhaust vent. Operations that use over 10 tons must reduce emissions in the sterilization chamber vent, the aeration room vent, and the chamber exhaust vent. Facilities that use less than one ton have no controls, but must meet recordkeeping requirements.

The deadline for compliance with these emission standards was December 8, 1997 (U.S. EPA, 1996). Sources which use one ton, but are not major or located at major sources, may be deferred by the applicable Title V permitting authority from the Title V permitting requirements for five years until December 9, 1999. However, due to explosions of several ethylene oxide commercial sterilization and fumigation facilities, which may be attributable to emission scrubbers, this compliance was deferred for one year, until December 8, 1998 (62 FR 64736, July 1998).

FDA regulates ethylene oxide as a food additive under the Food, Drug, and Cosmetic Act (FD&CA), and finds that it is the common practice in the drug industry to contract out the performance of ethylene oxide sterilization. FDA allows denture adhesives to be composed of an ethylene oxide homopolymer, alone or with carboxymethyl cellulose sodium or karaya. Tolerances for residues of ethylene oxide on agricultural commodities have been established under FD&CA; however, FDA is re-evaluating its established regulations governing ethylene oxide residues, in light of recent toxicity data and information concerning the formation of 1,4-dioxane.

Ethylene oxide was the subject of a Special Hazard Review performed by NIOSH, which has recommended an exposure limit of 0.1 ppm (0.18 mg/m^3) as an 8-hr TWA and 5 ppm (9 mg/m^3) ceiling concentration (10-minute). OSHA lowered the permissible exposure limit (PEL) from 50 ppm to 1 ppm as an 8-hr TWA in 1984 and established an STEL of 5 ppm during a 15-minute period in 1988. OSHA regulates ethylene oxide under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
EPA	40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/23/71. U.S. Code: 42 U.S.C. 7401, 7411, 7413, 7414, 7416, 7429, 7601 and 7602.	
	40 CFR 60—Subpart VV—Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry. Promulgated: 48 FR 48335, 10/18/83.	The provisions of this subpart apply to affected facilities in the synthetic organic chemicals manufacturing industry that commences construction or modification after January 5, 1981.
	40 CFR 60.489—Sec. 60.489 List of chemicals produced by affected facilities.	Ethylene oxide is produced, as an intermediate or final product, by process units covered under this subpart. Ethylene oxide is designated as a potential human health hazard.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 60—Subpart III—Standards of Performance for Volatile Organic Compound (VOC) Emissions From the Synthetic Organic Chemical Manufacturing Industry (SOCMI) Air Oxidation Unit Processes. Promulgated: 55 FR 26922, 06/29/90.	The provisions of this subpart apply to each affected facility that produces ethylene oxide as a product, co-product, by-product, or intermediate; however, if the facility has a total resource effectiveness (TRE) index value > 4.0, it is exempt from all provisions of this subpart except for 60.612, 60.614(f), 60.615(h), and 60.615(l).
	40 CFR 60—Subpart NNN—Standards of Performance for Volatile Organic Compound (VOC) Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Promulgated: 55 FR 26942, 06/29/90.	The provisions of this subpart apply to each affected facility that produces ethylene oxide as a product, co-product, by-product, or intermediate; exceptions do apply.
	40 CFR 60—Subpart RRR—Standards of Performance for Volatile Organic Compound Emissions From Synthetic Organic Chemical Manufacturing Industry (SOCMI) Reactor Processes. Promulgated: 58 FR 45962, 08/31/93.	The provisions of this subpart apply to each affected facility that produces ethylene oxide as a product, co-product, by-product, or intermediate; exceptions do apply.
	40 CFR 61—PART 61—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS. Promulgated: 38 FR 8826, 04/06/73. U.S. Code: 42 U.S.C. 7401, 7412, 7413, 7414, 7416, 7601 and 7602.	
	40 CFR 61—Subpart A—General Provisions.	
	40 CFR 61.1—Sec. 61.1 Lists of pollutants and applicability of part 61.	Ethylene oxide is a substance for which a Federal Register notice has been published that included consideration of the serious health effects, including cancer, from ambient air exposure to the substance.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 42 U.S.C. 7401 et seq.	
	40 CFR 63—Subpart DD—National Emission Standards for Hazardous Air Pollutants from Off-Site Waste and Recovery Operations. Promulgated: 61 FR 34158, 07/01/96.	Ethylene oxide is listed as a hazardous air pollutant (HAP).
	40 CFR 63—Subpart JJ—National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95.	Ethylene oxide is listed as a volatile HAP (VHAP), a pollutant excluded from use in cleaning and washoff solvents, and a VHAP of potential concern ("nonthreshold" and "high-concern" pollutant).
	40 CFR 63—Subpart D—Regulations Governing Compliance Extensions for Early Reductions of Hazardous Air Pollutants. Promulgated: 57 FR 61992, 12/29/92, as amended at 58 FR 62543, 11/29/93; 59 FR 53110, 10/21/94.	The provisions of this subpart apply to an owner or operator of an existing source who wishes to obtain a compliance extension from a standard issued under section 112(d) of the CAA. Listed as a high-risk pollutant, ethylene oxide has a chemical weighting factor of 10.
	40 CFR 63—Subpart F—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94.	Ethylene oxide is listed as a synthetic organic chemical manufacturing industry chemical, an organic HAP, and an organic HAP subject to cooling tower monitoring requirements in section 63.104.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 63—Subpart G—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry for Process Vents, Storage Vessels, Transfer Operations, and Wastewater. Promulgated: 59 FR 19468, 04/22/94.	Ethylene oxide is subject to the wastewater provisions for process units at new and existing sources, with a corresponding fraction removed (Fr) value of 0.98. The fraction measured (Fm) and fraction emitted (Fe) are 1.00 and 0.50, respectively, in wastewater streams. Ethylene oxide is also a compound used for compliance demonstrations for enhanced biological treatment processes.
	40 CFR 63—Subpart O—Ethylene Oxide Emissions Standards for Sterilization Facilities. Promulgated: 59 FR 62589, 12/06/94. [Effective Date Note: At 62 FR 64738, 12/09/97, this subpart is suspended from Dec. 4, 1997, until Dec. 6, 1998.]	All sterilization sources using 1 ton per year in sterilization or fumigation operations are subject to the emissions standards in section 63.362, except as specified in paragraphs (b)-(e) of this section. Standards for ethylene oxide commercial sterilizers and fumigators are summarized in Table 1. This subpart does not apply to ethylene oxide sterilization operations at stationary sources such as hospitals, doctors' offices, clinics, or other facilities whose primary purpose is to provide medical services to humans or animals.
	40 CFR 63—Subpart U—National Emission Standards for Hazardous Air Pollutant Emissions: Group I Polymers and Resins. Promulgated: 62 FR 46925, 09/05/96.	Ethylene oxide is listed as a known organic HAP from elastomer products.
	40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISIONS. Promulgated: 59 FR 4493, 01/31/94. U.S. Code: 42 U.S.C. 7412(r), 7601(a)(1), and 7661-7661f.	
	40 CFR 68—Subpart F—Regulated Substances for Accidental Release Prevention.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 68.130—Sec. 68.130 List of substances.	Ethylene oxide is a regulated toxic substance. Its threshold quantity for accidental release prevention is 10,000 lb. Its toxic endpoint (for analyses of offsite consequences, toxics) is 0.09 mg/L.
	40 CFR 180—PART 180—TOLERANCES AND EXEMPTION FROM TOLERANCES FOR PESTICIDE CHEMICALS IN FOOD. Promulgated: 41 FR 4537, 01/30/76.	Ethylene oxide is listed as a pesticide chemical.
	40 CFR 180—Subpart C—Specific Tolerances.	
	40 CFR 180.151—Sec. 180.151 Ethylene oxide; tolerances for residues.	A tolerance of 50 ppm is established for residues of the antimicrobial agent and insecticide ethylene oxide, when used as a postharvest fumigant in or on the following raw agricultural commodities: Black walnut meats, copra, whole spices.
	40 CFR 180—Subpart D—Exemptions from Tolerances.	
	40 CFR 180.1001—Sec. 180.1001 Exemptions from the requirement of a tolerance.	Residues of ethylene oxide are exempted from the requirement of a tolerance when used in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest.
	40 CFR 185—PART 185—TOLERANCES FOR PESTICIDES IN FOOD. Promulgated: 40 FR 14156, 03/28/75. Redesignated at 41 FR 26568, 06/28/76, and 53 FR 24667, 06/29/88. U.S. Code: 21 U.S.C. 346a and 348.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 185—Subpart B—Food Additives Permitted in Food for Human Consumption.	
	40 CFR 185.2850—Sec. 185.2850 Ethylene oxide.	Ethylene oxide may be safely used as a fumigant for the control of microorganisms and insect infestation in ground spices and other processed natural seasoning materials, except mixtures to which salt has been added, provided that either alone or admixed with carbon dioxide or dichlorodifluoromethane, it shall be used in amounts not to exceed that required to accomplish the intended technical effects, and residues of ethylene oxide in ground spices from both postharvest application to the raw agricultural commodity whole spices and application to the ground spices do not exceed the established tolerance of 50 ppm in whole spices.
	40 CFR 192—PART 192—HEALTH AND ENVIRONMENTAL PROTECTION STANDARDS FOR URANIUM AND THORIUM MILL TAILINGS. Promulgated: 48 FR 602, 01/05/83. U.S. Code: 42 U.S.C. 2022, as added by the Uranium Mill Tailings Radiation Control Act of 1978, as amended.	Ethylene oxide is listed as a constituent of this part.
	40 CFR 261—PART 261—IDENTIFICATION AND LISTING OF HAZARDOUS WASTE. Promulgated: 45 FR 33119, 05/19/80. U.S. Code: 42 U.S.C. 6905, 6912(a), 6921, 6922, 6924(y), and 6938.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 261—Subpart D—Lists of Hazardous Wastes.	Ethylene oxide is listed as a toxic substance and is subject to the small quantity generator exclusion defined in section 261.5 (a) and (g). It is also listed as a hazardous waste; the hazardous waste number is U115.
	40 CFR 266—PART 266—STANDARDS FOR THE MANAGEMENT OF SPECIFIC HAZARDOUS WASTES AND SPECIFIC TYPES OF HAZARDOUS WASTE MANAGEMENT FACILITIES. Promulgated: 50 FR 666, 01/04/85. U.S. Code: 42 U.S.C 1006, 2002(a), 3004, 3014, 6905, 6906, 6912, 6922, 6923, 6924, 6925, 6934, and 6937, effective 07/11/96.	
	40 CFR 266—Subpart M—Military Munitions. Promulgated: 62 FR 6654, 02/12/97.	The regulations in this subpart identify when military munitions become a solid waste, and, if these wastes are also hazardous under this subpart or part, the management standards that apply to these wastes. The risk specific dose of ethylene oxide is 0.1 $\mu g/m^3$. The concentration limit for residues of ethylene oxide is 3×10^{-4} mg/kg.
	40 CFR 261.11, 261.33. Promulgated 5/19/80. RCRA 3001-3004: Subjects waste products, off-specification batches, and spill residues in excess of 1,000 kg to handling and report/recordkeeping requirements. Also designates ethylene oxide as a hazardous constituent of waste, and subjects wastes known to contain it to the same requirements.	Based on toxic effects other than acute. The EPA Carcinogen Assessment Group has included this chemical on its list of potential carcinogens. As a result of this listing, ethylene oxide is regulated under the hazardous waste disposal rule of RCRA.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Code: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	
	40 CFR 302.4—Sec. 302.4 Designation of hazardous substances.	The statutory final reportable quantity (RQ) for ethylene oxide is 10 lb (4.54 kg).
	40 CFR 355—PART 355—EMERGENCY PLANNING AND NOTIFICATION. Promulgated: 52 FR 13395, 04/22/87. U.S. Code: 42 U.S.C. 11002, 11004, and 11048.	Ethylene oxide is listed as an extremely hazardous substance, and its threshold planning quantity is 1,000 lb.
	40 CFR 372—PART 372—TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11023 and 11048.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA of 1986, which is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, to aid in the development of regulations, guidelines, and standards, and for other purposes.
	40 CFR 372—Subpart D—Specific Toxic Chemical Listings.	
	40 CFR 372.65—Sec. 372.65 Chemicals and chemical categories to which this part applies.	The effective date for reporting for ethylene oxide was January 1, 1987.
	40 CFR 721—PART 721—SIGNIFICANT NEW USES OF CHEMICAL SUBSTANCES. Promulgated: . U.S. Code: 15 U.S.C. 2604, 2607, and 2625(c).	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
EPA	40 CFR 721—Subpart E—Significant New Uses for Specific Chemical Substances. [Sections were promulgated in 57 FR in 1992.]	This subpart lists several ethylene oxide adducts and a chemical substance identified generically as a polymer of disodium maleate, allyl ether, and ethylene oxide, which are subject to reporting for significant new uses. Specific requirements, recordkeeping requirements, and limitations or revocation of certain notification requirements are also given.
	40 CFR 721.3680—Sec. 721.3680 Ethylene oxide adduct of fatty acid ester with pentaerythritol. Promulgated: 57 FR 46466, Oct. 8, 1992, as amended at 58 FR 34204, June 23, 1993]	The chemical substance is subject to reporting under this section for the significant new uses of releases to water. Specific requirements, recordkeeping requirements, and limitations
FDA	21 CFR 172—PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14491, 03/15/77. U.S. Code: 21 U.S.C. 321, 341, 342, 348, 371, and 379e.	
	21 CFR 172—Subpart H—Other Specific Usage Additives.	
	21 CFR 172.770—Sec. 172.770 Ethylene oxide polymer.	The polymer of ethylene oxide may be safely used as a foam stabilizer in fermented malt beverages provided that it has a minimum viscosity of 1,500 centipoises in a 1% aqueous solution at 25 °C.
	21 CFR 172—Subpart I—Multipurpose Additives.	
	21 CFR 172.808—Sec. 172.808 Copolymer condensates of ethylene oxide and propylene oxide. Promulgated: 42 FR 14491, 03/15/77, as amended at 46 FR 57476, 11/24/81.	Copolymer condensates of ethylene oxide may be safely used in food provided that certain prescribed conditions listed in this section are met.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 172.820—Sec. 172.820 Polyethylene glycol (mean molecular weight 200-9,500).	Polyethylene glycol may be safely used in food provided that the additive is an addition polymer of ethylene oxide and water with a mean molecular weight of 200 to 9,500.
	21 CFR 175—PART 175—INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS. Promulgated: 42 FR 14534, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, 379e.	This part gives substances containing ethylene oxide permitted for use in adhesives
	21 CFR 176—PART 176—INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS. Promulgated: 42 FR 14554, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 346, 348, 379e.	This part gives substances containing ethylene oxide that may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food.
	21 CFR 176.210—Sec. 176.210 Defoaming agents used in the manufacture of paper and paperboard.	Substances permitted to be used in the formulation of defoaming agents include fatty triglycerides, and marine oils, and the fatty acids and alcohols reacted with ethylene oxide, with or without dehydration, to form esters and ethers.
	21 CFR 178—PART 178—INDIRECT FOOD ADDITIVES: ADJUVANTS, PRODUCTION AIDS, AND SANITIZERS. Promulgated: 42 FR 14609, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, 379e.	This part concerns ethylene oxide use with other substances as a sanitizing solution, antistatic and/or antifogging agent in food-packaging materials, food contact surface lubricant, and industrial starch modifier.
	21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998, 03/27/75. U.S. Code: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 358, 360, 360b, 360gg-360ss, 371, 374, 379e; 42 U.S.C. 216, 241, 262, 264.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 201—Subpart A—General Labeling Provisions.	
	21 CFR 201.1—Sec. 201.1 Drugs; name and place of business of manufacture, packer, or distributor.	FDA finds that it is the common practice in the drug industry to contract out the performance of certain manufacturing operations, which include ethylene oxide sterilization.
	21 CFR 872—PART 872—DENTAL DEVICES. Promulgated: 52 FR 30097, 08/12/87. U.S. Code: 21 U.S.C. 351, 360, 360c, 360e, 360j, 371.	
	21 CFR 872—Subpart D—Prosthetic Devices.	
	21 CFR 872.3410—Sec. 872.3410 Ethylene oxide homopolymer and/or carboxymethyl cellulose sodium denture adhesive. Promulgated: 52 FR 30097, 08/12/87, as amended at 59 FR 63008, 12/07/94.	An ethylene oxide homopolymer and/or carboxymethyl cellulose sodium denture adhesive is a device intended to be applied to the base of a denture before the denture is inserted in a patient's mouth to improve denture retention and comfort.
	21 CFR 872.3450—Sec. 872.3450 Ethylene oxide homopolymer and/or karaya denture adhesive. Promulgated: 52 FR 30097, 08/12/87, as amended at 59 FR 63008, 12/07/94.	An ethylene oxide homopolymer and/or karaya denture adhesive is a device intended to be applied to the base of a denture before the denture is inserted in a patient's mouth to improve denture retention and comfort.
	21 CFR 880—PART 880—GENERAL HOSPITAL AND PERSONAL USE DEVICES. Promulgated: 45 FR 69682-69737, 10/21/80. U.S. Code: 21 U.S.C. 351, 360, 360c, 360e, 360j, 371.	This part sets forth the classification of general hospital and personal use devices intended for human use that are in commercial distribution.
	21 CFR 880—Subpart G—General Hospital and Personal Use Miscellaneous Devices.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
FDA	21 CFR 880.6100—Sec. 880.6100 Ethylene oxide gas aerator cabinet.	An ethylene oxide gas aerator cabinet is a device that is intended for use by a health care provider and consists of a cabinet with a ventilation system designed to circulate and exchange the air in the cabinet to shorten the time required to remove residual ethylene oxide from wrapped medical devices that have undergone ethylene oxide sterilization. (Class II—performance standards)
	21 CFR 880.6860—Sec. 880.6860 Ethylene oxide gas sterilizer.	An ethylene gas sterilizer is a nonportable device intended for use by a health care provider that uses ethylene oxide to sterilize medical products. (Class II—performance standards)
NIOSH	8/77. Special Occupational Hazard Review with Control Recommendations for the Use of Ethylene Oxide as a Sterilant in Medical Facilities. NIOSH Publication 77-200. NTIS No. PB 274795.	Summary of current NIOSH recommendation: exposure limits—Ca, 0.1 ppm (0.18 mg/m^3) 8-hr TWA, 5 ppm (9 mg/m^3) ceiling concentration (10-minute).
	5/81. Recommended that ethylene oxide be treated as a potential human carcinogen, occupational exposure should be decreased, and OSHA should reexamine PEL.	Recommended 5-ppm (9 mg/m^3) ceiling (10 min/day).
	5/22/81. Current Intelligence bulletin #35—Ethylene Oxide (EtO): Evidence of Carcinogenicity.	
	4/2/82. Comments Regarding the OSHA Advanced Notice of Proposed Rulemaking—Occupational Exposure to Ethylene Oxide.	
	6/22/83. Comments on OSHA's Proposed Rule on Occupational Exposure to Ethylene Oxide.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
NIOSH	7/20/83. Testimony on OSHA's Proposed Rule on Occupational Exposure to Ethylene Oxide. 7/18/84. Comments to OSHA on the Evaluation of the OMB Comments on OSHA's Draft STEL for Ethylene Oxide. 3/19/85. Comments to OSHA on Short-Term Exposures to Ethylene Oxide. 4/30/85. Comments to OSHA on Proposed Rule: Occupational Exposure to Ethylene Oxide; Labeling Requirements. 9/26/85. Comments to OSHA on a Short-Term Exposure Limit for Ethylene Oxide. 2/22/88. Comments on OSHA's Proposed Rule on Occupational Exposure to Ethylene Oxide. 7/13/89. Current Intelligence Bulletin #52—Ethylene Oxide Sterilizers in Health Care Facilities; Engineering Controls and Work Practices.	Blood monitoring and medical counseling recommended.
OSHA	29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74. U.S. Code: 29 U.S.C. 653, 655, and 657. 29 CFR 1910—Subpart B—Adoption and Extension of Established Federal Standards. U.S. Code: 29 U.S.C. 653, 655, 657; 41 U.S.C. 35 et seq.; 41 U.S.C. 351 et seq.; 40 U.S.C. 333; 33 U.S.C. 941; and 20 U.S.C. 951 et seq.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
OSHA	29 CFR 1910.19—Sec. 1910.19 Special provisions for air contaminants.	This section states that section 1910.1047 shall apply to the exposure of every employee to ethylene oxide in every employment and place of employment covered by construction work, shipyard employment, and longshoring and marine terminals, in lieu of any different standard on exposure to ethylene oxide which would otherwise be applicable by virtue of those sections.
	29 CFR 1910—Subpart H—Hazardous Materials.	Ethylene oxide is listed as a toxic and reactive highly hazardous chemical which presents a potential for a catastrophic event at or above the threshold quantity of 5000 lb.
	29 CFR 1910—Subpart Z—Toxic and Hazardous Substances.	
	29 CFR 1910.1047—Sec. 1910.1047 Ethylene Oxide. Promulgated: 49 FR 25796, 06/22/84 through 63 FR 1292, 01/08/98. Establishes PEL of 1 ppm as an 8-hr TWA and an STEL of 5 ppm sampled over 15 min.	This section applies to all occupational exposures to ethylene oxide (EtO), but does not apply to the processing, use, or handling of products containing EtO where objective data are reasonably relied upon that demonstrate that the product is not capable of releasing EtO in airborne concentrations at or above the action level, and may not reasonably be foreseen to release EtO in excess of the excursion limit.
	29 CFR 1910.1200—Sec. 1910.1200 Hazard Communication. Promulgated: 11/25/83. U.S. Code: also includes 5 U.S.C. 553.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication Program to include labels, material safety data sheets, and worker training.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
O S H A	29 CFR 1910.1450—Sec. 1910.1450 Occupational exposure to hazardous chemicals in laboratories. Promulgated: 55 FR 3327, 01/31/90 through 55 FR 12111, 03/30/90. OSH Act: Final rule. 29 CFR 1916—PART 1926—SAFETY AND HEALTH REGULATIONS FOR CONSTRUCTION. Promulgated: 44 FR 8577, 02/09/79; 44 FR 20940, 04/06/79. 29 CFR 1926—Subpart Z—Toxic and Hazardous Substances. 29 CFR 1926.1147—Sec. 1916.1147 Ethylene oxide. Promulgated: 61 FR 31434, 06/20/96.	As a select carcinogen (IARC Group 2A), ethylene oxide is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan. The requirements applicable to construction work under this section are identical to those set forth in section 1910.1047.

[a]The regulations in this table have been updated through the 1998 issues of Code of Federal Regulations titles 21, 29, and 40.

3.0 HUMAN STUDIES

3.1 Studies Evaluated by IARC (1994)

The IARC Working Group for consideration of ethylene oxide concluded that there is limited evidence in humans for the carcinogenicity of ethylene oxide (IARC, 1994). The majority of reviewed studies evaluated the risk from occupational ethylene oxide exposure. These studies provided some evidence that the risk of lymphatic and hematopoietic cancer was increased among workers exposed to ethylene oxide.

Lymphatic and hematopoietic cancer were the most frequently reported cancers associated with exposure to ethylene oxide in epidemiological studies (IARC, 1994). Study populations were either workers using ethylene oxide as a sterilant or chemical workers involved in production of the compound or its derivatives. The study with the largest number of sterilization personnel included only workers in the United States (Steenland et al., 1991). Three European studies of sterilization workers found non-significant excesses of lymphatic and hematopoietic cancer.

An increase in these cancers was also shown in a study of chemical workers at two production plants in the United States, but the increase was only seen in a small subgroup with occasional exposure to low levels of ethylene oxide (Benson and Teta, 1993; cited by IARC, 1994). In six other studies of chemical workers involving fewer deaths, four found increases in

lymphatic and hematopoietic cancer (two of which showed statistical significance) and two found no excess deaths compared to the control group (IARC, 1994).

A study of U.S. sterilization workers (Steenland et al., 1991) reported mortality in a cohort of over 18,000 workers from 14 industrial plants that regularly used ethylene oxide to sterilize medical supplies or spices. Vital status through the end of 1987 was determined. The average length of exposure was 4.9 years and the average length of follow-up was 16.1 years. Vital status was determined for 95.5% of the cohort. Overall, there was no association with hematopoietic cancers as a group (Standardized Mortality Ratio [SMR] = 1.06; 95% CI = 0.75-1.47). An elevated, but imprecise SMR was found for lymphosarcoma-reticulosarcoma (SMR = 1.52; 95% CI = 0.65-3.00). The SMR for leukemia was not elevated. An increased risk of death from kidney cancer (SMR = 1.80; 95% CI = 0.96-3.08) was found. Potential exposure to ethylene oxide was determined from job title and department at each plant. Analysis by duration of exposure to ethylene oxide did not show any consistent pattern of increased risk of death (including hematopoietic cancers). The analysis by length of time since first exposure to ethylene oxide, however, did show a trend toward an increased risk of death from several hematopoietic cancers including leukemia, Hodgkin's disease and non-Hodgkin's lymphoma. However, the short follow-up period and the lack of precise estimates for some exposure categories limit the interpretation.

An expanded analysis of this cohort study included the use of an industrial hygiene-based regression model to estimate individual cumulative exposure to ethylene oxide (Stayner et al., 1993). The mean of the individual time-weighted exposure values from the facilities for 1976 through 1985 was calculated for exposure categories based on the aggregation of jobs with similar potential for exposure. A regression model was then derived using a number of predictors such as exposure category, sterilizer volume, product age, and engineering controls. This model was used to estimate individual cumulative ethylene oxide exposure for workers in each facility. Life-table methods and the Cox proportional hazards model were used to evaluate cancer mortality. An elevated SMR was found for all hematopoietic neoplasms in the highest cumulative exposure category, but a dose-response gradient was not apparent. Non-Hodgkin's lymphoma also showed an excess risk in the highest exposure category. The Cox regression analysis showed a significant relationship between cumulative exposure and mortality from the combined group of "lymphoid" cancers (lymphocytic leukemia and Non-Hodgkin's lymphoma). Weaker dose-response effects were found for non-Hodgkin's lymphoma and all hematopoietic cancers. The positive association appeared to be limited to male workers. Other exposure measures (duration, average, and maximum exposure) were not as strongly associated as the cumulative exposure measure. A lagged model estimated a rate ratio of about 1.2 for the lymphatic and hematopoietic neoplasms at the current OSHA standard level for ethylene oxide. This study was large and included a relatively sophisticated exposure estimation model. Nonetheless, a definitive conclusion is limited by some analyses based on a small number of deaths and the apparent specificity by gender. However, the results provide additional support for an association between ethylene oxide and hematopoietic cancers.

Shore et al. (1993) reported the findings of a meta-analysis of 10 cohort mortality studies published from 1979-1993 that included workers potentially exposed to ethylene oxide. A total of 29,800 workers (2,540 deaths) were included. The studies were reviewed and their quality assessed, although quality scores were not incorporated in the meta-analysis. The summary SMR for leukemia was 1.06 (95% CI = 0.73-1.48). Overall, there were no significant trends

based on duration or intensity of exposure. The summary SMR for non-Hodgkin's lymphoma was elevated (SMR = 1.35; 95% CI = 0.93-1.90), but there did not appear to be any trend in risk with frequency or intensity, duration of exposure, or latency. The summary SMR was 1.28 (95% CI = 0.98-1.65) for stomach cancer, but after removal of the one study responsible for the significant test of heterogeneity, the SMR decreased to 1.11. There were no significant gradients in risk accounting for intensity, duration of exposure, or latency. However, studies that evaluated brain cancer (with a total of 19 deaths and SMRs from 0.0 to 12.7) showed a significant heterogeneity. The summary 95% confidence interval, accounting for the heterogeneity, was relatively wide (0.39-2.04). There was no suggestion of a trend in exposure intensity or duration. Pancreatic cancer was not associated with an elevated summary SMR (SMR=0.98; 95% CI = 0.69-1.36).

The authors concluded that there did not appear to be strong evidence for an increased risk of death from selected cancers, although they recommended continued investigation of leukemia and non-Hodgkin's lymphoma. The meta-analysis was limited by the fact that even with the pooling of studies, many of the risk estimates for specific cancers were imprecise and the follow-up period inadequate. In addition, individual exposure estimates were not available in most studies, and confounding exposures could not be ruled out in some studies.

3.2 Studies Post-IARC (1994)

A study reported that the incidence of breast cancer was elevated in a cohort of workers at a plant that used ethylene oxide as a sterilant (Norman et al., 1995). An earlier study found an increased incidence of sister chromatid exchange (SCE) in workers at this plant compared to community controls (Stolley et al., 1984; cited by Norman et al., 1995 and IARC, 1994). The cohort consisted of 1,132 persons employed between 1974 and 1980, the period of potential ethylene oxide exposure at the plant. Cancer incidence was determined through 1987. The 8-h time TWA exposure concentrations for sterilizer operators was 50-200 ppm, based on three 2-h samples obtained in March 1980; samples taken subsequent to corrective action showed exposures ranging from 5-20 ppm.

Cancer incidence was ascertained from a variety of sources, including a health exam or interview. A total of 79% of the workers participated in either one exam or interview. The number of observed cancers was compared with the expected number based on incidence rates reported by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program. Twelve of the 28 cancers observed were breast cancers. The standardized morbidity ratio for breast cancer among regular female employees ranged from 2.55 (95% CI = 1.31-4.98; 8 observed, 3.14 expected; p = 0.02) to 1.70 (95% CI = 0.89-3.23; 9 observed, 5.28 expected; p = 0.09) depending on calendar year of follow-up, assumptions about completeness of follow-up, and the reference rates used. None of the breast cancer cases were discovered from screening observations at the worksite, and there was no unusual distribution of risk factors for breast cancer (e.g., nulliparity, family history of breast cancer) among the cases. Incorporating a 2-yr latency period into the analysis produced similar results. No statistically significant excess of breast cancer was found for temporary employees, and there was no increase in cancer incidence at other sites associated with ethylene oxide in previous studies. Because of the size of the study, the statistical power to detect significant increases at these sites was very low. The latency, length of follow-up, and absence of detailed exposure data are additional study limitations.

An update of the cohort study of Swedish sterilant workers reported by Hagmar et al. in 1991 did not find a significant positive association between ethylene oxide exposure and cancer risk (Hagmar et al., 1995). The cohort (n = 2,170) comprised workers employed at least one year before 1986 in two plants that produced disposable medical equipment sterilized with ethylene oxide. Cancer incidence was assessed for 1972 to 1990 and 1976 to 1990 using a national registry. No worker was lost to follow-up. Only 13% of the cohort had direct exposure to ethylene oxide. Peak exposure concentrations when unloading autoclaves were estimated to be 500-1,000 ppm until 1973. The median estimated cumulative exposure for the cohort was 0.13 ppm-yr. Increased Standardized Incidence Ratios (SIRs) for lymphohematopoietic tumors (SIR = 1.78; 95% CI = 0.65-3.88; 6 observed, 3.37 expected) and leukemia (SIR = 2.44; 95% CI = 0.3-8.81; 2 observed, 0.82 expected) were found but were not statistically significant. Among those workers with exposure greater than the median and with a minimum induction period of 10 years, the SIR for leukemia was increased, but imprecise (SIR = .14; 95% CI = 0.87- 25.8). The study, based on cancer incidence, included individual exposure estimates and complete follow-up. However, the cohort was not large, many workers did not have a high level of exposure, and the follow-up time was relatively short.

Workers in plants that produced ethylene oxide by the ethylene chlorohydrin process were followed-up for mortality and associated cancer incidence (Olsen et al., 1997). The cohort consisted of male workers (n = 1,361) from 1940-1992 in process areas within several U.S. plants. The production work areas were used for synthesis of ethylene chlorohydrin and ethylene oxide, but analyses of work area or personal samples were not reported. In later years, all of these plants were converted for production of propylene chlorohydrin and propylene oxide, and many cohort members were also exposed to these substances. The overall SMR for lymphopoietic and haematopoietic cancer, with a 25-yr induction latency period, was 1.44 (95% CI = 0.52-3.12). The Mantel-Haenszel relative risk increased to 3.56 (95% CI = 1.23-10.29) with 10-20 years employment, but decreased for more than 20 years duration. The analysis of workers only in the ethylene chlorohydrin production process showed elevated risks of lymphopoietic and haematopoietic cancer (SMR = 1.49; 95% CI = 0.60-3.07) overall, and with a 25-yr latency induction period (SMR = 1.94; 95% CI = 0.71-4.23). There were no lymphopoietic and hematopoietic cancer deaths observed among both the propylene and ethylene chlorohydrin production process workers. The overall mortality and mortality associated with other cancers was not significantly increased. The study was limited by the length of the follow-up period (mean of 24.5 years) and the imprecision of the SMR estimates.

The apparent discrepancy between the positive studies identified by IARC (1994) and these later studies may be attributed to the relatively small size of the cohorts and short time of follow-up, as well as limitations of the earlier studies.

Table 3-1. Post-IARC (1994) Ethylene Oxide Human Studies

Design	Population Group	Exposure	Effects	Comments	Reference
Cohort	1,132 Workers employed from 1974-1980 observed cancers compared to expected cancers based on age- and sex-specific cancer rates in the Surveillance, Epidemiology, End Results (SEER) database	Ethylene oxide only major process chemical used from mid-1970s to 1980; documentation of ethylene oxide leaks on several occasions; elevated SCE in workers compared to controls from community The 8-h TWA exposure concentrations for sterilizer operators was 50-200 ppm, based on three 2-h samples obtained in March 1980. Samples taken subsequent to corrective action showed 5-20 ppm.	Calculated breast cancer standardized morbidity ratios (SMRs) with 95% CI; no. observed/no. expected; p value 2.55 (1.31-4.98; 8/3.14; p=0.02) for regular female employees and follow-up to date of last interview, to death, or to first diagnosis 1.70 (0.89-3.23; 9/5.28; p=0.09) for regular female employees through 1987 with later SEER rates Excess diminished over time. No excess at any time for temporary female employees.	SMRs varied with calendar yr of follow-up, assumptions about completeness of follow-up, and reference rates.	Norman et al. (1995)
Cohort update	2,170 Workers employed at least 1 yr in two plants that produced equipment sterilized with ethylene oxide	Peak exposure concentrations were estimated to be 500-1000 ppm, up to 1973; individual cumulative exposure (ppm-yr) estimated; median value 0.13 ppm-yr; median calendar year start of exposure was 1978; median employment duration from start of exposure was 5.6 yr until 1985	Calculated cause-specific standardized incidence ratios (SIRs) with 95% CI; no. observed/no. expected <u>Lymphohematopoietic tumors:</u> 1.78 (0.65-3.88; 6/3.37) for entire cohort and no latency period <u>Leukemia:</u> 2.44 (0.30-8.81; 2/0.82) for entire cohort and no latency period 7.14 (0.87-25.8; 2/0.28) for cumulative exposure to more than 0.13 ppm-yr and 10-yr latency No SIRs were significant.	Cohort was small, many workers did not have high level of exposure, follow-up time was relatively short.	Hagmar et al. (1995)

Table 3-1. Post-IARC (1994) Ethylene Oxide Human Studies (Continued)

Design	Population Group	Exposure	Effects	Comments	Reference
Cohort	1,361 Workers from 1940-1992 in U.S. plants that produced ethylene oxide by the ethylene chlorohydrin process	Work areas used for synthesis of ethylene chlorohydrin and ethylene oxide; analyses of work area or personal samples n.p.; some workers also later exposed to propylene chlorohydrin and propylene oxide	Calculated SMRs and Mantel-Haenszel relative risks (RRs) with 95% CI Lymphopoietic and haematopoietic cancer: 1.44 (0.52-3.12) SMR overall with 25-yr latency period 3.56 (1.23-10.29) RR with 10-20 yr employment 1.72 (0.24-12.21) RR with > 20 yr employment 1.49 (0.6-3.07) SMR overall for workers only in the ethylene chlorohydrin production areas 1.94 (0.7-4.23) SMR for workers only in the ethylene chlorohydrin production areas and a 25-yr induction no lymphopoietic and haematopoietic cancer deaths among propylene chlorohyrin production workers	Limited by length of follow-up (mean = 24.5 yr) and imprecise estimates	Olsen et al. (1997)

Abbreviations: CI = confidence interval; n.p. = not provided; SCE = sister chromatid exchange

4.0 EXPERIMENTAL CARCINOGENICITY

4.1 Studies Reviewed by IARC (1994)

4.1.1 Mice

In mice, local sarcomas were induced by subcutaneous injection of ethylene oxide (Dunkelberg, 1981; cited by IARC, 1994). Female NMRI mice, aged 6-8 wk, were injected with ethylene oxide (99.7% pure) in tricaprylin at doses of 0.1, 0.3, or 1.0 mg/mouse, once/wk for 95 wk. A significant dose-dependent increase in tumor incidence occurred at the injection site (4/200 vehicle only, 5/100 low-dose, 8/100 mid-dose, 11/100 high-dose), but not at other sites. Survival was reduced in the high-dose group.

When administered by inhalation, ethylene oxide increased the incidences of tumors at several sites in male and female mice (NTP 326, 1987). Groups of male and female B6C3F1 mice, aged 8 wk, were exposed to 0, 50, or 100 ppm (0, 92, or 183 mg/m^3) ethylene oxide (>99% pure) for 6 h/day, 5 days/wk for up to 102 wk. The survival of treated groups was similar to that of the control groups. The combined incidences of lung tumors were increased in males (11/50 control, 19/50 low-dose, 26/50 high-dose) and females (2/49 control, 5/48 low-dose, 22/49 high-dose). Harderian gland papillary cystadenoma incidences were also significantly increased in males (1/43 control, 9/44 low-dose, 8/42 high-dose) and females (1/46 control, 6/46 low-dose, 8/47 high-dose). In females, the incidences of malignant lymphomas (9/49 control, 6/48 low-dose, 22/49 high-dose), uterine adenocarcinomas (0/49 control, 1/47 low-dose, 5/49 high-dose) and mammary gland carcinomas (1/49 control, 8/48 low-dose, 6/49 high-dose) were significantly increased.

No skin tumors were observed in mice treated by skin application (Van Duuren et al., 1965; cited by IARC, 1994). ICR/Ha Swiss female mice were painted with ethylene oxide (99.7% pure) in acetone (~100 mg of a 10% solution), three times/wk from age 8 wk until death (median survival time = 493 days).

4.1.2 Rats

Rats administered ethylene oxide by inhalation showed increased incidences of gliomas of the brain and mononuclear cell leukemia in both sexes, and males showed increased incidences of peritoneal mesotheliomas in the testicular region and subcutaneous fibromas (Snellings et al., 1984; Garman et al., 1986; both cited by IARC, 1994). Groups of male and female Fischer 344 rats, aged 8 wk, were exposed to ethylene oxide (>99.9% pure) at 10, 33, or 100 ppm (18, 59, or 180 mg/m^3) for 6 h/day, five days/wk for two yr. Survival was reduced in groups exposed to the mid- and high dose. Glioma incidences were greater in males (1/181 controls, 0/92 low-dose, 3/86 mid-dose, 6/87 high-dose) and females (0/187 controls, 1/94 low-dose, 2/90 mid-dose, 2/78 high dose). Mononuclear cell leukemia was also seen in males (5/48 control I, 8/49 control II 9/51 low-dose, 12/39 mid-dose, 9/30 high-dose) and females (5/60 control I, 6/56 control II, 11/54 low-dose, 14/48 mid-dose, 15/26 high-dose). In males, peritoneal mesothelioma (1/48 control I, 1/49 control II 2/51 low-dose, 4/39 mid-dose, 4/30 high-dose) and subcutaneous fibromas (1/48 control I, 2/49 control II, 9/51 low-dose, 1/39 mid-dose, 11/30 high-dose).

Mononuclear-cell leukemia, peritoneal mesothelioma, and glioma were also observed in another inhalation study with Fischer 344 rats (Lynch et al., 1984; cited by IARC, 1994). Male weanling rats were exposed to 0, 50, or 100 ppm (92 or 180 mg/m^3) ethylene oxide (99.7% pure),

7 h/day five days/wk for two yr. Mortality was greater in both treated groups compared to controls. The incidence of mononuclear-cell leukemia was significantly increased in the low-dose group (24/77 control, 38/79 low-dose), but excess mortality in the group exposed to 100 ppm prevented the evaluation of leukemia incidence at a high dose. Peritoneal mesothelioma incidence was significantly increased in the high-dose group (3/78 control, 9/79 low-dose, 21/79 high-dose). In addition, the incidence of glioma was significantly increased in rats exposed to the high dose (0/76 control, 2/77 low-dose, 5/79 high-dose).

When administered by gavage, ethylene oxide induced local tumors, mainly squamous-cell carcinomas of the forestomach, in female rats (Dunkelberg, 1982; cited by IARC, 1994). Groups of female Sprague-Dawley rats, aged ~100 days, were given ethylene oxide (99.7% pure) in vegetable oil at 7.5 or 30 mg/kg by gastric intubation twice/wk for 107 wk. Survival was lessened in rats given the high-dose, compared to control groups. Malignant tumors of the stomach were found in a total of 31/50 treated rats, while no tumors were identified in untreated or vehicle controls.

Based on the studies with mice and rats, IARC (1994) concluded that there is sufficient evidence for the carcinogenicity of ethylene oxide in experimental animals.

4.2 Studies Post-IARC (1994)

No post-IARC (1994) studies were identified.

5.0 GENOTOXICITY

Studies on the genotoxic effects of ethylene oxide published prior to 1994 have been reviewed by IARC (1994, pp. 122-136; see Appendix A). More recent studies are summarized below and in Table 5-1. Ethylene oxide has been reported to induce genotoxic damage in prokaryotic, lower eukaryotic, higher plant, and *in vitro* and *in vivo* mammalian systems, including exposed workers. The types of genetic damage induced by ethylene oxide in exposed animals and workers include biomarkers of exposure (e.g., hemoglobin adducts, DNA breaks, SCE) and biomarkers of effect (e.g., chomosomal aberrations, mutations). The former endpoints are indicative of the ability of ethylene oxide to interact with DNA in cells of exposed animals and humans, while the latter endpoints are indicative of the ability of ethylene oxide to induce heritable cellular DNA changes. In human studies, nucleated blood cells have been used most frequently to evaluate ethylene oxide-induced genotoxic damage. These cells are informative in regard to exposure, but not as a direct indicator of future adverse health outcomes. Such information can only be obtained from cells in the tissue(s) at risk for tumor formation. The fact that the level of damage in blood cells of ethylene oxide-exposed animals and humans declines and returns to control levels with increasing time between the cessation of exposure and sampling is consistent with the repair of ethylene oxide-induced DNA damage and/or the dilution of damaged cells due to cell turnover. However, the decline in genetic damage in nonproliferating blood cells does not preclude the potential for heritable tumor-initiating events from occurring in proliferating cells in other tissues during exposure to ethylene oxide.

5.1 Genotoxicity Studies Reviewed by IARC (1994)

In bacterial systems, ethylene oxide induced an increase in *his* gene mutations in *Salmonella typhimurium* strains TA100 and TA1535 (with and without S9 activation) and TA102 (with S9), but not in strains TA1537 and TA1538 (with and without S9) or TA98

(without S9). Similarly, an increase in forward mutations was observed in *Escherichia coli* strains KMBL 3835, WP2 uvr A, WP6 (pol A), and WU36-10-89, as well as *Bacillus subtilis* (strain not provided), all tested only in the absence of metabolic activation. It was listed as negative only for reverse mutations in *Streptomyces griseoflavus* tested in the absence of S9 activation.

In lower eukaryotes, ethylene oxide induced gene conversions and reverse mutations in *Saccharomyces cerevisiae*, forward mutations in *Schizosaccharo-myces pombe* and *Aspergillus nidulans*, and reverse mutations in *Neurospora crassa*. It was negative for genetic crossing over in *A. nidulans*. Somatic and sex-linked recessive mutations as well as heritable translocations were induced in *Drosophila melanogaster*.

In higher plants, ethylene oxide induced chlorophyll and waxy mutations and chomosome aberrations in *Hordeum* (barley) species, gene mutations in *Oryza sativa* (rice) and *Glycine max* (soy beans), and chromosome aberrations in *Trandescantia* species.

Calf thymus DNA exposed to ethylene oxide *in vitro* produces 7-(2-hydroxyethyl)guanine (7-HEG) as the main DNA adduct; smaller amounts of O6-(2-hydroxyethyl)guanine and N3-(2-hydroxyethyl)adenine were also identified. Using cultured mammalian cells, ethylene oxide induced an increase in SCE in human lymphocytes and fibroblasts, both tested in the absence of metabolic activation. Unscheduled DNA synthesis (UDS) was also induced in human lymphocytes without added S9. Gene mutations at the *hprt* locus were caused by ethylene oxide in Chinese hamster ovary (CHO) cells in the presence and absence of S9 activation, and in lung V79 cells tested in the absence of S9 activation. Similarly, tk gene mutations were induced in mouse lymphoma L5178Y cells without S9. Ethylene oxide induced chromosome aberrations in transformed human amniotic cells, both micronuclei and chromosome aberrations in Chinese hamster V79 cells, and cell transformation in mouse C3H10T1/2 and Syrian hamster embryo (SHE) SA7 cells.

IARC (1994) described numerous acute and subchronic inhalation studies conducted *in vivo* with ethylene oxide. DNA adducts (primarily 7-HEG) were found in liver and kidney of mice and the liver, brain, and lung of rats exposed to ethylene oxide. O6-(2-hydroxyethyl)guanine and N3-(2-hydroxyethyl)adenine were also identified in exposed rats. Ethylene oxide was mutagenic at the *hprt* locus in T-lymphocytes of B6C3F1 mice exposed by i.p. injection. Analysis of the *hprt* mutants indicated that ethylene oxide mutagenesis involves modification of guanine and adenine bases. Ethylene oxide induced DNA damage, as measured by alkaline elution, in mouse (strain not provided) spermatids and sperm (administered i.p.). It was found to bind covalently to mouse and rat DNA exposed via i.p. injection or inhalation. Ethylene oxide caused an increase in SCE in the lymphocytes of rats, rabbits, and cynomolgus monkeys, the bone marrow cells of mice and rats, and the spleens of rats. Dominant lethal mutations were induced in both mice and rats, and heritable translocations in mice using both inhalation and i.p. exposures. Chromosome aberrations were induced in the bone marrow of mice and rats exposed via inhalation, p.o. (rats only), or i.p. (mice only), the lymphocytes of cynomolgus monkeys, and the spermatocytes of mice. Similarly, ethylene oxide induced an increase in bone marrow micronuclei in mice and rats treated both i.v. and via inhalation. It was negative for specific locus mutations in mouse postspermatogonia and other stages, for aneuploidy in 10-day-old mouse fetuses, and for chromosome aberrations in rat lymphocytes.

Fourteen studies reported an increase in the frequency of SCE in the peripheral blood lymphocytes of exposed workers, while four studies reported equivocal or negative results. In

three separate papers, ethylene oxide was reported to be negative for DNA strand breaks, positive for DNA crosslinks, and weakly positive for unscheduled DNA synthesis in human lymphocytes. Differing results were obtained for micronuclei induction in several tissues of exposed workers. Three studies reported an increase in micronuclei in bone marrow, peripheral blood lymphocytes, and nasal cells, while four studies found no increase in lymphocytes, buccal cells, or nasal cells of exposed workers. Ethylene oxide induced an increase in chromosome aberrations in the blood lymphocytes of exposed workers in eight studies, a weak positive response in two studies, and a negative result in four.

5.2 Genotoxicity Studies Published Post-IARC (1994)

5.2.1 Prokaryotic Systems

An investigation of factors that may modify ethylene oxide mutagenicity in *S. typhimurium* was reported in a meeting abstract (Oesch and Hengstler, 1997). Factors investigated were human erythrocytes and theophylline, which were added to the preincubation mixture. Erythrocytes enhanced the mutagenic activity of ethylene oxide by two- to fourfold, while theophylline inhibited erythrocyte-induced enhancement. One experiment used erythrocytes from drinkers of black tea, and controls drank water. Erythrocytes from tea drinkers caused a significantly smaller increase in ethylene oxide mutagenicity compared to controls. The specific factor in erythrocytes that stimulated ethylene oxide mutagenicity was not identified.

5.2.2 Lower Eukaryotic Systems

de Serres and Brockman (1995) reported a reproducible increase in specific locus mutations at the adenine-3 region of *N. crassa*. The two-component heterokaryon H-12 was exposed in solution to five ethylene oxide concentrations ranging from 0.1% to 0.35% for 3 h. Ethylene oxide induced a high percentage of point mutations (97%) and a low percentage of multilocus deletions (3%).

Vogel and Nivard (1997) found an increase in the level of sex-linked-recessive-lethal (SLRL) mutations in repair-defective *D. melanogaster*. Wild-type males were exposed via inhalation to ethylene oxide at doses ranging from 31.3 to 500 ppm for 24 h followed by mating to Basc females competent (NER+) or deficient (NER-) in nucleotide excision repair. No increase in SLRL mutations were seen in post meiotic germ cells when matings were to NER+ females; however, a 17-fold increase in SLRL mutations was obtained with NER- females, indicating the ethylene oxide 2-hydroxyethyl adduct is repaired in the wild-type strains.

5.2.3 Mammalian Systems *In Vitro*

Because *in vitro* reaction of ethylene oxide gave rise to the 3-(2-hydroxyethyl)deoxyuridine (3-HE-dU) adduct, Bhanot et al. (1994) constructed a 55-nucleotide DNA template containing this adduct, and analyzed mutagenic bypass (i.e., DNA products synthesized from this template). The lesion could be bypassed only by DNA polymerases Pol I (Klenow fragment) or T7 Pol lacking a 3′-5′ exonuclease proofreading activity. Both dA and dT were incorporated opposite the dC-derived 3-HE-dU adduct, indicating that G:C to A:T or G:C to T:A mutagenesis results from the 3-HE-dU adduct.

A sensitive method of measuring ethylene oxide-DNA adducts using high performance liquid chromatography/mass spectrometry (HPLC/MS) was developed by Leclercq et al. (1997).

Calf thymus DNA or human blood was incubated with ethylene oxide at doses ranging from 0.1 to 100 mM (DNA) or 2.5 to 10 mM (blood) for 3 h at 37 °C. A clear dose-dependent increase in the level of 7HEG adducts was observed in both systems with lower concentrations observed in blood.

Incubation of human mononuclear cells with ethylene oxide (0.5-10 mM) resulted in a dose-dependent increase in DNA single strand breaks (Hengstler et al., 1994). In another study, Hengstler et al. (1997) compared ethylene oxide-induced DNA damage in human blood *in vitro*, using both the standard (isolated leukocytes) and a faster, more sensitive direct (whole blood) method of alkaline elution. Whole blood was exposed to 0.5 and 2.0 mM gaseous ethylene oxide (injected into sealed culture tubes) for one hour followed by either direct processing or isolation of leukocytes. A statistically significant increase in the direct method over the standard method was observed at the top dose tested.

Human diploid fibroblasts were used in an *in vitro* study of specific genetic changes caused by ethylene oxide (Lambert et al., 1994). Various *hprt* gene mutations, including chromosomal, gross structural alterations, and point mutations were examined after exposure of fibroblasts to ethylene oxide. The mutant frequency increased linearly with 2.5-10 mM ethylene oxide. The most significant mutation was large genomic deletions; 48% of mutant clones showed these deletions compared to 10% in the background. Point mutations were also induced by ethylene oxide.

DNA damage, including single and double strand breaks, was induced in human fibroblasts exposed to ethylene oxide for one hour in suspension or in monolayer (Nygren et al., 1994). The induction rates of DNA breaks depended on the analytical method and treatment conditions.

5.2.4 Mammalian Systems *In Vivo*

The frequency of *hprt* gene mutations was significantly increased in both thymus and spleen T-lymphocytes of ethylene oxide-exposed transgenic mice (Walker et al., 1997). Male B6C3F$_1$ Big Blue® lacI transgenic mice were exposed to 50, 100, or 200 ppm (6 h/day, 5 days/week) for four weeks and necropsied two weeks post-exposure for thymus and eight weeks for spleen.

Using the same mice, Sisk et al. (1997) reported an increase in lacI mutants in the lung but not in the spleen, bone marrow, or germ cells. The mutant frequency in the lung was significantly increased at 8 weeks post-exposure to 200 ppm ethylene oxide. Only DNA of spleens from the 200-ppm-exposed mice was sequenced. Single base substitution accounted for 85% of the mutants (76% transitions/24% transversions). The authors hypothesized that since ethylene oxide is known to induce deletion mutations in other systems, the lack of a response in lacI was likely due to the inability to recover large deletion mutants in the lambda-based shuttle vector.

Mutations were induced at the *hprt* locus of thymus and spleen T-cells in rats and mice exposed to ethylene oxide by inhalation (Walker et al., 1997 abstr.). Mutations were a combination of base substitutions, frameshifts, and small deletions. Transitions and transversions were observed at both GC and AT base pairs.

Vergnes and Pritts (1994) found a significant increase in micronuclei in bone marrow cells of rats and mice exposed to ethylene oxide for four weeks.

5.2.5 Human Studies

Workers in a Brazilian industry that used ethylene oxide as an intermediate showed signs of DNA damage (Ribeiro et al., 1994). Exposure to ethylene oxide was indicated by air analyses and measurement of increased levels of ethylene oxide-hemoglobin adducts (HOEtVal). Ambient air measurements made during a three month sampling period indicated that workers were exposed to 2-5 ppm TWA for an eight hour working day. Blood samples were taken from 75 workers and 22 controls (no exposure to ethylene oxide) matched for sex, age, and smoking habits. Exposed workers exhibited a significant increase in chromosomal aberrations and micronuclei (scored in binucleated lymphocytes), but no increase of micronuclei in buccal exfoliated cells.

In humans, a significant increase in DNA damage, as measured by alkaline elution, was found in peripheral mononuclear blood cells of ethylene oxide exposed sterilization workers in Germany (Oesch et al., 1995). A dose-dependent increase in DNA damage was detected in both smokers and nonsmokers exposed to <0.1-2.0 mg/m^3 (0.055-1.1 ppm). The majority of nonsmokers showed a more sensitive response than smokers at doses ranging from 0.5-2.0 mg/m^3 (0.28-1.1 ppm).

A study of ethylene oxide exposed disinfection workers at 15 German hospitals [25 workers (15 female, 10 male), ages 31-57 years] did not show a significantly elevated frequency of SCE in lymphocytes over historical controls (Popp et al., 1994). However, a significant decrease in DNA alkaline elution rates (indicative of DNA-protein crosslinks) was observed in exposed workers. Peak levels of ethylene oxide in air were 417 ppm during the first eight minutes after opening the sterilization units.

Tates et al. (1995) found no increase in lymphocyte *hprt* mutations, SCEs, or micronuclei in ethylene oxide-exposed workers at a chemical manufacturing plant in the Netherlands. Lymphocytes from 28 workers (7 acutely exposed to ethylene oxide, 7 workers employed for <5 years, 7 workers employed for >15 years, and 7 control workers) were analyzed. Blood samples from the acutely exposed workers were collected and analyzed 89-180 days post exposure. For the acutely exposed group, exposures were estimated to be 52-785 mg/m^3 [29-436 ppm], based on hemoglobin adduct levels. Average exposures in the second and third groups were estimated to range from <0.01-0.06 mg/m^3 (<0.1 ppm). No statistically significant difference between any of the groups was detected at any endpoint. The authors cite repair of preclastogenic and premutagenic lesions as well as the small group sizes as possible explanations for the lack of any observable effect.

Schulte et al. (1995) reported an increase in the frequency of SCE and micronuclei in blood lymphocytes from ethylene oxide-exposed female workers. Sixty-eight women (mean age 43.6 years for U.S. women and 29.4 for Mexican women) from one Mexican and nine 9 U.S hospitals were sampled and divided into 0, <32, and >32 ppm-h categories based on a 4-month cumulative exposure estimate from 2-4 day exposure monitoring data. A significant increase in SCE (in both dose groups) and micronuclei (at the high dose group) was observed among the 46 U.S. women, but none at either endpoint in the 22 Mexican workers. The increase in SCEs corresponded with an increase in hydroxyethyl hemoglobin adducts. The authors speculated that the lack of response in the Mexican subjects could be due to various factors including environmental effects, a one-time sampling, the smaller sample size, and the longer time from sampling until processing (up to 20 h).

Fuchs et al. (1994) analyzed single strand breaks in the DNA of peripheral mononuclear blood cells of 97 male and female hospital workers exposed to ethylene oxide. Ethylene oxide was detected in the air of working areas, up to a maximum 4-h TWA concentration of 16 mg/m^3 (9 ppm) or 1-h TWA of 49.5 mg/m^3 (27.5 ppm). DNA single stand breaks were significantly increased in nonsmoking workers.

Occupational exposure of nurses to ethylene oxide in Hungary was associated with increased frequencies of SCE and chromosome aberrations in peripheral blood lymphocytes (Major et al., 1996). Ethylene oxide concentrations in ambient air samples ranged from 5-100 mg/m^3 (2.8-55.6 ppm).

No human studies of ethylene oxide DNA adducts were located.

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post -IARC (1994)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response na/e Activation	Comments	Reference
Prokaryotic Systems							
Salmonella typhimurium TA 1535	reverse mutation	no S9 used	NG	test of factors that may modify EO mutagenicity; human erythrocytes and theophylline added to preincubation mixture; one experiment used erythrocytes from drinkers of black tea while controls drank water	Erythrocytes enhanced the mutagenicity of ethylene oxide by two to fourfold; erythrocytes from tea drinkers caused a significantly smaller increase in EO mutagenicity compared to controls; theophylline inhibited the increase in mutagenicity caused by erythrocytes	Factor in erythrocytes that stimulates EO mutagenicity not identified	Oesch and Hengstler (1997 abstr.)
Lower Eukaryotic Systems							
Neurospora crassa heterokaryon H-12	specific locus mutations in the *adenine-3* region	na	NG	0.1, 0.2, 0.25, 0.3, & 0.35% for 3 h	positive	A high percentage of gene/point mutations (97%) and a low percentage of multilocus deletions (3%) were induced. The authors classified ethylene oxide as a moderate mutagen.	de Serres and Brockman (1995)
Drosophila melanogaster nucleotide excision repair deficient strain	sex-linked recessive lethal (SLRL) mutations	na	NG	31.3, 62.5, 125, 250, & 500 ppm via inhalation for 24 h	positive	No increase in SLRL mutations in post meiotic germ cells when matings were to NER^+ females, however, a 17-fold increase in SLRL mutations was obtained with NER^- females indicating the EO 2-hydroxyethyl adduct is repaired in the wild-type strains.	Vogel and Nivard (1997)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response +/- Activation	Comments	Reference
Mammalian Systems *in vitro*							
55-nucleotide DNA template	DNA synthesis across DNA adduct	na	NG	na	positive	A 55-nucleotide DNA template containing the 3-HE-dU adduct at a single site was made and DNA products synthesized from this template were analyzed. The lesion could be bypassed only by DNA polymerases Pol I (Klenow fragment) or T7 Pol lacking a 3′-5′ exonuclease proofreading activity. Both dA and dT were incorporated opposite the dC derived 3-HE-dU adduct, indicating G:C to A:T or G:C to T:A mutagenesis.	Bhanot et al. (1994)
calf thymus DNA and human blood lymphocytes	DNA adducts (detected via HPLC/MS)	na	NG	0.1, 1.0, 10, & 100 mM EO (in calf thymus DNA) or 1.0, 2.0, 5.0, & 10 mM EO (in blood) for 3 h at 37°C	positive in both systems	A clear dose-dependent increase in the level of 7-(2-hydroxyethyl)guanine (7HEG) adducts was observed in both systems with lower amounts observed in blood. Detection levels were on the order of approximately 3 modified bases per 10^8 nucleotides.	LeClercq et al. (1997)
human whole blood and isolated leukocytes	DNA single strand breaks (SSBs) using standard and direct alkaline elution methods	na	99.8%	0.5 and 2.0 mM gaseous ethylene oxide injected into sealed culture tubes for 1 h	positive with both methods	A statistically significant increase in sensitivity with the direct method (using whole blood directly) over the standard method (using isolated leukocytes) was observed at the top dose tested.	Hengstler et al. (1997)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Known Purity	Doses Used	Endpoint Response and Activation	Comments	Reference
normal human diploid fibroblasts	hypoxanthine phosphoribosyl transferase (*hprt*) gene mutations (chromosomal, gross structural alterations, point mutations)	na	NG	EO exposure levels and duration NG; mutant clones selected after expression time of 8-10 days	positive; 48% showed large genomic deletions of the whole or part of the gene, compared to 10% in background; base substitutions, small deletions and insertions, and splice mutations were also identified, but the frequency was less than background		Lambert et al. (1994)
normal human diploid fibroblasts	DNA SSBs and double strand breaks (DSBs)	na	NG	2.5-30 mM EO; cells exposed for 1 h in suspension or in monolayer	positive	Induction rates of SSBs and DSBs depended on analytical method and treatment conditions (cells in monolayer or suspension)	Nygren et al. (1994)
Mammalian Systems *In Vivo*							
male Fischer 344/CR/BR rats	SCE in spleen and bone marrow	na	99.7%	100 ppm EO for 6 h/day, 300 ppm for 2 h/day, or 600 ppm for 1 h/day for up to 9 months	positive in both tissues	Following 3 months, all treated groups had a significantly higher level of SCE in spleen, but bone marrow SCE were only higher in the low-dose group. After 6 and 9 months, all dose groups were higher than controls for both spleen and bone marrow with the low- and high-dose groups significantly higher then the medium dose group. The authors theorized that the spleen was more sensitive due to cell-cycle kinetics and tissue function.	Ong et al (1993)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Purity and Purity	Doses Used	Endpoint Response with Activation	Comments	Reference
male B6C3F1 Big Blue® *lacI* transgenic mice	*hprt* mutations in thymus and spleen	na	99.99%	200 ppm (6 h/day, 5days/wk) for 4 wk - necropsied 2 h and 2 and 8 wk post exposure for time course; for dose response 50, 100, and 200 ppm for 4 wk; necropsied 2 wk post exposure for thymus, 8 wk for spleen.	positive in both tissues	A dose-dependent increase in the frequency of *hprt* gene mutations was observed in the T-lymphocytes of both thymus (at 2 wk) and spleen (at 8 wk).	Walker et al. (1997)
male B6C3F1 Big Blue® *lacI* transgenic mice	*lacI* mutations in lung, spleen, bone marrow, and germ cells	na	99.99%	50, 100, and 200 ppm for 4 wk - necropsied 2 wk and 8 wk post exposure.	positive for lung only	The mutant frequency in the lung was significantly increased at 8 wk post exposure. Only spleens from the 200 ppm exposed mice were sequenced. Single base substitution accounted for 85% of the mutants (76% transitions/24% transversions). The authors hypothesized that since ethylene oxide is known to induce deletion mutations in other systems, the lack of response here was likely due to the inability to recover deletion mutants in the lambda-based shuttle vector.	Sisk et al. (1997)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response with Activation	Comments	Reference
1) male F344 rats 2) male B6C3F1 mice 3) male B6C3F1 Big Blue® *lacI* transgenic mice	mutations in *hprt* exon 3 of T-cells	na	NG	rats and nontransgenic mice exposed to 0 and 200 ppm EO; transgenic mice exposed to 0, 50, 100, and 200 ppm EO for 4 wk (6 h/day, 5days/wk)	*Hprt* mutant frequencies significantly increased in thymus and spleen of transgenic mice compared to controls, and in nontransgenic rats and mice necropsied 5 and 8 wk post-exposure, respectively. Mutations were a combination of base substitutions, frameshifts, and small deletions.	The authors concluded that this and other data indicate similar mutagenic mechanisms of EO in rats and mice.	Walker et al. (1997 abstr.)
male Fischer 344 rats and male B6C3F1 mice	micronuclei in bone marrow	na	>99.9%	200 ppm EO (target) inhalation exposure 4 wk	positive		Vergnes and Pritts (1994)
Human Studies							
75 workers in a Brazilian industry that used EO as an intermediate; 22 controls from same plant	1) chromosomal aberrations 2) micronuclei in lymphocytes 3) micronuclei in buccal exfoliated cells 4) EO-hemoglobin adducts (HOEtVal)	na	NG	Analyses of air sample pumps showed workers exposed to EO levels of 2-5 ppm TWA for an 8 h working day during 3 mo sampling	significant increase in chromosomal aberrations, micronuclei in binucleated lymphocytes, and hemoglobin adducts in exposed populations compared to controls		Ribeiro et al. (1994)
German sterilization workers (number not provided in source used)	DNA damage (alkaline elution) in mononuclear blood cells	na	NG	<0.1, 0.1-0.5, 0.5-2.0 mg/m^3 in smokers and nonsmokers	positive	A dose-dependent increase in DNA damage was detected in both smokers and nonsmokers. The majority of nonsmokers showed a more sensitive response than smokers at doses ranging from 0.5-2.0 mg/m^3	Oesch et al. (1995)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Dose Used	Endpoint Response S9/+ Activation	Comments	Reference
25 disinfection workers (15 female , 10 male, ages 31-57 years) at 15 German hospitals	SCEs and DNA-protein cross-links (alkaline elution) in lymphocytes	na	NG	peak levels up to 417 ppm immediately after opening sterilization units	negative (SCE), positive (cross-links)	Exposed workers did not show a significantly elevated frequency of lymphocyte SCE over historical controls; however, a significant decrease in their DNA alkaline elution rates (indicative of DNA-protein cross-links) was observed.	Popp et al. (1994)
28 workers at a Netherlands chemical manufacturing plant	*hprt* gene mutations, SCE, and micronuclei induction	na	NG	7 workers acutely exposed to 52-785 mg/m^3 (calculated from hemoglobin adducts), 7 workers employed for <5 years, 7 workers employed for >15 years, and 7 control workers	negative	No statistically significant difference between any of the groups was detected at any endpoint. The tests for the acutely exposed workers were performed 89-180 days post exposure. The authors cite lesion repair as well as the small group sizes as possible explanations for the lack of any observable effect.	Tates et al. (1995)
68 female workers in 9 U.S. hospitals and 1 Mexican hospital	SCE and micronuclei in blood lymphocytes	na	NG	0, >0-32, and >32 ppm-h groups based on a 4-month cumulative exposure	positive for both endpoints (U.S.), negative (Mexico)	A significant increase in SCE (in both dose groups) and micronuclei (at the high-dose group) was observed among the 46 U.S. women but none in the 22 Mexican workers. The authors speculated that the lack of response in the Mexican subjects may be due to various factors including environmental effects, a one-time sampling, the smaller sample size, and the longer time until processing (up to 20 h).	Schulte et al. (1995)
hospital worker cohort follow-up	somatic cell mutant frequency	na	na	previously reported (Tomkins et al., 1993)	negative		Tomkins et al. (1996 abstr.)

Table 5-1. Summary of Ethylene Oxide (Eo) Genotoxicity Studies Published Post-IARC (1994) (Continued)

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response -/+ Activation	Comments	Reference
97 male and female workers in hospitals and plants that used EO to sterilize medical equipment	DNA SSBs in peripheral mononuclear blood cells	na	na	EO detected in air of working areas with a mean of 1.47 $\pm$ 0.52 mg/m^3; max. concn. 16.5 mg/m^3 as 4-h TWA	Significant increase in SSB among nonsmoking workers exposed to 0.1-2 mg/m^3 vs. nonsmoking workers exposed to air with EO below the detection limit (0.1 mg/m^3)	Nonsmoking workers were classified into two subpopulations based on sensitivity to EO.	Fuchs et al. (1994)
exposed hospital nurses in Hungary: Budapest (n=9); Eger (n=27) hospital controls in Hungary: Budapest (n=14); Eger (n=10)	1) SCE 2) chromosome aberrations (CA) in peripheral blood lymphocytes	na	na	ethylene oxide in ambient air samples ranged from 5-100 mg/m^3 (2.8-55.6 ppm)	Significant increase in SCE among Eger nurses Significant increase in CA frequencies in Eger and Budapest nurses	Leukocyte count and mean age showed significant effect on CA in Eger exposed and on SCE in historical controls	Major et al. (1996)

Abbreviations: na = not applicable; NG = not given

6.0 OTHER RELEVANT DATA

6.1 Absorption, Distribution, Metabolism, and Excretion in Humans

Gaseous ethylene oxide in fabric was found to be absorbed by human skin *in vitro*, in a study designed to model skin/fabric exposure to any potentially hazardous gas (Wester et al., 1997). [1,2-^{14}C]Ethylene oxide was introduced into a sealed glass container with fabric discs. After removal, the discs were placed on human skin mounted in glass diffusion cells for measurement of absorption. When the fabric/skin surface was open to surrounding air, the percutaneous absorption was 1.3% of the dose. When the surface was occluded with latex glove material, the percutaneous absorption was 46.0% of the dose. Absorption of intact ethylene oxide occurred within the first 0-4 hours of the assay. Other studies show aqueous ethylene oxide solutions can penetrate human skin *in vivo* (IARC, 1994).

Once inhaled, ethylene oxide is readily absorbed by the lungs. Approximately 20 to 25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound and 75-80% is absorbed by the lungs and metabolized (Brugnone et al., 1986; cited by IARC, 1994).

Ethylene oxide is a metabolite of ethene (ethylene), a substance produced endogenously. A study of workers exposed to ethene found that 0.5% of absorbed ethene was metabolized to ethylene oxide (Granath et al., 1996). In a study of sterilization personnel exposed to ethylene oxide (0.3 to 52 ppm [0.55 to 95.2 mg/m^3] [8-hour TWA readings]), the mean concentration of ethylene glycol in the blood of exposed workers (90 mg/L) after three days of exposure was twice that of non-exposed workers (45 mg/L) (Wolfs et al., 1983; cited by IARC, 1994).

Ethylene oxide is eliminated by hydrolysis and by conjugation with glutathione, and excreted in the urine mainly as thioethers (IARC, 1994). When the concentration of thioethers excreted in urine was analyzed in workers at the end of sterilization processes, the concentrations were twice as high in nonsmoking personnel exposed to peak concentrations of 1 to 200 ppm ethylene oxide (1.83-366 mg/m^3) as compared to unexposed workers (Burgaz et al., 1992; cited by IARC, 1994).

The elimination half-life for ethylene oxide in humans has been calculated variously as 14, 39, 42, and 198 minutes (Osterman-Golkar and Bergmark, 1988; Filser et al., 1992; Beliles and Parker, 1987; all cited by IARC, 1994). Using a human data set, Osterman-Golkar and Bergmark (1988; cited by IARC, 1994) calculated the elimination half-life to be 14 minutes and Filser et al. (1992; cited by IARC, 1994) calculated it to be 42 minutes. Using rat data, elimination half-life was calculated for humans on the basis of allometric scaling with body surface factors. Beliles and Parker (1987) and Filser et al. (1992) (both cited by IARC, 1994) calculated the half-life of ethylene oxide in humans to be 3.3 hours and 39 minutes, respectively.

Conjugators (75% of the population), as defined by a standardized conjugation of methyl bromide and glutathione, eliminate ethylene oxide from blood three to six times faster than those who are not conjugators (25% of the population) (Hallier et al., 1993; cited by IARC, 1994).

6.2 Absorption, Distribution, Metabolism, and Excretion in Experimental Systems

Using male Sprague-Dawley rats, Filser and Bolt (1984; cited by IARC, 1994) found that ethylene oxide is absorbed rapidly by the lungs after inhalation. About 50% of the amount inhaled was exhaled without becoming systemically available. Maximal accumulation of ethylene oxide in the body of Sprague-Dawley rats was examined as the thermodynamic partition coefficient whole body:air (Filser et al., 1993; cited by IARC, 1994). Based on the metabolic

elimination rate of ethylene oxide in rats, the whole body:air concentration ratio was estimated to be 1.88, a value that is similar to the coefficient for venous blood:environmental air in exposed workers (Filser, 1992; cited by IARC, 1994). In male Fischer 344 rats, tissue:air partition coefficients are similar for most organs, indicating an almost uniform distribution of ethylene oxide within their bodies (Krishnan et al., 1992; cited by IARC, 1994).

Blood ethylene oxide concentrations in male B6C3F$_1$ mice after a single 4-hr nose-only inhalation exposure increased linearly with concentrations up to 200 ppm but increased more rapidly (sublinear dosimetry) at exposure concentrations greater than 200 ppm (Brown et al., 1998). Tissue measurements of GSH indicated that the deviation from linear dosimetry was due to GSH depletion.

In Sprague-Dawley rats (Filser and Bolt, 1984; cited by IARC, 1994) and Fischer 344 rats (Krishnan et al., 1992; cited by IARC, 1994), elimination of ethylene oxide was described by first-order kinetics. These studies indicated that, at steady state, about 95% of the systemic ethylene oxide was eliminated by metabolism while 5% was exhaled.

To determine metabolites of ethylene oxide, male Sprague-Dawley rats were administered an i.p. injection of ethylene oxide labeled with ^{14}C. The percentages of radioactivity excreted in the urine were 9% *S*-(2-hydroxyethyl)cysteine and 33% *N*-acetyl-*S*-(2-hydroxyethyl)cysteine. In addition, 1.5% of the dose was exhaled as $^{14}CO_2$ and 1% was exhaled as unchanged ethylene oxide (Jones and Wells, 1981; cited by IARC, 1994).

Ethylene glycol, 2-hydroxymercapturic acid, 2-methylthioethanol, and 2-mercaptoethanol were determined as metabolites in the urine of male Wistar rats (Koga et al., 1987; cited by IARC, 1994). Significant levels of thiodiacetic acid were detected in the urine of Sprague-Dawley rats and NMRI mice after inhalation exposure to ethylene oxide, compared to levels in urine collected prior to exposure (Scheick et al., 1997).

Marked species differences were noted in the pattern of excretion of ethylene oxide metabolites in mice, rats, and rabbits. Metabolites resulting from the conjugation of ethylene oxide with glutathione were found in the urine of male Swiss CD-1 mice and male Sprague-Dawley rats, but not in the urine of rabbits. *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine was excreted in mice and rat urine. *S*-(2-Hydroxyethyl)cysteine and *S*-(carboxymethyl)cysteine were found only in mouse urine. Ethylene glycol (reaction product of the hydrolytic pathway of ethylene oxide) was found in the urine of all three species (Tardif et al., 1987; cited by IARC, 1994).

6.3 Glutathione Depletion

After 4-hour exposure to ethylene oxide at concentrations ranging from 100-900 ppm (mice) and 100-1200 ppm (rats), concentration-related decreases in glutathione levels were found in the kidney, heart, lung, brain, stomach, spleen, testis, and liver of male Swiss-Webster mice and male Fischer 344 rats (McKelvey and Zemaitis, 1986; cited by IARC, 1994). Additionally, there were decreases in the glutathione levels in the blood of mice, but not of rats, and in bone marrow examined in rats only. The reduction in glutathione levels in both species was more marked in the liver, lung, and stomach than in other organs. A study with male B6C3F1 mice also demonstrated tissue glutathione depletion that was dependent on ethylene oxide exposure concentration (Brown et al., 1998).

6.4 Binding to DNA and Hemoglobin

Studies analyzing adduct formation caused by ethylene oxide were reviewed and summarized by IARC (1994). Ethylene oxide forms adducts with proteins in both humans and experimental animals and with DNA in experimental animals.

The formation of a DNA adduct (7-hydroxyethylguanine; 7-HEG) was shown in rats exposed to 3 and 10 ppm ethylene oxide (Swenberg et al., 1995). Other DNA adducts of ethylene oxide found in tissues from exposed rats are O6-(2-hydroxyethyl) guanine (in brain, kidney, lung, and spleen) and N3-(2-hydroxyethyl) adenine (in spleen) (IARC, 1994).

Ethylene oxide also forms hemoglobin adducts, as do other alkylating agents (Farmer et al., 1993). Blood levels of the hemoglobin adduct *N*-(2-hydroxyethyl)valine (HOEtVal) were higher in an urban population than in a rural control population (Cordero et al., 1995).

When hemoglobin adducts were used for biomonitoring of workers exposed to ethylene oxide, a significant correlation was found between cumulative exposure over four months and levels of *N*-terminal hydroxyethylvaline in hemoglobin of exposed workers (Schulte et al., 1995). Hydroxyethylvaline was formed at an increment of 12-16 pmol/g hemoglobin per ppm-h ethylene oxide (Ehrenberg and Törnqvist, 1995).

Notably, hydroxyethyl adducts of DNA and hemoglobin (7-HEG) were found in humans and animals even in the absence of known exposure to ethylene oxide (Swenberg et al., 1995; La and Swenberg, 1996). Most of the background hemoglobin adducts of ethylene oxide are believed to arise from the endogenous metabolism of ethene (Törnqvist, 1996).

An analysis of experimentally determined data sets on hemoglobin adducts and DNA adducts of ethylene oxide in lymphocytes from humans and animals indicated that normal background (endogenous) levels are similar across species. Background DNA adduct levels also appeared to be consistent among tissues (Bolt, 1996).

6.5 Structure-Activity Relationships (SAR)

6.5.1 1,2-Propylene oxide

Propylene oxide is a structural analog of ethylene oxide that also displays alkylating activity (DFG, 1993). It was mutagenic and clastogenic in several *in vitro* assays, but only showed mutagenic activity *in vivo* after injection of high doses. Recessive lethal mutations were observed in *D. melanogaster* (Hardin et al., 1983; cited by DFG, 1993), and positive results were reported in Ames assays (Pfeiffer and Dunkelberg, 1980; cited by DFG, 1993). Human lymphocytes treated *in vitro* showed chromosome damage (Bootman et al., 1979; cited by DFG, 1993), as did cultured rat hepatocytes (Dean and Hodson-Walker, 1979; cited by DFG, 1993). Mice showed no effects in a dominant lethal test after oral administration of moderate and high doses (50 mg/kg; 250 mg/kg), or in a micronucleus assay following oral administration of twice 500 mg/kg (Bootman et al., 1979; cited by DFG, 1993). A similar dose (300 mg/kg) administered to mice by i.p. injection produced positive results in a micronucleus test, but lower doses (75 mg/kg; 150 mg/kg) gave negative results (Bootman et al., 1979; cited by DFG, 1993).

Propylene oxide also exhibited carcinogenic activity in experimental animals. Forestomach carcinoma developed in female Sprague-Dawley rats given propylene oxide by gavage for almost three years; at study termination, the tumor incidence was 40% and 4% in the treated and control groups respectively (Dunkelberg, 1982; cited by DFG, 1993). B6C3F_1 mice chronically exposed to 400 ppm propylene oxide by inhalation (6 h/day, 5 days/wk for 103

weeks) had a significant increase in hemangioma and hemangiosarcoma of the nasal cavity (NTP, 1983; cited by DFG, 1993).

6.5.2 1,3-Butadiene

1,3-Butadiene is metabolized to butadiene monoepoxide or monoxide (BMO), also known as epoxybutene, with human isoforms 2E1 and 2A6 exhibiting the highest oxidation rates of all active cytochrome P450 isoforms (Elfarra et al., 1996). The metabolism of butadiene to BMO and oxidation of BMO to diepoxybutane (BDE) was demonstrated in human liver microsomes (Csanády et al., 1996; Seaton et al., 1995).

1,3-Butadiene, its epoxide metabolites, and ethylene oxide have similar mutational spectra at the *hprt* locus in mouse lymphocytes (Walker and Skopek, 1993; Cochrane and Skopek 1994). All these compounds form N7-alkylguanine adducts and are associated with a high percentage of frameshift mutations. Occupational exposure to 1,3-butadiene has been consistently associated with excess mortality from lymphatic and hematopoietic cancers. 1,3-Butadiene is carcinogenic at multiple organ sites in rats and mice.

6.5.3 Styrene oxide

In vitro studies showed binding of styrene oxide to all nucleic acid bases and nucleotides except uracil (Hemminki et al., 1980; cited by Segerback, 1994). However, the primary adduct was from alkylation at N7 of guanine (Vodicka and Hemminki, 1988; cited by Segerback, 1994). N7-Alkylguanine adducts were detected in mice after treatment with radiolabeled styrene oxide (Byfalt Nordqvist et al., 1985; cited by Segerback, 1994).

Styrene oxide also produced tumors in experimental animals. The incidence of squamous-cell carcinoma of the forestomach was significantly increased in B6C3F_1 mice and Fischer 344/N rats chronically (> 100 wk) administered styrene-7,8-oxide in corn oil by gastric intubation (Lijinsky, 1986; cited by IARC, 1994). A shorter term (52-wk) administration of styrene-7,8- oxide in olive oil by gastric intubation also resulted in a significant increase in squamous-cell carcinoma of the forestomach in Sprague-Dawley rats (Maltoni et al., 1979; Conti et al., 1988; both cited by IARC, 1994).

6.6 IARC (1994) Evaluation

In making an overall evaluation of the carcinogenicity of ethylene oxide, the Working Group took into consideration that ethylene oxide is a directly acting alkylating agent that induces a sensitive, persistent dose-related increase in the frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes and micronuclei in bone marrow cells of exposed workers; has been associated with malignancies of the lymphatic and hematopoietic system in both humans and experimental animals; induces a dose-related increase in the frequency of hemoglobin adducts in exposed humans and dose-related increases in the numbers of adducts in both DNA and hemoglobin in exposed rodents; induces gene mutations and heritable translocations in germ cells of exposed rodents; and is a powerful mutagen and clastogen at all phylogenetic levels. Based on the overall evaluation, IARC (1994) concluded that ethylene oxide is carcinogenic to humans (Group 1).

7.0 MECHANISMS OF CARCINOGENESIS

Ethylene oxide is an electrophile and a direct-acting mutagen in plants, microorganisms, insects, and mammals (IARC, 1994). In their overall evaluation, IARC (1994) considered genetic changes in exposed workers to be important supporting evidence of the carcinogenicity of ethylene oxide in humans. Ethylene oxide induces a dose-related increase in the frequency of chromosomal aberrations and SCE in peripheral lymphocytes and micronuclei in bone marrow cells of exposed humans. In addition, ethylene oxide is associated with a dose-related increase in the frequency of hemoglobin adducts in exposed humans and dose-related increases in numbers of adducts in both DNA and hemoglobin of exposed rodents. More recently published studies on the genetic activity of ethylene oxide are consistent with the IARC (1994) report. The proposed cancer mechanisms for 1,3-butadiene, invoking the alkylating potential of the epoxide metabolites, can be compared to ethylene oxide. This comparison is valuable because epidemiology studies have consistently found an association between occupational exposure to 1,3-butadiene and excess mortality from lymphatic and hematopoietic cancers, and because there are several common sites of tumor induction by ethylene oxide and 1,3-butadiene in experimental animals.

Ethylene oxide is eliminated by hydrolysis and by conjugation with glutathione, but the untransformed epoxide is reactive with DNA and proteins. 1,3-Butadiene, a chemical considered to be a probable human carcinogen (IARC, 1992) or a known human carcinogen (NTP, 1999), is metabolized by liver microsomal cytochrome P-450 enzymes to monoepoxide and diepoxide intermediates. There are striking similarities between the genotoxic activity of ethylene oxide and 1,3-butadiene. The most likely mechanism for the carcinogenicity of 1,3-butadiene is related to DNA damage by one or more reactive metabolites (Melnick and Kohn, 1995). Exposure to 1,3-butadiene resulted in increased levels of DNA damage and mutations in experimentally exposed animals and occupationally exposed workers.

In mice, exposure by inhalation to 1,3-butadiene resulted in increased levels of DNA-DNA and DNA-protein cross-links in liver and lung, N7-alkylguanine adducts in liver DNA, SCE and chromosomal aberrations in bone marrow cells, micronucleated erythrocytes detected in peripheral blood, *hprt* mutations in lymphocytes, dominant lethal mutations, and sperm abnormalities. At the *hprt* locus in mouse lymphocytes, the mutational spectra of 1,3-butadiene, its metabolite epoxybutene, and ethylene oxide are similar, suggesting a common mechanism of mutagenesis (Walker and Skopek, 1993; Cochran and Skopek, 1994).

In workers, occupational exposure to 1,3-butadiene induced a significant increase in hemoglobin adducts, and (in some but not all studies) sister chromatid exchange, *hprt* mutations, and chromosomal aberrations in lymphocytes (Au et al., 1996; IARC, 1992; Sorsa et al., 1996; Ward et al., 1996).

The mutagenic and carcinogenic effects of ethylene oxide appear to mimic those of the epoxide intermediates of 1,3-butadiene metabolism. The carcinogenic effects of these compounds most likely result from their mutagenic and clastogenic effects that have been observed at all phylogenetic levels.

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APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 60 (Some Industrial Chemicals) Ethylene Oxide pp. 73-159, 1994

ETHYLENE OXIDE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976), June 1984 (IARC, 1985) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

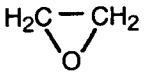
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-21-8
Replaced CAS Reg. No.: 19034-08-3; 99932-75-9
Chem. Abstr. Name: Oxirane
IUPAC Systematic Name: Oxirane
Synonyms: Dihydrooxirene; dimethylene oxide; 1,2-epoxyethane; epoxyethane; ethene oxide; EtO; ETO; oxacyclopropane; oxane; oxidoethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_4O Relative molecular mass: 44.06

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless gas (Rebsdat & Mayer, 1987)
(*b*) *Boiling-point*: 13.2 °C at 746 mm Hg [99.4 kPa] (Lide, 1991); 10.8 °C at 760 mm Hg [101.3 kPa] (Rebsdat & Mayer, 1987)
(*c*) *Melting-point*: –111 °C (Lide, 1991)
(*d*) *Density (liquid)*: 0.8824 at 10 °C/10 °C (Lide, 1991)
(*e*) *Spectroscopy data*: Infrared [prism, 1109] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
(*f*) *Solubility*: Soluble in water, acetone, benzene, ethanol and diethyl ether (Lide, 1991)

(*g*) *Volatility*: Vapour pressure, 145.6 kPa at 20 °C (Rebsdat & Mayer, 1987; Hoechst Celanese Corp., 1992); relative vapour density (air = 1), 1.5 at 20 °C (Hoechst Celanese Corp., 1992)

(*h*) *Stability*: Reacts readily with acids (Cawse *et al.*, 1980); reactions proceed mainly via ring opening and are highly exothermic; explosive decomposition of vapour may occur at higher temperatures if heat dissipation is inadequate (Rebsdat & Mayer, 1987). Lower explosive limit, 2.6–3.0% by volume in air (Rebsdat & Mayer, 1987; Dever *et al.*, 1994)

(*i*) *Octanol–water partition coefficient (P)*: log P, –0.30 (Sangster, 1989)

(*j*) *Conversion factor*: $mg/m^3 = 1.80 \times ppm$[a]

1.1.4 *Technical products and impurities*

Ethylene oxide of high purity (99.5–99.95%) is available from several sources with the following typical specifications: acidity (as acetic acid), 0.002% max.; aldehydes (as acetaldehyde), 0.001–0.01% max.; chlorides (as Cl), 0.005%; water, 0.02–0.03% max.; acetylene, 0.0005%; carbon dioxide, 0.001–0.002%; and residue, 0.005–0.01 g/100 ml max. (Rebsdat & Mayer, 1987; Hoechst Celanese Corp., 1988; Dow Chemical Co., 1989; Union Carbide, 1993).

Ethylene oxide for use as a fumigant and sterilizing agent is available in mixtures with nitrogen, carbon dioxide or dichlorodifluoromethane. Mixtures of 8.5–80% ethylene oxide/91.5–20% carbon dioxide (Allied Signal Chemicals, 1993) and 12% ethylene oxide in dichlorodifluoromethane are commonly used (Cawse *et al.*, 1980). As a result of concern about the role of chlorofluorocarbons in causing depletion of stratospheric ozone, they are being replaced in such mixtures by nitrogen and other flame retardant diluent gases (Dever *et al.*, 1994).

1.1.5 *Analysis*

Ethylene oxide in workplace air can be determined by packed column gas chromatography (GC) with an electron capture detector (ECD). The sample is adsorbed on hydrobromic acid-coated charcoal and desorbed with dimethylformamide. The sample is derivatized to 2-bromoethylheptafluorobutyrate for analysis. This method (NIOSH Method 1614) has an estimated limit of detection of 1 μg ethylene oxide per sample (Eller, 1987a). A similar method is reported by the US Occupational Safety and Health Administration, in which the sample is adsorbed on charcoal, desorbed with a benzene:carbon disulfide solution, converted to 2-bromoethanol and analysed by GC/ECD (Tucker & Arnold, 1984; Cummins *et al.*, 1987; European Commission, 1989). In another method (NIOSH Method 3702), a portable gas chromatograph is used with a photoionization detector. The sample is either drawn directly into a syringe or collected as a bag sample; it is then injected directly into the gas chromatograph for analysis. The estimated limit of detection is 2.5 pg/ml injection (0.001 ppm [0.002 mg/m^3]) (Eller, 1987b).

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[a]Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101.3 kPa)

Methods for the analysis and quantification of ethylene oxide in emissions from production plants and commercial sterilizers by GC with flame ionization detection have been reviewed (Gray *et al.*, 1985; Steger, 1989; Margeson *et al.*, 1990). Passive methods for personal sampling of ethylene oxide in air have also been reported (Kring *et al.*, 1984; Puskar & Hecker, 1989; Puskar *et al.*, 1990, 1991; Szopinski *et al.*, 1991).

Biological monitoring of occupational exposure to ethylene oxide has been conducted by analysis of alveolar air and blood (Brugnone *et al.*, 1986). Several methods have been reported for the determination of *N*-(2-hydroxyethyl) adducts in haemoglobin, with cysteine, valine and histidine: a radioimmunological technique, a modified Edman degradation procedure with GC/mass spectrometry; a GC method with selective ion monitoring mass spectrometry and a GC/ECD method (Gray *et al.*, 1985; Farmer *et al.*, 1986; Bailey *et al.*, 1987; Bolt *et al.*, 1988; Föst *et al.*, 1991; Kautiainen & Törnqvist, 1991; Sarto *et al.*, 1991; van Sittert *et al.*, 1993).

Methods have been reported for the detection of residues of ethylene oxide used as a sterilant: headspace GC (Marlowe *et al.*, 1987) and GC (Wojcik-O'Neill & Ello, 1991) for analysis of medical devices, capillary GC for analysis of drugs and plastics (Danielson *et al.*, 1990) and headspace GC for analysis of packaging materials and for ethylene oxide in ethoxylated surfactants and demulsifiers (Dahlgran & Shingleton, 1987). Methods have also been developed for the determination of ethylene oxide residues in processed food products. In one such method, ethylene oxide is converted to ethylene iodohydrin and analysed by GC/ECD (Jensen, 1988).

1.2 Production and use

1.2.1 *Production*

Ethylene oxide was produced from 1914 by the chlorohydrin process, in which ethylene chlorohydrin is prepared by reacting ethylene with hypochlorous acid (chlorine in water) and is converted to ethylene oxide by reaction with calcium oxide (Cawse *et al.*, 1980). This method is no longer used on an industrial scale, at least in the USA. The process was inefficient, as most of the chlorine that was used was lost as calcium chloride and unwanted organochlorine by-products were generated. Since 1931, that process has been gradually replaced by the direct vapour phase oxidation process, in which ethylene is oxidized to ethylene oxide with air or oxygen and a silver catalyst at 10–30 atm (1–3 MPa) at 200–300 °C (Rebsdat & Mayer, 1987; Berglund *et al.*, 1990).

Table 1 gives production volumes in Germany, Japan and the USA. It has been estimated that worldwide production of ethylene oxide exceeds 5500 thousand tonnes per year (WHO, 1985). Information available in 1991 indicated that ethylene oxide was produced by eight companies in the USA, seven in Germany, five in Japan, four each in China and the United Kingdom, two each in Belgium, Brazil, Canada and Spain and one each in Australia, Bulgaria, the former Czechoslovakia, France, India, Italy, Mexico, the Netherlands, the Republic of Korea, Singapore, Sweden and Venezuela (Chemical Information Services Ltd, 1991).

Table 1. Production of ethylene oxide in selected countries, 1982–92 (thousand tonnes)

Country	Year					
	1982	1984	1986	1988	1990	1992
Germany[a]	393	474	498	626	629	630
Japan	471	533	489	510	674	721
USA	2262	2585	2463	2700	2429	2522[b]

From Anon. (1985, 1989, 1993a); Japan Petrochemical Industry Association (1993)
[a]Western
[b]Preliminary

1.2.2 *Use*

Ethylene oxide is an important raw material for making major consumer goods in virtually all industrialized countries. Table 2 presents the pattern of use of ethylene oxide as a chemical intermediate in the USA, which is typical of that elsewhere in the world. It is used directly in the gaseous form as a disinfectant, sterilizing agent, fumigant and insecticide (see Table 3), either alone or in nonexplosive mixtures with nitrogen, carbon dioxide or dichlorofluoromethane. It is used as a fumigant to remove pests and microorganisms from spices and seasonings, furs, furniture, nut meats, tobacco, books, drugs, leather, motor oil, paper, soil, animal bedding, clothing and transport vehicles; and as a sterilant for foodstuffs, cocoa, flour, dried egg powder, coconut, fruits, dehydrated vegetables, cosmetics and dental, medical and scientific supplies (Popp *et al.*, 1986; US Environmental Protection Agency, 1986; Rebsdat & Mayer, 1987).

Table 2. Use patterns (%) for ethylene oxide in the USA

Use	Year				
	1981	1984	1987	1990	1993
Ethylene glycol	62	62	59	59	61
Non-ionic surfactants	12	12	14	13	16
Ethanolamines	5	7	8	8	8.5
Glycol ethers	6	7	6	6	5
Diethylene glycol	NR	NR	6	6	5
Triethylene glycol	NR	NR	2	2	2
Miscellaneous[a]	15	12	5	6	2.5

From Anon. (1981, 1984, 1987, 1990, 1993b); NR, not reported
[a]Includes higher glycols (polyethylene glycol), urethane polyols and exports

Table 3. Use of ethylene oxide as a fumigant and sterilant in the USA, 1983

Site and use	Amount used (tonnes)
Manufacturing facilities (production of sterile disposable items for medical use)	1500–2600
Medical facilities	500–550
Hospitals	400–450[a]
Medical clinics	50
Dental clinics	29.7
Doctors' surgeries (private)	16.8
Dentists' surgeries (private)	3.3
Veterinarians (private and clinical)	0.045
Museums	0.3
Libraries, archives	0.86
Research laboratories	277–446
Animal breeding	22.7
Drugs and medical devices	250–410
Microbiological, cancer	2.3–11.4
USDA high-containment laboratories	2.0
Railroad cars	1.0
Beehives	0.68–0.9
USDA quarantine port of entry	0.3
Spices	340
Black walnuts	1.5
Cosmetics	11
Dairy packaging	14.5
Total	2600–3900

From US Environmental Protection Agency (1986); USDA, US Department of Agriculture
[a]1976 value

Most ethylene oxide is converted into other products, including ethylene glycol; glycol ethers; ethanolamine; ethoxylation products of long-chain alcohols and amines, alkyl phenols, cellulose, starch and poly(propylene glycol); and ethylene carbonate. Ethylene glycol is used principally as an intermediate in the production of terephthalate polyester resins for fibres, films and bottles and in automotive antifreeze. Ethoxylation products of long-chain alcohols and alkylphenols are used as nonionic surfactants in household and industrial detergents. Glycol ethers, made by the addition of ethylene oxide to short-chain alcohols (including ethylene glycol to give di-, tri- and polyethylene glycols), are used as solvents, intermediates and in many other applications (Cawse *et al.*, 1980).

1.3 Occurrence

1.3.1 *Natural occurrence*

Ethylene oxide occurs as a metabolite of ethylene (see the monograph on ethylene, section 4.1). It is reactive in the environment. Its estimated atmospheric residence time, the time required for a given quantity to be reduced to 37% of its original level, is 5.8 days. In water, ethylene oxide reacts with anions such as chloride and carbonate; its half-life in fresh water (pH 7, 25 °C) is two weeks, and that in salt water is four days (US Environmental Protection Agency, 1986).

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health in the USA between 1981 and 1983 indicated that 270 000 US employees were potentially exposed at work to ethylene oxide (US National Institute for Occupational Safety and Health, 1993). Of this number, 22% were estimated to be exposed to ethylene oxide and 78% to materials containing ethylene oxide. Workers in hospitals and in the chemicals and allied products industry (plastics, synthetic materials and drugs manufacture) accounted for half of the number. The estimate is based on a survey of US companies and did not involve actual measurements of exposure. People with relatively high exposure include approximately 96 000 exposed in hospitals and 21 000 exposed during commercial sterilization of medical supplies, pharmaceutical products and spices (Steenland *et al.*, 1991). Most of the data on occupational exposure is related to the production of ethylene oxide and its use in industrial and hospital sterilization.

(*a*) *Production of ethylene oxide and its derivatives*

Rough estimates of exposure to ethylene oxide have been made for a Swedish company where ethylene oxide and derivatives were produced by the chlorohydrin process. Average exposure was estimated to be to less than 25 mg/m^3 during the period 1941–47 and 10–50 mg/m^3 during the 1950s and early 1960s, with occasional peaks above the odour threshold of 1300 mg/m^3. After manufacture of ethylene oxide was stopped in 1963, exposure to 1–10 mg/m^3 (with occasional higher values) continued to occur because of its use in the manufacture of other compounds (Hogstedt *et al.*, 1979a).

At another Swedish plant, where ethylene oxide was produced by oxygenation of ethylene, the 8-h time-weighted average (TWA) exposure to ethylene oxide was 9–15 mg/m^3 in 1963–76 and 2–4 mg/m^3 in 1977–82 during production of ethylene oxide and ethylene glycol, 6 mg/m^3 in 1963–76 and 2 mg/m^3 in 1977–82 in processing of ethylene oxide and 2–6 mg/m^3 in 1963–76 and 1–3 mg/m^3 in 1977–82 in maintenance and technical service work. Certain workers in each category are reported to have had higher exposures, up to 600–1800 mg/m^3, during periods of minutes (Hogstedt *et al.*, 1986).

Area samples taken in the 1960s throughout a US plant where ethylene oxide was produced by direct oxidation of ethylene contained 0–55 ppm [0–100 mg/m^3]; the majority contained 3–20 ppm [5–36 mg/m^3]. On the basis of these results, the general long-term exposure of operators was estimated to be 5–10 ppm [9–18 mg/m^3] (Joyner, 1964).

The following exposures were estimated for production workers in two US plants where manufacture and use of ethylene oxide started in 1925: 1925–39, > 14 ppm [25 mg/m^3]; 1940–56, 14 ppm [25 mg/m^3]; 1957–73, 5–10 ppm [9–18 mg/m^3]; and 1974–88, < 1 ppm [1.8 mg/m^3], with frequent peaks of several hundred parts per million in the earliest period and some peaks of similar intensity in the 1940s to mid-1950s. The chlorohydrin process was used from 1925 to 1957 (Teta *et al.*, 1993). Although the results of environmental monitoring in these plants since 1976 indicated that the 8-h TWA was less than 1 ppm [1.8 mg/m^3], it was generally between 1 and 5 ppm [1.8 and 9 mg/m^3] for maintenance employees and could go up to 66 ppm [119 mg/m^3] (Greenberg *et al.*, 1990).

Area and personal samples were taken in five US plants where ethylene oxide and its derivatives were produced, including the two described above, by the US National Institute for Occupational Safety and Health during 1977 and 1978. In most of the 95 personal samples taken, representative of a shift, the concentration of ethylene oxide was below the detection limits (which varied from 0.1 to 8 mg/m^3), although a few contained between 1 and 148 mg/m^3 (82 ppm). Similarly, in most area samples, the concentration was below the detection limits or was in the range < 1–1.5 ppm [2–3 mg/m^3], apart from exceptional situations such as leaks (Lovegren & Koketsu, 1977a,b,c; Oser *et al.*, 1978a,b, 1979). The fact that full-shift concentrations in these plants were usually well below the standards of the time (50 ppm, or 90 mg/m^3) has been attributed to three main factors: use of completely closed systems for the storage, transfer and production of ethylene oxide; implementation of measures to prevent fire; and operation out of doors, resulting in dilution by natural air (Morgan *et al.*, 1981).

In one US chemical manufacturing complex, two groups of employees may have been exposed to ethylene oxide: during its production and during production of ethylene glycol, glycol ethers and ethanolamines. Yearly TWA exposures (1977–80) were reported to have been to less than 1 ppm [1.8 mg/m^3] in all jobs except loading, where technicians were exposed to up to 1.7 ppm [3 mg/m^3] yearly and 5.7 ppm [10 mg/m^3] individually. Peak exposures were usually to less than 20 ppm [36 mg/m^3], except in loading where exposure was to up to 235 ppm [420 mg/m^3] (Currier *et al.*, 1984).

The typical average daily exposures of workers in a 1979 survey of US plants where ethylene oxide was manufactured and used were 0.3–4 ppm [0.5–7.3 mg/m^3]; worst-case peak exposures of maintenance workers were up to 9600 ppm [17 300 mg/m^3] (Flores, 1983).

Under the sponsorship of the Chemical Manufacturers Association, company data were collected on current exposures of workers to ethylene oxide in 11 ethylene oxide production units and 24 ethoxylation units in the USA in 1987 (Table 4). Respirators were reported to be used in specific operations, such as rail car loading and unloading, maintenance and product sampling, where engineering controls are not feasible (Heiden Associates, 1988a).

In a German plant where ethylene oxide is manufactured, 2-h area samples taken in 1978–79 contained less than 5 ppm [9 mg/m^3] under normal working conditions. Concentrations rose to as much as 1900 ppm [3400 mg/m^3] for several minutes in exceptional cases during plant breakdown (Thiess *et al.*, 1981a).

In a Dutch ethylene oxide manufacturing plant, geometric mean concentrations in 8-h personal samples were calculated to be < 0.01 ppm [< 0.02 mg/m^3] for 1974, 1978 and 1980

and 0.12 ppm [0.2 mg/m^3] for 1981, with individual values ranging overall from not detected (< 0.05 ppm [< 0.1 mg/m^3]) to 8 ppm [14 mg/m^3] (van Sittert *et al.*, 1985).

Table 4. Worker exposure to ethylene oxide by type of unit and job category in the US chemical manufacturing industry, 1987

Unit and job category	No. of samples	8-h TWA (mg/m^3)		No. of samples	Short-term (10–150 min) exposure (mg/m^3)	
		Mean[a]	Range		Mean[a]	Range
Ethylene oxide production						
Production workers	402	0.7	0.11–3.2	171	7.7	1.62–19.8
Maintenance workers	439	1.3	0.14–5.6	59	19.6	0.20–35.3
Supervisors	123	0.2	0.04–0.18	3	1.3	1.3–1.4
Distribution workers	218	2.9	0.36–6.8	111	11.7	3.6–17.6
Laboratory workers	189	0.7	0.12–4.3	39	1.4	0.4–2.2
Other workers	97	0.2	0.05–0.72			
Ethoxylation						
Production workers	640	0.4	0.12–1.26	172	2.0	0.02–9.9
Maintenance workers	191	1.1	0.02–4.7	56	13.3	0.11–54.9
Supervisors	54	0.4	0.05–0.72	5	8.6	0.9–23.8
Distribution workers	105	0.7	0.20–2.7	100	3.4	0.9–21.6
Laboratory workers	52	0.4	0.02–0.9	19	5.0	0.4–11.0
Other workers	24	0.4	0.18–0.54			

Adapted from Heiden Associates (1988a); TWA, time-weighted average
[a]Weighted by number of workers exposed

In the former Czechoslovakia, the 8-h TWA concentrations of ethylene oxide measured in 1982–84 in the working environment of an ethylene oxide production plant were 0–8.25 mg/m^3 (Karelová *et al.*, 1987).

Gardner *et al.* (1989) reported that monitoring since 1977 in four British plants where ethylene oxide and derivatives were produced indicated average exposures to less than 5 ppm [9 mg/m^3] in almost all jobs and to < 1 ppm [1.8 mg/m^3] in many jobs; occasional peaks up to several hundred parts per million occurred as a result of operating difficulties. In earlier years, peak exposures above the odour threshold of 700 ppm [1260 mg/m^3] were reported.

In industries where ethylene oxide and its derivatives are manufactured, exposure may occur to a large variety of chemicals other than ethylene oxide, depending on the types of processes and jobs. They include unsaturated aliphatic hydrocarbons (e.g. ethylene, propylene), other epoxides (e.g. propylene oxide), chlorohydrins (e.g. epichlorohydrin, ethylene chlorohydrin), chlorinated aliphatic hydrocarbons (e.g. dichloromethane, dichloroethane), glycols and ethers (e.g. ethylene glycol, glycol ethers, bis(2-chloroethyl)ether), aldehydes (e.g. formaldehyde), amines (e.g. aniline), aromatic hydrocarbons (e.g. benzene, styrene), alkyl sulfates and other compounds (Shore *et al.*, 1993).

(*b*) *Use of ethylene oxide for industrial sterilization*

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of products, such as medical equipment and products (e.g. surgical products, single-use medical devices), disposable health care products, pharmaceutical and veterinary products, spices and animal feed.

In an extensive survey of the industry in the USA, conducted by the National Institute for Occupational Safety and Health, exposure to ethylene oxide was estimated on the basis of data collected in 1976–85 by 21 out of 36 companies, most of which were involved in sterilization of medical supplies and spices. Individual 8-h TWA concentrations in workers' personal breathing zones, collected by active sampling on charcoal tubes, were included in a model in which regression analysis was used to link exposure concentration to seven significant variables: year of operation, volume of sterilizer or treatment vessel, period since product was sterilized, product type, aeration procedure, presence of a rear exhaust valve in the sterilizer, and exposure category (sterilizer, chamber area, maintenance, production, warehouse, clean room, quarantine and laboratory; Stayner *et al.*, 1993) (Greife *et al.*, 1988). When the model was applied in a cohort study to the job histories of exposed workers in 13 of the companies, the estimated historical average exposure concentrations ranged from 0.05 to 77.2 ppm [0.1–139 mg/m^3], with a mean of 5.5 ppm [9.9 mg/m^3] and a median of 3.2 ppm [5.8 mg/m^3] (Stayner *et al.*, 1993). Wong and Trent (1993) used the industrial hygiene data from the same companies and estimated that sterilizer operators were exposed to an 8-h TWA concentration of 16 ppm [29 mg/m^3] before 1978 and 4–5 ppm [7–9 mg/m^3] after 1978, while production workers were exposed to about 5 ppm [9 mg/m^3] before 1978 and 2 ppm [3.6 mg/m^3] after that year.

Engineering controls and new work practices designed to lower workers' exposure were generally adopted by ethylene oxide users in the USA in 1978 and 1979 (Steenland *et al.*, 1991). Stolley *et al.* (1984) estimated that the 8-h TWA concentrations of sterilizer operators in three US facilities before 1980 had been 0.5, 5–10 and 5–20 ppm [1, 9–18 and 9–36 mg/m^3], while data collected in the two plants that were still operating in 1980–82 indicated concentrations of less than 1 ppm [2 mg/m^3].

Under the sponsorship of the Health Industry Manufacturers Association, company data were collected on current exposures of workers to ethylene oxide in 71 facilities in the USA in 1987 where medical devices and diagnostic products were sterilized. The workers included sterilizer operators, maintenance workers, supervisors, warehouse workers, laboratory workers and quality control personnel. Respirators were reported to be used in specific operations, such as unloading the sterilizer, maintenance, quality control sampling, emergencies, loading aeration, and changing ethylene oxide bottles, cylinders and tanks. Concentrations were measured outside the respirators. The routine 8-h TWA concentration, occurring two or more days per week, was > 1 ppm (> 1.8 mg/m^3) for 12.6% of workers, 0.5–1 ppm (0.9–1.8 mg/m^3) for 13.9%, 0.3–0.5 ppm (0.5–0.9 mg/m^3) for 26.7% and < 0.3 ppm (< 0.5 mg/m^3) for 46.8%. Short-term sampling (for 5–120 min; average, 28 min; except in one factory where sampling was for 210 min for workers in other jobs) showed routine short-term exposures of > 10 ppm (> 18 mg/m^3) for 10.7% of workers, 5–10 ppm (9–18 mg/m^3) for 17.1% and < 5 ppm (< 9 mg/m^3) for 72.2%. Non-routine short-term

exposure, occurring one day per week or near areas where there was exposure was > 10 ppm (> 18 mg/m^3) for 5.1% of workers, 5–10 ppm (9–18 mg/m^3) for 2.6% and < 5 ppm (< 9 mg/m^3) for 92.3% (Heiden Associates, 1988b).

In a Swedish factory where hospital equipment was sterilized, area samples taken in 1977 in the storage area showed concentrations of ethylene oxide ranging from 2 to 70 ppm [3.6–126 mg/m^3]; the 8-h TWA concentration in the breathing zone of workers in the same area was 20 ppm [36 mg/m^3] (Hogstedt *et al.*, 1979b). In a Swedish factory evaluated in 1978, full-shift personal sampling indicated that sterilizing room operators had an exposure concentration of 2.4 ppm [4.3 mg/m^3]; area sampling indicated an exposure of 1.3 ppm [2.3 mg/m^3]. Personal sampling showed a concentration of 0.1 ppm [0.2 mg/m^3] in the packing room, and area sampling showed a concentration of 0.8 ppm [1.4 mg/m^3] in the stockroom (Högstedt *et al.*, 1983). In another Swedish study, sterilizers and a laboratory technician in the production of disposable medical equipment were reported to have been exposed to bursts of ethylene oxide at concentrations of 5–10 ppm [9–18 mg/m^3] for a total of 1 h per working day, while packers were exposed at an average of 0.5–1 ppm [1–2 mg/m^3] for the entire week (Pero *et al.*, 1981). Sterilizers, packers and truck drivers at another Swedish factory, where single-use medical equipment was produced, were reported to be exposed to an 8-h TWA concentration of 0.5–1 ppm [1–2 mg/m^3] (Pero *et al.*, 1982). In two Swedish disposable medical equipment plants, sterilizers and packers were the most heavily exposed, but levels decreased steadily from 35–40 ppm [about 70 mg/m^3] in 1970 to < 0.2–0.75 ppm [< 1.5 mg/m^3] in 1985; the average exposures of store workers and development engineers decreased from 5–20 ppm [9–36 mg/m^3] to < 0.2 ppm [< 0.4 mg/m^3] in the same period, while those of people in other job categories (repairmen, laboratory technicians, controllers and foremen) decreased from 1–4 ppm [2–7 mg/m^3] to < 0.2 ppm [< 0.4 mg/m^3] (Hagmar *et al.*, 1991).

In a plant in eastern Germany where disposable medical equipment was sterilized, workers were found to have been exposed to an average concentration of about 60 mg/m^3 in 1985 and about 30 mg/m^3 from 1989 onwards (Tates *et al.*, 1991a).

In Belgium, 12 workers involved in industrial sterilization in three plants were exposed to 8-h TWA concentrations of 0.1–9.3 ppm [0.2–16.7 mg/m^3], with averages per plant of 1.7 ppm [3.1 mg/m^3], 3.7 [6.7] and 4.5 [8.1] (Wolfs *et al.*, 1983).

Other substances to which workers involved in sterilizing medical products may be exposed include gases present with ethylene oxide in the sterilizing mixture, such as chlorofluorocarbons and carbon dioxide (Heiden Associates, 1988b) and methyl formate in Sweden (Hagmar *et al.*, 1991).

(c) *Use of ethylene oxide in hospitals*

Ethylene oxide is used widely in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come into contact with biological tissues (Babich, 1985). The US National Institute for Occupational Safety and Health estimated that there were more than 10 000 sterilizers in use in US health care facilities. Large sterilizers are found in central supply areas of most hospitals, and smaller sterilizers are found in clinics, operating rooms, tissue banks and research facilities (Glaser, 1979).

Exposure to ethylene oxide may result during any of the following operations and conditions: changing pressurized ethylene oxide gas cylinders; leaking valves, fittings and piping; leaking sterilizer door gaskets; opening of the sterilizer door at the end of a cycle; improper ventilation at the sterilizer door; improperly or unventilated air gap between the discharge line and the sewer drain; removal of items from the sterilizer and transfer of the sterilized load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; passing near sterilizers and aerators during operation (Mortimer & Kercher, 1989).

The US National Institute for Occupational Safety and Health conducted a series of studies over 10 years to document the exposure of US hospital sterilization staff to ethylene oxide. The main results are summarized in Table 5. Levels found in other studies in the USA and in other countries are presented in Table 6.

In a unit in Argentina equipped with old gas sterilizers with no mechanical ventilation, the 8-h TWA concentration was 60–69 ppm [108–124 mg/m^3] (Lerda & Rizzi, 1992).

In most studies, exposure appears to result mostly from peak emissions during such operations as opening the door of the sterilizer and unloading and transferring sterilized material. Proper engineering controls and work practices are reported to result in full-shift exposure levels of less than 0.1 ppm [0.18 mg/m^3] and short-term exposure levels of less than 2 ppm [3.6 mg/m^3] (Mortimer & Kercher, 1989). In a survey of 125 US hospitals, however, use of personal protective equipment was found to be limited to the wearing of various types of gloves while transferring sterilized items. No respirators were used (Elliott *et al.*, 1988).

Other substances to which sterilizer operators in hospitals may be exposed include other gases, such as chlorofluorocarbons (e.g. dichlorodifluoromethane) and carbon dioxide present in the sterilizing mixture (Wolfs *et al.*, 1983; Deschamps *et al.*, 1989). Some operating room personnel exposed to ethylene oxide may also be exposed to anaesthetic gases and X-rays (Sarto *et al.*, 1984a), and some may have occasional exposure to low concentrations of formaldehyde (Gardner *et al.*, 1989).

(*d*) *Other uses*

In a US waste-water treatment plant in the starch processing area, where ethylene oxide is used as a reaction chemical to modify starch, full-shift personal breathing zone concentrations ranged from undetectable to 0.43 mg/m^3 for operators and from undetectable to [2.5 mg/m^3] for mechanics (McCammon *et al.*, 1990).

1.3.3 *Air*

Estimated ethylene oxide emissions in member states of the European Union in the mid-1980s are presented in Table 7 (Bouscaren *et al.*, 1987). In 1985, US emissions of ethylene oxide were estimated to have been approximately 5000 tonnes per year. Sterilization and fumigation sites accounted for 57% of total emissions, production and captive use for 31%, medical facilities for 8% and ethoxylation for 4%. Most emissions from producer and ethoxylator sites are due to equipment leaks. Less than 0.1% of the ethylene oxide produced is used in sterilizer and fumigator processes, but nearly all of the ethylene oxide used for this purpose is released into the atmosphere or mixed with water and routed to a sewer system (Markwordt, 1985).

Table 5. Exposure of hospital sterilizer operators to ethylene oxide (personal samples) in studies conducted by the US National Institute for Occupational Safety and Health, 1977–90

No. of hospitals	Operation or conditions	Duration of sampling	No. of samples	Concentration (mg/m^3)	Period of measurements	Reference
12	Good engineering controls and good work practice	8-h TWA	4	ND	[1984–85]	Elliott *et al.* (1988)
		Short-term (2–30 min)	3	ND		
	Good engineering controls and poor work practices	8-h TWA	15	[ND–0.29]		
		Short-term (2–30 min)	19	[ND–5.4]		
	No engineering controls and good work practices	8-h TWA	14	[ND–0.83]		
		Short-term (2–30 min)	4	[0.43–7.2]		
	No engineering controls and poor work practices	8-h TWA	24	[ND–8.3]		
		Short-term (2–30 min)	8	[0.43–186]		
8		Full-shift TWA (6–8 h)	50	[ND–0.5]	1984–86	Mortimer & Kercher (1989)
		Short-term (1–30 min)	59	[ND–10.4]		
1	Decontamination room	8-h TWA	2	[0.58–0.77]	1987	Boeniger (1988a)
	Sterile room	8-h TWA	6	[0.02–1.37]		
1		8-h TWA	8	[< 0.02]	1988	Newman & Freund (1989)
1	Before installation of controls (1984)	Full-shift TWA	NR	[0.43] (average)		Kercher & Mortimer (1987)
		Short-term (15–20 min)	NR	[3.4] (average)		
		Short-term (1–2 min)	NR	[4.3] (average)		
	After installation of controls (1985)	Full-shift TWA	NR	[< 0.1] (average)		
		Short-term (15–20 min)	NR	[< 0.4] (average)		
		Short-term (1–2 min)	NR	[1]		
1	Full shift	4–7-h TWA	8	[0.04–0.40]	1987	Boeniger (1988b)
	Cracking sterilizer door open	30 sec	6	[< 0.05–7.7]		
	Transferring load to aerator	30 sec	15	[0.23–18.9]		
1		6–8-h TWA	3	[< 0.02]	1991	Shults & Seitz (1992)

ND, not detected; NR, not reported

Table 6. Ethylene oxide concentrations observed in hospitals in various countries

Country	No. of hospitals	Year of sampling	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m^3)		Reference
						Range	Mean	
Belgium	3		Sterilizer operators	8-h TWA	28	0.4–4.5	0.5–2.9	Wolfs *et al.* (1983)
	1		Sterilizer operators; leaking equipment	8-h TWA	16	0.5–32.9	14.0	
	1		Sterilizer operators; box sterilizer with capsules	8-h TWA	5	16.2–95.2	27.0	
Former Czechoslovakia		1984	Sterilization workers; area sampling	8-h TWA	NR	0–4.8		Karelová *et al.* (1987)
Finland	24	1981	Sterilizer operators	8-h TWA	NR	0.2–0.9		Hemminki *et al.* (1982)
				Peaks	NR	≤ 450		
	24	1981	Sterilizing chamber open	20 min	NR	9–18		Hemminki *et al.* (1982)
France	4[a]		Loading, sterilizing, unloading, aerating; area sampling	Few min	270	0.9–414		Mouilleseaux *et al.* (1983)
				6–8-h TWA	14	0.1–9		
	5	1983–86	Opening sterilizer and handling sterilized material; personal sampling	2.5–102 min	10	0.4–70		Deschamps *et al.* (1989)
Italy	1		Sterilization workers	8-h TWA	10 subjects	1.90–4.71		Brugnone *et al.* (1985)
	1		Sterilizer operators	7–8-h TWA	4 subjects	11.5–16.7	14.3	Sarto *et al.* (1987)
			Helpers	7–8-h TWA	4 subjects	6.8–9.0	7.7	
	6		Old sterilizers					Sarto *et al.* (1984a)
			Opening sterilizer; area sampling	5 min	NR	23–288	113	
			One sterilization cycle; personal sampling	Variable	NR	6.7–63.9	28.4	
			Standard working day; personal sampling	8-h TWA	19 subjects	6.7–36	19.3	
	2		Second-generation sterilizers					
			Opening sterilizer, area sampling	5 min	NR	9–47	15.5	
			One sterilization cycle; personal sampling	Variable	NR	0.5–4.7	2.0	
			Standard working day; personal sampling	8-h TWA	NR	0.4–0.9	0.63	

Table 6 (contd)

Country	No. of hospitals	Year of sampling	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m^3)		Reference
						Range	Mean	
Italy (contd)	1		Sterilization workers	6.5-h TWA	5 subjects	0.68[b]		Sarto *et al.* (1991)
			Preparation workers	6.5-h TWA	5 subjects	0.045		
Mexico	1		Sterilizer operators	8-h TWA	22 subjects	0–2.4		Schulte *et al.* (1992)
USA	1		Sterilizer workers	8-h TWA	14	< 0.13–7.7		Hansen *et al.* (1984)
			Sterilizer unloading; personal sampling	15 min	17	< 4.3–81		
			Sterilizer unloading; maximum	Instantaneous	13	4–1430		
	1	1985–86	Sterilizer operators; personal sampling	8-h TWA	34 subjects	≤ 4.3		Mayer *et al.* (1991)
		1987			NR	< 1.8		
		1988			31	< 0.18]		
	9		Sterilizer operators	8-h TWA	51 subjects	0–0.54		Schulte *et al.* (1992)

[a]One was a municipal sterilization and disinfection facility
[b]Each has the same concentration.

Table 7. Estimated ethylene oxide emissions in member states of the European Union

Country	Emissions (thousand tonnes/year)	
	From chemical industry	Other sources
Belgium	0.41	NR
France	0.40	NR
Germany	0.8	0.45
Italy	0.5	0.28
Netherlands	0.2	0.23
Spain	0.12	NR
United Kingdom	0.41	NR
Total	2.8	

From Bouscaren *et al.* (1987); NR, not reported

Emissions of ethylene oxide reported to the US Environmental Protection Agency by industrial facilities in the USA declined from approximately 2900 tonnes in 1987 to 835 tonnes in 1991 (US National Library of Medicine, 1993).

1.3.4 *Other occurrence*

Of 204 food products from Danish retail shops in 1985 examined for ethylene oxide residues, 96 samples were found to have concentrations of ethylene oxide ranging from 0.05 to 1800 mg/kg. The food products surveyed included herbs and spices (14–580 mg/kg); dairy (0.06–4.2 mg/kg), pickled fish (0.08–2.0 mg/kg), meat (0.05–20 mg/kg) and cocoa (0.06–0.98 mg/kg) products; and black and herb teas (3–5 mg/kg; one sample contained 1800 mg/kg). In a follow-up survey of 59 honey samples, no ethylene oxide residue was detected (Jensen, 1988).

Ethylene oxide has also been reported to be a product of incomplete combustion and has been identified in automobile and diesel exhaust and in tobacco smoke (Gray *et al.*, 1985).

Patients on dialysis units sterilized with ethylene oxide showed allergic symptoms due to sensitization to residual ethylene oxide (see section 4.2.1).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for ethylene oxide in a number of countries are presented in Table 8.

A tolerance of 50 ppm (mg/kg) has been established in the USA for residues of ethylene oxide when used as a postharvest fumigant in or on raw black walnut meats, copra and whole spices (US Environmental Protection Agency, 1992a).

Ethylene oxide, either alone or admixed with carbon dioxide or dichlorodifluoromethane, is permitted in the USA as a fumigant for the control of microorganisms and insect

infestation in ground spices and other processed natural seasoning materials, except mixtures to which salt has been added. Residues of ethylene oxide in ground spices must not exceed the established tolerance of 50 ppm (mg/kg) in whole spices (US Environmental Protection Agency, 1992b).

The US Food and Drug Administration (1993) permits the use of ethylene oxide in various products that may come into contact with food.

Table 8. Occupational exposure limits and guidelines for ethylene oxide

Country or region	Year	Concentration (mg/m^3)	Interpretation
Argentina	1991	2	TWA; suspected of having carcinogenic potential
Australia	1983	2	TWA; suspected human carcinogen
Austria	1982	18	TWA
Belgium	1984	1.8	TWA; probable human carcinogen
Brazil	1978	70	TWA
Canada	1986	2	TWA; suspected human carcinogen
Chile	1983	16	TWA
Denmark	1988	1.8	TWA; suspected carcinogen
Finland	1993	1.8	TWA; suspected of having carcinogenic potential
France	1993	2	TWA; suspected carcinogen
		10	STEL
Germany	1993	None	Carcinogenic in animals; skin
Hungary	1978	1	Ceiling (30-min); probable human carcinogen; irritant; sensitizer
Indonesia	1978	90	TWA
Italy	1978	60	TWA; sensitizer
Japan	1991	1.8	TWA; suspected of having carcinogenic potential (tentative)
Mexico	1989	2	TWA
Netherlands	1986	90	TWA
Poland	1982	1	TWA
Romania	1975	30	Average
		60	Maximum
Sweden	1991	2	TWA; probable human carcinogen; skin
		9	STEL
Switzerland	After 1987	2	TWA; suspected carcinogen; skin
Taiwan	1981	90	TWA
United Kingdom	1992	10	TWA; maximum exposure limit
USA			
ACGIH (TLV)	1994	1.8	TWA; suspected human carcinogen[a]
OSHA (PEL)	1992	1.8	TWA
		9	STEL
NIOSH (REL)	1992	0.18	TWA
		9	Ceiling

Table 8 (contd)

Country or region	Year	Concentration (mg/m^3)	Interpretation
Venezuela	1978	90	TWA
		135	Ceiling

From Arbeidsinspectie (1986); Cook (1987); Arbejdstilsynet (1988); ILO (1991); Health and Safety Executive (1992); US Occupational Safety and Health Administration (OSHA) (1992); US National Institute for Occupational Safety and Health (NIOSH) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993); TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; skin, absorption through the skin may be a significant source of exposure

[a]Substance identified by other sources as a suspected or confirmed human carcinogen

2. Studies of Cancer in Humans

2.1 Case reports

Hogstedt *et al.* (1979b) reported three cases of haematopoietic cancer that had occurred between 1972 and 1977 in workers at a Swedish factory where 50% ethylene oxide and 50% methyl formate had been used since 1968 to sterilize hospital equipment. Attention had been drawn to the case cluster by the factory safety committee. One woman with chronic myeloid leukaemia and another with acute myelogenous leukaemia had worked in a storage hall where they were exposed for 8 h per day to an estimated 20 ± 10 (SD) ppm [36 ± 18 mg/m^3] ethylene oxide. The third case was that of a man with primary macroglobulinaemia (morbus Waldenström) who had been manager of the plant since 1965 and had been exposed to ethylene oxide for an estimated 3 h per week. [The Working Group noted that Waldenström's macroglobulinaemia is classified in ICD 10 as a malignant immunoproliferative disease.]

2.2 Cohort studies

Two hundred and three workers employed for at least one year at the above factory were subsequently followed up for mortality (Hogstedt *et al.*, 1986). During 1978–82, five deaths occurred (4.9 expected), of which four were from cancer (1.6 expected). Two of the deaths were from lymphatic and haematopoietic cancer (0.13 expected), but one of these decedents had been part of the original case cluster that had prompted the study.

In a second study, Hogstedt *et al.* (1979a, 1986) and Hogstedt (1988) examined workers at a Swedish chemical plant where ethylene oxide had been produced by the chlorohydrin process. The cohort comprised men who had taken part in a medical survey in 1960–61 and included 89 operators with regular exposure to ethylene oxide, 86 maintenance staff with intermittent exposure and 66 unexposed men. All of the men had been exposed or employed

for at least one year. Average exposures to ethylene oxide during 1941–47 were estimated to have been below 25 mg/m^3 but with occasional peaks above the odour threshold of 1300 mg/m^3. During the 1950s and through to 1963, an average concentration of 10–50 mg/m^3 was estimated. In 1963, production of ethylene oxide ceased, but the compound continued to be used in manufacturing processes, and random samples showed ethylene oxide concentrations in the range 1–10 mg/m^3, with occasional higher values. Other exposures in the plant included chloroform, chlorinated acetals, chloral, DDT, ethylene glycol, surfactants, cellulose ethers, ethylene, ethylene chlorhydrin, ethylene dichloride, bis(2-chlorethyl)ether and propylene oxide. The cohort was followed from January 1961 to December 1985. With no adjustment for any latency from first exposure, there were 34 deaths from all causes among the ethylene oxide operators (25.0 expected), including 14 cancer deaths (6.1 expected) of which five were due to stomach cancer (0.6 expected) and two to leukaemia (0.2 expected). There was no overall excess mortality from cancer among the maintenance staff with intermittent exposure or among the unexposed workers; however, four of the maintenance men had died of stomach cancer (0.6 expected) and one from leukaemia (0.2 expected).

The above reports also describe a second cohort of Swedish workers exposed to ethylene oxide in a plant where the process used was based on direct oxidation of ethylene (Hogstedt *et al.*, 1986; Hogstedt, 1988). The cohort comprised 128 workers employed in the production of ethylene oxide or ethylene glycol, who had almost pure exposure to ethylene oxide; 69 workers employed in the processing of ethylene oxide and propylene oxide to nonionic surfactants and polyols, whose principal exposure was to ethylene oxide and propylene oxide but who had also been exposed to various amines, sodium nitrate, formaldehyde and 1,2-butene oxide; and 158 maintenance and technical personnel with multiple exposures including ethylene oxide. Analyses of air samples and interviews with experienced staff indicated 8-h TWA exposures to ethylene oxide of 1–8 ppm [1.8–14.4 mg/m^3] during 1963–76, which fell to 0.4–2 ppm [0.7–3.6 mg/m^3] during 1977–82. Expected numbers of cancers and deaths were calculated from five-year age-, sex- and calendar year-specific rates for the national population. During follow-up from 1964 to 1981, eight deaths were observed in the entire cohort as compared with 11.6 expected; one man in the maintenance and repair group died of chronic myeloid leukaemia, but no additional incident cases of leukaemia were recorded. The expected number of incident leukaemia cases was 0.16. During extended follow-up to 1985, a fatal case of reticular-cell sarcoma was recorded among the production workers [expected number not given]. [The Working Group noted that the cohort was not defined precisely.]

Hogstedt (1988) summarized the findings of the three cohort studies described above. After exclusion of the three cases in the initial cluster at the sterilizing plant, seven lymphatic and haematological malignancies were observed during follow-up for cancer incidence to 1983 (2.2 expected) [standardized incidence ratio [SIR], 3.2; 95% confidence interval [CI], 1.3–6.6], including five cases of leukaemia (0.8 expected) [SIR, 6.3; 95% CI, 2.0–15.0].

Morgan *et al.* (1981) reported a retrospective cohort study of 767 men employed at a chemical plant in eastern Texas, USA, between 1955 and 1977 where ethylene oxide was produced. All of the men had worked at the factory for at least five years and were 'potentially exposed' to the compound. Potential exposure to ethylene oxide was determined

by personnel at the company on the basis of work histories. In an industrial hygiene survey in 1977, all samples taken in the ethylene oxide production area contained less than 10 ppm [18 mg/m^3]. Vital status was ascertained for more than 95% of cohort members from a combination of plant records, 'personal knowledge' and telephone follow-up. Altogether, 46 deaths were recorded, whereas 80 were expected on the basis of US vital statistics. Death certificates were obtained for 42 of the 46 deceased subjects. Eleven deaths were from cancer (15.2 expected), and nonsignificant excesses were seen of cancers of the pancreas (3/0.8) and brain and central nervous system (2/0.7) and of Hodgkin's disease (2/0.4); no death from leukaemia was found. [The Working Group noted that details were missing on the nature of the manufacturing process, on the extent to which exposure readings were representative of earlier conditions in the plant and on potential confounding exposures.]

The results of an extended follow-up of this cohort to 1985 were presented at a meeting and reported by Shore *et al.* (1993) as part of a meta-analysis of cohort studies on ethylene oxide. The follow-up rate was 99.7%. Three deaths were observed from brain cancer (1.1 expected), three from lymphatic and haematopoietic cancer (3.0 expected), none from leukaemia (1.1 expected) and none from stomach cancer [expected number not given].

Thiess *et al.* (1981b) examined the mortality of 602 active and former male employees of a company in western Germany who had worked for at least six months in an area of alkylene oxide production. Until 1965, ethylene oxide had been made from ethylene chlorohydrin, but thereafter it was produced by direct oxidation of ethylene. Propylene oxide had been made since 1959 by a propylene chlorohydrin process. Industrial hygiene measurements in 1978 showed that the average concentration of ethylene oxide was < 4 ppm [7.2 mg/m^3], but no earlier measurement was available. Discussions with long-standing employees indicated that exposures in the past would have been higher. Other potential exposures included propylene oxide, butylene oxide, dioxane, epichlorohydrin, dichloropropane, ethylene chlorohydrin, propylene chlorohydrin, aniline, piperazine, cyclohexylamine, cyclohexane, formaldehyde, isobutyraldehyde, ethylene-imine, hydrocyanic acid, hydrogen sulfide, aluminium chloride, benzene, phenol, cyanuric acid, acrylic acid and acetylene alcohols. The first worker was employed in 1928, and follow-up was from that year until 30 June 1980. Follow-up of former German employees was 97.6% successful, but 30/66 non-German ex-employees were lost to follow-up. The expected numbers of deaths in the cohort were calculated for each five-year age group by the person-years method, using mortality rates for the populations of Ludwigshafen and Rhinehessia-Palatinate during 1970–75 and of Germany during 1971–74 as reference. In addition, an internal comparison group of 1662 persons employed in a styrene production facility on the same site was used. During follow-up, 56 deaths were recorded in the exposed cohort, whereas the expected numbers were 71.5 (Ludwigshafen), 73.4 (Rhinehessia-Palatinate), 76.6 (Germany) and 57.9 (styrene cohort). Fourteen of the deaths were due to cancer, whereas 16.6 were expected from national statistics. The deaths from cancer included one case of myeloid leukaemia and one case of lymphatic sarcoma. [The Working Group noted that no indication is given of the completeness with which the cohort was ascertained, and the methods of follow-up are not stated. It is not clear how losses to follow-up were handled in the analysis.]

Most of the above cohort was included in a larger study of employees from six chemical companies in western Germany (Kiesselbach *et al.*, 1990). The 2658 cohort members had

been exposed to ethylene oxide for at least 12 months before 31 December 1982. The year of their first exposure ranged from 1928 to 1981, but most had first been exposed after 1950. Other possible exposures included benzene, 4-aminobiphenyl and 2-naphthylamine, but no information was given on the extent of exposure to those substances. Subjects who had left employment were traced through local registries and, in the case of foreigners who had returned home, by letter or by asking fellow countrymen who were still working in the plant. Of the cohort members, 97.6% were traced successfully to 31 December 1982. For those who had died, the cause of death was ascertained from death certificates (27.6% of all deaths), lay statements, the physician who last treated the patient or hospital reports. Mortality was compared with that expected from five-year age-, sex- and calendar period-specific rates in the national population; no statistics were available for periods before 1951, so the rates for 1951 were used. Altogether, 268 deaths were observed, with 307.6 expected. There were 68 cancer deaths (69.9 expected), including three from oesophageal cancer (1.5 expected), 14 from stomach cancer (10.2 expected) and five from lymphatic and haematopoietic cancer (5.0 expected). Two deaths were ascribed to leukaemia (2.4 expected). When expected numbers were calculated on the basis of rates in the states in which each plant was situated, the findings were much the same. Mortality ratios based on calculations in which the first 10 years of exposure for each subject were ignored were similar to those in the main analysis. It was possible to classify the ethylene oxide exposure of 67.2% of subjects as 'weak', 'medium' or 'high'. The excess of stomach cancer was greatest in those with weak or medium exposure and with less than 15 years of total exposure. When foreign workers were excluded from the analysis, there was no change in the observed number of deaths and mortality ratios were only slightly increased. [The Working Group noted that the definition of the cohort was imprecise, no data were given on likely levels of exposure to ethylene oxide or on the nature of the processes on which subjects worked, and calculation of expected numbers from death certificate data may have been a source of bias since certificates were available for only about one-quarter of deaths in the cohort.]

Gardner *et al.* (1989) studied 2876 workers in four British chemical companies where ethylene oxide or its derivatives had been manufactured and in eight hospitals where ethylene oxide had been used as a sterilant. In one company, ethylene oxide had been produced by the chlorohydrin process during 1950–60 and by direct oxidation of ethylene from 1959 onwards; in the second company, the chlorohydrin process was used during 1955–70 and direct oxidation thereafter; in the third company, ethylene oxide was produced during 1960–81 only by direct oxidation; in the fourth company, ethylene oxide had been used in the manufacture of derivatives since 1959. The eight hospitals had started using ethylene oxide between 1962 and 1972. The cohort comprised all workers at each factory and hospital with likely exposure to ethylene oxide during specified periods for which employment records were complete. Sixteen subjects had to be excluded because information about them was incomplete. Jobs held by cohort members at the factories were classified as having involved definite, probable or possible exposure to ethylene oxide. At the hospitals, jobs were classed as involving continual, intermittent or possible exposure. Environmental and personal monitoring since 1977 had shown a TWA concentration of < 5 ppm [9 mg/m^3] in almost all jobs, but with occasional peaks of exposure up to several hundred parts per million as a result of operating difficulties in the chemical plants and

during loading and unloading of sterilizers in the hospitals. Exposures were thought to have been higher in earlier years, and peak exposures above the odour threshold of 700 ppm were reported both by the chemical manufacturers and at the hospitals. Cohort members at the manufacturing plants were potentially exposed to many other chemicals, including chlorohydrin, propylene oxide, styrene and benzene; some of the hospital workers had occasionally been exposed to formaldehyde and carbon tetrachloride. The cohort was followed up to 1987 through National Health Service and Social Security records, and tracing was 98% successful. Expected numbers of deaths were calculated from national sex-, age- and five-year calendar period-specific rates. Among the 1471 factory employees (all but one were male), there were 157 deaths from all causes (172.0 expected) and 53 deaths from cancer (46.6 expected). The latter included three cases of stomach cancer (4.3 expected), two of non-Hodgkin's lymphoma (1.0 expected) and three of leukaemia (1.3 expected). Two of the leukaemias were acute myeloid and the other was lymphatic unspecified. All three of the leukaemia cases were classed as having had definite exposure to ethylene oxide (0.86 expected), and in each case the death occurred after a latency of at least 20 years from first exposure. On the basis of their job histories, none was thought likely to have been exposed to benzene. Among the 1405 hospital employees (394 men and 1011 women), there were 69 deaths from all causes (86.9 expected) and 32 from cancer (30.0 expected). These included two deaths from stomach cancer (1.7 expected), two from non-Hodgkin's lymphoma (0.6 expected) and none from leukaemia (0.8 expected). Adjustment for local differences in mortality rates had little effect on the expected numbers of leukaemia in the cohort. In the cohort as a whole, there were slight excesses of oesophageal cancer (5/2.2), lung cancer (29/24.6) and bladder cancer (4/2.0), but these were not significant.

A series of studies was carried out on a cohort of 2174 male employees at two chemical plants in West Virginia, USA, where ethylene oxide had been produced and used (Greenberg *et al.*, 1990; Benson & Teta, 1993; Teta *et al.*, 1993). It was produced by the chlorohydrin process during 1925–57 and by direct oxidation from 1937–71. After 1971, the plants continued to use ethylene oxide brought in from elsewhere. The cohort comprised men employed at the plants during 1940–78 and assigned at any time before 1979 to a chemical production department in which ethylene oxide was judged to have been manufactured or used at the time of the assignment. The first large-scale environmental monitoring project at the plant began in 1976. The 8-h TWA concentration of ethylene oxide in departments where it was used was less than 1 ppm [1.8 mg/m^3] but ranged up to 66 ppm [120 mg/m^3]. The authors estimated that the 8-h TWA concentration in ethylene oxide production by direct oxidation in the 1960s ranged from 3 to 20 ppm and that exposures during production by the chlorohydrin process were probably rather higher. Departments were classified as having high, medium or low exposure concentrations according to the operations carried out, and the classification was validated by reference to reported incidents of acute exposure. The cohort was followed to the end of 1988, and vital status was ascertained for more than 98% of subjects. Death certificates were obtained for 99% of decedents, and expected numbers of deaths were calculated on the basis of national five-year age- and calendar period-specific rates in white males.

A total of 278 men had worked in a chlorohydrin unit which primarily produced ethylene chlorohydrin, with ethylene dichloride and bischloroethyl ether as by-products (Benson &

Teta, 1993). For part of the time, propylene chlorohydrin was also made. Ethylene oxide was handled only sporadically and in small volumes. Of these men, 147 died, with 140.8 expected. The deaths included 40 from cancer (30.8 expected), eight from lymphatic and haematopoietic cancer (2.7 expected) and eight from pancreatic cancer (1.6 expected). In a comparison with workers from other plants in the same locality, the risks for cancers of all types, for lymphatic and haematopoietic cancer, leukaemia and pancreatic cancer increased with duration of assignment to the chlorohydrin unit.

Among the 1896 men who had never been assigned to the chlorohydrin unit, there were 431 deaths, whereas 547.7 were expected (Teta *et al.*, 1993). The numbers of observed and expected deaths were 110/128.1 for cancer at any site, 8/5.0 from stomach cancer, 4/6.6 from pancreatic cancer, 6/4.0 from cancers of the brain and nervous system, 7/11.8 from lymphatic and haematopoietic cancer, 2/2.0 from lymphosarcoma and reticulosarcoma (ICD9 200), 5/4.7 from leukaemia and aleukaemia and 0/1.2 from Hodgkin's disease. No significant excess mortality was observed for any cause of death. There were no excesses of leukaemia or stomach cancer among men who had spent two or more years in high-exposure departments. Comparison with death rates of workers from plants in the same location who had never been assigned to ethylene oxide production or use showed no significant trend with duration of assignment for all cancer, leukaemia or pancreatic, brain or stomach cancers; but a two- to three-fold increase in risk for leukaemia (based on three cases) was observed among workers with more than 10 years of assignment to ethylene oxide departments. These studies confirmed and amplified the findings of an earlier case–control study at the same plants (Ott *et al.*, 1989).

Steenland *et al.* (1991) followed up 18 254 employees at 14 US industrial plants where ethylene oxide had been used to sterilize medical supplies or spices or in the testing of sterilizing equipment. The plants were selected because they held adequate records on personnel and exposure and their workers had accumulated at least 400 person-years at risk before 1978. Only workers with at least three months of exposure to ethylene oxide were included in the cohort. Forty five per cent of the cohort were male, 79% were white, 1222 were sterilizer operators and 15 750 were employed before 1978. Analysis of 627 8-h personal samples indicated that average exposure during 1976–85 was 4.3 ppm [7.7 mg/m^3] for sterilizer operators; the average level for other exposed workers, on the basis of 1888 personal samples, was 2.0 ppm [3.6 mg/m^3]. Many companies began to install engineering controls in 1978, and exposures before that year were thought to have been higher. There was no evidence of confounding exposure to other occupational carcinogens. The cohort was followed to 1987 through the national death index and records of the Social Security Administration, the Internal Revenue Service and the US Postal Service, and 95.5% were traced successfully. The expected numbers of deaths were calculated from rates in the US population, stratified according to age, race, sex and calendar year. In total, 1177 cohort members had died (1454.3 expected), including 40 for whom no death certificate was available. There were 343 deaths from cancer (380.3 expected). The observed and expected numbers of deaths were 36/33.8 from all lymphatic and haematopoietic cancer, including 8/5.3 from lymphosarcoma–reticulosarcoma [ICD9 200], 4/3.5 from Hodgkin's disease, 13/13.5 from leukaemia, 8/6.7 from non-Hodgkin's lymphoma [ICD9 202] and 3/5.1 from myeloma; 6/11.6 from cancer of the brain and nervous system; 11/11.6 from cancer of the

stomach; 16/16.9 from cancer of the pancreas; 8/7.7 from cancer of the oesophagus; and 13/7.2 from cancer of the kidney. Mortality ratios for subjects first exposed before 1978 were virtually identical to those for the full cohort. No significant trend in mortality was observed in relation to duration of exposure, but the mortality ratios for leukaemia (1.79 based on five deaths) and non-Hodgkin's lymphoma (1.92 based on five deaths) were higher after allowance for a latency of more than 20 years. Among the sterilizer operators, mortality ratios (and observed numbers of deaths) were 2.78 (two) for leukaemia and 6.68 (two) for lymphosarcoma/reticulosarcoma; no death from stomach cancer was seen.

In a further analysis of the same study (Stayner *et al.*, 1993), a regression model was used to estimate individual exposures to ethylene oxide at 13 of the facilities studied; information about the other facility was inadequate. Mortality from lymphatic and haematopoietic cancer was greatest in the highest category of cumulative exposure to ethylene oxide (> 8500 ppm-days) (standardized mortality ratio [SMR], 124; 95% CI, 66–213; 13 deaths), but the trend across three categories of cumulative exposure was weak (χ^2, 0.97; $p = 0.32$). A similar pattern was observed for non-Hodgkin's lymphoma, but not for leukaemia. The Cox proportional hazards model was also used to examine cumulative exposure (ppm-days), average exposure (ppm), maximal exposure (ppm) and duration of exposure (days) to ethylene oxide. A significant positive trend in risk with increasing cumulative exposure to ethylene oxide was observed for all neoplasms of the lymphatic and haematopoietic tissues ($p < 0.05$, two-tailed). This trend was strengthened when analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukaemia, ICD9 204; non-Hodgkin's lymphoma, ICD9 200, 202). The exposure–response relationship between cumulative exposure to ethylene oxide and leukaemia was positive but nonsignificant. The regression coefficients for neoplasms of the lymphatic and haematopoietic tissues for duration, average and maximal exposure were either weakly positive or negative. Rate ratios for neoplasms of the lymphatic and haematopoietic tissues corresponding to a working lifetime (45 years) of exposure to ethylene oxide at a level of 1 ppm were also estimated. The results given in Table 9 are shown for the best fitting regression models, in which exposures were 'lagged' from 5 to 10 years. Lagging was used in order to discount exposures occurring in previous years that might not be etiologically relevant to the occurrence of the disease. Significantly increased rate ratios of about 1.2 were found for all neoplasms of the lymphatic and haematopoietic tissues, non-Hodgkin's lymphoma and neoplasms of lymphoid cell origin. In this analysis, no significant increase was found for cancers of the stomach, pancreas, brain or kidney.

Wong and Trent (1993) subsequently reported a separate analysis of mortality in much the same population (Steenland & Stayner, 1993), with similar results. The cohort comprised 18 728 employees, and follow-up was to the end of 1988. [The Working Group noted that this report adds little useful information to that provided by Steenland *et al.* (1991).]

Hagmar *et al.* (1991) studied employees at two Swedish plants where disposable medical equipment sterilized with ethylene oxide was produced. In plant A, a 50:50 mixture of ethylene oxide and methyl formate had been used since 1970. In 1973, personal sampling for two packers indicated an exposure to ethylene oxide of 24 ppm [43 mg/m^3]. After 1981, monitoring carried out annually over one to three days for sterilizers and packers showed a continuous decrease in exposure such that, after 1985, only sterilizers were exposed to

Table 9. Results from Cox proportional hazards models for mortality due to lymphatic and haematopoietic neoplasms in which cumulative exposures to ethylene oxide were lagged

Neoplasm	Lag period (years)	β	Standard error	χ^2	Rate ratio for 45 ppm-years	95% CI
All haematopoietic cancers	10	1.12×10^{-5}	4.24×10^{-6}	4.96	1.20	1.05–1.38
Leukaemia	10	1.20×10^{-5}	7.73×10^{-6}	2.07	1.24	0.96–1.58
Non-Hodgkin's lymphoma	10	1.29×10^{-5}	5.36×10^{-6}	3.98	1.24	1.04–1.47
Lymphoid	5	1.20×10^{-5}	3.31×10^{-6}	8.44	1.22	1.09–1.35

From Stayner *et al.* (1993). The results presented are those from models including a lag period that maximizes the goodness of fit (i.e. minimizes the -2 log likelihood). Results from all models were controlled for calendar year, age at risk, sex and race. CI, confidence interval. Confidence intervals for the rate ratios were estimated by computing the upper and lower bound estimates of the regression coefficients ($\beta \pm$ SE) and substituting those bounds into the rate ratio formula. Rate ratios for a particular exposure level were estimated from the formula: $\exp(\beta,\chi)$, where χ is the cumulative exposure in ppm-days. For example, the rate ratio for all haematopoietic neoplasms corresponding to 45 years of exposure at 1 ppm is $\exp[(1.2 \times 10^{-5})$ (45 ppm-years)(365 days/year)].

concentrations greater than 0.2 ppm [0.4 mg/m^3] (the limit of detection of the method used). In plant B, a 50:50 mixture of ethylene oxide and methyl formate was used from 1964 but was replaced by an ethylene oxide:carbon dioxide mixture in 1978. In 1975, personal monitoring indicated exposures of 4–5 ppm [7–9 mg/m^3] ethylene oxide for four packers. After 1985, the 8-h TWA concentration was < 0.2 ppm [0.4 mg/m^3] for all employees except sterilizers and store workers. The authors estimated that sterilizers were exposed to up to 75 ppm [135 mg/m^3] in the earliest years of operation at this plant. On the basis of estimates of exposures in different job categories and time periods, the authors calculated individual cumulative exposures for 97% of subjects at plant A and 89% at plant B. The cohort comprised 594 men and 557 women who had been employed at plant A for at least 12 months between 1970 and 1985 and who were still working after 1 June 1975, and 267 men and 752 women employed at plant B for at least 12 months between 1964 and 1985 and still working after 1 January 1972. These subjects were followed to 1986 for mortality and from 1972 to 1985 for cancer registration. None was lost to follow-up. Expected mortality was calculated on the basis of calendar year-, sex- and five-year age-specific rates (censored at age 80) for the county in which the plants were situated, and expected cancer incidence from corresponding registration rates in the same area. Fifteen deaths were observed (25.7 expected), including eight from cancer (9.0 expected), two from gastrointestinal cancer (2.1 expected) and one from haematopoietic and lymphatic cancer (1.0 expected). The observed and expected numbers of incident cancers were 21/26.8 cancers at any site, no case of stomach cancer (0.5 expected), 1/1.6 for brain cancer, 2/1.3 for lymphoma and myeloma and one case of polycythaemia vera with 0.7 cases of leukaemia, polycythaemia vera and myelofibrosis expected. Among subjects with more than 1 ppm-year of cumulative exposure to ethylene oxide, there were two cases of cancer (3.3 expected) and none of lymphatic or haematopoietic cancer (0.2 expected).

Bisanti *et al.* (1993) studied a cohort comprising all 1971 male chemical workers in the Lombardy and Piedmont regions of Italy who had held a licence to handle ethylene oxide for at least one year during 1938–84; 637 had held licences for ethylene oxide only and 1334 for other toxic gases as well. Some workers may have been exposed to ethylene oxide before getting a licence. The cohort was followed from 1 January 1940 to 31 May 1984, and vital status was ascertained at the census office at each subject's place of residence. Sixteen subjects (0.8%) who were lost to follow-up were considered to be still living. Expected numbers of deaths were calculated from five-year age-, sex- and calendar period-specific rates for the regional (Lombardy) population. Seventy-six deaths were recorded (98.8 expected), including 43 from cancer (33.0 expected). The observed and expected numbers of deaths were 5/4.1 from stomach cancer, 3/1.2 from cancer of the pancreas, 1/0.6 from cancer of the kidney, 4/0.6 from lymphosarcoma and reticulosarcoma and 2/1.0 from leukaemia. The two deaths from leukaemia occurred among men with fewer than five years' exposure and after a latency of fewer than 10 years since first exposure to ethylene oxide. Among the men who had held licences only for ethylene oxide, there were 27 deaths (30.1 expected), 15 from cancer (10.5 expected), including one from stomach cancer (1.3 expected), three from lymphosarcoma and reticulosarcoma (0.2 expected) and two from leukaemia (0.3 expected). Results obtained with national mortality rates as the basis for expected numbers were similar. [The Working Group noted that no data were available on levels of exposure to ethylene oxide or on exposure to other chemicals.]

Epidemiological findings on ethylene oxide are summarized in Table 10.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 50 female Sprague-Dawley rats, about 100 days old, were administered ethylene oxide (purity, 99.7%) at 7.5 or 30 mg/kg bw in a commercial vegetable oil [composition unspecified] by gastric intubation twice weekly for 107 weeks (average total dose, 1186 or 5112 mg/kg bw, respectively). Control groups consisted of 50 untreated female rats and 50 female rats treated with vegetable oil alone. The survival rate of rats in the high-dose group was lower than that of the control groups. Treatment with ethylene oxide resulted in a dose-dependent increase in the incidence of forestomach tumours, which were mainly squamous-cell carcinomas. Such tumours were not found in the untreated or vehicle controls. In total, 31/50 treated animals developed malignant tumours of the stomach; 29 were squamous-cell carcinomas of the forestomach and two were fibrosarcomas, one of which was located in the glandular stomach. In addition, 4/50 had carcinomas *in situ* and 11/50 had papillomas, hyperplasia or hyperkeratosis of the squamous epithelium of the forestomach. In the low-dose group, 8/50 animals developed squamous-cell carcinomas, four had carcinomas *in situ* and nine had papillomas, hyperplasia or hyperkeratosis in the forestomach. Of the 37 squamous-cell carcinomas found in the two dose groups, 10 metastasized or grew invasively into neighbouring organs. There was no increase in the incidence of tumours at other sites in the treated animals over that in controls (Dunkelberg, 1982).

Table 10. Summary of epidemiological findings on ethylene oxide

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Hogstedt *et al.* (1986); Hogstedt (1988) (Sweden)	Production of sterilized supplies; 1978–82; 203 subjects; 1 year; 100%	5	4 2	[2.5] [15]	[0.68–6.4] [1.9–56]	All neoplasms L&H	Estimated average past exposure in storage room was 20 ppm; one leukaemia was part of a cluster which had originally prompted the study.
Hogstedt *et al.* (1979a, 1986); Hogstedt (1988) (Sweden)	Ethylene oxide production plant (one facility); 1961–85; 241 subjects, of which 89 'full-time operators'; 1 year; 100%	34	14 5 2	[2.3] [8.3] [10]	[1.3–4.8] [2.9–21] [1.2–36]	All neoplasms Stomach Leukaemia	Estimated average exposure before 1963, 5–25 ppm; mortality rates shown only for 'full-time operators' (high-exposure group); no overall excess tumour mortality among workers with intermittent exposure or those unexposed; excess mortality from stomach cancer (4 deaths, SMR, 6.67) and from leukaemia (1 death; 0.2 expected) among workers with intermittent exposure
Hogstedt *et al.* (1986) (Sweden)	Ethylene oxide production (one plant); 1964–81; 355 subjects; 1 year; 100%	8	1[a]	–	–	Leukaemia	The one case of leukaemia (0.16 expected) was in a maintenance worker with multiple exposures; average exposure in 1963–76, 1–8 ppm; after 1977, 0.4–2 ppm
Morgan *et al.* (1981) (USA)	Production of ethylene oxide; 1955–77; 767 men; 5 years; around 95%	46	11 2 0	0.72 5.7 0	0.36–1.3 0.64–21 0–5.2	All neoplasms Hodgkin's disease Leukaemia	High percentage of deaths of unknown cause (9%); limited information on manufacturing processes and exposure concentrations; exposures probably below 10 ppm with occasional peaks to 6000 ppm; nonsignificant excess risks from cancer of the pancreas and cancers of the central nervous system
Divine (unpublished); reported by Shore *et al.* (1993) (USA)	Updating of Morgan *et al.* (1981); 1955–85; 99.7%	Not applicable	3 0	[1.0] [0]	[0.21–2.9] [0.0–3.4]	Hodgkin's disease Leukaemia	
Kiesselbach *et al.* (1990) (Germany)	Chemical plants (8 facilities); 1928–82; 2658 men; 1 year; 97.6%	268	68 14 5 2	0.97 1.4 1.0 0.85	0.76–1.2 0.75–2.3 0.32–2.3 0.10–3.1	All neoplasms Stomach L&H Leukaemias	No information on exposure concentrations or on nature of production processes; most of study population of Thiess *et al.* included.

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Gardner *et al.* (1989) (UK)	Production or use of ethylene oxide (4 facilities); 1956–87; 1471 subjects; no minimal employment; around 98%	157	53 3 3 2	1.1 0.7 2.3 [1.9]	[0.85–1.5] [0.15–2.1] [0.47–6.6] [0.23–7.0]	All neoplasms Stomach Leukaemia Non-Hodgkin's lymphoma	Average exposure after 1977 was to less than 5 ppm (< 1 ppm in many jobs), with occasional peak exposures of several hundred ppm; highest mortality from leukaemia among subjects with definite exposure to ethylene oxide; risk increased with latency of exposure; non-significant excess risks for cancers of the oesophagus, lung and bladder.
	Hospital sterilization units (8 hospitals); 1964–87; 1405 subjects; no minimal exposure; around 98%	69	32 2 0 2	1.1 1.2 0 [3.5]	0.73–1.5 0.15–4.3 0–4.9 [0.42–13]	All neoplasms Stomach Leukemia Non-Hodgkin's lymphoma	
Benson & Teta (1993) (USA)	Work in a chlorohydrin unit and potential exposure to ethylene oxide (2 facilities); 1940–88; 278 men; no minimal employment; 98%	147	40 1 8 4	1.3 [0.7] 2.9 [3.5]	0.93–1.8 0.02–3.9 1.3–5.8 0.96–8.9	All neoplasms Stomach L&H Leukaemia	Updating of study by Greenberg *et al.* (1990), including only workers ever employed in the chlorohydrin department; excess of pancreatic cancer (8 deaths, SMR, 4.9; 95% CI, 1.6–11).
Teta *et al.* (1993) (USA)	Production or use of ethylene oxide (2 facilities); 1940–88; 1896 men; no minimal employment; 99%	431	110 8 7 5	0.86 1.6 0.59 1.1	0.71–1.0 0.69–3.2 0.24–1.2 0.35–2.5	All neoplasms Stomach L&H Leukaemia	Average exposure in production departments < 1 ppm, but occasionally up to 66 ppm 8-h TWA. Updating of study by Greenberg *et al.* (1990), excluding workers ever employed in the chlorohydrin department; in an internal comparison with workers in the same complex, a two- to three-fold increase in leukaemia risk was observed for workers exposed for more than 10 years to ethylene oxide.
Steenland *et al.* (1991); Stayner *et al.* (1993) (USA)	Production of sterilized medical supplies and spices (14 facilities); 1943–87; 18 254 subjects; 3 months; 95.5%	1117	343 11 36 13 [16]	0.90 0.95 1.06 0.97 [1.3]	0.81–1.0 0.45–1.7 0.75–1.5 0.52–1.7 [0.76–2.2]	All neoplasms Stomach L&H Leukaemia [Non-Hodgkin's lymphoma; ICD9 200, 202]	Recent average exposure of sterilizer operators was 4.3 ppm, that of other workers was 2.0 ppm; no significant trend in mortality from L&H with duration of exposure; mortality from L&H increased with latency (SMR at ≥ 20 years since first exposure, 1.8 [95% CI, 0.94–3.0]); test for linear trend, $p = 0.03$; increased risk for L&H with cumulative exposure (for results by cumulative exposure, see Table 9); mortality from kidney cancer was also elevated (SMR, 1.8, 13 deaths) and increased with latency

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Hagmar *et al.* (1991) (Sweden)	Production of disposable medical equipment (2 facilities); 1964–86; 2170 subjects; 1 year; 98.2%	15	21[a] 3 0	0.78 1.5 0	0.49–1.2 0.32–4.5 0–7.4	All neoplasms L&H Stomach	Average estimated exposure of sterilizers, around 40 ppm in 1970–72, less than 1 ppm in 1985; packers, around 35–40 ppm in 1970–72, less than 0.2 after 1985; no trend in risk with increasing cumulative exposure but only 0.2 expected cases of L&H in 'high' exposure group (> 1 ppm-year).
Bisanti *et al.* (1993) (Italy)	Workers licenced to handle ethylene oxide; 1940–84; 1971 men; 1 year with licence; 99.2%	76	43 6 2 4 5	1.3 2.5 1.9 6.8 1.2	0.94–1.8 0.91–5.5 0.23–7.0 1.9–17 0.40–2.9	All neoplasms L&H Leukaemias Lympho- and reticulosarcoma Stomach	Increased mortality from all types of cancer; no increase in risk for L&H with latency or duration of exposure; risk for L&H highest among workers licenced only for ethylene oxide (5 deaths; SMR, 7.0; 95% CI, 2.3–16); no information on exposure levels

RR, risk estimate: standardized mortality ratio, SMR, unless otherwise specified; CI, confidence interval; L&H, neoplasms of the lymphatic and haematopoietic tissues

[a]Cancer cases, standardized incidence ratio

3.2 Inhalation

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, eight weeks of age, were exposed by inhalation to 0, 50 or 100 ppm (0, 92 or 183 mg/m^3) ethylene oxide (> 99% pure) for 6 h per day on five days per week for up to 102 weeks, at which time the experiment was terminated. Mean body weights of treated males and females were similar to those of controls. At the end of the study, 28/50 control males, 31/50 low-dose males and 34/50 high-dose males, and 25/50 control females, 24/50 low-dose females and 31/50 high-dose females were still alive. The incidences of alveolar/bronchiolar carcinomas in male mice were 6/50 control, 10/50 low-dose and 16/50 high-dose ($p = 0.017$, incidental tumour test for trend). A slight increase in the incidence of alveolar/bronchiolar adenomas also occurred. The combined incidences of lung tumours were 11/50 control, 19/50 low-dose and 26/50 high-dose ($p = 0.002$, incidental tumour test for trend). In females, the incidences of alveolar/bronchiolar adenomas (2/49 control, 4/48 low-dose and 17/49 high-dose) and alveolar/bronchiolar carcinomas (0/49 control, 1/48 low-dose and 7/49 high-dose) and the combined incidence of lung tumours (2/49 control, 5/48 low-dose and 22/49 high-dose) were all significantly increased ($p < 0.001$, incidental tumour test for trend). The incidence of papillary cystadenoma of the Harderian gland increased significantly in animals of each sex (males: 1/43 control, 9/44 low-dose and 8/42 high-dose; females: 1/46 control, 6/46 low-dose and 8/47 high-dose; $p < 0.05$, incidental tumour test for trend). In addition, one papillary cystadenocarcinoma of the Harderian gland was observed in a high-dose male mouse and one in a low-dose female mouse. In females, the incidences of malignant lymphomas were 9/49 control, 6/48 low-dose and 22/49 high-dose ($p = 0.023$, life-table test for trend). An increase in the incidence of uterine adenocarcinomas was observed: 0/49 control, 1/47 low-dose and 5/49 high-dose ($p = 0.019$, incidental tumour test for trend). In females, the incidences of mammary gland carcinomas were 1/49 control, 8/48 low-dose ($p = 0.012$, incidental pair-wise tumour test) and 6/49 high-dose ($p = 0.087$, incidental pair-wise tumour test) (US National Toxicology Program, 1987).

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to development of this neoplasm, groups of 30 female strain A/J mice, eight to ten weeks of age, were exposed by inhalation to ethylene oxide (at least 99.7% pure) at 0, 70 or 200 ppm (0, 128 or 366 mg/m^3) for 6 h per day on five days per week for up to six months in two independent experiments; in the second experiment, the 70 ppm group was omitted. Two groups of 30 female mice were exposed to room air and served as negative controls, and two groups of 20 animals received a single intraperitoneal injection of urethane (1000 mg/kg bw) and served as positive controls for both experiments. At the end of the sixth month, the survivors were killed and examined for pulmonary adenomas. In the first experiment, survival was 30/30 (0 ppm), 28/30 (70 ppm), 29/30 (200 ppm) and 19/20 (urethane); that in the second was 29/30 (0 ppm), 28/30 (200 ppm) and 19/20 (urethane). The numbers of animals with pulmonary adenomas among survivors in the first experiment were: untreated controls, 8/30 (0.46 ± 0.38 adenomas/mouse); low-dose, 16/28 (0.86 ± 0.45); high-dose, 25/29 (2.14 ± 0.49); and urethane-treated, 19/19 (20.1 ± 1.77); the numbers in the second experiment were: untreated controls, 8/29 (0.22 ± 0.38); ethylene oxide-treated,

12/28 (0.73 ± 0.98); and urethane-treated, 19/19 (23.5 ± 6.49). In the first experiment, the number of lung tumour-bearing animals increased significantly in a dose-dependent manner [$p < 0.0001$ Cochran-Armitage trend test]; in the second, a slight, nonsignificant increase was observed, although the high dose was the same as that used in the first experiment. The number of tumours per surviving mouse increased significantly in each experiment ($p < 0.05$, Duncan's new multiple-range test) (Adkins *et al.*, 1986).

3.2.2 *Rat*

Groups of 120 male and 120 female Fischer 344 rats, eight weeks of age, were exposed by inhalation to ethylene oxide (purity, > 99.9%) vapour at 10, 33 or 100 ppm (18, 59 or 180 mg/m^3) for 6 h per day on five days per week for two years. Two control groups, each of 120 male and 120 female rats, were exposed in inhalation chambers to room air. All animals that died or were killed when moribund and those killed at scheduled intervals of 6, 12, 18 and 24–25 months were examined. During month 15 of exposure, mortality increased in both treated and control groups due to a viral sialodacryoadenitis. Mortality was higher in the groups inhaling 33 and 100 ppm ethylene oxide than in the other groups and was more frequent in females than in males near the fifteenth month. Up to 18 months of exposure, no significant increase in tumour incidence was observed. In treated rats killed after 18 months, the incidence of tumours in the brain classified as 'gliomas, malignant reticulosis and granular-cell tumours' was increased for animals of each sex. The incidences of glioma among rats killed at 18 and 24–25 months were: males: 1/181 (controls), 0/92 (10 ppm), 3/86 (33 ppm) and 6/87 (100 ppm) ($p < 0.05$, trend analysis and Fisher's exact test for high dose *versus* control); and females: 0/187 (controls), 1/94 (10 ppm), 2/90 (33 ppm) and 2/78 (100 ppm) ($p < 0.05$, trend analysis). In females killed after 24 months of exposure, mononuclear-cell leukaemia was found in 5/60 (control I), 6/56 (control II), 11/54 (10 ppm), 14/48 (33 ppm) and 15/26 (100 ppm) animals; the incidence of leukaemia was reported by the authors to be significantly increased in the 100-ppm group ($p < 0.001$) and in a mortality-adjusted trend test ($p < 0.005$). In males, mononuclear-cell leukaemia was found in 5/48 (control I), 8/49 (control II), 9/51 (10 ppm), 12/39 (33 ppm) and 9/30 (100 ppm) animals ($p < 0.05$ in a mortality-adjusted trend test). Peritoneal mesotheliomas originating in the testicular serosa were found in 1/48 (control I), 1/49 (control II), 2/51 (10 ppm), 4/39 (33 ppm) and 4/30 (100 ppm) males ($p < 0.005$ trend test). The incidence of subcutaneous fibromas in male rats of the high-dose group was also significantly increased: 1/48 (control I), 2/49 (control II), 9/51 (10 ppm), 1/39 (33 ppm) and 11/30 (100 ppm) ($p < 0.001$) (Snellings *et al.*, 1984a; Garman *et al.*, 1985, 1986).

Groups of 80 male weanling Fischer 344 rats were exposed by inhalation to ethylene oxide (purity, 99.7%) vapour at 0 (control; filtered air), 50 or 100 ppm (92 or 180 mg/m^3) for approximately 7 h per day on five days per week for two years. The mortality rate was increased in the two treated groups over that in controls, and the increase was significant for the high-dose group ($p < 0.01$). Mononuclear-cell leukaemia was observed in 24/77 control rats, 38/79 exposed to 50 ppm ethylene oxide and 30/76 exposed to 100 ppm. The overall increase in the incidence of mononuclear-cell leukaemia was significant ($p = 0.03$) in the low-dose group, but the increase could not be ascertained in the high-dose group owing to excessive mortality. Peritoneal mesotheliomas in the region of the testis developed in 3/78

control, 9/79 low-dose and 21/79 high-dose rats; the increase was significant for the high-dose group ($p = 0.002$). Gliomas were found in 0/76 control, 2/77 low-dose and 5/79 high-dose animals ($p < 0.05$, pair-wise comparison for the high dose). Focal proliferation of glial cells, termed 'gliosis', was observed in two rats exposed to 50 ppm and in four rats exposed to 100 ppm ethylene oxide. The incidences of other neoplasms were comparable in the control and treated groups and were not associated with exposure to ethylene oxide. A high incidence of proliferative lesions described as 'multifocal cortical hyperplasia' and 'cortical nodular hyperplasia' was observed in the adrenal cortex of animals exposed to ethylene oxide (Lynch *et al.*, 1984a).

3.3 Skin application

Mouse: Thirty female ICR/Ha Swiss mice, eight weeks of age at the start of treatment, were painted with about 100 mg of a 10% solution of ethylene oxide (purity, 99.7%) in acetone per application on the clipped dorsal skin three times per week for life. The median survival time was 493 days. No skin tumour was observed (Van Duuren *et al.*, 1965).

3.4 Subcutaneous administration

Mouse: Groups of 100 female NMRI mice, six to eight weeks old, received subcutaneous injections of ethylene oxide (purity, 99.7%) in tricaprylin at 0.1, 0.3 or 1.0 mg/mouse once per week for 95 weeks (mean total dose, 7.3, 22.7 and 64.4 mg/mouse). Groups of 200 untreated and 200 tricaprylin-treated mice served as controls. The survival rate of the group given the highest dose was reduced. Ethylene oxide induced a dose-dependent increase in the incidence of tumours at the injection site: 0/200 untreated controls, 4/200 animals treated with tricaprylin alone, and 5/100 (0.1 mg), 8/100 (0.3 mg) and 11/100 (1 mg) ethylene oxide-treated animals [$p < 0.001$, Cochran-Armitage test for trend]. No significant increase in the incidence of tumours at other sites was observed (Dunkelberg, 1981).

3.5 Induction of enzyme-altered foci in a two-stage liver system

Rat: Groups of male and female Sprague-Dawley rats, three to five days of age, were exposed by inhalation to ethylene oxide [purity unspecified] at 0 ppm (5 male and 9 female rats), 33 ppm (60 mg/m^3, 10 females), 55 ppm (100 mg/m^3, 4 males and 7 females) or 100 ppm (183 mg/m^3, 4 males and 8 females) for 8 h per day on five days per week for three weeks. One week later, the rats were administered 10 mg/kg bw Clophen A 50 (a mixture of polychlorinated biphenyls [not otherwise specified]) orally by gavage twice a week for up to eight additional weeks (promotion), at which time the experiment was terminated. The livers were examined for ATPase-deficient and γ-glutamyltranspeptidase (GGT)-positive foci. In females receiving the two highest doses, but not in males, the number and total area of ATPase-deficient foci increased significantly ($p < 0.05$, t test) in comparison with the controls receiving Clophen A 50 only. There was no significant difference between controls and animals given the high dose of ethylene oxide in the number or total area of GGT-positive foci (Denk *et al.*, 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

(a) Ethylene oxide

Ethylene oxide is readily taken up by the lungs. A study on workers exposed to ethylene oxide revealed an alveolar retention of 75–80%, calculated from hourly determinations of ethylene oxide concentrations in environmental air ranging from 0.2 to 22.5 mg/m^3 [0.11–12.3 ppm] and in alveolar air from 0.05 to 7 mg/m^3 [0.03–3.8 ppm] (Brugnone *et al.*, 1985, 1986). At steady state, therefore, 20–25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound and 75–80% is taken up by the body and metabolized. Blood samples taken from workers 4 h after the work shift and later gave venous blood:alveolar air coefficients of 12–17 and venous blood:environmental air coefficients of 2.5–3.3. The difference from the value of 90 determined for the blood:air partition coefficient *in vitro* was explained by incomplete saturation of tissues and limitation of the metabolic rate by the lung uptake rate (Brugnone *et al.*, 1986).

The data set of Brugnone *et al.* (1986) was used by two groups to estimate the elimination rate constant for ethylene oxide. A value of 3/h, corresponding to a 14-min half-life of ethylene oxide in the body, was calculated by Osterman-Golkar and Bergmark (1988) on the basis of an alveolar retention of 80%, a venous blood:environmental air coefficient of 3.3 (see above) and the following assumptions: (i) steady-state conditions; (ii) elimination according to first-order kinetics; (iii) equal distribution of ethylene oxide within the body; and (iv) alveolar ventilation of 0.2 L/min per kg bw. Filser *et al.* (1992) calculated a 42-min half-life of ethylene oxide in the body, corresponding to an elimination rate constant of 1/h, by fitting an exponential function to the time dependence of the mean alveolar air:environmental air ratio given for ethylene oxide by Brugnone *et al.* (1986). The procedure chosen by Filser *et al.* (1992) relied on two assumptions: (i) steady-state conditions; and (ii) elimination according to first-order kinetics.

Using data obtained by Filser and Bolt (1984) in studies of rats, the half-life of ethylene oxide has been calculated as 3.3 h (Beliles & Parker, 1987) and 39 min (Filser *et al.*, 1992) on the basis of allometric scaling with body surface factors (two-thirds body weight). The difference is due to the scaling methods: the lower value was calculated on the basis of the scaled elimination clearance (Filser, 1992), and the higher value was scaled directly from the half-life. Using the latter method for data obtained in studies on dogs (Martis *et al.*, 1982), Beliles and Parker (1987) estimated a half-life of 2.4 h.

Pharmacokinetic data obtained in animals have been used to calculate the internal dose of ethylene oxide in man derived from daily exposure. For a man exposed for 8 h to ethylene oxide at an air concentration of 1.8 μg/L [1 ppm], the area under the concentration–time curve in blood plasma was estimated to be 18.8 μg×h/ml on the basis of data for rats and 14.3 μg×h/ml on the basis of data for dogs (Beliles & Parker, 1987).

The pharmacokinetics of ethylene oxide as a metabolite of ethylene are summarized in the monograph on ethylene.

(*b*) *Metabolites*

Ethylene oxide is eliminated metabolically by hydrolysis and by conjugation with glutathione. Blood concentrations of ethylene glycol were determined at the end of day 3 of a normal working week in sterilization personnel exposed to ethylene oxide. TWA concentrations determined over 8 h ranged from 0.3 to 52 ppm [0.55–95.2 mg/m^3] (overall mean, 4.2 ppm [7.7 mg/m^3]). The mean concentrations of ethylene glycol in blood of exposed subjects were twice as high (90 mg/L) as those in unexposed ones (45 mg/L) (Wolfs *et al.*, 1983).

The concentration of thioethers excreted in urine collected at the end of sterilization processes was found to be twice as high in nonsmoking personnel (10.2 mmol/mol creatinine) exposed to peak concentrations of 1–200 ppm ethylene oxide [1.83–366 mg/m^3] as in unexposed workers (5.46 mmol/mol creatinine). The concentration of ethylene oxide in air was not monitored routinely (Burgaz *et al.*, 1992).

The disappearance of ethylene oxide was investigated in the gas phase of closed vials containing glutathione and cytoplasm of erythrocytes obtained from a study population (n = 36 [not further specified]). Ethylene oxide was eliminated three to six times faster in samples from the three-quarters of the population who were so-called conjugators (defined by a standardized conjugation of methyl bromide and glutathione) than in those from the remaining quarter, in whom disappearance did not differ from that of controls (Hallier *et al.*, 1993).

(*c*) *Binding to haemoglobin and DNA*

Ethylene oxide, an electrophilic agent, alkylates nucleophilic groups in biological macromolecules. Haemoglobin (Hb) adducts have been used to monitor tissue doses of ethylene oxide (Calleman *et al.*, 1978; Farmer *et al.*, 1987; Osterman-Golkar, 1988; Ehrenberg, 1991; Ehrenberg & Törnqvist, 1992). Sensitive methods involving gas chromatography–mass spectrometry, gas chromatography–electron capture detection and radioimmunoassay have been developed for the determination of *N*-2(hydroxyethyl) (HOEt) adducts at histidine-N$^\tau$ (HOEtHis) and at N-terminal valine (HOEtVal) in Hb of humans occupationally exposed to ethylene oxide (Farmer *et al.*, 1986; Mowrer *et al.*, 1986; Törnqvist *et al.*, 1986; Wraith *et al.*, 1988; Kautiainen & Törnqvist, 1991; Törnqvist *et al.*, 1992).

HOEtHis levels were investigated in workers engaged for 1–14 years in ethylene oxide manufacture. Concentrations measured between 1974 and 1981 were generally below the detection limit of 0.05 ppm [0.09 mg/m^3], with occasional transient concentrations of up to 8 ppm [14.6 mg/m^3]. Without taking smoking habits into consideration, van Sittert *et al.* (1985) determined a mean HOEtHis level of 2080 pmol/g globin, which did not differ significantly from the level of 1590 pmol/g globin found in unexposed controls (van Sittert *et al.*, 1985).

Higher concentrations of HOEtVal adducts were measured in workers occupationally exposed to ethylene oxide than in controls, and workers in loading operations had higher concentrations than those in manufacture (van Sittert & van Vliet, 1994).

In workers exposed to low, intermediate and high concentrations of ethylene oxide [not further specified], the HOEtHis levels (pmol/g Hb) were 550–1000, 2000 and 5300, and 2000 and 8000, while the HOEtVal levels (pmol/g Hb) were 20–410, 980 and 4600, and 1500 and

7700. Hb adduct concentrations in controls were 530–1600 pmol/g Hb for HOEtHis and 30–930 pmol/g Hb for HOEtVal. A linear correlation was found between HOEtHis and HOEtVal, with a slope of 1; HOEtHis levels were on average 600 pmol/g Hb higher than the corresponding HOEtVal levels (Farmer *et al.*, 1986; Bailey *et al.*, 1987). The reason for the high background level of HOEtHis is unknown. Two speculations seem reasonable: trace amounts of HOEtHis might be formed during the analytical procedure; alternatively, during protein synthesis, HOEtHis may be incorporated as such (Farmer *et al.*, 1986).

Workers at one plant who were exposed daily to ethylene oxide at concentrations of < 8–312 ppm×h [14.6–571 mg/m^3×h] had concentrations of HOEtHis ranging from 400 to 14 300 pmol/g Hb. In two other plants, where daily exposures were estimated to be 8–16 ppm×h [14.6–29.2 mg/m^3×h], HOEtVal concentrations were 700 to about 10 000 pmol/g Hb. These data were used to estimate the first-order rate constant of ethylene oxide elimination from the organism. Taking into consideration the life-time of erythrocytes, the reaction constant of ethylene oxide with histidine-N$^\tau$ in Hb and the HOEtHis concentrations, the elimination rate constant was calculated to be < 2.6–54/h [corresponding half-lives, > 16 and 0.8 min]. Values obtained using the HOEtVal concentrations and the reaction constant of ethylene oxide with the N-terminal valine in Hb were ~1–≤ 8.8/h [corresponding half-lives, ~42 and ≥ 4.7 min] (Osterman-Golkar & Bergmark, 1988).

Background levels of HOEtVal in nonsmokers not exposed to ethylene oxide have been reported to be 11–188 pmol/g Hb (see monograph on ethylene, p. 54). Högstedt *et al.* (1990) investigated HOEtVal levels in two groups of workers in the same factory. One group, referred to as sterilizers, was exposed during 8-h shifts to atmospheric ethylene oxide at a concentration of about 2 ppm [3.7 mg/m^3]; the other group assembled electronic equipment about 100 m from the sterilizer. The HOEtVal concentrations were 1900–10 000 pmol/g Hb in the sterilizers and 850–2300 pmol/g Hb in the assemblers; the concentration in a group exposed only to propylene oxide was 20–870 pmol/g Hb. The results did not indicate an influence of smoking.

A cohort of workers exposed to ethylene oxide at 0.02, 0.1 and > 0.2 ppm [0.037, 0.18 and > 0.37 mg/m^3], estimated by personal air monitoring, had HOEtVal concentrations of 50, 230 and 1380 pmol/g Hb. On the basis of the previous finding that HOEtVal is formed at a rate of 2400 pmol/g Hb per ppm of exposure to ethylene oxide during a work shift of 8 h, the measured HOEtVal concentrations were used to estimate the corresponding concentrations of ethylene oxide at the workplace, after correction for smoking habits and for a background level of 20 pmol/g Hb in controls. The predicted values, 0.02, 0.1 and 0.7 ppm, were in agreement with the ranges estimated from personal air monitoring (Hagmar *et al.*, 1991).

HOEtVal concentrations were determined in three groups of nonsmokers exposed occupationally to ethylene oxide. One group was exposed once or twice a week for about 10 min to ethylene oxide at air concentrations ranging from 22 to 72 ppm [40–132 mg/m^3]; the other groups were exposed to an average concentration of about 17 ppm [31 mg/m^3] ethylene oxide either daily or occasionally. The mean concentrations of HOEtVal increased from 32 pmol/g Hb in matched controls to 80 pmol/g Hb in subjects in the first group and from 32 pmol/g Hb in matched controls to 13 200 pmol/g Hb in subjects exposed daily and to 2720 pmol/g Hb in subjects exposed occasionally. On the basis of the relationship between

adduct and exposure levels used by Hagmar *et al.* (1991), the 40-h TWA concentration of ethylene oxide was calculated as 0.025 ppm [0.046 mg/m^3] at the low dose and as 5 ppm [9.2 mg/m^3] at the high dose. These values were several times lower than those measured in air samples. The use of gas masks was discussed as a possible reason for this discrepancy (Tates *et al.*, 1991a, 1992).

An 8-h TWA exposure concentration of about 0.046 ppm [0.084 mg/m^3] was estimated by air monitoring for plant workers exposed to ethylene oxide. The HOEtVal concentration in nonsmokers in the group (about 139 μmol/mol Hb [2160 pmol/g Hb]) differed significantly from that in controls (45 μmol/mol Hb [700 pmol/g Hb]). An 8-h TWA exposure concentration of 0.008 ppm [0.015 mg/m^3] was estimated for the control group by personal monitoring (Mayer *et al.*, 1991). The values for HOEtVal are one order of magnitude higher than would have been expected from the relationship between adduct level and ethylene oxide concentration. The discrepancy is due to the use of d_4-hydroxyethylvaline instead of d_4-hydroxyethylated globin as internal standard. A 10-fold lower yield from the free amino acid was noted in a subsequent inter-laboratory comparison of methods (Törnqvist *et al.*, 1992) .

At nine US and one Mexican hospital, workers exposed to ethylene oxide were divided according to four-month cumulative exposures of > 0–32 ppm×h [> 0–59 mg/m^3×h] and > 32 ppm×h [> 59 mg/m^3×h]. The mean exposures were estimated to be 12.8 and 105.2 ppm×h [23.4 and 193 mg/m^3×h] in the US hospitals and 10.5 and 349.1 ppm×h [19.2 and 639 mg/m^3×h] in the Mexican hospital. The corresponding 8-h TWA concentrations, weighted by duration of each job task, were estimated to be 0.04 and 0.16 ppm [0.07 and 0.29 mg/m^3] and 0.02 and 0.54 ppm [0.037 and 1 mg/m^3]. After adjustment for confounding factors, including smoking habits, the mean concentrations of HOEtVal were determined by radioimmunoassay to be 90 and 160 pmol/g Hb in the USA and 60 and 160 pmol/g Hb in the Mexican workers. In a US hospital control group, the mean background level of HOEtVal was found to be 60 pmol/g Hb; the level in one Mexican worker not exposed to ethylene oxide was 140 pmol/g Hb. A significant correlation was seen between cumulative dose of ethylene oxide and HOEtVal concentration in both groups of workers (Schulte *et al.*, 1992).

In a study of workers at an ethylene oxide producing plant, concentrations of HOEtVal were determined during three successive annual health assessments (van Sittert *et al.*, 1993). The median increments in HOEtVal concentration were determined by radioimmunoassay and gas chromatography–mass spectrometry to be 145, 238 and 53 pmol/g Hb. Using the relationship between adduct and exposure levels of Hagmar *et al.* (1991), the median four-month 8-h TWA concentrations of ethylene oxide were estimated to be 0.056, 0.1 and 0.02 ppm [0.1, 0.18 and 0.037 mg/m^3]. These results are consistent with measurements made during normal plant operations which showed 8-h airborne ethylene oxide concentrations to be below 0.5 ppm. The authors concluded that determination of HOEtVal in Hb is a sensitive method for monitoring low, time-integrated levels of ethylene oxide.

In the population of 36 subjects, of whom 27 were characterized as 'conjugators' and nine as 'non-conjugators' (see p. 105) in terms of the enzymic conjugation of ethylene oxide with glutathione in erythrocytes (Hallier *et al.*, 1993), blood was taken from three individuals in each group and incubated at 37 °C over 4 h with ^{14}C-ethylene oxide. Radioactivity bound to blood plasma and erythrocytes was determined in the low-relative-molecular-mass

fractions (< 10 kDa), containing glutathione, and in the high-relative-molecular-mass fractions (> 10 kDa), containing proteins, such as albumin and Hb. Counts in blood from conjugators were significantly higher in both low-relative-molecular-mass fractions and significantly lower in the high-relative-molecular-mass fraction of erythrocytes than in blood from non-conjugators. No significant difference between conjugators and non-conjugators was seen in the amount of radioactivity associated with the high-relative-molecular-mass fraction of blood plasma. Radioactivity counts in lymphocytes, ascribed to DNA adducts of ^{14}C-ethylene oxide, were similar in the two groups (Föst *et al.*, 1991; Gansewendt *et al.*, 1991).

4.1.2 *Experimental systems*

(*a*) *Ethylene oxide*

The permeation rate of a solution of 1% ethylene oxide in water (w/v) through excised human skin at 30 °C was determined to be 0.125 mg/(cm^2×h) (Baumbach *et al.*, 1987).

The pharmacokinetics of inhaled ethylene oxide have been investigated in male Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al.*, 1992). The studies were carried out in closed exposure chambers of 6.4 and 9.5 L occupied by two and three rats, respectively. The initial concentrations of ethylene oxide vapour in the chamber atmospheres were up to about 1100 ppm [2000 mg/m^3]. Filser and Bolt (1984) showed that ethylene oxide is rapidly taken up by the lungs, as the clearance due to uptake, reflecting the rate of transfer of ethylene oxide from the atmosphere into the organism, was 11 100 ml/h (185 ml/min) for two Sprague-Dawley rats of 500 g bw. Johanson and Filser (1992) calculated a value of 58 ml/min for one animal of 250 g bw by allometric scaling, according to the method of Filser (1992). This value represents 50% of the alveolar ventilation (117 ml/min; Arms & Travis, 1988), indicating that about 50% of the amount inhaled into the lung is exhaled again without becoming systemically available via the bloodstream. A possible explanation for this finding is that there is a 'wash in–wash out' effect in the upper airways (Johanson & Filser, 1992), which may be more effective in rodents than in humans (Filser *et al.*, 1993). The maximal accumulation of ethylene oxide in the body of Sprague-Dawley rats, determined as the thermodynamic partition coefficient whole body:air, was 30. Owing to fast metabolic elimination, the concentration ratio at steady-state whole body:air, calculated for two animals of 500 g bw, was only 1.52 over the entire dose range. A recalculation of this parameter according to Filser (1992) for one rat weighing 250 g bw yielded a value of 1.88, which is similar to the coefficient for venous blood:environmental air found in workers exposed to ethylene oxide under steady-state conditions (see above).

An almost uniform distribution of ethylene oxide within the body was concluded from the similar tissue:air partition coefficients for organs of male Fischer 344 rats determined *in vitro*: fat, 44.1; muscle, 48.3; brain, 58.7; lung, 60.9; liver, 61.6; blood, 64.1; testes, 83 (Krishnan *et al.*, 1992).

Elimination of ethylene oxide was described by first-order kinetics over the whole concentration range examined, in both Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al.*, 1992). At steady state, the clearance due to metabolism in relation to the concentration in the atmosphere (Cl_{tot} of Filser & Bolt, 1984) was 10 600 ml/h (177 ml/min) for two Sprague-Dawley rats weighing 500 g bw. Recalculation for one rat of

250 g bw according to the method of Filser (1992) gives a value of almost 55 ml/min. This indicates that at steady state about 95% of systemic ethylene oxide is eliminated unchanged by metabolism and only 5% by exhalation, as calculated on the basis of values for clearance due to uptake and clearance due to metabolism in relation to the concentration in the atmosphere. On the basis of the finding that clearance due to metabolism in relation to the concentration in the atmosphere is nearly identical to clearance due to uptake, uptake of ethylene oxide by inhalation was concluded to be the rate-limiting step for metabolism of this compound. The alveolar retention in one Sprague-Dawley rat of 250 g bw was calculated as 47% on the basis of the ventilation rate of 117 ml/min (see above) and the clearance of metabolism in relation to the concentration in the atmosphere of 55 ml/min. The half-life was reported in two animals weighing 500 g bw to be 6 min (Bolt & Filser, 1987). Recalculation for one Sprague-Dawley rat of 250 g bw according to the method of Filser (1992) gives a similar value.

In male Fischer 344/N rats exposed by nose only for 60 min to 5 ppm [9.2 mg/m^3] ethylene oxide, a steady-state blood level of about 60 ng/g was reached after 15 min (Maples & Dahl, 1993).

(*c*) *Metabolites*

After intraperitoneal injection of ethylene oxide labelled uniformly with ^{14}C (2 mg/kg bw) to male Sprague-Dawley rats, 9% of the radioactivity was excreted in urine as *S*-(2-hydroxyethyl)cysteine and 33% as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine within 18 h; 1.5% of the dose was exhaled as $^{14}CO_2$ and 1% as unchanged ethylene oxide within 6 h (Jones & Wells, 1981).

Exposure of male Sprague-Dawley rats for 6 h by inhalation to ethylene oxide at concentrations of 1–200 ppm [1.83–366 mg/m^3] resulted in urinary excretion of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine. The amounts excreted within 24 h correlated linearly with the concentration of ethylene oxide in air; the average amount was 0.27 μmol/ppm [0.15 μmol/mg per m^3] for a rat weighing 200 g bw (Gérin & Tardif, 1986). A value of 0.21 μmol/ppm [0.11 μmol/mg per m^3] can be calculated from the clearance of metabolism in relation to the concentration in the atmosphere (55 ml/min per 250 g bw = 44 ml/min per 200 g bw) (Filser & Bolt, 1984), a molar gas volume of 25 L, and the finding that 33% is excreted as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine in urine (Jones & Wells, 1981).

After intravenous injection of 1 and 10 mg/kg ethylene oxide to male Sprague-Dawley rats, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine was excreted as a constant percentage of the dose: about 30% from 0 to 12 h and 5% from 12 to 24 h. With 100 mg/kg ethylene oxide, the equivalent percentages were 16% and 5%. These results indicate that at the high dose the capacity for glutathione conjugation could have been exceeded within the first 12 h (Gérin & Tardif, 1986).

Ethylene glycol, 2-hydroxymercapturic acid, 2-methylthioethanol and 2-mercaptoethanol were identified as metabolites in the urine of male Wistar rats exposed for 6 h to ethylene oxide at 500 ppm [915 mg/m^3] (Koga *et al.*, 1987). The amounts of ethylene glycol in the urine of male Wistar rats collected during 6-h exposures to ethylene oxide at 50, 100, 200, 300 and 500 ppm [91.5, 183, 366, 549 and 915 mg/m^3] and up to 20 h thereafter were 0.2, 0.35, 1.0, 2.5 and 4.2 mg (means read from a figure), thus increasing disproportionately to the

exposure concentrations (Koga *et al.*, 1985). The findings might indicate a relative decrease in glutathione conjugation.

The pattern of excretion of ethylene oxide metabolites in mice, rats and rabbits was investigated in urine collected 24 h after treatment with ethylene oxide, either intravenously (20 and 60 mg/kg) or by inhalation for 6 h (about 200 ppm [366 mg/m^3]. Marked species differences were seen (Table 11), as metabolites resulting from conjugation of ethylene oxide with glutathione were found in the urine of male Swiss CD-1 mice and male Sprague-Dawley rats but not in that of rabbits [strain not given]. *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine was excreted in the urine of mice and rats, but *S*-(2-hydroxyethyl)cysteine and *S*-(carboxymethyl)cysteine were present only in the urine of mice. Ethylene glycol, the reaction product of the hydrolytic pathway of ethylene oxide, was found in the urine of animals of all three species (Tardif *et al.*, 1987).

Table 11. Urinary excretion of ethylene oxide metabolites within 24 h after treatment intravenously or by inhalation of mice, rats and rabbits with ethylene oxide

Treatment	Urinary metabolites (μmol/100 g bw) (mean values)			
	N-Acetyl-*S*-(2-hydroxyethyl)cysteine	*S*-(2-Hydroxyethyl)cysteine	*S*-(Carboxymethyl)cysteine	Ethylene glycol
20 mg/kg intravenously				
Mouse	3.75	2.62	0.85	1.48
Rat	14.00	ND	ND	2.68
Rabbit	ND	ND	ND	0.95
60 mg/kg intravenously				
Mouse	9.53	6.80	4.30	3.55
Rat	32.28	ND	ND	8.59
Rabbit	ND	ND	ND	3.76
200 ppm, 6 h inhalation [366 mg/m^3]				
Mouse	4.63	2.62	2.83	0.77
Rat	19.61	ND	ND	1.84
Rabbit	ND	ND	ND	2.56

Adapted from Tardif *et al.* (1987)
ND, not detected

(*c*) *Glutathione depletion*

Treatment of animals with ethylene oxide lowered the concentration of glutathione in various tissues. Immediately after a 4-h exposure of male Swiss-Webster mice and male Fischer 344 rats to ethylene oxide at atmospheric concentrations of 100, 400 and 900 ppm [183, 732 and 1647 mg/m^3] (mice) and 100, 600 and 1200 ppm [183, 1098 and 2196 mg/m^3]

(rats), there were concentration-related decreases in glutathione levels in kidney, heart, lung, brain, stomach, spleen, testis and liver of both species, in blood of mice but not of rats, and in bone marrow which was examined in rats only. In both species, the glutathione levels were reduced more in liver, lung and stomach than in other organs. After exposure to the highest concentrations, glutathione levels in the tissue were depressed to 20–30% of the control values (McKelvey & Zemaitis, 1986).

The concentrations of glutathione in hepatic cytosol of male Wistar rats decreased to 37% of that of controls after a single exposure (4 h) to 500 ppm [915 mg/m^3], to 10% after exposure to 1500 ppm [2745 mg/m^3] (Katoh *et al.*, 1990), to 10% after exposure to 1300 ppm [2379 mg/m^3] (Katoh *et al.*, 1991) and to 5% after exposure to 2500 ppm [4575 mg/m^3] (Nakashima *et al.*, 1987). Immediately after the last of a series of repeated exposures of male Wistar rats (6 h/day, three days per week, six weeks) to 500 ppm [915 mg/m^3] ethylene oxide, the hepatic glutathione concentration was diminished by 50%. Control values were reached again 12 h thereafter (Katoh, T. *et al.*, 1989).

(*d*) *Binding to haemoglobin and DNA*

Binding of ethylene oxide to Hb and DNA has been reviewed (European Chemical Industry Ecology and Toxicology Centre, 1989; Walker *et al.*, 1990; Uziel *et al.*, 1992).

^{14}C-Ethylene oxide was reacted *in vitro* (30 min, 37 °C, pH 7.4) with Hb in washed erythrocytes obtained from CBA mice, Fischer rats and humans. The second-order rate constants (Table 12) were about the same for N^2-valine, N^{π}-histidine and N^{τ}-histidine and did not differ between the three species; however, large species differences were seen with respect to *S*-cysteine.

Table 12. Second-order rate constants for in-vitro binding of ethylene oxide to S-cysteine, N^2-valine, N^{π}-histidine and N^{τ}-histidine in human, mouse and rat haemoglobin

Species	Rate constant [L/(g Hb) per h] (mean values)			
	S-Cysteine	N^2-Valine	N^{π}-Histidine	N^{τ}-Histidine
Man	0.06×10^{-4}	0.45×10^{-4}	0.38×10^{-4}	0.37×10^{-4}
Mouse	0.70×10^{-4}	0.32×10^{-4}	0.37×10^{-4}	0.21×10^{-4}
Rat	10×10^{-4}	0.46×10^{-4}	0.62×10^{-4}	0.27×10^{-4}

From Segerbäck (1990)

After male Sprague-Dawley rats had been exposed for several hours to a constant concentration of ethylene oxide in air, a correlation was seen between estimated dose taken up and 7-(2-hydroxyethyl)guanine (7-HOEtGua) in hepatic DNA. In DNA extracted from blood of untreated rats, the mean background level of HOEtGua was 5600 pmol/g DNA (Föst *et al.*, 1989).

Comparative studies were performed in male B6C3F1 mice and male Fischer 344 rats in order to investigate the applicability of Hb adducts for monitoring DNA adducts in various tissues. Rats were killed after a 6-h exposure by nose only to atmospheric ^{14}C-ethylene oxide

at concentrations of 1, 10 and 33 ppm [1.83, 18.3 and 60.4 mg/m^3], and hydroxyethyl adducts were determined at S-cysteine, N-terminal valine and N^{π}- and N^{τ}-histidine of Hb and at 7-guanine of DNA from brain, lung, liver, spleen, kidney or testis. Linear relationships were seen between formation of hydroxyethyl adducts in both Hb and DNA and the exposure concentration (Table 13). The mean ratios of the hydroxyethyl adducts to S-cysteine : N-terminal valine : N^{π}-histidine : N^{τ}-histidine were 16 : 1.6 : 1.9 : 1.0. Alkylation frequencies determined in DNA were similar in all tissues studied, except for testis in which they were 60% lower. There was no evidence of saturation kinetics (Potter *et al.*, 1989).

Table 13. Hydroxyethyl adducts to N^{π}-histidine of Hb and 7-guanine of DNA of rats exposed to atmospheric ethylene oxide for 6 h

Ethylene oxide ppm (mg/m^3)	N^{π}-(Hydroxyethyl)-histidine (pmol/g Hb)	7-(2-Hydroxyethyl)guanine (pmol/g DNA)	
		In testis	In other tissues[a]
1 (1.83)	136	65	79–118
10 (18.3)	1030	466	777–964
33 (60.4)	4640	2000	3030–3660

Adapted from Potter *et al.* (1989)
[a]Ranges in brain, lung, liver, spleen and kidney

Male B6C3F1 mice and male Fischer 344 rats were exposed repeatedly (6 h/day, five days/week, four weeks) to atmospheric concentrations of 0, 3, 10, 33 and 100 ppm ethylene oxide [5.5, 18.3, 60.4 and 183 mg/m^3] and rats also to 300 ppm [549 mg/m^3]. In both species, HOEtVal concentrations in Hb after the end of exposure (Table 14) increased linearly with exposure concentration up to 33 ppm [60.4 mg/m^3], with an identical slope (mean) of about 1100 pmol HOEtVal/g globin per ppm ethylene oxide for these conditions of exposure. At concentrations between 33 and 100 ppm [60.4 and 183 mg/m^3] (mice) and 33 and 300 ppm [60.4 and 549 mg/m^3] (rats), the mean slopes were higher, at about 1440 and 1330 pmol HOEtVal/g globin per ppm per h ethylene oxide, calculated from the figures presented by Walker *et al.* (1992a). The authors compared their results with those of Osterman-Golkar *et al.* (1983), who investigated the concentrations of HOEtHis in Hb of male Fischer 344 rats exposed repeatedly (6 h/day, five days/week, two years) to atmospheric ethylene oxide at concentrations of 0, 10, 33 and 100 ppm [18.3, 60.4, 183 mg/m^3]. Similar adduct levels were found in the two studies up to 33 ppm [60.4 mg/m^3] ethylene oxide. The adduct levels determined by Osterman-Golkar *et al.* (1983) were, however, almost directly proportional to the 6-h exposure concentrations up to 100 ppm [183 mg/m^3], with a slope of about 1000 pmol HOEtVal/g globin per ppm per h ethylene oxide, calculated from published data. After cessation of exposure to the highest concentrations (300 ppm in rats; 100 ppm in mice), the initial loss of HOEtVal was faster than expected on the basis of the normal erythrocyte life span (Walker *et al.*, 1992a). It was suggested that these findings indicate removal of older, more heavily alkylated populations of erythrocytes, accompanied by a burst of erythropoiesis.

Table 14. Hydroxyethyl adducts to N-terminal valine of haemoglobin of rats and mice exposed repeatedly (6 h/day, 5 days/week, 4 weeks) to atmospheric ethylene oxide

Ethylene oxide (ppm)	HOEtVal (pmol/g Hb)	
	Rat	Mouse
0	42	58
1	3 500	3 400
10	11 200	11 100
33	33 400	37 900
100	133 000	144 000
300	397 000	–

Adapted from Walker *et al.* (1992a)

Male B6C3F1 mice and male Fischer 344 rats were exposed repeatedly (6 h/day, five days/week, four weeks) to atmospheric ethylene oxide at concentrations of 0–100 ppm [0–183 mg/m^3] (mice) and 0–300 ppm [0–549 mg/m^3] (rats), as described above. 7-HOEtGua in DNA was determined in various tissues immediately after the end of exposure. Similar adduct levels were found among the tissues, the lowest values being found in testis and the highest in lung. After equivalent exposures to ethylene oxide, the 7-HOEtGua levels were two- to three-fold lower in mice than in rats (Table 15). In order to allow a comparison between species, the data were expressed as picomoles per micromole guanine, taking into account differences in the guanine content of DNA in mouse and rat tissues (28% and 22%, respectively). The slopes of the curves representing the levels of 7-HOEtGua in DNA in various tissues in relation to ethylene oxide exposure concentration increased with increasing concentration, as was observed for HOEtVal levels in Hb (see above) (Walker *et al.*, 1992b).

Removal of 7-HOEtGua from tissue DNA was investigated in mice and rats exposed repeatedly (6 h/day, five days/week, four weeks) to ethylene oxide at concentrations of 100 ppm [183 mg/m^3] and 300 ppm [549 mg/m^3], respectively. It disappeared slowly from DNA of mouse kidney (half-life, 6.9 days) and rat brain and lung (half-lives, 5.4–5.8 days). The authors suggested that the disappearance rate was consistent with a loss due mainly to chemical depurination and that the more rapid removal in other tissues from mice (liver, testis, spleen, brain, lung; half-lives, 1.0–2.3 days) and rats (spleen, white blood cells, kidney, liver, testis; half-lives, 2.9–4.8 days) indicated DNA repair in addition to depurination. Two further DNA adducts of ethylene oxide were found in tissues of rats exposed to 300 ppm ethylene oxide [549 mg/m^3]: O^6-HOEtGua in brain, kidney, lung and spleen and 3-(2-hydroxyethyl)adenine in spleen, which reached a steady-state level of about 1000 pmol/g DNA, 250- to 300-fold less than the corresponding level of 7-HOEtGua (Walker *et al.*, 1992b).

Walker *et al.* (1993) presented a comparison of their results on adduct formation of ethylene oxide to Hb and DNA. On the basis of the observation in laboratory animals that the relationships between HOEtVal in Hb and 7-HOEtGua in DNA vary with length of

exposure, interval since exposure, species and tissue, the authors concluded that the HOEtVal adduct in human Hb was unlikely to provide accurate predictions of DNA adducts in tissues under conditions in which the actual exposure concentration of ethylene oxide is unknown.

Table 15. Hydroxyethyl adducts to 7-guanine in DNA of various tissues from mice and rats exposed repeatedly (6 h/day, 5 days/week, 4 weeks) to an atmospheric ethylene oxide concentration of 100 ppm [183 mg/m^3]

Tissue	Mean 7-HOEtGua (pmol/μmol guanine [nmol/g DNA])	
	Mouse	Rat
Lung	38 [34]	105 [75]
Brain	38 [34]	87 [62]
Spleen	33 [30]	81 [58]
Kidney	33 [30]	55 [39]
Liver	31 [28]	49 [35]
Testis	21 [19]	44 [31]

Adapted from Walker *et al.* (1992b); 7HOEtGua, 7-(2-hydroxyethyl)guanine

The effects of different rates of exposure (300 ppm [549 mg/m^3] for 1 h, 150 ppm [275 mg/m^3] for 2 h, 75 ppm [137 mg/m3] for 4 h) to [1,2-^{3}H]ethylene oxide on incorporation of radioactivity in Hb and DNA of testis were studied in (C3H/Rl×Bl10/Rl)F_1 hybrid male mice. Animals were killed 90 min and one, three and six days after the end of the exposures. The radioactivity count in Hb (averaged over the four time points) was 1.5 times higher after the high exposure rate than after the lowest. A clear effect of exposure rate on radioactivity counts in DNA of testis was observed only 90 min after the end of exposure: incorporation of radioactivity was 2.9-fold higher after the highest exposure rate than after the lowest. The concentration of 7-HOEtGua in DNA of testis showed a first-order decline with a half-life of 2.8 days after exposure to 300 ppm for 1 h (Sega *et al.*, 1991).

A physiologically based pharmacokinetic model has been developed for dosimetry of inhaled and intravenously injected ethylene oxide in rats (Krishnan *et al.*, 1992). The model makes it possible to describe tissue distribution, metabolic pathways, i.e. hydrolysis by epoxide hydrolase and conjugation with glutathione by glutathione *S*-transferase, depletion of hepatic and extrahepatic glutathione and binding of ethylene oxide to Hb and DNA. The biochemical parameters used in the model were obtained by fitting data obtained after inhalation of ethylene oxide in closed chambers (see above) to data on tissue glutathione concentrations (McKelvey & Zemaitis, 1986) and on levels of hydroxyethyl adducts in Hb and tissue DNA (Potter *et al.*, 1989). The model was validated by comparing simulated and published data on urinary excretion of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine after inhalation and intravenous administration of ethylene oxide (Gérin & Tardif, 1986; Tardif *et al.*, 1987)

and on levels of hydroxyethyl adducts in Hb and tissue DNA after exposure (6 h) to 300 ppm [549 mg/m^3] ethylene oxide (Walker *et al.*, 1990, 1992a). The second-order rate constants obtained for the binding of ethylene oxide to amino acid residues in Hb are similar to those published by Segerbäck (1990). According to the model, adduct formation in Hb and DNA accounted for 0.25% and 0.001% of the inhaled dose, respectively. After exposure to atmospheric concentrations of up to 500 ppm [915 mg/m^3] ethylene oxide, the model predicted first-order kinetics for whole-body elimination but nonlinearity in individual metabolic pathways and exhalation. Comparison of the predictions for low and 500-ppm exposures indicated that the share of glutathione conjugation decreased from 38 to 27%, whereas the share of hydrolysis increased from 31 to 36% and that of exhalation from 23 to 28% (Krishnan *et al.*, 1992).

4.2 Toxic effects

The toxicology of ethylene oxide has been reviewed (European Chemical Industry Ecology and Toxicology Centre, 1984; US Occupational Safety and Health Administration, 1984; WHO, 1985; US Environmental Protection Agency, 1985; Golberg, 1986; Henschler, 1993).

4.2.1 *Humans*

(*a*) *Acute effects*

Burns on the hands were attributed to gloves containing residual traces of ethylene oxide used for sterilization (Fisher, 1988). Eye and skin irritation in sterilizer operators were associated with personal exposures to ethylene oxide in air at concentrations up to 10.7 ppm [19.6 mg/m^3] (Bryant *et al.*, 1989). Five sterilizer operators were exposed accidentally to atmospheric ethylene oxide at concentrations high enough to be smelt (odour threshold: 700 ppm [1280 mg/m^3]) for periods up to 0.5 h. Two of the subjects were moderately intoxicated, with headache and diarrhoea as acute symptoms, which disappeared after about 70 h. More severe intoxication was seen in the three other subjects, who had a variety of immediate clinical symptoms including irritation of eyes and throat, mouth dryness, pruritus, headache, vertigo and myasthenia. Indigestion appeared on the day after exposure. All of these symptoms had disappeared by day 21. Haemolysis diagnosed on days 9–11 lasted until day 16 (Deleixhe *et al.*, 1986). Following accidental exposure (4 h/day, four days) to concentrations of ethylene oxide high enough to be smelt, one worker out of five developed persistent nonimmunological asthma, probably induced by extensive epithelial injury which led finally to fibrosis (Deschamps *et al.*, 1992).

(*b*) *Chronic effects*

In two studies of workers engaged in ethylene oxide manufacture for at least six months and between one and 14 years, respectively, no significant differences in selected immunological, haematological and biochemical parameters were observed when comparison was made with matched control personnel unexposed to ethylene oxide (Currier *et al.*, 1984; van Sittert *et al.*, 1985). In a cohort of workers exposed to TWA concentrations of ethylene oxide in air that were generally below 10 ppm and mostly below 1 ppm [18.3 and 1.83 mg/m^3], the

prevalence of proteinuria was increased significantly (Currier *et al.*, 1984). In a cohort exposed to ethylene oxide at air concentrations generally below 0.05 ppm [0.09 mg/m^3] but transiently up to 8 ppm [14.6 mg/m^3], a differential white blood cell count revealed that duration of employment was correlated positively with the percentage of neutrophils and negatively with the percentage of lymphocytes. The values remained within the limits of a control population and were therefore considered to have no significance for health (van Sittert *et al.*, 1985).

People working in a sterilization unit were exposed for 0.6–13 years to ethylene oxide in air at mean concentrations of < 0.25–9.2 ppm [< 0.46–16.8 mg/m^3] measured during seven working days. No haematological, hepatological, nephrological or immunological abnormalities were observed (Wagner & Kollorz, 1987).

In an epidemiological study, the toxicity of ethylene oxide to the lens was investigated in sterilizer operators exposed to atmospheric concentrations varying from 0.06 ppm [0.11 mg/m^3] for 97 min to 39 ppm [70 mg/m^3] for 2.5 min. The prevalence of cataract (but not of lens opacities in the absence of reduced visual acuity) was significantly higher in exposed (aged over 45) than in unexposed, matched subjects. There was, however, no correlation with concentration of ethylene oxide (Deschamps *et al.*, 1990a,b). A regression analysis showed that cumulative exposure to ethylene oxide (years employed × working hours per week × ppm ethylene oxide) was associated with decreased numbers of white blood cells (Deschamps *et al.*, 1990b).

(*c*) *Sensitization*

The sensitizing effects of ethylene oxide have been reviewed (Bommer & Ritz, 1987; Bousquet & Michel, 1991).

A broad spectrum of IgE-mediated allergic symptoms, including anaphylactic reaction, has been observed among dialysis patients, which is due to the use of ethylene oxide for sterilization of dialysis equipment (Bommer *et al.*, 1985; Röckel *et al.*, 1985; Piazolo & Brech, 1986; Kessler *et al.*, 1988; Röckel *et al.*, 1989; Lemke *et al.*, 1990). In these patients, IgE and IgG antibodies were found to be directed against ethylene oxide–human serum albumin conjugates (Marshall *et al.*, 1984; Caruana *et al.*, 1985; Grammer *et al.*, 1985a,b; Marshall *et al.*, 1985; Rumpf *et al.*, 1985; Nicholls, 1986; Grammer & Patterson, 1987; Lemke, 1987; Pearson *et al.*, 1987; Rumpf *et al.*, 1987; Wass *et al.*, 1988).

Exposure to residual ethylene oxide in fluid administration sets induced IgE antibodies against ethylene oxide–human serum albumin conjugate in a few donors undergoing repeated plateletpheresis or plasmapheresis (Leitman *et al.*, 1986; Muylle *et al.*, 1986; Dolovich *et al.*, 1987; Strobel *et al.*, 1988). Cases of allergic asthma have been observed among nurses in haemodialysis centres, who may show a combined IgE-dependent sensitization to ethylene oxide after handling ethylene oxide-sterilized equipment (Balland *et al.*, 1990; Meurice *et al.*, 1990; Dugue *et al.*, 1991; Jacson *et al.*, 1991).

(*d*) *Neurotoxicity*

In several studies, chronic occupational exposure of sterilizer operators to ethylene oxide has been associated with symptoms of peripheral and central neurotoxicity (Schröder *et al.*, 1985; Fukushima *et al.*, 1986; Estrin *et al.*, 1987; Crystal *et al.*, 1988; Estrin *et al.*, 1990;

Klees *et al.*, 1990; Grober *et al.*, 1992). Exposures over 0.5–20 years were characterized by a few daily short-term peaks of air concentrations of 250–700 ppm [458–1281 mg/m^3] ethylene oxide. Eight-hour TWA concentrations ranged from < 1 to 4.7 ppm [< 1.83–8.6 mg/m^3] ethylene oxide. The symptoms and pathological features found in cases of peripheral neuropathy include numbness in the feet and fingers, muscular weakness in the lower limbs, reduction in sural nerve velocity, nerve fibre degeneration and demyelination. Toxic effects were concluded to have occurred on the central nervous system on the basis of personality dysfunction or cognitive impairment.

4.2.2 *Experimental systems*

(*a*) *Acute effects*

The acute effects of a 4-h exposure to ethylene oxide were investigated in male and female B6C3F1 mice exposed at air concentrations up to 1600 ppm [2928 mg/m^3]. At 800 ppm [1464 mg/m^3], all males and four of five females died within six days; at 1600 ppm [2928 mg/m^3], all animals died within 4 h. Lachrymation and dyspnoea occurred at 800 ppm [1464 mg/m^3] and semiconsciousness, severe dyspnoea and diarrhoea at 1600 ppm [2928 mg/m^3] (US National Toxicology Program, 1987).

(*b*) *Subchronic effects*

Subchronic effects of ethylene oxide in animals are summarized in Table 16. Reductions in erythrocyte lifespan and increased erythrocyte fragility have been noted (Popp *et al.*, 1986; Mori *et al.*, 1989, 1990a), which may explain the rapid elimination of Hb adducts in ethylene oxide-exposed animals (Walker *et al.*, 1992a).

(*c*) *Chronic effects*

In a chronic study, male Wistar rats and male cynomolgus monkeys were exposed (7 h/day, five days/week, two years) to air concentrations of 50 and 100 ppm [91.5 and 183 mg/m^3] ethylene oxide. Exposed rats had higher incidences of inflammatory lesions of the lungs, nasal cavities, trachea and internal ear than controls. Furthermore, proliferative and degenerative lesions of the adrenal cortex were found which were characterized by vacuolation and hyperplasia or hypertrophy of the cells of the zona fascicularis. Skeletal myopathy consisting of multifocal areas of atrophy and degeneration without neural changes was observed at 100 ppm [183 mg/m^3] ethylene oxide. In exposed monkeys, the incidence of cataracts was elevated. Decreased nerve conduction velocity was measured in two of 12 monkeys exposed to the higher concentration. Neuropathological examination of two animals in each group revealed demyelination in the very distal portion of the fasciculus gracilis in one animal in each exposure group (Lynch *et al.*, 1984a,b).

Exposure (6 h/day, five days/week, 102 weeks) of male and female B6C3F1 mice to atmospheric concentrations of ethylene oxide up to 100 ppm [183 mg/m^3] did not result in treatment-related clinical signs (US National Toxicology Program, 1987).

Table 16. Subchronic effects in rodents exposed to atmospheric ethylene oxide

Species	Exposure	Effects	Reference
General toxicity			
Wistar rats, males	0, 500 ppm [915 mg/m^3] 6 h/day, 3 days/week, 13 weeks	Decrease in glutathione reductase in liver and brain, increase in lipid peroxidation (malondialdehyde level) in liver	Katoh *et al.* (1988, 1989)
		Disturbance of porphyrin–haem metabolism, decrease in hepatic cytochrome P450, decrease in haemoglobin concentration, normocytic and normochromic anaemia	Fujishiro *et al.* (1990a)
		Decrease in glutathione reductase and glutathione in erythrocytes	Mori *et al.* (1990a)
		Decrease in glutatione reductase in lens	Fujishiro *et al.* (1991)
Wistar rats, males and females	0, 250 ppm [458 mg/m^3] 6 h/day, 5 days/week, 17 weeks	Males: decrease in hepatic cytochrome P450; females: increase in hepatic NADPH–cytochrome c reductase	Fujishiro *et al.* (1990b)
		Females: increase in liver weight; males and females: decrease in glutathione reductase and increase in glutathione-*S*-transferase in the liver; males: increase in hepatic glutathione peroxidase	Mori *et al.* (1990b)
B6C3F1 mice, males and females	0–250 ppm [0–458 mg/m^3] 6 h/day, 5 days/week, 10 (males) and 11 (females) weeks	100 ppm [183 mg/m^3]: decrease in spleen weight in females; 250 ppm [458 mg/m^3]: decrease in spleen weight, increase in relative liver weight in females, decrease in absolute testicular weight and slight decrease in haemoglobin concentration and erythrocyte count	Snellings *et al.* (1984b)
B6C3F1 mice, males and females	0–600 ppm [1098 mg/m^3] 6 h/day, 5 days/week, 14 weeks	Dose-related epithelial damage in the nasal portion of the respiratory tract; 100–400 ppm [183–732 mg/m^3]: renal tubular degeneration; 200–600 ppm [366–1098 mg/m^3]: rhinitis of nasal cavity; 600 ppm [1098 mg/m^3]: renal tubular necrosis; lymphocytic necrosis of thymus and spleen in males	US National Toxicology Program (1987)
C57BL/6J mice, males	0, 255 ppm [467 mg/m^3] 6 h/day up to 16 days; 6 h/day, 5 days/week, 4–10 weeks	Haematological damage: general depression of cellularity in blood and bone marrow, with large fluctuations, however; transient increase in granulocytes	Popp *et al.* (1986)
ddY mice, males	0, 400 ppm [732 mg/m^3] 6 h/day, 3 days/week, 13 weeks	Macrocytic anaemia; hepatic cytochrome P450 increased two fold; increase in ferricyanide reductase; decrease in glutathione reductase and glutathione peroxidase in liver; increase in hepatic glutathione-*S*-transferase	Fujishiro *et al.* (1992)

Table 16 (contd)

Species	Exposure	Effects	Reference
Neurotoxicity			
B6C3F1 mice, males and females	0–250 ppm [0–58 mg/m^3] 6 h/day, 5 days/week, 10 (males) and 11 (females) weeks	Dose-related trend in reduction in locomotor activity and in abnormal reflexes; no microscopic findings	Snellings *et al.* (1984b)
Wistar rats, males	0, 250 ppm [458 mg/m^3] 6 h/day, 5 days/week, 9 months	Preferential distal axonal degeneration of myelinated fibres in sural nerves and gracile fascicles	Ohnishi *et al.* (1986)
Wistar rats, males and females	0, 250 ppm [458 mg/m^3] 6 h/day, 5 days/week, 17 weeks	Paresis of hindlegs; degeneration of myelinated fibres in the peroneal nerve, in the nerve of the soleus muscle and in gracile fascicles; no sex difference	Mori *et al.* (1990c)
Wistar rats, males	0, 500 ppm [915 mg/m^3] 6 h/day, 3 days/week, 4–13 weeks	Ataxic gait after six weeks; preferential distal axonal degeneration of myelinated fibres in hindleg nerves and gracile fascicles; decrease in creatine kinase activity in serum, brain and spinal cord after four weeks	Ohnishi *et al.* (1985); Matsuoka *et al.* (1990, 1993)

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Hemminki *et al.* (1982) reported the results of a retrospective study of all female sterilizing staff employed in hospitals in Finland in 1980. Nursing supervisors from approximately 80 hospitals identified the study participants and the exposure status of each with regard to specific sterilizing agents, which included ethylene oxide, glutaraldehyde and formaldehyde. The 1443 pregnancy outcomes that occurred between the early 1950s and 1981 were categorized as 'exposed' or 'unexposed' on the basis of the work history at the beginning of each pregnancy, established by answers to questionnaires from the study participants. A control group was established, consisting of 1179 pregnancies among female nursing auxiliaries who had had no exposure to sterilizing agents, anaesthetic gases or X-rays. The rates of spontaneous abortion were adjusted for age, parity, decade of pregnancy, coffee consumption, alcohol consumption and smoking habits. The most marked increase was observed for women who had been exposed during pregnancy to ethylene oxide alone: 16.1% of 82 exposed *versus* 7.8% of 1068 unexposed ($p < 0.01$) and 10.5% of 1179 controls. The rates of spontaneous abortion among women exposed to glutaraldehyde and formaldehyde were similar to those among unexposed women. Similar results were obtained in a comparison of pregnancy outcomes identified from hospital discharge registries for sterilizing staff and controls in Finland in 1973–79. In a subsequent analysis (Hemminki *et al.*, 1983), the authors applied a stricter age adjustment and restricted attention among controls to pregnancies that began during hospital employment. The rates were 11.3% of 721 pregnancies for the controls and 20.4% ($p < 0.05$) for exposure to ethylene oxide alone; the rate was also increased for women exposed to glutaraldehyde but not for those exposed to formaldehyde.

4.3.2 *Experimental systems*

The reproductive and prenatal effects of ethylene oxide have been reviewed (Kimmel *et al.*, 1984), and only the most important papers published up to that date are highlighted. All papers published after 1984 are reviewed here. The reproductive toxicity of ethylene oxide has been studied in mice, rats and rabbits following oral, intravenous and inhalational exposure.

In CD-1 mice, intravenous administration of 0, 75 or 150 mg/kg bw ethylene oxide in 5% dextrose solution on days 4–6, 6–8, 8–10 or 10–12 of gestation significantly increased the incidences of craniofacial defects and of fusions of vertebrae in high-dose animals exposed on days 6–8 (19.3%) and 10–12 (9.5%). The incidence ranged from 0 to 2.3% in the control groups. The high-dose level resulted in maternal mortality after treatment on days 4–6, 8–10 and 10–12 (LaBorde & Kimmel, 1980).

Female Sprague-Dawley rats were exposed by inhalation for 7 h per day on five days per week on days 7–16 of gestation, on days 1–16 of gestation or for three weeks prior to mating and then daily until day 16 of gestation to 150 ppm (measured concentration was within 10% of target) ethylene oxide (99.7% pure) vapour. An increased incidence of resorptions (13.6% *versus* 5.4% in controls) was reported in the third group. Pregestational exposure appeared to be important, as similar effects were not found in females exposed during gestation only.

Maternal weight gain and fetal growth were reduced in all groups (Hackett *et al.*, 1982; Hardin *et al.*, 1983).

Male and female Fischer 344 rats were exposed by inhalation to 10, 33 or 100 ppm [18, 60.4 or 183 mg/m^3] ethylene oxide vapour for 6 h per day on five days per week for 12 weeks and then mated; exposure was continued during mating on seven days per week, and females continued to be exposed through to day 19 of gestation. Fewer implantation sites per female, a smaller ratio of fetuses born to number of implants, a decreased number of pups born per litter and a tendency for longer gestation were observed only in animals exposed to 100 ppm. No treatment-related effect was found at the two lower dose levels. It was not determined whether the effects seen were due to treatment of the males or females or both. When lactating females were subsequently exposed from day 5 to 21 of lactation, no adverse effect was seen on pup growth rate or survival (Snellings *et al.*, 1982a).

Snellings *et al.* (1982b) also reported a study of teratogenic effects in Fischer 344 rats exposed to ethylene oxide (> 99.9% pure). Groups of 22 pregnant rats were exposed to 10, 33 or 100 ppm [18, 60.4 or 183 mg/m^3] ethylene oxide for 6 h per day on days 6–15 of gestation; two control groups were exposed to air only. Fetuses were delivered for examination on day 20. All were examined grossly, and then the control group and that exposed to the highest dose were examined for visceral and skeletal defects. No toxicity was observed in the dams and no treatment-related adverse effect was observed, except for a small but significant reduction in fetal weight at the highest dose. There was no evidence of any teratogenic effect.

Exposure of rabbits by inhalation to 150 ppm [274.5 mg/m^3] ethylene oxide (99.7% pure) vapour for 7 h per day on days 7–19 or 1–19 of gestation resulted in no evidence of maternal toxicity, embryotoxicity or teratogenicity (Hackett *et al.*, 1982; Hardin *et al.*, 1983).

It has been believed for a long time that chemicals cannot induce congenital malformations during the preimplantation period of development. Exposure at that time either results in cell death or allows the remaining, undamaged cells to go on to produce a normal embryo: the concept of 'totipotency' of the cells. A series of publications by Generoso and his coworkers has demonstrated, however, that mutagens can induce fetal malformations and death when administered around the time of fertilization.

Generoso *et al.* (1987) first demonstrated that exposure of (C3H×C57Bl)F1 or (SEC×C57Bl)F1 female mice mated with (C3H×C57Bl)F1 males to ethylene oxide gas (1200 ppm [2196 mg/m^3] for 1.5 h) could produce different results, depending on the timing of exposure. Females were exposed 1, 6, 9 or 25 h after carefully timed 30-min matings, these intervals corresponding, respectively, to time of sperm penetration, early pronuclear stage (before DNA synthesis), pronuclear DNA synthesis and early two-cell stage. Exposure at 1 or 6 h increased the number of midgestational and late fetal deaths, but few such effects were seen after exposure at 9 h and none after 25 h. A large proportion of fetuses that survived after exposure at 6 h had a range of congenital malformations, including omphalocoele, hydropia, open thorax and limb and tail defects (37% *versus* 2% in controls). Malformations were also seen in fetuses exposed at 1 h but not in those exposed at 9 or 25 h. In a later study (Rutledge & Generoso, 1989), with identical exposure protocols but more detailed fetal examination, an increased incidence of malformations was found after exposure at 1, 6, 9 and 25 h. Other females exposed to ethylene oxide for up to 14 days before mating had mainly an

increase in early embryonic death around the time of implantation, probably as a result of dominant lethal mutations. In a subsequent publication (Generoso *et al.*, 1988), ethyl methane-sulfonate (EMS), which has mutagenic activity, was shown to produce similar effects on midterm and late fetal deaths and malformations in mice after exposure 6 h after mating.

The mechanism involved in the induction of fetal malformations so early in gestation was further investigated by Katoh, M. *et al.* (1989) in mated female (C3H×C57Bl)F1 mice exposed to ethylene oxide at 1200 ppm [2196 mg/m^3] for 1.5 h or to 250 mg/kg bw EMS intraperitoneally beginning 6 h after the end of the 30-min mating period. Reciprocal zygote transfer to treated or untreated recipient mice 3–9 h after treatment with EMS or buffer resulted in midterm and late fetal deaths and malformations only when the donor females had received EMS, so that the effect was mediated on the zygote and was not secondary to effects on the dam. Analysis of chromosomes of exposed embryos from the late zygote and two-cell stages through to 14 days (only the 10-day embryos had been exposed to ethylene oxide, the others to EMS) showed no increase in either numerical or structural aberrations in the early embryonic stages, nor evidence of aneuploidy in the later embryos. Since the effect is on the zygote but is not associated with chromosomal aberrations, it may be a consequence of gene mutation or reflect an epigenetic effect on gene expression. Postnatal survival of live-born fetuses to weaning was also reduced (79% *versus* 94% in controls) in (SEC×C57Bl)F1 mice treated with 1200 ppm [2196 mg/m^3] ethylene oxide for 1.5 h starting 1 or 6 h after mating. The surviving males were tested for heritable translocations by examining them for sterility or semisterility; none was found in 131 offspring tested (Rutledge *et al.*, 1992).

Mori *et al.* (1991) exposed groups of six male Wistar rats to 50, 100 or 250 ppm [91.5, 183 or 457.5 mg/m^3] ethylene oxide for 6 h per day on five days per week for 13 weeks; there were 12 unexposed controls. In the 250-ppm dose group, epididymal but not testicular weight was reduced, there was slight degeneration in some seminiferous tubules, reduced sperm count in the body and tail but not the head of the epididymis and an increase in sperm head abnormalities, due mainly to the presence of immature sperm. An increase in malformed sperm heads unrelated to dose was observed in all treated groups over that in controls (15% *versus* 2%).

4.4 Genetic and related effects

The mutagenicity of ethylene oxide has been reviewed (Dellarco *et al.*, 1990).

4.4.1 *Humans*

(*a*) *DNA adducts*

The background level of 7-HOEtGua in DNA of peripheral lymphocytes from eight people not occupationally exposed to ethylene or ethylene oxide was 8.5 ± 5.7 nmol/g DNA. The sources of the adduct were not discussed (Föst *et al.*, 1989). No data were available on the formation of DNA adducts in humans exposed to ethylene oxide.

(*b*) *Mutation and allied effects* (see also Tables 17 and 18)

A review of the mutagenicity of ethylene oxide (Dellarco *et al.*, 1990) contains a section on cytogenetic studies of the somatic cells of humans exposed to ethylene oxide. Detailed summaries of many of the studies considered in that analysis are given in section 4.4.2.

Many studies have been carried out to evaluate the effect of exposure to ethylene oxide on the incidences of chromosomal aberrations (including micronuclei) and sister chromatid exchange in peripheral blood lymphocytes of workers exposed occupationally to ethylene oxide. These include workers at hospital and factory sterilization units and those working at ethylene oxide manufacturing and processing plants. The results, summarized in Table 17, show that ethylene oxide induces chromosomal damage in exposed humans. In general, the degree of damage is correlated with the level and duration of exposure. The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is the formation of adducts, chromosomal aberrations or micronuclei. Alkali-labile sites and DNA single-strand breaks (Table 18) were not observed in lymphocytes of sterilization workers, but the induction of DNA cross-linking was reported in one study.

Concentrations of ethylene oxide are often reported as 8-h TWA levels, which do not necessarily reflect the actual concentration to which workers are exposed. During certain operations (e.g. unloading sterilizers), workers may be exposed to short bursts of ethylene oxide at concentrations as high as 400 ppm [720 mg/m^3], while the 8-h TWA may be as low as 5 ppm [9 mg/m^3] (Tates *et al.*, 1991a). Furthermore, the length of time that an individual is exposed to ethylene oxide may be an important factor in determining the relationship between genetic effects and exposure (Thiess *et al.*, 1981a).

Four informative studies (Yager *et al.*, 1983; Stolley *et al.*, 1984; Galloway *et al.*, 1986; Tates *et al.*, 1991a) of genetic end-points in exposed workers are described in detail below.

Yager *et al.* (1983) reported an increased incidence of sister chromatid exchange in peripheral blood lymphocytes of 14 hospital sterilization workers exposed to 1 ppm ethylene oxide (8-h TWA) over that in 13 unexposed controls. In order to evaluate the relationship between exposure and sister chromatid exchange induction, workers were divided into a high-exposure group (five subjects) and a low-exposure group (nine subjects) on the basis of a six-month cumulative dose of ethylene oxide determined by measuring air concentrations during specified tasks and multiplying this value by the number of times each task was performed. The high-exposure group, which received an average cumulative dose of 501 mg ethylene oxide, showed a significant increase in sister chromatid exchange frequency over that in controls and in the low-exposure group (average cumulative dose, 13 mg ethylene oxide). Sister chromatid exchange frequency did not differ significantly between the low-exposure group and the controls.

Tates *et al.* (1991a) compared the frequencies of sister chromatid exchange in nine hospital workers and 15 workers from factory sterilization units occupationally exposed to ethylene oxide and in two respective control groups matched for age, sex and smoking habits (eight donors from administrative personnel working in the neighbourhood and 15 from the same factory). Exposure was measured by gas chromatography in the sterilization rooms (20–25 ppm) and in front of the sterilizer after opening (mean, 50 ppm) for the hospital workers and was monitored during four months (period covering the erythrocyte lifespan) for

the factory workers. Additionally, HOEtVal concentrations were determined in two laboratories. Sister chromatid exchanges were analysed in independent cultures in two different laboratories. The mean frequency of sister chromatid exchange was significantly elevated by 20% in the hospital workers and by almost 100% in the factory workers; moreover, the frequency was clearly greater in daily than in occasionally exposed workers in the factory population.

Other investigators have also reported increased incidences of sister chromatid exchange in lymphocytes of workers exposed to ethylene oxide in hospital sterilization units (Garry *et al.*, 1979; Abrahams, 1980; Laurent *et al.*, 1984; Sarto *et al.*, 1984a,b, 1987, 1991; Lerda & Rizzi, 1992; Schulte *et al.*, 1992). The results from two studies (Högstedt *et al.*, 1983; Hansen *et al.*, 1984) showed that sister chromatid exchanges were not induced in workers who were exposed to less than 1 ppm ethylene oxide.

In a longitudinal study (Stolley *et al.*, 1984), 61 sterilization workers from three work sites were evaluated for induction of sister chromatid exchange at 6, 12 and 24 months. At work site I there was low exposure (0.5 ppm TWA), at work site II there was moderate exposure (5–10 ppm) and at worksite III there was high exposure (5–20 ppm at the time of sampling; action had been taken six months previously to reduce the TWA from 50–200 ppm). Workers at each site were further divided with regard to low and high potential for exposure on the basis of job classification and proximity to sterilizer operations and controls. Controls were primarily randomly selected site personnel (53) considered to have no exposure; community controls (29) were also included. Initial exposures were confirmed by measurements of ethylene oxide in breathing zones. After the initial sampling, blood was taken at each of three sampling times at the work sites and at 6 and 18 months for community controls. The effects on sister chromatid exchange frequency of age, sex, smoking habits and cytogenetic scorer were taken into account. The results showed no increase in sister chromatid exchange frequency for any exposure at work site I or for the workers with potentially low exposure at work site II. Pair-wise comparisons between groups at work site II indicated that the group with potentially high exposure had significantly higher mean frequencies of sister chromatid exchange than the group with potentially low exposure initially ($p = 0.003$), at 12 months ($p < 0.001$) and at 24 months ($p = 0.023$). Similarly, the differences in mean sister chromatid exchange frequency between the group with potentially high exposure and control groups were increased significantly initially and at 12 and 24 months ($p = 0.011, p < 0.001$ and $p = 0.018$, respectively). At work site III, the mean sister chromatid exchange frequency in the group with potentially low exposure differed significantly from those in the work site control group at the initial ($p = 0.024$) and six-month ($p = 0.008$) testings, but not subsequently. Subjects at work site III did not continue to receive exposure after the initial blood samples were taken. These results indicate that the induction of sister chromatid exchange in workers exposed to ethylene oxide is related to the concentration of ethylene oxide at the workplace and that it persists up to six months after cessation of exposure.

Galloway *et al.* (1986) evaluated chromosomal aberration frequencies in lymphocytes from the same group studied by Stolley *et al.* (1984). The results showed no increase in chromosomal aberration frequencies at work sites I or II in any of the samples. Frequencies were significantly elevated in two samples from the group with potentially high exposure at

work site III in comparison with controls and with the group with potentially low exposure taken at 6 and 24 months. Significance was achieved for total structural aberrations only at 24 months ($p = 0.018$) and when data were pooled over time ($p = 0.003$). The 24-month samples from the group with potentially low exposure at work site III had significantly higher numbers of chromosomal aberrations than those from the community controls but not those from the site controls. The authors indicate that the work site III controls may have been exposed accidentally to low levels of ethylene oxide during a leak in 1980, which would account for the higher levels of aberrations than in the other work site controls. The effects of possible confounding variables on the control aberration frequencies were analysed: There was no detectable effect of sex; smoking and age had small but significant effects on the frequencies of chromatid aberrations and chromosomal exchanges, respectively. Exposures at levels of 5 ppm [9 mg/m^3] or above (8-h TWA) are thus required for ethylene oxide to induce chromosomal aberrations in peripheral blood lymphocytes. Similar results were reported from other studies (Pero *et al.*, 1981; Sarto *et al.*, 1984b; Mayer *et al.*, 1991; Tates *et al.*, 1991a).

Tates *et al.* (1991a) reported a significant increase in the frequency of micronuclei in lymphocytes from factory workers exposed to ethylene oxide at concentrations ranging from 14 to 400 ppm [25–720 mg/m^3]; the 40-h TWA was estimated to be 5 ppm on the basis of measurements of Hb adducts. Four other studies (Högstedt *et al.*, 1983; Mayer *et al.*, 1991; Sarto *et al.*, 1991; Schulte *et al.*, 1992) showed no significant increase in the incidence of micronuclei in lymphocytes from workers exposed to ethylene oxide. Högstedt *et al.* (1983) did show, however, that micronuclei were induced in erythroblasts and polychromatic erythrocytes in bone-marrow samples from factory workers who had been exposed to less than 1 ppm ethylene oxide for six months to eight years. Sarto *et al.* (1990) showed increased frequencies of micronucleated exfoliated nasal mucosa cells in two of three workers who had been acutely exposed to ethylene oxide during accidental leakage.

Associations between different genetic end-points were analysed in two studies. Galloway *et al.* (1986) reported a weak overall association between the frequencies of chromosomal aberration and sister chromatid exchange in 61 employees in three work sites and in 304 unexposed controls. The correlation was significant ($p < 0.001$) in potentially exposed groups but not in control groups, and, for any individual, one observation could not be used to predict the other. Tates *et al.* (1991a) confirmed the correlation ($p < 0.001$) between chromosomal aberration and sister chromatid exchange frequencies in pooled data for 9 hospital and 15 factory workers. Additionally, sister chromatid exchange frequencies were shown to correlate better with HOEtVal levels than with chromosomal aberration frequencies which, in turn, correlated better with HOEtVal levels than with micronucleus formation frequency.

hprt Mutations were found in circulating lymphocytes of factory workers exposed to ethylene oxide in a single study (Tates *et al.*, 1991b). The sensitivity of this end-point is considered to be lower than that of Hb adducts and cytogenetic end-points.

Table 17. Cytogenetic observations in people occupationally exposed to ethylene oxide

No. exposed	No. of referents	Exposure time (years)		Ethylene oxide in air (ppm [mg/m^3])		Cytogenetic effects[a]			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
12	8			0–36[b]				+	Garry *et al.* (1979)
75	41				≤ 50	+		+	Abrahams (1980)
12	11	1–8	4	0.5–1		–			Pero *et al.* (1981)
5	11	0.8–3	1.6	5–10		+			
9	13 (low-dose task)				13[c]			–	Yager *et al.* (1983)
5	13 (high-dose task)				501[c]			+	
18	11 (factory I)	0.5–8	3.2		< 1	+	+[d]	–	Högstedt *et al.* (1983)
10	9 (factory II)	0.5–8	1.7		< 1	+	–	–	
13	12 (work site I)		3.2	0.5[e]		–		–	Stolley *et al.* (1984);
22 (21)[f]	19 (20) (work site II)		3.1	5–10[e]		–		(+)	Galloway *et al.* (1986)
26 (25)	22 (21) (work site III)		4	5–20[e]		(+)		+	
10	15 (nonsmokers)	0.5–10	5.7 }	[36–225]				+	Laurent *et al.* (1984)
15	7 (smokers)	0.5–10	4.5 }					+	
14	14			< 0.07–4.3[e]				–	Hansen *et al.* (1984)
22	22 (low exposure)	1–4	3	0.2–0.5[e]	0.35	(+)		+	Sarto *et al.* (1984b)
10	10 (moderate exposure)			0–9.3[e]	1.84			+	Sarto *et al.* (1987)
19	19 (high exposure)	1.5–15	6.8	3.7–20[e]	11	+		+	Sarto *et al.* (1984b)
56	141	1–10		1–40[e]		+		+	Richmond *et al.* (1985)
36	35	1–14		0.1–8	0.05	–			van Sittert *et al.* (1985)
18	10 (sterilization unit)			0–2.7		+			Karelová *et al.* (1987)
21	20 (factory workers)			0–4		+			
14	10 (laboratory workers)			0–5		+			
11	10 (laboratory workers)			0–2.4		–			
9	27	0.5–12	5	0.025–0.38[e]			–		Sarto *et al.* (1990)
3	27			> 0.38[g]			+		

Table 17 (contd)

No. exposed	No. of referents	Exposure time (years)		Ethylene oxide in air (ppm [mg/m^3])		Cytogenetic effects[a]			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
5	10	0.1–4	2		0.025		–	–	Sarto *et al.* (1991)
5	10	4.11	8.6	< 1–4.4	0.38		–	+	
9	8 (hospital workers)	2–6	4	20–25 [36–45]	0.125[h]	+	–	+	Tates *et al.* (1991a)
15	15 (factory workers)	3–27	12	17–33 [30–60]	5[h]	+	+	+	
34	23		8	0.008–2.4[e]	< 0.3	–	–	+	Mayer *et al.* (1991)
32	8		5.1	0–0.3[e]	0.04		–	+	Schulte *et al.* (1992)
11	8		9.5	0.13–0.3[e]	0.16		–	+	
10	10		3	60–69		+		+	Lerda & Rizzi (1992)
47	47				< 1			–	Tomkins *et al.* (1993)

Blanks, not studied

[a]CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange

[b]Maximal concentration measured during purge cycle

[c]Average six-month cumulative dose in mg ethylene oxid

[d]Positive for erythroblasts and polychromatic erythrocytes; negative for peripheral blood lymphocytes

[e]Time-weighted average (TWA)

[f]Numbers in parentheses are for chromosomal aberrations evaluated by Galloway *et al.* (1986)

[g]Exposed acutely from sterilizer leakage

[h]Estimated 40-h TWA based on haemoglobin adducts

4.4.2 *Experimental systems* (see also Table 18)

(*a*) *DNA adducts*

The reaction of ^{14}C-ethylene oxide *in vitro* (3 h, 37 °C, pH 7.4) with calf thymus DNA yielded 7-HOEtGua as the main product; O^6-HOEtGua and 3-(2-hydroxyethyl)adenine occurred as 0.5 and 4.4% of the amount of 7-HOEtGua (Segerbäck, 1990).

After 50 mmol ethylene oxide had been incubated (10 h, 37 °C, 0.05 mol/L phosphate buffer, pH 7–7.5) in screw-cap flasks with calf thymus DNA (3 mg/ml), several adducts were found: 7-HOEtGua at 330 nmol/mg DNA, 3-(2-hydroxyethyl)adenine at 39 nmol/mg DNA, N^6-(2-hydroxyethyl)adenine at 6.2 nmol/mg DNA, 3-(2-hydroxyethyl)cytosine at 3.1 nmol/mg DNA, 3-(2-hydroxyethyl)thymine at 2 nmol/mg DNA and 3-(2-hydroxyethyl)uracil at 0.8 nmol/mg DNA. 3-(2-Hydroxyethyl)deoxyuridine was formed from 3-(2-hydroxyethyl)-cytosine by hydrolytic deamination of the imino group at C4 (Li *et al.*, 1992).

7-Guanine has commonly been identified and quantified as a hydroxylated base *in vivo*, and many studies have also used 7-HOEtGua as a measure of tissue dose (Segerbäck, 1983). [The Working Group calculated the covalent binding index—(μmol adduct per mol DNA nucleotide)/(mmol chemical per kg bw)—from the data of Segerbäck (1983) in order to evaluate DNA binding potency. Five hours after intraperitoneal injection of ethylene oxide to mice, a covalent binding index of 6.4 was calculated for liver DNA.] For example, it has been observed that exposure of male Sprague-Dawley rats over several hours to a constant atmospheric concentration of ethylene oxide results in a correlation between estimated uptake and 7-HOEtGua in hepatic DNA. A mean background level of 5600 pmol/g DNA was found in DNA extracted from blood of untreated rats (Föst *et al.*, 1989). In studies of tissue dose, Hb adducts have frequently been used as a surrogate for DNA adducts, so that when tissue dose was the objective, studies of DNA and Hb were described in section 4.1.2.

In mouse kidney and rat brain and lung, there is a slow loss of 7-HOEtGua from DNA, with half-lives of 5.4–6.9 days; there is somewhat more rapid loss from other tissues, with half-lives of 1.0–2.3 days in mice and 2.9–4.8 days in rats (Walker *et al.*, 1992b). The authors concluded that the differences were due to the extent of DNA repair in the various tissues superimposed upon chemical depurination.

Other adducts identified *in vivo* in rats exposed to 300 ppm [549 mg/m^3] ethylene oxide are O^6-HOEtGua in brain, kidney, lung and spleen and 3-(2-hydroxyethyl)adenine in spleen. Steady-state levels of about 1000 pmol/g DNA were attained, which were 250- to 300-fold lower than the corresponding levels of 7-HOEtGua (Walker *et al.*, 1992b).

(*b*) *Mutations and allied effects*

Ethylene oxide caused DNA damage and gene mutation in bacteria. It induced gene conversion in yeast and gene mutation in yeast and fungi. In plants, it caused gene mutation and chromosomal aberrations. Ethylene oxide induced somatic cell and sex-linked recessive lethal mutations and heritable translocations in *Drosophila melanogaster*. Gene mutation, micronuclei, chromosomal aberrations and cell transformation were induced in rodent cells *in vitro*. Ethylene oxide caused unscheduled DNA synthesis and sister chromatid exchange in human lymphocytes, gene mutation and sister chromatid exchange in human fibroblasts and chromosomal aberrations in transformed human amniotic cells *in vitro*.

Exposure to ethylene oxide *in vivo* induced *hprt* locus mutation in mouse spleen T lymphocytes, sister chromatid exchange in rat, rabbit and cynomolgus monkey lymphocytes, in mouse and rat bone marrow and in rat spleen, micronuclei in mouse and rat bone-marrow cells and chromosomal aberrations in mouse and rat bone-marrow cells and in cynomolgus monkey but not rat lymphocytes. Aneuploidy was not induced in cells from 10-day-old mouse fetuses from dams exposed to ethylene oxide for 1.5 h beginning 6 h after mating.

Ethylene oxide induced alkali-labile sites and DNA single-strand breaks in mouse sperm and spermatids, as measured by alkaline elution of DNA from polycarbonate filters. It also induced dominant lethal effects in mice and rats, chromosomal aberrations in mouse spermatocytes and heritable translocations in mice. In two studies on offspring of male mice exposed to ethylene oxide by inhalation, under similar exposure conditions but using different mating regimens and examining different genetic events, no significant increase in the frequency of specific locus mutations was seen in one study (Russell *et al.*, 1984), while dominant visible and electrophoretically detected mutations were observed in another (Lewis *et al.*, 1986).

(*c*) *Mutational spectra*

The mutational spectrum of *hprt* locus mutants was studied in B6C3F1 mice given intraperitoneal injections of ethylene oxide from day 12 after birth on alternate days until eight weeks after the first treatment (Walker & Skopek, 1993). After selection of splenic T-lymphocytes for 6-thioguanine resistance, DNA was extracted and the exon 3 region of *hprt* was sequenced. Of the 123 *hprt*$^-$ mutants analysed, 18 were located in exon 3; 11 of the 18 mutants were base-pair substitutions at eight different sites. Four AT transversions, three AT transitions, two GC transversions and two GC transitions were observed. Three of the substitutions (two AT → CG, one AT → GC) occurred at a single base (203) in a single mouse. The remaining seven mutations, isolated from four different mice, had the same +1 frameshift mutation in a run of six consecutive guanine bases (207–212) in exon 3. Thus, ethylene oxide mutagenesis in mice involves both modified guanine and adenine bases.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Ethylene oxide has been produced since the early 1900s, originally by the reaction of ethylene chlorohydrin with base and in recent years more commonly by catalytic oxidation of ethylene. It has been used as a chemical intermediate in the production of ethylene glycol, glycol ethers, nonionic surfactants and other industrial chemicals. Although much smaller amounts are used in sterilizing medical instruments and supplies in hospitals and industrially and for the fumigation of spices, it is during these uses that the highest occupational exposure levels have been measured.

Table 18. Genetic and related effects of ethylene oxide

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BRD, *Bacillus subtilis*, differential toxicity	(+)	0	480.0000	Tanooka (1979)
BPF, Bacteriophage, forward mutation	-	0	14500.0000	Cookson *et al.* (1971)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	220.0000	Pfeiffer & Dunkelberg (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.0000	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	1.0000[c]	Simmon (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	125.0000[d]	Hughes *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.0900[c]	Victorin & Ståhlberg (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	0	132.0000	Agurell *et al.* (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	0	+	500.0000[d]	Hughes *et al.* (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	220.0000	Pfeiffer & Dunkelberg (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.0000	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	1.0000[c]	Simmon (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	132.0000	Agurell *et al.* (1991)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	0	2200.0000	Pfeiffer & Dunkelberg (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	-	-	0.0000	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	-	-	0.0000	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	0	2200.0000	Pfeiffer & Dunkelberg (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	-	0.0000	De Flora (1981)
ECF, *Escherichia coli* KMBL 3835, forward mutation	+	0	220.0000	Kolman (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	0	440.0000	Kolman & Näslund (1987)
EC2, *Escherichia coli* WP2, reverse mutation	+	0	440.0000	Kolman & Näslund (1987)
ECR, *Escherichia coli* WU36-10-89, reverse mutation	+	0	220.0000	Kolman & Näslund (1983)
ECR, *Escherichia coli* WU36-10 and WU-10-89, reverse mutation	+	0	220.0000	Kolman (1984)
ECR, *Escherichia coli* WP6 (*pol*A), reverse mutation	+	0	220.0000	Kolman & Näslund (1987)
ECR, *Escherichia coli* WU36-10, reverse mutation	+	0	440.0000	Kolman *et al.* (1989a)
BSM, *Bacillus subtilis*, multigene test	(+)	0	580.0000[c]	Jones & Adams (1981)
SCG, *Saccharamyces cerevisiae* D7, gene conversion	+	0	880.0000	Agurell *et al.* (1991)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ANG, *Aspergillus nidulans*, genetic crossing-over	-	0	88000.0000	Morpurgo (1963)
SCR, *Saccharamyces cerevisiae* D7, reverse mutation	+	0	880.0000	Agurell *et al.* (1991)
SGR, *Streptomyces griseoflavus*, reverse mutation	-	0	9.0000	Mashima & Ikeda (1958)
AZF, *Schizosaccharomyces pombe*, forward mutation	+	+	22.0000	Migliore *et al.* (1982)
ANF, *Aspergillus nidulans*, forward mutation	(+)	0	88000.0000	Morpurgo (1963)
NCR, *Neurospora crassa*, reverse mutation	+	0	1100.0000	Kölmark & Westergaard (1953)
NCR, *Neurospora crassa*, reverse mutation	+	0	6170.0000	Kilbey & Kølmark (1968)
NCR, *Neurospora crassa*, reverse mutation	+	0	66.0000	Kølmark & Kilbey (1968)
HSM, *Hordeum* species, chlorophyll mutation	+	0	0.5300[c]	Ehrenberg *et al.* (1956)
HSM, *Hordeum* species, chlorophyll mutation	+	0	1200.0000	Ehrenberg & Gustafsson (1957)
HSM, *Hordeum* species, chlorophyll mutation	+	0	750.0000	Ehrenberg *et al.* (1959)
HSM, *Hordeum* species, waxy mutation	+	0	0.1800[c]	Šulovská *et al.* (1969)
PLM, *Oryza sativa*, gene mutation	+	0	900.0000	Jana & Roy (1975)
PLM, Soya beans, gene mutation	+	0	500.0000	Sichkar (1980)
HSC, *Hordeum* species, chromosomal aberrations	+	0	900.0000	Moutschen-Dahmen *et al.* (1968)
TSC, *Tradescantia* species, chromosomal aberrations	+	0	14.0000[c]	Smith & Lotfy (1954)
DMM, *Drosophila melanogaster*, somatic mutation	+		5000.0000	Fahmy & Fahmy (1970)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		100000.0000	Rapoport (1948)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		441.0000, inj.	Fahmy & Fahmy (1956)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		5000.0000, inj.	Bird (1952)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		4000.0000, inj.	Nakao & Auerbach (1961)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		4000.0000	Watson (1966)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		880.0000	Zijlstra & Vogel (1988)
DMH, *Drosophila melanogaster*, heritable translocation	+		4000.0000, inj.	Nakao & Auerbach (1961)
DMH, *Drosophila melanogaster*, heritable translocation	+		4000.0000	Watson (1966)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus	+	+	88.0000	Tan *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus	+	0	12.0000[c]	Zamora *et al.* (1983)
G9H, Gene mutation, Chinese hamster V79 cells, *hprt* locus	+	0	2.2000[c]	Hatch *et al.* (1986)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9O, Gene mutation, Chinese hamster V79 cells, ouabain resistance	+	0	2.2000[c]	Hatch *et al.* (1986)
G5T, Gene mutation, mouse L5178Y cells, *tk* locus	+	0	0.0000[e]	Krell *et al.* (1979)
MIA, Micronucleus formation, Chinese hamster V79 cells	+	0	22.0000[c]	Zhong *et al.* (1992)
CIC, Chromosomal aberrations, Chinese hamster V79 cells *in vitro*	+	0	6.0000[c]	Zhong *et al.* (1992)
TCM, Cell transformation, mouse C3H10T1/2 cells	+	0	110.0000	Kolman *et al.* (1989b)
TCM, Cell transformation, mouse C3H10T1/2 cells	+	0	110.0000	Kolman *et al.* (1990)
T7S, Cell transformation, SA7/SHE cells	+	0	1.1000[c]	Hatch *et al.* (1986)
UHL, Unscheduled DNA synthesis, human lymphocytes *in vitro*	+	0	44.0000	Pero *et al.* (1981)
GIH, Gene mutation, human fibroblasts *in vitro*	+	0	110.0000	Kolman *et al.* (1992)
GIH, Gene mutation, human fibroblasts *in vitro*	+	0	200.0000	Bastlová *et al.* (1993)
SHF, Sister chromatid exchange, human fibroblasts *in vitro*	+	0	36.0000	Star (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	10.0000	Garry *et al.* (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	70.0000[c]	Tucker *et al.* (1986)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	0	110.0000	Agurell *et al.* (1991)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[f]	0	10.0000	Hallier *et al.* (1993)
CHT, Chromosomal aberrations, transformed human amniotic cells *in vitro*	+	0	220.0000	Poirier & Papadopoulo (1982)
DVA, DNA single strand breaks, mouse spermatids *in vivo*	+		190.0000 inhal. 1 h	Sega *et al.* (1988)
DVA, DNA single strand breaks, mouse sperm *in vivo*	+		25.0000×1 ip	Sega & Generoso (1988)
GVA, Gene mutation, mouse spleen T-lymphocytes, *hprt* locus *in vivo*	+		100.0000×2 ip	Walker & Skopek (1993)
SLP, Mouse specific locus, postspermatogonia *in vivo*	–		160.0000, inhal. 6 h/d[g]	Russell *et al.* (1984)
SLO, Mouse specific locus, other stages *in vivo*	–		160.0000, inhal. 6 h/d[g]	Russell *et al.* (1984)
SLO, Mouse specific locus, other stages *in vivo*	+		125.0000, inhal. 6 h/d[h]	Lewis *et al.* (1986)
SVA, Sister chromatid exchange, rabbit lymphocytes *in vivo*	+		40.0000, inhal. 6 h/d, 12 w	Yager & Benz (1982)
SVA, Sister chromatid exchange, rat lymphocytes *in vivo*	+		19.0000, inhal. 6 h	Kligerman *et al.* (1983)
SVA, Sister chromatid exchange, monkey lymphocytes *in vivo*	+		14.0000, inhal. 7 h/d[i]	Lynch *et al.* (1984c)
SVA, Sister chromatid exchange, rabbit lymphocytes *in vivo*	+		26.0000, inhal. 0.5 h/d[j]	Yager (1987)
SVA, Sister chromatid exchange, monkey lymphocytes *in vivo*	+		14.0000, inhal. 7 h/d[i]	Kelsey *et al.* (1988)
SVA, Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		30.0000, ip×1	Farooqi *et al.* (1993)
SVA, Sister chromatid exchange, rat bone-marrow cells *in vivo*	+		38.0000, 6 h/d, 3 mo	Ong *et al.* (1993)
SVA, Sister chromatid exchange, rat spleen *in vivo*	+		38.0000, 6 h/d, 3 mo	Ong *et al.* (1993)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		100.0000×1 iv	Appelgren *et al.* (1978)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		10.0000×2 ip	Conan *et al.* (1979)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		150.0000×1 ip	Jenssen & Ramel (1980)
MVM, Micronucleus formation, mouse bone-marrow cells *in vivo*	+		30.0000 mmol/kg ip×1	Farooqi *et al.* (1993)
MVR, Micronucleus formation, rat bone-marrow cells *in vivo*	+		38.0000, inhal. 6 h/d[k]	Hochberg *et al.* (1990)
MVR, Micronucleus formation, rat bone-marrow cells *in vivo*	+		100.0000×1 iv	Applegren *et al.* (1978)
CBA, Chromosomal aberration, rat bone-marrow cells *in vivo*	+		9.0000×1 po	Strekalova (1971)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		26.0000, inhal. 6 h/d×2	Fomenko & Strekalova (1973)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		1.0000, inhal. 66 d	Strekalova *et al.* (1975)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		127.0000, inhal. 6 h/d	Ribeiro *et al.* (1987a)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		30.0000×1 ip	Farooqi *et al.* (1993)
CLA, Chromosomal aberrations, rat lymphocytes *in vivo*	-		170.0000, inhal. 6 h/d×3	Kligerman *et al.* (1983)
CLA, Chromosomal aberrations, monkey lymphocytes *in vivo*	+		28.0000, inhal. 7 h/d[i]	Lynch *et al.* (1984c)
CCC, Chromosomal aberrations, mouse spermatocytes treated *in vivo*, spermatocytes observed	+		127.0000, inhal. 6 h/d	Ribeiro *et al.* (1987a)
DLM, Dominant lethal mutation, mouse *in vivo*	-		100.0000×1 iv	Appelgren *et al.* (1977)
DLM, Dominant lethal mutation, mouse *in vivo*	+		150.0000×1 ip	Generoso *et al.* (1980)
DLM, Dominant lethal mutation, mouse *in vivo*	+		160.0000, inhal. 6 h/d[l]	Generoso *et al.* (1983)
DLM, Dominant lethal mutation, mouse *in vivo*	+		190.0000, inhal. 6 h/d×4	Generoso *et al.* (1986)
DLM, Dominant lethal mutation, mouse *in vivo*	+		130.0000, inhal. 6 h/d[m]	Generoso *et al.* (1990)
DLR, Dominant lethal mutation, rat *in vivo*	+		1.0000, inhal. 66 d	Strekalova *et al.* (1975)
DLR, Dominant lethal mutation, ras *in vivo*	+		250.0000, inhal. 4 h	Embree *et al.* (1977)
MHT, Mouse heritable translocation	+		30.0000×1 ip	Generoso *et al.* (1980)
MHT, Mouse heritable translocation	+		100.0000, inhal. 6 h/d[m]	Generoso *et al.* (1990)
AVA, Aneuploidy, mouse fetus *in vivo*	-		228.0000, inhal. 1.5 h	Katoh *et al.* (1989)
DVH, DNA strand breaks, human lymphocytes *in vivo*	-		0.0700	Mayer *et al.* (1991)
DVH, DNA cross-links, human lymphocytes *in vivo*	+		0.0000	Popp *et al.* (1992)
UVH, Unscheduled DNA synthesis, human lymphocytes *in vivo*	(+)		0.5000	Pero *et al.* (1981)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
*, Gene mutation, human lymphocytes *in vivo*, *hprt* locus	+		1.2000	Tates *et al.* (1991b)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		9.0000[n]	Garry *et al.* (1979)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0000[n]	Abrahams (1980)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	(+)		0.0000	Lambert & Lindblad (1980)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	–		0.2500[n]	Högstedt *et al.* (1983)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0600[n]	Yager *et al.* (1983)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	–		1.0000[n]	Hansen *et al.* (1984)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.2000[n]	Laurent *et al.* (1984)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.1000[n]	Sarto *et al.* (1984b)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		1.2500[n]	Stolley *et al.* (1984)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0000[n]	Richmond *et al.* (1985)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.4000[n]	Sarto *et al.* (1987)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0000	Laurent (1988)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0700	Mayer *et al.* (1991)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	?		0.0800[n]	Sarto *et al.* (1991)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0030[n]	Tates *et al.* (1991a)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		20.0000[n]	Lerda & Rizzi (1992)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	+		0.0400[n]	Schulte *et al.* (1992)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	–		1.2500[n]	Tomkins *et al.* (1993)
MVH, Micronucleus formation, human bone-marrow cells *in vivo*	+		0.2500[n]	Högstedt *et al.* (1983)
MVH, Micronucleus formation, human nasal cells *in vivo*	+°		0.0000	Sarto *et al.* (1990)
MVH, Micronucleus formation, human buccal and nasal cells *in vivo*	–		0.0000	Sarto *et al.* (1990)
MVH, Micronucleus formation, human lymphocytes *in vivo*	–		0.0700	Mayer *et al.* (1991)
MVH, Micronucleus formation, human buccal cells and lymphocytes *in vivo*	–		0.0800[n]	Sarto *et al.* (1991)
MVH, Micronucleus formation, human lymphocytes *in vivo*	+		1.2000[n]	Tates *et al.* (1991a)
MVH, Micronucleus formation, human lymphocytes *in vivo*	–		0.0400[n]	Schulte *et al.* (1992)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.0000[n]	Abrahams (1980)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	(+)		0.5000	Pero *et al.* (1981)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.0000	Thiess *et al.* (1981a)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	–		0.2500[n]	Högstedt *et al.* (1983)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CLH, Chromosomal aberration, human lymphocytes *in vivo*	(+)		0.1000[n]	Sarto *et al.* (1984b)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	–		0.0020[n]	Clare *et al.* (1985)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.0000[n]	Richmond *et al.* (1985)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	–		0.0300[n]	van Sittert *et al.* (1985)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		1.2000[n]	Galloway *et al.* (1986)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.0000	Karelová *et al.* (1987)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.5000, inhal. 8 h/d	Högstedt *et al.* (1990)[p]
CLH, Chromosomal aberration, human lymphocytes *in vivo*	–		0.0700	Mayer *et al.* (1991)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		0.0030[n]	Tates *et al.* (1991a)
CLH, Chromosomal aberration, human lymphocytes *in vivo*	+		20.0000[n]	Lerda & Rizzi (1992)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	0	590.0000	Segerbäck (1990)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	0	88000.0000	Li *et al.* (1992)
BIP, Binding (covalent) to haemoglobin *in vitro*	+	0	590.0000	Segerbäck (1990)
BVD, Binding (covalent) to mouse DNA *in vivo*	+		0.2000 inhal. 2 h	Ehrenberg *et al.* (1974)
BVD, Binding (covalent) to rat DNA *in vivo*	+		0.9000×1 ip	Osterman-Golkar *et al.* (1983)
BVD, Binding (covalent) to mouse DNA *in vivo*	+		2.0000×1 ip	Segerbäck (1983)
BVD, Binding (covalent) to rat DNA *in vivo*	+		4.0000, inhal. 6 h	Potter *et al.* (1989)
BVD, Binding (covalent) to mouse DNA *in vivo*	+		32.0000, inhal. 1 h	Sega *et al.* (1991)
BVD, Binding (covalent) to mouse DNA *in vivo*	+		63.0000, inhal. 6 h/d[q]	Walker *et al.* (1992b)
BVD, Binding (covalent) to rat DNA *in vivo*	+		12.5000 inhal. 6 h/d[q]	Walker *et al.* (1992b)
Protein binding				
BVP, Binding (covalent) to mouse haemoglobin *in vivo*	+		2.0000×1 ip	Segerbäck (1983)
BVP, Binding (covalent) to rat haemoglobin *in vivo*	+		4.0000, inhal. 6 h	Potter *et al.* (1989)
BVP, Binding (covalent) to mouse haemoglobin *in vivo*	+		32.0000, inhal. 1 h	Sega *et al.* (1991)
BVP, Binding (covalent) to mouse haemoglobin *in vivo*	+		6.3000, inhal. 6 h/d[q]	Walker *et al.* (1993)
BVP, Binding (covalent) to rat haemoglobin *in vivo*	+		3.8000, inhal. 6 h/d[q]	Walker *et al.* (1993)
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		23.0000[n]	Calleman *et al.* (1978)
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		0.0000	Farmer *et al.* (1986)
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		0.0000	Hagmar *et al.* (1991)
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		0.0000	Mayer *et al.* (1991)

Table 18 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		0.0050[n]	Sarto *et al.* (1991)
BHP, Binding (covalent) to human haemoglobin *in vivo*	+		0.0400[n]	Schulte *et al.* (1992)
SPM, Sperm morphology, mouse *in vivo*	+		127.000, inhalation 6 h/d ×5	Ribeiro *et al.* (1987b)

*Not on profile
[a]+, positive; (+), weak positive; –, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)
[b]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw
[c]Atmospheric concentration in exposure chamber (μg/ml)
[d]Incubated in Tedlar bags
[e]Cells cultured in ethylene oxide-sterilized polycarbonate flasks
[f]Single concentration, positive only for non-conjugators of glutathione
[g]Sixty days total over a five-month period
[h]Five days/week; six to seven months; mating started 7th week of exposure and continued throughout exposure period
[i]Five days/week; two years (study group from Lynch *et al.*, 1984b)
[j]Five days/week; 16 days
[k]Five days/week; three, six and nine months
[l]Five days/week; two or 11 weeks
[m]Five days/week; six weeks then daily 2.5 weeks
[n]Inhalation; dose based on time-weighted average (TWA) concentration in work area
[o]Positive in two of three workers exposed by accidental leakage
[p]No controls (not on profile)
[q]Five days/week; four weeks

5.2 Human carcinogenicity data

In epidemiological studies of exposure to ethylene oxide, the most frequently reported association has been with lymphatic and haematopoietic cancer. The populations studied fall into two groups—people using ethylene oxide as a sterilant and chemical workers manufacturing or using the compound. In general, people involved in sterilization are less likely to have occupational exposure to other chemicals.

Of the studies of sterilization personnel, the largest and most informative is that conducted in the USA. Overall, mortality from lymphatic and haematopoietic cancer was only marginally elevated, but a significant trend was found, especially for lymphatic leukaemia and non-Hodgkin's lymphoma, in relation to estimated cumulative exposure to ethylene oxide. For exposure at a level of 1 ppm [1.8 mg/m^3] over a working lifetime (45 years), a rate ratio of 1.2 was estimated for lymphatic and haematopoietic cancer. Three other studies of workers involved in sterilization (two in Sweden and one in the United Kingdom) each showed nonsignificant excesses of lymphatic and haematopoietic cancer.

In a study of chemical workers exposed to ethylene oxide at two plants in the USA, the mortality rate from lymphatic and haematopoietic cancer was elevated, but the excess was confined to a small subgroup with only occasional low-level exposure to ethylene oxide. Six other studies in the chemical industry (two in Sweden, one in the United Kingdom, one in Italy, one in the USA and one in Germany) were based on fewer deaths. Four found excesses of lymphatic and haematopoietic cancer (which were significant in two), and in two, the numbers of such tumours were as expected from control rates.

Because of the possibility of confounding occupational exposures, less weight can be given to the positive findings from the studies of chemical workers. Nevertheless, they are compatible with the small but consistent excesses of lymphatic and haematopoietic cancer found in the studies of sterilization personnel.

Some of the epidemiological studies of workers exposed to ethylene oxide show an increased risk for cancer of the stomach, which was significant only in one study from Sweden.

5.3 Animal carcinogenicity data

Ethylene oxide was tested for carcinogenicity in one experiment by oral administration in rats, in two experiments by inhalation in mice and two experiments by inhalation in rats. It was also tested in single studies in mice by skin application and by subcutaneous injection.

In the experiment by intragastric intubation in rats, ethylene oxide produced tumours of the forestomach, which were mainly squamous-cell carcinomas. In one study in mice, inhalation of ethylene oxide resulted in increased incidences of alveolar/bronchiolar lung tumours and tumours of the Harderian gland in animals of each sex and of uterine adenocarcinomas, mammary carcinomas and malignant lymphomas in females. In a bioassay of pulmonary tumours in strain A mice, inhalation of ethylene oxide increased the number of pulmonary adenomas per mouse. In the two experiments in which rats of one strain were exposed by inhalation, ethylene oxide increased the incidences of mononuclear-cell leukaemia and brain tumours in animals of each sex and of peritoneal mesotheliomas in the

region of the testis and subcutaneous fibromas in males. Ethylene oxide produced local sarcomas in mice following subcutaneous injection. In a limited study in mice treated by skin application, no skin tumours were observed.

5.4 Other relevant data

Inhaled ethylene oxide is readily taken up in man and rat, and aqueous ethylene oxide solutions can penetrate human skin. Ethylene oxide is uniformly distributed throughout the body of rats. Its half-life has been estimated as between 14 min and 3.3 h in the human body and about 6 min in rats. Exposure of rats to 5 ppm [9 mg/m^3] resulted in steady-state ethylene oxide levels in blood of 60 ng/g. Whole-body elimination of ethylene oxide from rats is described by first-order kinetics. It is excreted mainly in the urine as thioethers; at high doses, the proportion of thioethers is reduced, while the proportion of ethylene glycol increases. Rats conjugate ethylene oxide with glutathione to a greater extent than mice, while rabbits do not appear to be capable of this reaction.

Ethylene oxide was not teratogenic to rats or rabbits exposed by inhalation to concentrations up to 150 ppm [270 mg/m^3]. It was teratogenic to mice after intravenous injection in a single study. Surprisingly, brief exposure of dams around the time of fertilization to a high concentration (1200 ppm [2160 mg/m^3]) of ethylene oxide by inhalation induced teratogenic effects in mice. The effect was shown to be due to a direct action on the zygote.

Ethylene oxide forms adducts with proteins in both man and experimental animals and with DNA in experimental animals. Haemoglobin adducts have been used for biomonitoring, as there is a significant correlation between cumulative exposure over four months and levels of N-terminal hydroxyethyl valine in haemoglobin of exposed workers. The increment of hydroxyethyl valine adduct formed is about 3.5 pmol/g haemoglobin per ppm-h ethylene oxide. Higher proportions of hydroxyethyl histidine are formed. Hydroxyethyl haemoglobin adducts are also found in the absence of known exposure to ethylene oxide. Greater numbers of haemoglobin and DNA adducts occur per unit of exposure in rats and mice at high concentrations (> 33 ppm) than at lower concentrations. 7-Hydroxyethyl guanine is quantitatively the most important DNA adduct formed. Its half-life varies from 1.0 to 6.9 days in mouse and rat tissues.

Studies of workers exposed to ethylene oxide in hospital and factory sterilization units and in ethylene oxide manufacturing and processing plants consistently showed chromosomal damage in peripheral blood lymphocytes, including chromosomal aberrations in 11 of 14 studies, sister chromatid exchange in 20 of 23 studies, micronuclei in three of eight studies and gene mutation in one study. Micronuclei were induced in the bone marrow of exposed workers in one study. In general, the degree of damage is correlated with level and duration of exposure. The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is that of either chromosomal aberrations or micronuclei. In one study, chromosomal aberrations were observed in the peripheral lymphocytes of workers two years after cessation of exposure to ethylene oxide, and sister chromatid exchanges six months after cessation of exposure.

Chromosomal aberrations and sister chromatid exchange were induced in cynomolgus monkeys exposed to ethylene oxide. Ethylene oxide also induced gene mutation, specific

locus mutation, sister chromatid exchange, chromosomal aberrations, micronuclei, dominant lethal mutation and heritable translocation in rodents treated *in vivo*. It induced unscheduled DNA synthesis, gene mutation, sister chromatid exchange and chromosomal aberrations in human cells and gene mutation, micronuclei, chromosomal aberrations and cell transformation in rodent cells *in vitro*.

Analogous genetic and related effects were observed in nonmammalian systems.

5.5 Evaluation[1]

There is *limited evidence* in humans for the carcinogenicity of ethylene oxide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethylene oxide.

In making the overall evaluation, the Working Group took into consideration the following supporting evidence. Ethylene oxide is a directly acting alkylating agent that:

(i) induces a sensitive, persistent dose-related increase in the frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes and micronuclei in bone-marrow cells of exposed workers;

(ii) has been associated with malignancies of the lymphatic and haematopoietic system in both humans and experimental animals;

(iii) induces a dose-related increase in the frequency of haemoglobin adducts in exposed humans and dose-related increases in the numbers of adducts in both DNA and haemoglobin in exposed rodents;

(iv) induces gene mutations and heritable translocations in germ cells of exposed rodents; and

(v) is a powerful mutagen and clastogen at all phylogenetic levels.

Overall evaluation

Ethylene oxide *is carcinogenic to humans (Group 1)*.

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[1]For definition of the italicized terms, see Preamble, pp. 27–30.

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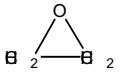
ETHYLENE OXIDE
CAS No. 75-21-8

First Listed in the *Fourth Annual Report on Carcinogens* as *Reasonably Anticipated to be a Human Carcinogen* updated to *Known to be a Human Carcinogen* in the *Ninth Report on Carcinogens*



CARCINOGENICITY

Ethylene oxide is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans, involving a combination of epidemiological and mechanistic investigations which indicate a causal relationship between exposure to ethylene oxide and human cancer.

Ethylene oxide is a direct-acting alkylating agent that has been used as a starting material in the production of other chemicals, and as a disinfectant and sterilant. The DNA damaging activity of ethylene oxide provides its effectiveness as a sterilant, and it is this same property that accounts for its carcinogenic risk to humans. Epidemiological evidence demonstrating this risk has come from studies of workers using ethylene oxide as a sterilant for medical devices and spices, and in chemical synthesis and production. Evidence for a common mechanism of carcinogenesis in humans and experimental animals comes from studies that have demonstrated similar genetic damage in cells of exposed animals and workers.

In 1985, ethylene oxide was first listed in the Fourth Report on Carcinogens as "reasonably anticipated to be a human carcinogen" based on limited evidence of its carcinogenicity in humans and sufficient evidence in experimental animals. Several epidemiological studies, some of which were reviewed in support of the 1985 listing of ethylene oxide as a reasonably anticipated human carcinogen, reported an association between exposure to ethylene oxide and increased leukemia and stomach cancer risk (Hogstedt et al., 1979, 1986; Hogstedt, 1988); however, other studies found no significant excesses in cancer risk (Morgan et al., 1981; Kiesselbach et al., 1990; Teta et al., 1993; Steenland et al., 1991; Hagmar et al., 1991; Bisanti et al., 1993). In most studies, information about the extent of actual ethylene oxide exposure was limited. The most frequently reported association in exposed workers has been for lymphatic and hematopoietic cancer. A meta-analysis of 10 distinct cohort studies of workers exposed to ethylene oxide found no association between exposure to ethylene oxide and increased risk of pancreatic or brain cancers. There was a suggestive risk for non-Hodgkin's lymphoma and for stomach cancer (Shore et al., 1993).

The largest study of U.S. workers exposed to ethylene oxide at plants producing sterilized medical supplies and spices (Steenland et al., 1991) found no increase in mortality from any cause of death; however, an increase in mortality from all hematopoietic neoplasms, concentrated in the subcategories lymphosarcoma, reticulosarcoma, and non-Hodgkin's lymphoma, was observed among males. An analysis of the exposure-response data from the study by Steenland et al. (1991) found a positive trend in risk with increasing cumulative exposure to ethylene oxide and mortality from lymphatic and hematopoietic neoplasms. This trend was strengthened when analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukemia and non-Hodgkin's lymphoma combined). The relationship between cumulative exposure to ethylene oxide and leukemia was positive, but nonsignificant (Stayner et

al., 1993).

In the study by Teta et al. (1993), leukemia risk was increased in workers exposed for more than 10 years to ethylene oxide. A more recent study found an increased incidence of breast cancer in a cohort of workers who used ethylene oxide as a sterilant (Norman et al., 1995). The occupational groups most studied are workers who use ethylene oxide as a sterilant and those who work in the production of ethylene oxide and its derivatives. The likelihood of confounding occupational exposures to other chemicals is generally lower in sterilization workers than in chemical synthesis and production workers.

The evidence that ethylene oxide is a human carcinogen is supported by experimental studies in laboratory animals that have demonstrated that ethylene oxide is carcinogenic at multiple organ sites in rats and mice, likely due to its direct alkylating activity. Sites of tumor induction in mice included the hematopoietic system, lung, Harderian gland, mammary gland, and uterus (NTP 326, 1987). Sites of tumor induction in rats included the hematopoietic system, brain, and mesothelium (Snellings et al., 1984; Garman et al., 1985; Lynch et al., 1984). An IARC (V.60, 1994) evaluation noted that ethylene oxide is associated with malignancies of the lymphatic and hematopoietic system in both humans and experimental animals, and concluded that ethylene oxide was carcinogenic to humans. No additional cancer studies of ethylene oxide in experimental animals have been reported since the IARC (V.60, 1994) review.

ADDITIONAL INFORMATION RELEVANT TO CARCINOGENESIS OR POSSIBLE MECHANISMS OF CARCINOGENESIS

Ethylene oxide is a direct-acting alkylating agent that forms adducts with biological macromolecules including hemoglobin and DNA. Measurements of hemoglobin adducts (hydroxyethyl histidine and hydroxyethyl valine) have been used to monitor worker exposure to ethylene oxide. IARC (V.60, 1994) noted that ethylene oxide induces a dose-related increase in the frequency of hemoglobin adducts in exposed humans and rodents.

The major DNA adduct of ethylene oxide is N7-(2-hydroxyethyl)guanine. Dose-related increases in this adduct, as well as smaller amounts of O6-(2-hydroxyethyl)guanine and N3-(2-hydroxyethyl)adenine, have been measured in rodents exposed to ethylene oxide. Background levels of hemoglobin and DNA adducts of ethylene oxide in humans and experimental animals have been suggested to arise from endogenous production of ethene (ethylene) by gut flora or metabolism of unsaturated dietary lipids (Tornqvist, 1996).

Ethylene oxide is genotoxic at all phylogenetic levels, including prokaryotic and lower eukaryotic organisms, as well as *in vitro* and *in vivo* mammalian systems. Ethylene oxide induces gene mutations and heritable translocations in germ cells of exposed rodents. Significant dose-related increases in the frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes (Galloway et al., 1986; Lerda and Rizzi, 1992; Tates et al., 1991; Yager et al., 1983; Sarto et al., 1984; Stolley et al., 1984; Mayer et al., 1991; Schulte et al., 1992; ; Schulte et al., 1995; Major et al., 1996), of micronuclei in erythrocytes (Tates et al., 1991; Högstedt et al., 1983; ; Schulte et al., 1995), of DNA single-strand breaks in peripheral mononuclear blood cells (Fuchs et al., 1994; Oesch et al., 1995), and of *hprt* mutations in peripheral lymphocytes (Tates et al., 1991) have been observed in workers occupationally exposed to ethylene oxide. Similar genotoxic effects have been observed in rodents exposed to ethylene oxide. For direct-acting mutagenic chemicals, increases in chromosome aberration frequency appear to be a good predictor of increased human cancer risk. Thus, all measurable

genotoxic endpoints that are considered to be indicators of chemical carcinogenesis have been observed in both humans and experimental animals exposed to ethylene oxide.

PROPERTIES

Ethylene oxide (molecular weight, 44.06) is a colorless gas at room temperature and normal pressure, but is a liquid at or below 12 ^{o}C (Budavari, 1996). The liquid has a characteristic ether-like odor (Hoechst Celanese Polyester Intermediates et al., 1995). Ethylene oxide is completely miscible with water, ethanol, acetone, benzene, diethyl ether, and most organic solvents. It is relatively stable in aqueous solutions or when diluted with carbon dioxide or halocarbons, but it may undergo slow polymerization during storage. Ethylene oxide is highly reactive and potentially explosive when heated or in the presence of alkali metal hydroxides and highly active catalytic surfaces. Incomplete combustion releases carbon monoxide. It reacts readily with acids resulting in ring opening. Vapors may be flammable or explosive if there is inadequate heat dissipation (IARC V.60, 1994).

Ethylene oxide is available commercially in the United States as a high-purity chemical that contains a maximum of 0.03% water, 0.003% aldehydes as acetaldehyde, and 0.002% acidity as acetic acid. It has been sold as a mixture with either carbon dioxide or fluorocarbon 12 to reduce its fire hazard (HSDB, 1998). Because of the potential hazards of shipping bulk quantities of ethylene oxide, some producers have reportedly limited shipments to areas immediately proximate or within 50 miles of the producing point (Chem. Prod., 1988).

USE

The primary use of ethylene oxide is as an intermediate in the production of several industrial chemicals, most notably ethylene glycol. In 1986, 59% of the ethylene oxide produced was used to manufacture ethylene glycol. By 1995, the demand for ethylene oxide in ethylene glycol and polyester production was approximately half and half (Chem. Mark. Rep., 1995). Ethylene glycol is used primarily in automotive antifreeze and polyester is used in fibers, films, and bottles. Ethylene oxide was also used to produce nonionic surfactants (14%) in household and industrial detergents, ethanolamines (8%), glycol ethers (6%) used as solvents, intermediates, and for other purposes, diethylene glycol (6%), and triethylene glycol (2%) (Chem. Mark. Rep., 1987b). Less than 1 to 2% of the industrial production of ethylene oxide is used as a fumigant and sterilizing agent for a variety of purposes and materials, including hospital equipment and foods (NIOSH, 1976; ATSDR, 1990-H005). By the mid-1990s, ethylene oxide use for sterilization in hospitals was being replaced by other systems (Biomed. Mark. Newslett., 1995). The estimated 8 to 9 million lb used for sterilization and fumigation in 1996 represented about 0.1% of the total demand for ethylene oxide (SRIc, 1997c).

At one time, it was used in the production of acrylonitrile, but the process ended in 1966 (ATSDR, 1990-H005). Ethylene oxide has also been to accelerate the maturing of tobacco leaves. It has been investigated for use as an agent to improve wood durability (CHIP, 1982b; IARC V.11, 1976).

Other uses include ethoxylation products of long-chain alcohols and amines, alkyl phenols, cellulose, starch, poly(propylene glycol), and ethylene carbonate. Used directly in the gaseous form or in nonexplosive gaseous mixtures with nitrogen, carbon dioxide, or dichlorofluoromethane, ethylene oxide can serve as a disinfectant, fumigant, sterilizing agent, and insecticide. As a fumigant, ethylene oxide kills pests and microorganisms in spices and

seasonings, furs, furniture, nuts, tobacco, books, drugs, leather, motor oil, paper, soil, animal bedding, clothing, and transport vehicles. As a sterilizing agent, it purifies cocoa, flour, dried egg powder, coconut, fruits, dehydrated vegetables, cosmetics, and dental, medical, and scientific supplies (IARC V.60, 1994).

PRODUCTION

Ethylene oxide has been ranked among the top 50 largest volume chemicals produced in the United States for the past several years by *Chemical and Engineering News*. U.S. production from 1985 to 1997 ranged between 5.4 and 8.2 billion lb (Chem. Eng. News, 1996, 1998; USITC, 1985-1987, 1989, 1990). U.S. production from 1977 to 1984 ranged from 4.4 to 5.7 billion lb (USITC, 1978-1985). In the period 1985 to 1989, imports ranged from 12 to 33.6 million lb (average 26 million lb) and exports ranged from 12.1 to 62.5 million lb (average 31 million lb) (USDOC Imports, 1986, 1990; USDOC Exports, 1988, 1990; Chem. Prod., 1988). Compared to annual U.S. production, imports and exports are negligible (< 1%) (HSDB, 1997).

The 1979 TSCA Inventory identified 18 manufacturers producing 3.4 billion lb of ethylene oxide in 1977. The 1997 Directory of Chemical Producers identified 11 companies producing ethylene oxide at 13 plants (SRIa, 1997).

The current process for production of ethylene oxide is the direct vapor phase oxidation process (Hoechst Celanese Polyester Intermediates et al., 1995). The process oxidizes ethylene with air or oxygen in the presence of a silver catalyst at 10-30 atm (1-3 MPa) and 200-300 EC to give ethylene oxide (IARC V.60, 1994).

The chlorohydrin process used to be the primary process for ethylene oxide production. In this process, ethylene chlorohydrin is prepared by treating ethylene with hypochlorous acid (chlorine in water), which is then converted to ethylene oxide by reaction with calcium oxide. The chlorohydrin process has been phased out since 1931 and is not used on an industrial scale in the United States because of its inefficiency (IARC V.60, 1994).

EXPOSURE

The primary routes of potential human exposure to ethylene oxide are inhalation, ingestion, and dermal contact. A risk of potential occupational exposure exists for workers involved in ethylene oxide production, in the manufacture of its end products, or in the use of these compounds in occupational settings (ATSDR, 1990-H005). Because ethylene oxide is highly explosive and reactive, the process equipment containing it generally consists of tightly closed and highly automated systems, which decreases the risk of occupational exposure (NCI DCE, 1985h). Workers in the synthetic organic chemicals manufacturing industry using ethylene oxide are required to wear respirators when air concentrations exceed the PEL. Personnel in workplaces with up to 50 ppm ethylene oxide in the air should wear full facepiece respirators with an ethylene oxide-approved canister (Ludwig, 1994).

Ethylene oxide forms DNA and hemoglobin adducts. These adducts have been used to monitor human exposure to ethylene oxide.

Industries that may use only a small portion of the total ethylene oxide produced are responsible for high occupational exposures to many workers. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 107,450 workers in 74

job categories were potentially exposed to ethylene oxide in the workplace. This estimate was based on observations of the actual use of the compound and tradename products known to contain the compound (NIOSH, 1976). NIOSH estimated that approximately 75,000 health care workers employed in sterilization areas in the period 1972-1974 were potentially exposed to ethylene oxide, and that an additional 25,000 health care workers may have been exposed due to improper engineering and administrative controls (NIOSH 35, 1981). NIOSH conducted a limited field survey of hospitals and found that ethylene oxide concentrations near malfunctioning or improperly designed equipment may reach transitory levels of hundreds or even a few thousand parts per million, but time-weighted average (TWA) ambient and breathing zone concentrations were generally below the OSHA standard of 50 ppm (CHIP, 1982b).

In a separate survey, OSHA estimated that in 1983, 80,000 U.S. health care workers were directly exposed to ethylene oxide, and 144,000 medical device and related industry workers were incidentally exposed (NCI DCE, 1985h; IARC V.36, 1985). More recently, OSHA estimated that as many as 100,000 health care technicians may be exposed to ethylene oxide in the workplace. Health care technicians are typically exposed to quick, concentrated bursts of the gas when the door of a sterilizing machine is opened (Science, 1986). The National Occupational Exposure Survey (1981-1983) estimated that 107,450 workers, including 1,990 women, potentially were exposed to ethylene oxide (NIOSH, 1990). This estimate was derived from observations of the actual use of the compound (98% of total observations) and the use of the tradename products known to contain the compound (2% of total observations). A small population of workers may potentially be exposed to ethylene oxide during the fumigation of spices. OSHA estimated that 160 workers were directly exposed to the gas during spice manufacture (NCI DCE, 1985h).

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of products, such as medical equipment and products (surgical products, single-use medical devices, etc.), disposable health care products, pharmaceutical and veterinary products, spices, and animal feed. Although much smaller amounts of ethylene oxide are used in sterilizing medical instruments and supplies in hospitals and for the fumigation of spices, it is during these uses that the highest occupational exposure levels have been measured (IARC V.60, 1994). Measurements of worker exposure levels in U.S. hospitals, summarized below, showed a range of exposure concentrations (0-794 ppm), depending on operation, conditions, and duration of sampling.

In hospitals, ethylene oxide is used as a gaseous sterilant for heat-sensitive medical items, surgical instruments, and other objects and fluids coming in contact with biological tissues. Large sterilizers can be found in central supply areas of most hospitals and small sterilizers are used in clinics, operating rooms, tissue banks, and research facilities. Worker exposure may occur during the following operations and conditions: changing pressurized ethylene oxide gas cylinders; leaking valves, fittings, and piping; leaking sterilizer door gaskets; opening of the sterilizer door at the end of a cycle; improper ventilation at the sterilizer door; improperly or unventilated air gap between the discharge line and the sewer drain; removal of items from the sterilizer and transfer of the sterilized load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; and passing near sterilizers and aerators during operation (IARC V.60, 1994).

Exposure mostly results from peak emissions during operations such as opening the door of the sterilizer and unloading and transferring sterilized material. Short-term (2-30 min) exposure concentrations from below the level of detection to 186 mg/m^3 (103 ppm) were measured in personal samples from hospital sterilizer operators in studies conducted by NIOSH during 1977-1990. With the proper use of engineering controls and work practices, exposure

levels can be very low (full shift exposure, < 0.1 ppm; short-term exposure, < 2 ppm). However, the use of personal protective equipment in U.S. hospitals was generally limited to wearing gloves, with no use of respirators, when workers were transferring sterilized items (IARC V.60, 1994).

A recent study of hazardous materials incidents in Massachusetts found that most accidental releases at hospitals involved ethylene oxide (Kales et al., 1997). Detailed exposure data, including personal and area monitoring, were obtained for employees of Massachusetts hospitals during 1990-1992 (LaMontagne and Kelsey, 1997). During this period, 23% of hospitals exceeded the OSHA action level (0.5 ppm) at least once, 24% exceeded the short-term exposure limit (STEL = 5 ppm), and 33% reported accidental exposures to ethylene oxide in the absence of personal monitoring.

A study in a large tertiary-care hospital demonstrated that standard industrial hygiene practices can result in nearly "zero exposure" without personal protective equipment or prohibitive costs (Elias et al., 1993). Instantaneous measurements showed a reduction of peak levels from 500 ppm to 0-2.8 ppm from use of engineering and administrative controls.

Ethylene oxide was used as a reaction chemical to modify starch in the starch processing area of an industrial U.S. wastewater treatment plant. Exposures (personal breathing zone concentrations) for full shift operators ranged from undetectable to 0.43 mg/m^3 (0.24 ppm) and from undetectable to 2.5 mg/m^3 (1.4 ppm) for full shift mechanics. IARC (V.60, 1994) reviewed a number of studies of exposure at production facilities. Exposure data were collected in 1987 from 11 ethylene oxide production units in the United States. The highest mean 8-hr TWA was 2.9 mg/m^3 (1.6 ppm) with a range of 0.36 to 6.8 mg/m^3 (0.20 to 3,8 ppm); short-term mean exposure levels for maintenance workers were as high as 19.6 mg/m^3 (10.9 ppm). Respirators were used in operations where engineering controls were not feasible. The manufacture of ethylene oxide usually entails exposure to a variety of other chemicals, e.g., unsaturated aliphatic hydrocarbons, other epoxides, and chlorinated aliphatic hydrocarbons (IARC V.60, 1994).

Workers employed in a Brazilian industry using ethylene oxide as an intermediate were biologically monitored for exposure to ethylene oxide (Ribeiro et al., 1994). Ambient air measurements in the general area, made during a 3-month sampling period, indicated that workers were exposed to 2-5 ppm TWA for an 8-hour working day. Blood samples were taken from 75 workers and 22 controls (no occupational exposure to ethylene oxide) matched for sex, age, and smoking habits. Cytogenetic methods and analyses showed significant increases in chromosomal aberrations, micronuclei in binucleated lymphocytes, and hemoglobin adducts (HOEtVal) in the exposed group. However, the frequencies of micronucleated cells in buccal mucosa were not significantly different between the exposed and control groups.

In 1985, U.S. emissions of ethylene oxide in air were approximately 5,000 Mg (metric tons) per year. The following lists percentages of total air emissions by use: sterilization and fumigation sites, 57%; production and captive use, 31%; medical facilities, 8%; and ethoxylation, 4% (IARC V.60, 1994).

One entry route into the environment for ethylene oxide is as fugitive emissions lost during production, or as vented gases (ATSDR, 1990-H005). Fugitive emissions amounted to some 1.28 million lb in 1978. No information was available to indicate loss with solid waste. There is an estimated emission of 142,600 lb during storage. All ethylene oxide used as a fumigant (up to 10 million lb) is released into the environment. The EOIC estimated that about 3 million lb of ethylene oxide are released into the air each year. Additional sources of ethylene oxide in the environment include inadvertent production from combustion of hydrocarbon fuels

(estimated to be millions of pounds annually), cigarette smoke (from ethylene oxide-fumigated tobacco), ethylene oxide degradation products of certain bacteria, photochemical smog, and water disinfection (the latter source only minimal). It has been estimated that about 3 million lb per year were lost to the air and that about 800,000 lb per year were lost to water, representing 0.07% of the 1980 production. Most producers reported that water containing ethylene oxide is treated at a biopond before being discharged from the plant. Several producers stated that steps are underway to reduce the water-ethylene oxide discharges from the ethylene oxide plants to the waste treatment areas, so this number should decrease significantly in the near future. Those producers who have monitored ethylene oxide at the fence line reported nondetectable amounts in the water analyzed. Five ethoxylation companies reported that a total of 4,000 lb per year was lost to the air, while none was lost to water (CHIP, 1982b).

Significant gaseous releases of ethylene oxide to the environment are the result of uncontrolled industrial emissions (ATSDR, 1990-H005). These occur during the loading or unloading of transport tanks, product sampling procedures, and equipment maintenance and repair (CHIP, 1982b). Ethylene oxide emissions from commercial sterilization facilities in the United States were estimated from data in a 1985 survey of medical equipment suppliers, information provided to EPA (1986, 1988, 1989), and engineering judgment (USEPA, 1993). Emissions ranged from 520 to 20,000 kg per year per unit, depending upon chamber volume, number of facilities, and amount of ethylene oxide used. Emissions expected from mobile beehive fumigator units were not included in the estimation. The Toxic Chemical Release Inventory (EPA) listed 197 industrial facilities that produced, processed, or otherwise used ethylene oxide in 1988 (TRI88, 1990). In compliance with the Community Right-to-Know Program, the facilities reported releases of ethylene oxide to the environment which were estimated to total 4.7 million lb. By 1995, the total release to air was lower, 839,229 lb (157 facilities releasing at least 10 lb) (TRI95, 1997). The USEPA (1994) estimated that its final air toxics rule for controlling ethylene oxide emissions from commercial sterilization and fumigation operations would reduce ethylene oxide atmospheric emissions by 2 million lb annually from an estimated 114 sources.

The risk of potential consumer exposure to ethylene oxide occurs mainly through the use of products which have been sterilized with the compound. These include medical products; articles in libraries, museums, and research laboratories; beekeeping equipment; certain foods and dairy products; cosmetics; transportation vehicles; and articles of clothing (NIOSH 35, 1981). EPA reported that small amounts of ethylene oxide, used as a fumigant, were found in some food commodities, such as cocoa, flour, dried fruits and vegetables, and fish. Other sources, however, list ethylene oxide as a fumigant for only three foods: spices, black walnuts, and copra. Residual ethylene oxide may also be found in foods temporarily following fumigation. It may react with water and inorganic halides (Cl^- and Br^-) from foods, producing glycols and halohydrins. Researchers concluded that the persistence or disappearance of ethylene oxide and its by-products in fumigated commodities depends on the grain size, type of food aeration procedures, temperature, and storage and cooking conditions. Most fumigated commodities had levels of ethylene oxide below 1 ppm after 14 days in normal storage conditions (ATSDR, 1990-H005). Ethylene oxide residues were detected in the following food products sampled from Danish retail shops: herbs and spices (14-580 mg/kg), dairy (0.06-4.2 mg/kg), pickled fish (0.08-2.0 mg/kg), meat products (0.05-20 mg/kg), cocoa products (0.06-0.98 mg/kg), and black and herb teas (3-5 mg/kg; one sample contained 1,800 mg/kg). No ethylene oxide residue was detected in a follow-up study of 59 honey samples (IARC, V.60, 1994).

REGULATIONS

EPA regulates ethylene oxide under the Clean Air Act (CAA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Resource Conservation and Recovery Act (RCRA), Superfund Amendments and Reauthorization Act (SARA), and Toxic Substances Control Act (TSCA). Under CAA, ethylene oxide has been designated a hazardous air pollutant and potential human health hazard. Under CERCLA, a reportable quantity (RQ) of 10 lb has been established. It is regulated as a hazardous constituent of waste under RCRA. EPA subjects the compound to reporting requirements under SARA and TSCA. A Rebuttable Presumption Against Registration (RPAR) for ethylene oxide has been issued under FIFRA. EPA has changed labeling requirements for pesticide products containing ethylene oxide that are used for sterilization purposes. These changes will require modifications in workplace design and practice in hospitals and health care facilities.

Emission standards for ethylene oxide from commercial sterilizers/fumigators were implemented in 1994 (USEPA, 1994). Existing and new sources that use one to 10 tons must achieve a 99% emission reduction in the sterilization chamber vent, but no controls are required for the aeration room vent or chamber exhaust vent. Operations that use over 10 tons must reduce emissions in the sterilization chamber vent, the aeration room vent, and the chamber exhaust vent. Facilities that use less than one ton have no controls, but must meet recordkeeping requirements.

The deadline for compliance with these emission standards was December 8, 1997 (USEPA, 1996). Sources which use one ton, but are not major or located at major sources, may be deferred by the applicable Title V permitting authority from the Title V permitting requirements for five years until December 9, 1999. However, due to explosions of several ethylene oxide commercial sterilization and fumigation facilities, which may be attributable to emission scrubbers, this compliance was deferred for one year, until December 8, 1998 (62 FR 64736, July 1998).

FDA regulates ethylene oxide as a food additive under the Food, Drug, and Cosmetic Act (FD&CA), and finds that it is the common practice in the drug industry to contract out the performance of ethylene oxide sterilization. FDA allows denture adhesives to be composed of an ethylene oxide homopolymer, alone or with carboxymethyl cellulose sodium or karaya. Tolerances for residues of ethylene oxide on agricultural commodities have also been established under FD&CA; however, FDA is re-evaluating its established regulations governing ethylene oxide residues, in light of recent toxicity data and information concerning the formation of 1,4-dioxane.

Ethylene oxide was the subject of a Special Hazard Review performed by NIOSH, which has recommended an exposure limit of 0.1 ppm (0.18 mg/m^3) as an 8-hr TWA and 5 ppm (9 mg/m^3) ceiling concentration (10-minute). OSHA lowered the permissible exposure limit (PEL) from 50 ppm to 1 ppm as an 8-hr TWA in 1984 and established an STEL of 5 ppm during a 15-minute period in 1988. OSHA regulates ethylene oxide under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations summarized in Volume II, Table A-18.