

Hazard Assessment Report

Ver. 1.0

No. 36

Ethylene oxide

Cabinet order number in the gazetted list

(Law for PRTR and Promotion of Chemical Management): 1-42

CAS registry number: 75-21-8

New Energy and Industrial Technology Development

Organization (NEDO)

Outsourcer:

Chemicals Evaluation and Research Institute (CERI)

National Institute of Technology and Evaluation (NITE)

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1. Chemical substance identification information

1.1 Material name: Ethylene oxide

1.2 Class reference number in the gazetted list (Chemical Substance Control Law)

: 2-218

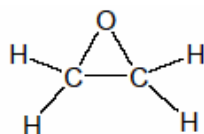
1.3 Cabinet order number in the gazetted list (Law for PRTR and Promotion of Chemical Management)

: 1-42

1.4 CAS registry number

: 75-21-8

1.5 Structural formula



1.6 Chemical formula

: C₂H₄O

1.7 Molecular weight

: 44.05

2. General information

2.1 Synonyms

Oxirane, Epoxyethane

2.2 Purity

>99% (General products)

(NITE, 2002)

2.3 Impurity

Acetaldehyde (<0.01%), nonvolatile component (<0.01%) (General products)

(NITE, 2002)

2.4 Additives/Stabilizers

Nitrogen inclusion (General products)

(NITE, 2002)

2.5 Current regulations in Japan

Law for PRTR and Promotion of Chemical Management: Class I PRTR Chemicals

Fire defense law: Chemicals required the notification of storage, etc.

Poisonous and Deleterious Substances Control Law: Deleterious substance

Labor Standards Law: Chemical Substances causing diseases

Industrial Safety and Health Law:

Group-2 specified chemical substances

Hazardous combustible gases

Harmful substances whose names, etc., are to be indicated

Harmful substances whose name, etc., are to be noticed

Control concentration: 1ppm

Air Pollution Control Law: Harmful air pollutant (Substances Requiring Priority Action)

Law on the Prevention of Marine Pollution and Maritime Disaster: Harmful liquid substances, Group C

Ship Safety Law:

High pressure gas (United Nations Number: 1040, 1041, 1952, 3070, 3297, 3298, 3299, 3300)

Inflammable liquid

Civil Aeronautics Law:

High pressure gas (United Nation Number: 1040, 1041, 1952, 3070, 3297, 3298, 3299, 3300)

Inflammable liquid (United Nation Number: 2983)

Port Regulation Law: High pressure gas, Inflammable liquid (United Nation Number: 2983)

Agricultural Chemicals Regulation Law: Registered agricultural chemicals

High Pressure Gas Safety Law: Flammable gas, toxic fume, liquefied gas

3. Physical-Chemical Properties

Appearance: Colorless gas (Merck, 2001)

Melting point: -111°C (Merck, 2001)

Boiling point: 10.7°C (Merck, 2001)

Flash point: -29°C (direct vent type) (NFPA, 2002)

Ignition point: 429°C (IPCS, 2002)

Explosion limit: 3-100 vol% (in air) (IPCS, 2002)

Specific gravity: 0.891 (4°C/4°C) (Merck, 2001)

Vapor density: 1.52 (Air=1)

Vapor pressure: 146 kPa (20°C) (IPCS, 2002)

Partition coefficient: Octanol/water Partition coefficient log Kow=-0.30 (measured), -0.05 (estimated) (SRC: KowWin, 2003)

Dissociation constant: No dissociation group

Spectrum: Major MS fragment

m/z 29 (base peak= 1.0), 44 (0.55), 42 (0.11) (NIST, 1998)

Adsorption/Desorption properties: Soil sorption coefficient Koc=1 (estimated) (SRC: KowWin,

2003)

Solubility:

Ethylene oxide/water: 1,000 g/L (25°C) (Merck, 2001)

Organic solvents, such as alcohol and ether: Soluble (Merck, 2001)

Henry's Constant: $15.0 \text{ Pa}\cdot\text{m}^3/\text{mol}$ ($1.48 \times 10^{-4} \text{ atm}\cdot\text{m}^3/\text{mol}$) (25°C, estimated) (SRC: KowWin, 2003)

Conversion factor: (Air, 20°C) 1 ppm= $1.83 \text{ mg}/\text{m}^3$, 1 mg= 0.546 ppm

4. Source information

4.1 Production and import

Production and import in FY2001: 100,000-1,000,000 tons (Ministry of Economy, Trade and Industry, 2003)

Production in 2001: 891,453 tons (Ministry of Economy, Trade and Industry, Research and Statistics Department, 2002)

4.2 Use information

Synthetic raw material (for ethylene glycol, ethylene glycol phenyl ether, ethylene glycol-tert-butyl ether, polyethyleneglycol, ethanolamine, N,N-dimethylethanolamine, dioxane, alkyl ethanol, choline chloride, polyethylene oxide, epichlorohydrin, ethylene chlorohydrin, hydroxyethylcellulose, and nonionic surfactant), fumigant for sterilization purposes, bactericide (Chemicals Evaluation and Research Institute, Japan, 2003)

4.3 Estimated release routes

According to the "FY2002 Survey Results on Reported Chemical Release and Transfer, and Non-reported Chemical Release" under the Law for PRTR and Promotion of Chemical Management (PRTR Law), in the year under review, the amount of ethylene oxide released or transferred by reporting operators nationwide was 279 tons into the air (119 tons in the chemical industry, 101 tons in the precision instrument industry, etc.), 20 tons into public water bodies (17 tons in the chemical industry, one ton in the rubber products industry, etc.), 111 tons as waste (84 tons in the chemical industry, 26 tons in the precision instrument industry, etc.), and 52 tons to sewage (42 tons in the chemical industry, 4 tons in the precision instrument industry, etc.) The amount of ethylene oxide released by non-reporting operators was estimated to be 32 tons in target industries and 185 tons in other industries (140 tons in the medical industry, and 46 tons in the contract sterilization industry). The amount of ethylene oxide released from households and movable bodies was not estimated. The percent distribution for ethylene oxide released by non-reporting

operators by type of medium (air, public water body, etc.) has not been publicly announced (Ministry of Economy, Trade and Industry, Ministry of the Environment, 2004a, b).

5. Environment fate

5.1 Stability in the atmosphere

a. Reaction with OH radical

In the troposphere, the reaction rate constant between ethylene oxide and the OH radical is 7.6×10^{-14} cm³/molecule/second (SRC:AopWin, 2003). Supposing the concentration of the OH radical is 5×10^5 to 1×10^6 molecules/cm³, the half-life of ethylene oxide is estimated to be four to seven months.

b. Reaction with ozone

As far as we know, there is no report available on the reaction of ethylene oxide with ozone.

c. Reaction with nitrate radicals

As far as we know, there is no report available on the reaction of ethylene oxide with nitrate radicals.

d. Degradation by direct sunlight

There is a report concluding that ethylene oxide is not degradable by direct sunlight in the atmosphere. (GDCh BUA, 1995)

5.2 Underwater stability

5.2.1 Nonbiodegradability

There is a report saying that the half-life of ethylene oxide when hydrolyzed is 12 to 14 days in fresh water and 9 to 11 days in seawater. (Gangolli, 1999) When ethylene oxide is hydrolyzed, ethylene glycol is generated in fresh water, while ethylene glycol and ethylene chlorohydrin is generated in seawater. (Conway et al., 1983)

5.2.2 Biodegradability

According to the results of a four-week test on biodegradability of ethylene oxide conducted in accordance with the Law Concerning the Examination and Regulation of Manufacture, etc. of

Chemical Substances under aerobic conditions using an improved culture jar designed for volatile substances, BOD-measured degradability of ethylene oxide at the concentration of 100 mg/L was found to be 107% (BOD: biochemical oxygen demand) when activated sludge was 30 mg/L. Thus, biodegradability of ethylene oxide was found to be high. When TOC-measured (TOC: total organic carbon), the degradation rate registered 96%, while the rate became 100% when GC-measured (GC: gas chromatography). (Ministry of International Trade and Industry 1995).

Another biodegradability test under aerobic conditions shows that BOD-measured degradation rate registered 3% after 5 days and 52% after 20 days. (Conway et al, 1983).

It has also been reported that ethylene oxide is biodegradable even under anaerobic conditions. (Howard et al., 1991)

5.3 Behavior in the natural water environment

Ethylene oxide takes the form of gas at a normal temperature (See Chapter 3). Supposing ethylene oxide volatilizes from the water surface, its estimated half-life when calculated based on the Henry constant is 5.9 hours in the environment of a model river (where depth is one meter, current velocity is one meter per second, and wind velocity is three meters per second) and 3.8 days in the environment of a model lake (where depth is one meter, current velocity is 0.05 meters per second, and wind velocity is 0.5 meters per second). (Lyman et al.1982)

Judging from the above data in this section and section 5.2, ethylene oxide discharged in rivers and other natural water environments can be assumed to disappear mainly through volatilization into the atmosphere, biodegradation and gradual hydrolyzation.

5.4 Bio-concentration

As far as we know, there is no report of any measured bio-concentration factor (BOF) of ethylene oxide. Judging from its octanol water partition coefficient (log Kow), steam pressure and water solubility (See Chapter 3), it can be assumed that ethylene oxide does not tend to be bio-accumulative. (Environmental Canada, Health Canada, 2001; Howard, 1989). Estimated BCF based on the octanol water partition coefficient of -0.30 is 3.16. (SRC:BcfWin, 2003)

6. Impact on living creatures in the environment

6.1 Impact on aquatic living creatures

6.1.1 Toxic impact on microorganisms

It has been reported that the 16-hour IC_{50} (concentration at which 50% suffer inhibited reproductive function) of ethylene oxide for microorganisms in activated sludge can be described as

10 to 100 mg/L (Conway et al., 1983).

6.1.2 Toxic impact on algae

As far as we know, there are no test reports available on the toxicity of ethylene oxide for algae.

6.1.3 Toxic impact on invertebrate animals

Table 6-1 shows the results of toxicity tests of ethylene oxide for invertebrate animals.

It should be noted that the reported results, which are for crustaceans including *Daphnia magna* (water flea) in fresh water and *Artemia salina* (brine shrimp) in seawater, do not take into consideration the substance's volatility. Through tests conducted in stagnant water in accordance with the U.S. EPA test guidelines, it was found that the substance's 24-hour LC₅₀ values range from 260 to 300 mg/L or more for *Daphnia magna* and 350 to 500 mg/L for *Artemia salina*, and that 48-hour LC₅₀ values range from 137 to 300 mg/L for *Daphnia magna* and 490 to 1,000 mg/L for *Artemia salina*. The said range covers three different results, as the tests were conducted three times for each exposure hour and each animal. (Conway et al., 1983)

It can be assumed that the concentration of ethylene oxide had been reduced during the above test procedure as ethylene oxide is supposed to have been hydrolyzed and decomposed into ethylene glycol and other substances. Through the process of decomposition of ethylene oxide, the principal substance to be generated is ethylene glycol. The 24-hour LC₅₀ values of ethylene glycol for *Daphnia magna* and *Artemia salina* are very high, exceeding 10,000 mg/L and 20,000 mg/L respectively. Thus, toxicity of ethylene glycol is significantly low, when compared to its parent compound. (Conway et al., 1983)

Table 6-1: Results of toxicity tests of ethylene oxide for invertebrate animals

Species	Endpoint	Concentration (mg/L)	Reference
Species in fresh water			
Daphnia magna (Crustacea, Water flea)	24-hour LC ₅₀	Lethal	260->300
	48-hour LC ₅₀		137-300 (n)
Species in seawater			
Artemia salina (Crustacea, Brine shrimp)	24-hour LC ₅₀	Lethal	350-500
	48-hour LC ₅₀		490-1,000 (n)

(n): Nominal value

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6.1.4 Toxic impact on fish

Table 6-2 shows results of toxicity tests of ethylene oxide for fish.

Reported results explain acute toxicity of the substance for fathead minnows (*Pimephales promelas*) and goldfish (*Carassius auratus*) in fresh water. Tests on fathead minnows, all conducted under the condition of stagnant water in accordance with the U.S. EPA test guidelines, resulted in 24-hour LC₅₀ values of 274mg/L when the test was conducted under ventilated conditions, 86 mg/L when conducted in a closed system, and 90 mg/L when conducted without ventilation. Judging from those different results, it can be assumed that ventilation can accelerate hydrolysis and increase the volatility of ethylene oxide, thus resulting in decreased toxicity. When the test was conducted without ventilation, 48-hour LC₅₀ and 96-hour LC₅₀ values registered 89 mg/L and 84 mg/L respectively. Those values are almost the same as the 24-hour LC₅₀ value obtained when conducted in a closed system (Conway et al., 1983). In a test on goldfish, ethylene oxide exhibited the 24-hour LC₅₀ value of 90 mg/L (Bridie et al., 1979b). It has also been reported that the 24-hour LC₅₀ value of ethylene glycol for fathead minnows is more than 10,000 mg/L, exhibiting very low toxicity when compared to its parent compound (Conway et al., 1983).

Table 6-2: Results of toxicity tests of ethylene oxide for fish

Species	Endpoint		Concentration (mg/L)	Reference
fresh water species				
<i>Pimephales promelas</i> (Fathead minnow)	24-hour LC ₅₀	Lethal (with ventilation)	274 (n)	Conway et al., 1983
	24-hour LC ₅₀	Lethal (in a closed system)	86 (n)	
	24-hour LC ₅₀	Lethal (without ventilation)	90	
	48-hour LC ₅₀		89	
	96-hour LC ₅₀		84 (n)	
<i>Carassius auratus</i> (Goldfish)	24-hour LC ₅₀	Lethal	90 (m)	Bridie et al., 1979b

(m): Measured value

(n): Nominal value

Closed system: A test vessel or tank is covered but has headspace.

6.1.5 Toxic impact on other aquatic living creatures

As far as we know, there are no test reports available on toxicity of ethylene oxide for

other aquatic living creatures (such as amphibians).

6.2 Impact on terrestrial living creatures

6.2.1 Toxic impact on microorganisms

Toxic impact of ethylene oxide on *Bacillus subtilis* was examined in a test where its spores were exposed to ethylene oxide at a concentration of 475 mg/L, to see how their germination was affected. It was found that after two hours of exposure, the germination rate was significantly decreased by 10 to 45% (Dadd and Rumbelow, 1986). According to another test report, the complete sterilization of 12 kinds of microbes in soil required 8 hours of fumigation when carbon dioxide containing 10% ethylene oxide was used under conditions of 65% humidity and 40°C (Gennari, 1987).

6.2.2 Toxic impact on plants

As far as we know, there are no test reports available on toxicity of ethylene oxide for plants.

6.2.3 Toxic impact on animals

A toxicity test of ethylene oxide for khapra beetles (*Trogoderma granarium*) was conducted by fumigating their eggs for a period of 24 hours with ethylene oxide at a concentration of 1,000 to 3,000 mg/m³ (546 to 1,638 ppm), to see how lethal the substance is to larvae or its reproduction-inhibiting impact on survivors. The result was that death occurred at a percentage of 24.5 to 98.6%. When examining those khapra beetles that survived fumigation, it was found that their reproductive function was not significantly affected (Rajendran, 1982). We obtained another toxicity test report on insects of the Bostrychoidea family (*Rhyzopertha dominica*), known to be harmful to rice and wheat. In the test, the insects were exposed to ethylene oxide of various concentrations (ranging from 150 to 1,500 mg/m³ (137 to 820 ppm)) for the period of 24 hours. It was found that the insects' reproductive function was significantly inhibited (the rate of reproduction decreased significantly) when the concentration was 500 mg/m³ (273 ppm) or more (Rajendran and Shivaramaiah, 1985). We have found no reliable data on impacts of ethylene oxide on birds or wild mammals.

6.3 Impact of ethylene oxide on living creatures in the environment (summary)

There is only a limited number of test reports available on the toxic impact of ethylene oxide on living creatures in the environment, because of the substance's physical characteristics

make it difficult to be tested for reliable data. In addition, we found little data to be reliable when taking into consideration the substance's tendency to be hydrolyzed or volatilize. There are no reports available on acute toxicity of ethylene oxide for algae or amphibians or chronic toxicity for aquatic living creatures.

Among the limited number of reports available, we found that the 16-hour IC_{50} toxicity of ethylene oxide for microorganisms in activated sludge was between 10 to 100 mg/L (concentration at which 50% suffer inhibited reproductive function.)

As for acute toxic impact on invertebrate animals, there is a report that 48-hour LC_{50} values range from 137 to 300 mg/L for *Daphnia magna* (water flea) and 490 to 1,000 mg/L for *Artemia salina* (brine shrimp). Those values put it outside the levels of acute toxicity under the GHS categorization.

Toxic impact on fish have been reported in the form of test results on fathead minnows conducted in accordance with the U.S. EPA test guidelines. Results show that 24-hour LC_{50} values registered 274mg/L when the test was conducted with ventilation, 86 mg/L when conducted in a closed system, and 90 mg/L when conducted without ventilation. Judging from those different results, it can be assumed that ventilation can accelerate hydrolysis and increase volatility of ethylene oxide, thus resulting in decreased toxicity. When the test was conducted without ventilation, 48-hour LC_{50} and 96-hour LC_{50} values registered 89 mg/L and 84 mg/L respectively. Those values are almost the same as the 24-hour LC_{50} value obtained when conducted in a closed system. As for the impact on goldfish, ethylene oxide exhibited the 24-hour LC_{50} value of 90 mg/L. Those values, on fathead minnows and goldfish, correspond to level III acute toxicity under the GHS categorization.

As for the impact on terrestrial living creatures, we obtained data on soil microbes and insects.

Judging from the above test reports, it can be concluded that ethylene oxide is harmful to fish to the degree of level III acute toxicity under the GHS categorization.

The lowest concentration which exhibits the substance's toxicity for aquatic living creatures was 84 mg/L for a 96-hour LC_{50} for fathead minnows.

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7. Human health effects

7.1 In vivo fate

Table 7-1 shows the metabolic pathways of ethylene oxide.

Ethylene oxide and ethylene are generated endogenously in test animals (Filser and Bolt, 1983). Ethylene oxide is also generated from ethylene in vivo (Ehrenberg et al., 1977).

a. Absorption

Ethylene oxide is highly soluble in blood and quickly absorbed from respiratory passages and gastrointestinal tracts (U.S. EPA., 1985). The absorption rate of ethylene oxide from the lungs depends on the ventilation rate and the concentration of ethylene oxide absorbed in lung alveoli (Ehrenberg et al., 1974). The rate of uptake of ethylene oxide was $1.1\mu\text{g}/\text{kg}$ body weight, per minute, for mice exposed to $1\text{mg}/\text{m}^3$ (0.546 ppm). This indicates nearly 100% absorption of ethylene oxide from 1.1 liter of air per minute and per kg body weight (Altman and Dittmer, 1974).

In the test of rats (300g) exposed to ^{14}C ethylene oxide for six hours, the rates of absorption and metabolism for those exposed to 18, 180, or $1,800\mu\text{g}/\text{L}$ (2.7, 20, 107 mg/kg) of ethylene oxide with respiratory volume of 50L/6 hours were 90, 68, and 36%, respectively (Beliles and Parker, 1987; Tyler and McKelvey, 1983).

b. Distribution

In the test of mice exposed to ^{14}C ethylene oxide (Appelgren et al., 1978), the concentration of radioactivity two minutes after intravenous injection was 3-4 times as high in liver, kidney, and pancreas as in blood, and ^{14}C ethylene oxide was distributed to all parts of the body at some time between 20 minutes and four hours after injection. The concentration of radioactivity in mice inhaling ^{14}C ethylene oxide peaked in the liver, kidney, and lungs shortly after inhalation. The concentration of radioactivity in liver and lung rapidly declined within four hours after inhalation, indicating rapid metabolism and excretion. In the test conducted by using the data on the alkylation of tissues (Ehrenberg et al., 1974) and hemoglobin (Hussian and Ehrenberg, 1975), the first-order clearance of ethylene oxide in tissues was 10 minutes for mice and rats as well as for humans (Calleman et al., 1978).

c. Metabolism and excretion

In humans and animals, there are two pathways for the metabolism of ethylene oxide, both of which are considered to be detoxification pathways. The first involves hydrolysis to ethylene glycol, with subsequent conversion to oxalic acid. The second involves conjugation with glutathione, with subsequent metabolic steps yielding S-(2-hydroxyethyl)cysteine [S-(2-carboxymethyl)cysteine] and SH derivatives (Wolfs et al., 1983; IPCS, 1985; ATSDR, 1990; Popp et al., 1994). In the metabolism of ethylene oxide, the second route appears to predominate in mice and rats; in larger species (rabbits and dogs), the first route is the most common (Gérin & Tardif, 1986; Jones & Wells, 1981; Martis et al., 1982; Tardif et al., 1987). Ethylene oxide is a substrate for the GSTT1 enzyme, and GSTT1 enzymatic activity is highest in mice, followed by rats and humans (Hallier et al., 1993; Hayes & Pulford, 1995; Pemble et al., 1994).

In humans and test animals, the metabolites of ethylene oxide were reported as different in

quantity but the same in quality (Tardif et al., 1987). Among rodents, the clearance of ethylene oxide from blood and tissues was about 3-4 times as high for mice as for rats (Brown et al., 1996). In dogs injected intravenously with ethylene oxide, the clearance of water-soluble ethylene oxide from the entire body was 20 mL/kg/minute (Osterman-Golkar et al., 1983).

In mice inhaling radiolabeled ethylene oxide, about 74% of the inhaled ethylene oxide was excreted as unidentified metabolites in the urine within 24 hours of inhalation, and only 4% was excreted in the next 24 hours (Eherenberg et al., 1974). Based on the above data, most of the metabolites of ethylene oxide are considered to be excreted in the urine within 24 hours of exposure (ACGIH, 2001).

In the test of four rats (male, F344) exposed to ¹⁴C ethylene oxide (10, 100, or 1,000 ppm) for six hours, the percentage of urinary radioactivity was lower for rats exposed to 1,000 ppm than for others. By contrast, the percentages of ¹⁴C-C2 and ¹⁴C-ethylene oxide in exhaled air were higher for rats exposed to 1,000 ppm (Tyler and McKelvey, 1983). On the basis of the above data, it was presumed that the metabolism and excretion of ethylene oxide would be saturated at 1,000 ppm (U.S. NTP, 1987).

Ethylene oxide is electrophilic, and alkylates nucleophilic substances in macromolecules (giant molecules) that contain DNA and protein. In hemoglobin, for example, adducts are formed at cysteine residues, N-terminal valine (Segerback, 1983). Ethylene oxide binding to DNA results primarily in the formation of 7-(2-hydroxyethyl)guanine (Fost et al., 1989; Li et al., 1992). This adduct was identified in humans and test animals (Garman and Snellings, 1986; Garman et al., 1985; Lynch et al., 1984a,b; Snellings et al., 1984b; U.S. NTP, 1987; Walker et al., 1992; Wu et al., 1999a). Human tissues contain endogenous 7-(2-hydroxyethyl)guanine 10-15 times as much as rodent tissues (Wu et al., 1999b). The formation of 7-(2-hydroxyethyl)guanine was slightly larger for rat tissues (lung, spleen, brain, and liver) than for mouse tissues. The increase in brain tumor frequency due to exposure to ethylene oxide was observed in rats rather than in mice. By contrast, the increase in lung tumor frequency was observed in mice rather than in rats, and the concentration of 7-(2-hydroxyethyl)guanine was not clearly correlated with the species-specific carcinogenic potency (Walker et al., 1992; Wu et al., 1999a). Whether or not DNA adducts and other factors affect the carcinogenicity of ethylene oxide in various tissues is still unknown (Environment Canada and Health Canada, 2001).

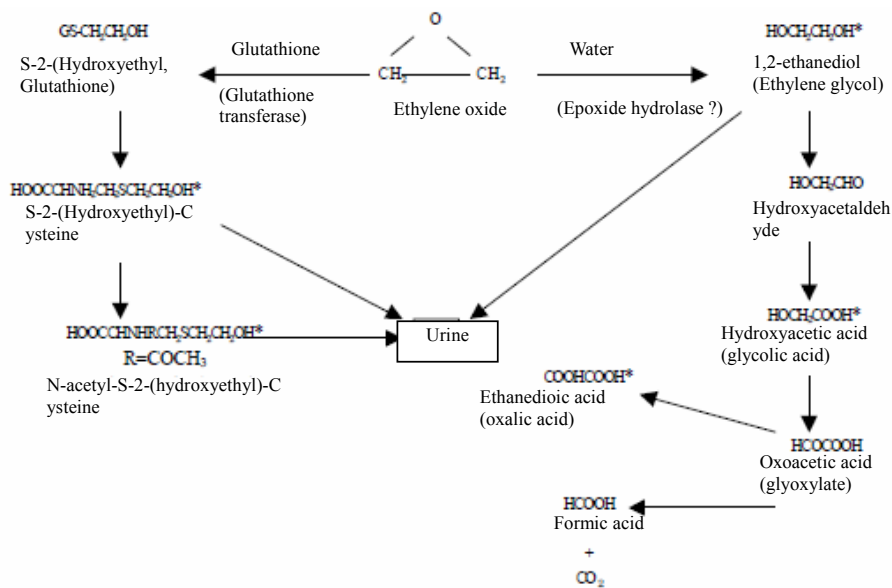


Fig. 7-1 Metabolic pathways of ethylene oxide (IPCS, 1985)

Note: Substances with an asterisk are metabolites experimentally identified by Gessner et al. (1961), Jones & Wells (1981), McChessney et al. (1971).

7.2 Epidemiological studies and case reports

a. Acute toxicity

Acute, short-term exposure to ethylene oxide causes nausea, headache, hyposthenia, vomiting, drowsiness, and incoordination. Skin contact with ethylene oxide causes dermatitis, bulla, edema, and frostbite, and symptoms range from mild to serious (U.S. NIOSH, 1988). Nausea and vomiting were observed in three workers accidentally exposed to 1% water-soluble ethylene oxide through skin (Sexton and Henson, 1949).

b. Irritant property and causticity

Ethylene oxide vapor irritates eyes, nose, and throat (ATSDR, 1990).

Bronchitis, and pulmonary edema and emphysema were detected in long-term exposed workers (Thiess, 1963), and mild irritation to skin was found in people dermally exposed to 1% water-soluble ethylene oxide (Sexton and Henson, 1949). Characteristic skin injuries are edema, erythema, and vesicles. Edema and erythema developed 1-5 hours after exposure, and vesicles developed subsequently. The seriousness of injury depends on the length of skin contact time and the

concentration of ethylene oxide (IPCS, 1985). Irritation to skin is also reported for those touching surgical gowns stained with ethylene oxide pesticide etc. (Biro et al., 1974; Bommer and Ritz, 1987; Fisher, 1988; Hanifin, 1971; LaDage, 1979; Lerman et al., 1995; Marx et al., 1969, Royce and Moore, 1955).

c. Sensitizing potential

Ethylene oxide is considered to have a high sensitizing potential, reacting with various types of chemicals.

Allergic reactions of Type I (anaphylaxis) and Type IV (contact dermatitis) were observed in humans exposed to ethylene oxide. Mild to serious anaphylactic reactions were observed in various types of dialysis patients (e.g.: hemodialysis, peritoneal dialysis, plasmapheresis, plateletpheresis) using the medical equipment sterilized by ethylene oxide (Bommer and Ritz, 1987). Occupational asthma caused by ethylene oxide exposure has also been reported (Dugue et al., 1991; Verraes and Michel, 1995).

d. Repeated dose toxicity

Chronic, long-term exposure to ethylene oxide causes nervous disorders and dermal sensitization. Nervous disorders have been reported most frequently (U.S. NIOSH, 1988).

d-1. Effects on the nervous system

Acute or chronic exposure to ethylene oxide causes sensorimotor polyneuropathy. In the previous case studies, the concentrations of ethylene oxide were reported to be 4.2-700 ppm (7.7-1,281 mg/m³) or more (Crystal et al., 1988; Finelli et al., 1983; Fukushima et al., 1986, Gross et al., 1979; Kuzuhara et al., 1983; Ristow and Cronelius, 1986; Schroder et al., 1985; Zampollo et al., 1984). The normal TWA (time-weighted average) exposure ranged from less than 1 to 4.7 ppm (less than 1.8 mg/m³ to 8.6 mg/m³) with the maximum of 250 ppm (458 mg/m³) (Estrin et al., 1987, 1990; Klees et al., 1990). The degeneration of sural nerve myelin and axon, and muscular degenerative atrophy were found in workers exposed to 700 ppm (1,281 mg/m³) or more (Kuzuhara et al., 1983). Damage to the central nervous system (e.g.: stroke) was observed in those acutely exposed to 500-700 ppm (915-1,281 mg/m³) of ethylene oxide (Gross et al., 1979; Salinas et al., 1981).

Ethylene oxide poisoning was reported for 4/6 persons involved in sterilization at medical equipment production plants in Japan (Fukushima et al., 1986). These workers were exposed to ethylene oxide (concentration unknown) while transporting sterilized products (8-10 times/day) and exchanging containers (once/day). Polyneuropathy, of which the major complaints were the sensory

impairment of lower limbs and titubation, was observed in all patients. The major symptom was the sensory impairment of lower limbs involving pallesthesia, which gradually extended to upper limbs and other body parts. All patients showed motoneuron disease, dorsal cord disorder, and cranial and autonomic disorders, and their symptoms were reversible.

In neuropsychological tests (psychomotor skill tests), chronic occupational exposure to ethylene oxide was correlated with performance disorder, and some cases showed a reduction in the conduction velocity of peripheral nerves. However, as these studies involved a small number of examinees, it is difficult to make a definite comparison between them. Performance was poor in all psychomotor skill tests for eight females who were involved in sterilization using ethylene oxide or worked nearby the sterilization site for 5-20 years (on average, 11.6 years: TWA 3 ppm [$5.5\text{mg}/\text{m}^3$]), and especially in hand-eye coordination tests, they gained considerably lower score ($P=0.03$) than unexposed workers (Estrin et al., 1987). In addition, in various psychomotor skill tests, 10 hospital workers chronically exposed to ethylene oxide (250 ppm [$458\text{ mg}/\text{m}^3$]) gained low scores, suggesting dysgnosia (Estrin et al., 1990).

d-2. Effects on blood

Reduced hematocrit and hemoglobin were found in 59 females employed at hospitals in the United States and Mexico who were exposed to the ethylene oxide leakage from sterilizers (On average, the cumulative exposure level for four months was 32 ppm for a hour or more.) (Schulte et al., 1995). No hematologic changes were observed in 36 male workers exposed to no more than 0.05 ppm (0.09 mg/m) for eight hours on a TWA basis (Van Sittert et al., 1985) and 84 male workers exposed to 1 ppm ($1.83\text{mg}/\text{m}^3$) or less (Currier et al., 1984).

e. Reproductive and developmental toxicity

Pregnancy disorder has been reported in males and females exposed to ethylene oxide. The increased miscarriage risk has also been reported in exposed females.

There is a study on the frequency of miscarriage that targeted medical staff in Finland who sterilized medical equipment by using ethylene oxide, glutaraldehyde, and formaldehyde (Hemminki et al., 1982). This study was conducted in 1980 for all sterilization workers employed at Finnish hospitals, especially 1,443 pregnant females (545 were exposed during pregnancy.). Since 1976, the maximum exposure level and eight-hour TWA in 24 Finnish hospitals has remained at 250 ppm ($458\text{ mg}/\text{m}^3$) and 0.1-0.5 ppm (0.2-0.9 mg/m^3), respectively. However, they might have been higher before 1976. The natural miscarriage rate was considerably ($P<0.001$) higher for sterilization workers (15.1%) than for those unexposed (4.6%). Considering ethylene oxide and other sterilizers, the increase in the number of natural miscarriages was observed only for those exposed to ethylene oxide during the initial period of pregnancy (those exposed (16.1%); those unexposed (7.8%);

P<0.01). The same pattern was observed in the data of hospitals. The natural miscarriage rates for controls, unexposed, and exposed groups were 9.2, 9.9, and 22.6%, respectively, and were significantly higher for the exposed group. In addition, comparisons were made between pregnant females in the exposed group who were working for hospitals and those in the control group, and the miscarriage rate was considerably (P<0.05) higher for those in the exposed group (20.4%) than for those in the control group (11.3%) (Hemminki et al., 1983).

In 1987, pregnancy outcomes were researched for 7,000 dental assistants aged 18-39 who were exposed to ethylene oxide. Targets were selected at random from the dental assistant register in California (Rowland et al., 1996). Out of 1,320 pregnant women whose age and exposure information during pregnancy were reliable, 32 were exposed to ethylene oxide during pregnancy (however, there is no detailed data regarding when and how much they were exposed during pregnancy). The risks of miscarriage, early delivery (21-37 weeks), and post-term delivery (42 weeks or more), for which age difference was taken into account, for pregnant women exposed to ethylene oxide were 2.5 (95% confidence limit (95% CI) = 1.0-6.3), 2.7 (95% CI = 8 - 8.8), 2.1 (95% CI = 0.7-5.9). With a logistic model, all of the adverse pregnancy risks (miscarriage, early delivery, post-term delivery) were 2.7 times (95% CI= 1.2-6.1) as high for pregnant women exposed as for those unexposed.

In Finland, a significant increase (P<0.05; odds ratio =4.7;95% CI = 1.2-18.4) in the risk of natural miscarriage was reported for pregnant women whose spouses were exposed to ethylene oxide (Lindholm et al., 1991). This study targeted 99,186 pregnant women. However, in this study, there was no available data about the exposure level. In addition, the number of pregnant women (n=0) whose spouses were exposed to ethylene oxide and the number of miscarriage cases (n=3) were insufficient, and confounding factors such as previous miscarriages, smoking habits, and alcohol consumption were not taken into account (Environment Canada and Health Canada, 2001).

f. Genotoxicity

Table 7-1 shows the test results for the genotoxicity of ethylene oxide in humans.

Chromosomal aberration, sister chromatid exchange, and DNA damage

Increases in chromosomal aberration were reported in those exposed to 5 ppm (9.2 mg/m³) or more of ethylene oxide. However, definite results were not available for those exposed at lower levels. Increases in the appearance frequency of micronucleate cells were observed in the bone marrow smears (Hogstedt et al., 1983) and peripheral blood of those exposed to ethylene oxide. Increases in the appearance frequency of micronuclei in peripheral blood were observed in workers exposed to relatively high levels of ethylene oxide (2-33 ppm (3.7-60.4 mg/m³)) (Ribeiro et al., 1994; Tates et al., 1991). However, in most of the previous studies, the appearance frequency of micronuclei was stable for those exposed at lower levels (Environment Canada and Health Canada,

2001).

Sister chromatid exchange increased in the peripheral lymphocytes of those exposed to ethylene oxide in the air (Abrahams, 1980; Garry et al., 1979; Laurent et al., 1984), indicating a relationship between ethylene oxide exposure and sister chromatid exchange. In order to identify the frequency of sister chromatid exchange in peripheral lymphocytes, a 24-month follow-up study was conducted for workers exposed to ethylene oxide (eight-hour TWA for workplace types I, II, III; exposure levels were 0.5, 5-10, 50-200 ppm (0.9, 9-18, 90-360 mg/m³)) (Stolley et al., 1984). The frequency of sister chromatid exchanges increased significantly among workplace types II and III. It was also very high among those exposed to high levels of ethylene oxide (Workplace type III, two workers) (on average, 32.3 sister chromatid exchanges/cell), and still remained high 24 months later (on average 21.1 sister chromatid exchanges/cell), indicating persistent DNA damage. Significant increases in sister chromatid exchanges were not found in those exposed to 0.5 ppm of ethylene oxide in workplace I (Stolley et al., 1984). However, there is a report of significant increases in sister chromatid exchanges in the peripheral lymphocytes of workers exposed at 0.35 ppm (Sarto et al., 1984). Another report says that the frequency of sister chromatid exchanges is considerably higher for 10 sterilization workers exposed to TWA 1.84 ppm of ethylene oxide than for 10 people in the control group (Sarto et al., 1987).

In a study targeting non-smoking males and females exposed to ethylene oxide in the sterilization of medical equipment (N = 4-12 persons/group), the amount of DNA single-strand breaks in peripheral mononuclear blood cells was 1.5 times as large for those exposed to 0.055-0.27 ppm (0.1-0.49 mg/m³) as for those exposed to no more than 0.055 ppm (0.1 mg/m³). As well, it was 2.2 times as large for those exposed to 0.27-1.1 ppm (0.5-2.0 mg/m³) as for those exposed to no more than 0.055 ppm (Fuchs et al., 1994).

Table 7-1 Test results for the genotoxicity of ethylene oxide in humans

Number of persons exposed to ethylene oxide	Number of persons in the control group	Period of exposure (year)		Concentration of ethylene oxide in the air		Results ²⁾			Reference
		Range	Average	Range	Average	CA	SCE	MN	
18	11 (factory I)	0.5 - 8	3.2		<1	+	-	+ ³⁾	Hogstedt et al., 1983
10	9 (factory II)	0.5 - 8	1.7		<1	+	-		
75	22	3 - 14	7	2 - 5 ⁴⁾		+		+	Ribeiro et al., 1994
9	8 (hospital worker)	2 - 6	4	20 - 25	0.125 ⁵⁾	+	+	-	Tates et al., 1991
15	9 (factory worker)	3 - 27	12	[36 - 45] 17 - 33 [30 - 60]	5 ⁵⁾	+	+	+	
75	41				≤50	+	+		Abrahams, 1980
12	8			0 - 36 ⁶⁾			+		Garry et al., 1979
10	15 (non-smoker)	0.5 - 10	5.7	} [36 - 225]			+		Laurent et al., 1984
15	7 (smoker)	0.5 - 10	4.5					+	
13	12 (workplace I)		3.2	0.5 ⁴⁾		-	-		Stolley et al., 1984
22 (21) ⁷⁾	19 (20) (workplace II)		3.1	5 - 10 ⁴⁾		-	(+)		
26 (25)	22 (21)(workplace III)		4	5 - 20 ⁴⁾		(+)	+		Galloway et al., 1986
22	22 (low exposure)	0.6 - 4	3	0.2 - 0.5 ⁴⁾	0.35	(+)	+		Sarto et al., 1984, 1987
10	10 (medium exposure)	1.5 - 15	6.8	0 - 9.3 ⁴⁾	1.84		+		
19	19 (high exposure)			3.7 - 20 ⁴⁾	10.7	+	+		
12	11	1 - 8	4	0.5 - 1		-			Pero et al., 1981
5	11	0.8 - 3	1.6	5 - 10		+			
9	13 (low exposure)				13 ⁸⁾		-		Yager et al., 1983
5	13 (high exposure)				501 ⁸⁾		+		
14	14			<0.07 - 4.3 ⁴⁾			-		Hansen et al., 1984
33	0	1 - 14		≤0.05 - 8	≤ 0.01 ⁹⁾	(+)			Clare et al., 1985
56	141	1 - 10		1 - 40 ⁴⁾		+	+		Richmond et al., 1985
36	35	1 - 14		0.1 - 8	0.05	-			van Sittert et al., 1985
18	10 (sterilization site)			0 - 2.7		+			Karelova et al., 1987
21	20 (production worker)			0 - 4		+			
14	10 (researcher)			0 - 5		+			

11	10 (researcher)			0 - 2.4		-			
9	27	0.5 - 12	5	0.025 - 0.38 ⁴⁾			-		Sarto et al., 1990
3	27			>0.38 ¹⁰⁾			+ ¹¹⁾		
5	10	0.1 - 4	2		0.025		- ¹²⁾		Sarto et al., 1991
5	10	4 - 12	8.6	<1 - 4.4	0.38		+ ¹²⁾		
34	23		8 ¹³⁾	<0.008 - 2.4 ⁴⁾	<0.3	-	+		Mayer et al., 1991
32	8		5.1	0 - 0.3 ⁴⁾	0.04		+		Schulte et al., 1992
11	8		9.5	0.13 - 0.3 ⁴⁾	0.16		+		
10	10		3	60 - 69 ⁴⁾		+	+		Lerda & Rizzi, 1992
47	47				<1		-		Tomkins et al., 1993
11	Smoker			0.5 - 417 ¹⁴⁾			-		Popp et al., 1994
14	Non-smoker			0.5 - 208 ¹⁴⁾			-		
	The total number of persons in the control group: 10								
7	The total number of Acciden			28 - 429 ⁴⁾			-	-	Tates et al., 1995
7	persons in the control t			<0.005 - 0.02			-	-	
7	group: 7		<5	<0.005 - 0.01			-	-	
			>15						
9	48 (low exposure)		4	2.7 - 10.9	2.7	+	-		Major et al., 1996
27	10 (high exposure)		15	2.7 - 82	5.5	+	+		
4-12	Sterilization of medical equipment by using ethylene oxide								
non-smoking	No more than 0.055 ppm (0.1 mg/m ³) 4-hour TWA								
males and	1. 0.055-0.27 ppm (0.1-0.49 mg/m ³)								
females	2. 0.27-1.1 ppm (0.5-2.0 mg/m ³)								
/group									
	The amount of DNA single-strand breaks (in peripheral mononuclear blood cells) a. 1.5 times b. 2.2 times as large as those exposed to no more than 0.055 ppm (0.1 mg/m ³)								

(Changes made to IARC ,1994: Environment Canada and Health Canada, 2001.)

- 1) 1 ppm = 1.83 mg/m³
- 2) CA: chromosomal aberration; SCE: sister chromatid exchange; MN: micronuclei
- 3) Positive for erythroblasts and polychromatic erythrocytes (negative for lymphocytes)
- 4) Eight-hour TWA
- 5) Forty-hour TWA based on hemoglobin adducts
- 6) Maximum concentration during the purge cycle
- 7) Figures in the parentheses are the number of persons studied by Galloway et al. (1986) for chromosomal aberration.
- 8) Average cumulative exposure (mg) for six months

- 9) Value obtained by linear extrapolation
- 10) Acute exposure due to leakage during sterilization
- 11) Nasal mucosa
- 12) Buccal cell
- 13) Maximum exposure period (year)
- 14) Maximum concentration

g. Carcinogenicity

Table 7-2 shows tumor risk assessment for ethylene oxide in epidemiological studies.

According to a cohort study in Sweden, the death rates for leukemia (standardized mortality ratio (SMR)= 9.21 (7 deaths), lymphoma/hematopoietic tumor (SMR=4.59 (9 deaths)) and stomach cancer (SMR= 5.46 (10 deaths) increased among 709 ethylene oxide production workers and sterilization workers (Hogstedt, 1988). The highest excess death rate was primarily observed in workers and engineers working during the period from 1941 to 1947 at old, sealed manufacturing plants where ethylene oxide was produced by the chlorohydrin method. In these plants, the exposure level of ethylene oxide was higher at the beginning. The average exposure level for the period from 1941 to 1947 was 14 ppm (26 mg/m³), and the highest level during that period was reported to be 400 ppm (732 mg/m³) which exceeded the odor threshold (Environmental Canada and Health Canada, 2001). 167 males out of 709 workers were working at old manufacturing plants, and SMRs for both stomach cancer and leukemia were about 7 (Hogstedt, 1988).

Greenberg et al. (1990) conducted a study on 2,174 workers employed at two ethylene oxide production plants in the United States, for which a 10-year update of this cohort, which excluded 278 chlorohydrin workers, was reported by Teta et al. (1993). Comparisons were made for the cause of death between the general population of the plants and unexposed workers. There were no increases in the death rate for those exposed to high levels of ethylene oxide. However, significant excess deaths due to stomach cancer were observed in those exposed at medium levels (SMR = 3.64, 95% CI = 1.02-9.57, four deaths), and deaths due to stomach cancer also increased in those exposed at low levels (no significant difference; SMR = 2.22, 95% CI = 0.61-5.75, four deaths). The relative risk for stomach cancer (2.77, 95% CI = 1.11-6.93, five deaths) increased among workers exposed to ethylene oxide for 2-9 years.

In a single, long-term cohort study (Steenland et al., 1991), Stayer et al. (1993) compared and analyzed the death rates for U.S. general population and 18,254 male and female workers exposed to ethylene oxide at U.S. factories of sterilized medical equipment and aroma chemicals. The SMR due to all types of hematopoietic tumors was 1.24 (no significant difference; 95%CI = 0.66-2.13) for those with the highest cumulative exposures, and there were no increases in the death rate for hematopoietic tumor. However, by gender, increases in the death rate for all types of hematopoietic tumors (SMR = 1.96; 95% CI = 1.01-3.43) were found in males of the highest exposure group. In addition, by the length of time that has elapsed since the first exposure, excess deaths due to hematopoietic tumor were found in workers exposed to ethylene oxide for 20 year or more (SMR = 1.55; 95% CI = 0.77-2.77), indicating a positive exposure-reaction relationship between cumulative exposure and leukemia (Stayner et al., 1993).

In addition, in the cohort study of 2,170 male and female workers exposed to ethylene oxide at two factories of disposable medical equipment in Sweden, the overall cancer risk did not increase,

while the risk of lymphoma/hematopoietic cancer increased (Hagmar et al., 1995). The level of cumulative exposure to ethylene oxide was slightly higher for leukemia patients than for other normal cohort members. In this study, the concentration of hemoglobin adducts was correlated well with the exposure level of ethylene oxide (Hagmar et al., 1995).

In Belgium, a case-referent study was conducted for 10 male workers with Hodgkin's disease who were employed at chemical production plants, and significant increases in the risk of Hodgkin's disease correlated with ethylene oxide exposure were observed (Swaen et al., 1996).

In further studies, the risks of lymphoma/hematopoietic cancer (Bisanti et al., 1993) and spleen cancer (Norman et al., 1995) were significantly high.

There is a report covering 13 epidemiological studies conducted from 1979 to 1993 (Shore et al., 1993). This report studied ethylene oxide-related issues such as exposure levels and frequency, and the length of exposure, in relation to relative risks for stomach, spleen, and brain cancers, leukemia, and non-Hodgkin's lymphoma. The average SMR (s SMR) for leukemia in 13 epidemiological studies was 1.06 (95% CI = 0.73-1.48) (Shore et al., 1993). A certain relationship was not observed among these studies in terms of exposure frequency and levels, but the risk of diseases increased linearly with the length of exposure. The s SMR for non-Hodgkin's lymphoma did not increase remarkably. Three studies were conducted to identify a relationship between the risk of diseases and exposure frequency or levels. In the most extensive study, the increasing tendency of risk of diseases due to cumulative exposure was observed (Environment Canada and Health Canada, 2001).

As outlined above, studies indicate that ethylene oxide has carcinogenicity to humans. However, carcinogenicity was not observed in some cases.

Tata et al. (1999) studied the carcinogenicity of ethylene oxide in the method similar to the one employed by Shore et al. (1993). This study was conducted by using the data of Hagmar et al. (1995) and Olsen et al. (1997), and the risks of leukemia, non-Hodgkin's lymphoma, and stomach/spleen/brain tumor did not increase remarkably. Significant increases in the risk of cancer were not observed (Gardner et al., 1989; Kiesselbach et al., 1990; Morgan et al., 1981; Olsen et al., 1997).

Table 7-2 Tumor risk assessment for ethylene oxide in epidemiological studies

Group exposed to ethylene oxide	Tumor	Risk assessment ¹⁾	Reference
Workers employed at ethylene oxide production plants and workers involved in the sterilization of medical equipment (males and females)	Stomach cancer	SMR ²⁾ = 5.46:10	Hogstedt, 1988
	Lymphoma/hematopoietic tumor	SMR= 4.59:9	
	Leukemia	SMR= 9.21:7	
Workers employed at old ethylene oxide production plants	Stomach cancer	SMR= 7.07:9	Teta et al., 1993
	Leukemia	SMR= 7.03:3	
Male workers producing or using ethylene oxide who were studied for 10 years by Greenberg, et al., (1990)	Stomach cancer	SMR= 1.60 (95%CI= 69-315):8	Stayner et al., 1993
	Spleen cancer	SMR= 0.61 (95%CI= 0.17-1.56):4	
	Brain and nervous system tumor	SMR= 1.50 (95%CI= 0.55-3.27):6	
	Leukemia	SMR= 1.06 (95%CI= 0.35-2.48):5	
Those exposed at medium levels	Stomach cancer	SMR= 3.64 (95%CI= 1.02-9.57):4*	Stayner et al., 1993
Those exposed at low levels	Stomach cancer	SMR= 2.22 (95%CI= 0.61-5.75):4	
Workers with cumulative exposure	Hematopoietic tumor	SMR= 1.24 (95%CI= 0.66-2.13):1	
	Non-Hodgkin's lymphoma	SMR= 1.92 (95%CI= 0.77-3.95):7	
	Leukemia	SMR= 0.75 (95%CI= 0.15-2.18):3	
Male workers with high cumulative exposure	Hematopoietic tumor	SMR= 1.96 (95%CI= 1.01-3.43):12*	
Male workers with medium cumulative exposure	Hematopoietic tumor	SMR= 1.43 (95%CI= 0.62-2.83):8	
Male workers with low cumulative exposure	Hematopoietic tumor	SMR= 0.95 (95%CI= 0.26-2.43):4	
Workers with cumulative exposure for 20 years or more	Hematopoietic tumor	SMR= 1.55 (95%CI= 0.77-2.77):4	
Male workers employed at chemical production plants	Hodgkin's disease	SIR= 4.97 (95%CI= 2.37-9.15):10*	Swaen et al., 1996
Male workers with a license for handling ethylene oxide and other chemicals	Non-Hodgkin's lymphoma	SMR= 6.82 (95%CI= 1.86-17.45):4*	Bisanti et al., 1993
	Leukemia	SMR= 1.93(95%CI= 0.23-6.99):2	
	Stomach cancer	SMR= 1.22 (95%CI= 0.40-2.87):5	
	Spleen cancer	SMR= 2.54 (95%CI= 0.52-7.44):3	
Male workers with a license for handling ethylene oxide only	Non-Hodgkin's lymphoma	SMR= 16.93 (95%CI= 3.49-49.53):3*	
Male and female workers who have used ethylene oxide as a pesticide.	Leukemia	SMR= 6.50 (95%CI= 0.79-23.49):2	Norman et al., 1995
	Leukemia	SMR= 1.85(p= 0.42):1	
	Spleen cancer	SMR= 3.92(p= 0.09):2	
	Leukemia	§SMR ⁴⁾ = 1.06 (95%CI= 0.73-1.48):31	Shore et al., 1993

Meta-analysis on the report released from 1979 to 1993.	Leukemia	$sSMR^4) = 1.06$ (95%CI= 0.73-1.48):31	Shore et al., 1993
	Non-Hodgkin's lymphoma	$sSMR = 1.35$ (95%CI= 0.93-1.90):31	
	Stomach cancer	$sSMR = 1.28$ (95%CI= 0.98-1.65):57	
	Spleen cancer	$sSMR = 0.98$ (95%CI= 0.69-1.36):34	
	Brain and central nervous system tumor	$sSMR = 0.89$ (95%CI= 0.55-1.36):19	
Assessment by Shore et al. (including two additional texts)	Leukemia	$mSMR^5) = 1.08$ (95%CI= 0.61-1.93):35	Teta et al., 1999
	Non-Hodgkin's lymphoma	$mSMR = 1.34$ (95%CI= 0.96-1.89):33	
	Stomach cancer	$mSMR = 1.23$ (95%CI= 0.71-2.13):59	
	Spleen cancer	$mSMR = 0.95$ (95%CI= 0.69-1.31):37	
	Brain tumor	$mSMR = 0.96$ (95%CI= 0.49-1.91):25	
Male and female workers involved in the sterilization of medical equipment	Lymphoma/hematopoietic tumor	$SIR^3) = 1.78$ (95%CI= 0.65-3.88): 6	Hagmar et al., 1995
Workers whose symptoms did not appear within at least 10 years after exposure (excluding those with no more than 0.13 ppm/year of cumulative exposure.)	Leukemia	$SIR = 2.44$ (95%CI = 0.3-8.81):2	
	Leukemia	$SIR = 7.14$ (95%CI= 0.87-25.8): 2	
	Brain tumor	$SIR = 3.80$ (95%CI= 0.78-11.1): 3	
Male workers producing ethylene chlorohydrin and propylene chlorohydrin	Brain and central nervous system tumor	$SMR = 1.23$ (95%CI = 0.25-3.58):3	Olsen et al., 1997
	Lymphoma/hematopoietic tumor	$SMR = 1.29$ (95%CI = 0.62 – 2.38):10	
Male workers producing ethylene chlorohydrin	Lymphoma/hematopoietic tumor	$SMR = 1.49$ (95%CI = 0.60-3.07):7	
Male workers producing ethylene chlorohydrin (including those whose symptoms did not appear for 25 years or more)	Lymphoma/hematopoietic tumor	$SMR = 1.94$ (95%CI = 0.71-4.23):6	
Males and females employed at ethylene oxide production plants or at facilities using ethylene oxide	Leukemia	$SMR = 2.25$ (95%CI = 0.47-6.59):3	Gardner et al., 1989
Males and females employed at hospitals	Stomach cancer	$SMR = 1.19$ (95%CI = 0.15-4.32):2	
Male workers employed at chemical production plants	Leukemia	$SMR = 0.85$ (95%CI = 0.10-3.07):2	Kiesselbach et al., 1990
	Stomach cancer	$SMR = 1.38$ (95%CI = 0.75-2.31):14	
Male workers employed at oil production plants	Spleen cancer	$SMR = 3.77$ (95%CI = 0.76-11.02):3	Morgan et al., 1981
	Brain and central nervous system tumor	$SMR = 2.85$ (95%CI = 0.32-10.30):2	
	Hodgkin's disease	$SMR = 5.70$ (95%CI = 0.64-20.58):2	

(Environmental Canada and Health Canada, 2001)

* = Statistically significant increase

1) Figures in italics show the numbers of deaths or diseases.

2) SMR: standardized mortality ratio, $SMR = \text{number of observed deaths} / \text{number of expected deaths}$

3) SIR: standardized incidence ratio

4) $sSMR$: summary SMR

5) $sSMR$: meta-SMK (Meta-analysis)

7.3 Toxicity to laboratory animals

7.3.1 Acute toxicity

The results of acute toxicity experiment on ethylene oxide are shown in Table 7-3. Lungs and the nervous system are target organs (Environment Canada and Health Canada, 2001).

The LD₅₀ by forced oral administration of ethylene oxide aqueous solution on rats, guinea pigs, and rabbits were 330 mg/kg, 270 mg/kg, and 631 mg/kg respectively (Smyth et al., 1941). All five samples that were given 200 mg/kg ethylene oxide in olive oil by forced oral administration died (Hollingsworth et al., 1956).

The acute toxicity is weak with inhalation exposure of ethylene oxide and the 4 hours LC₅₀ of mice, rats, and dogs were 835 ppm, 1,460 - 4,000 ppm, and 960 ppm (1,528 mg/m³, 2,672 - 7,320 mg/m³, and 1,757 mg/m³) respectively (Carpenter et al., 1949; Jacobson et al., 1956), and that of guinea pigs was 7,000 ppm (12,810 mg/m³) (Waite et al., 1930). The mortalities of both male and female mice were 80 to 100% by 800 ppm (1,463 mg/m³), but all subjects survived at 400 ppm (732 mg/m³) (U.S. NTP, 1987).

The acute symptoms observed first by inhalation exposure of animals are nose scratching, nasal mucus, watering eyes, ptyalism, and the symptoms observed next are lung congestion, edema, breathing difficulty, and gasping respiration. Lung infection and pneumonia were observed as secondary symptoms that resulted in death. Central nervous system effects such as ataxia, convulsion, and vomiting were also observed (Hollingsworth et al., 1956; Translation supervised by Hiroshi Naito and Noriko Yokote, 1999).

By intravenous administration of ethylene oxide, the LD₅₀ of rats and rabbits were 335 mg/kg and 178 mg/kg respectively. By intraperitoneal administration, that of mice, rats, and rabbits were 178 - 251 mg/kg, and by subcutaneous administration, that of rabbits was 200 mg/kg (Bruch, 1973; Woodward and Woodward, 1971).

Table 7-3 The results of acute toxicity experiments on ethylene oxide

	Mouse	Rat	Guinea pig	Rabbit	Dog
Oral administration LD50 (mg/kg)	ND	330	270	631	ND
Inhalation LC50 (ppm)	835 (4 hours)	1,460 – 4,000 (4 hours)	7,000 (4 hours)	ND	960 (4 hours)
Intravenous LD50 (mg/kg)	ND	335	ND	178	ND
Intraperitoneal LD50(mg/kg)	178	178	ND	251	ND

Subcutaneous LD50(mg/kg)	ND	ND	ND	200	ND
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ND: No data available

7.3.2 Stimulus and corrosion

By the experiment to apply the absorbent cotton soaked in 10% and 50% ethylene oxide aqueous solutions for 1 to 60 minutes to the surface of rabbit's skin where hair is shaved, acute inflammatory edema appeared (Hollingsworth et al., 1956).

With rabbits, for ethylene oxide of 1,000 ppm (1,800 mg/m³) or greater density, eye stimulus depending on the dosage was observed, and the maximum non-stimulus density of ethylene oxide for 6 hours exposure was 0.1% (McDonald et al., 1977).

7.3.3 Sensitization

With the experiment of local transdermal and subcutaneous administrations of 0.5 ml ethylene oxide of 1% solution applied 3 times per week for 3 weeks to guinea pigs, no sensitization was observed (Woodward and Woodward, 1971).

7.3.4 Repeat dose toxicity

The results of repeat dose toxicity tests on ethylene oxide are shown in Table 7-4. Concerning the repeated dose toxicity of ethylene oxide, there is little data available on exposure paths other than inhalation.

a. Oral administration

As the result of experiments in which 3 mg/kg, 10 mg/kg, and 30 mg/kg ethylene oxide were administered by oral administration to rats (female) for 5 days/week for 30 days, no effect was observed. As the result of experiments in which 100 mg/kg ethylene oxide was administered by oral administration for 5 days/week for 21 days, a decrease in weight, irritation in the stomach, and slight hepatopathy were observed (Hollingsworth et al., 1956).

b. Inhalation exposure

As the result of experiments in which 400 - 820 ppm (732 - 1,500 mg/m³) were administered to mice, rats, rabbits, guinea pigs, and monkeys for 10 days - 8 weeks by inhalation exposure, the mortality rate increased (Hollingsworth et al., 1956; Jacobson et al., 1956; Snellings, 1982; U.S. NTP, 1987). Toxicity was observed mainly in nerve and blood/lymphatic systems.

b-1 Neurotoxicity

In male and female B6C3F₁ mice, as the result of experiments in which 0 ppm, 10 ppm, 48 ppm, 104 ppm, and 236 ppm ethylene oxide were administered for 6 hours/day, 5 days/week, for 10 - 11 weeks by inhalation exposure, neuromuscular toxicity such as kyphotic gait, inhibition of locomotor activity, and decrease in righting reflex were observed in the groups administered 48 ppm or more (Snellings et al., 1984a).

In rats, as the result of experiments in which 500 ppm (915 mg/m³) ethylene oxide was administered for 6 hours/day, 3 days/week, for 13 weeks, hindlimb ataxia and axonopathy of myelinated fibers in the hindlimb nerves were observed (Hollingsworth et al., 1956; Matsuoka et al., 1990; Mori et al., 1990; Ohnishi et al., 1985, 1986).

In cynomolgus monkeys, as the result of experiments in which 50 ppm and 100 ppm (92 mg/m³ and 183 mg/m³) were administered intermittently for 2 years, delay in nerve conduction velocity, axonal dystrophy of the gracile nucleus in the medulla and demyelination of the axon terminal in fasciculus gracilis, atrophy of skeletal muscles were observed in both groups (Lynch et al., 1984a; b, Sprinz et al., 1982). In rabbits and guinea pigs, as the result of repetitive exposure experiments conducted for 176 - 226 days, paralysis and muscle atrophy were observed in those administered 204 ppm (373 mg/m³). In monkeys, as the result of repetitive exposure experiments conducted for 60 - 226 days, paralysis and muscle atrophy were observed in those administered 204 ppm (373 mg/m³) (Hollingsworth et al., 1956).

b-2 Hematotoxicity

In mice, as the result of experiments in which 255 ppm and 425 ppm (232 mg/m³ and 467 mg/m³) were administered for 6 hours/day, 5 days/week, for 10 - 13 weeks by inhalation exposure, a decrease in the number of erythrocytes (RBC), the quantity of hemoglobin (Hb), the hematocrit value (Ht), the bone marrow cell density, and the number of lymphocytes was observed (Popp et al., 1986). As the result of experiments in which they were exposed to higher density ethylene oxide (600 ppm, or 1,098 mg/m³, for example) for 14 weeks, lymphocyte necrosis in the thymus and spleen were observed, and they showed aplastic anemia and necrosis of lymphoid tissue (U.S. NTP, 1987).

In rats, as the result of experiments in which they were exposed to 100 - 500 ppm (183 - 915 mg/m³) for several weeks, all the exposed groups showed anemic symptoms such as a decrease in Hb, Ht, RBCs, the number of lymphocytes, as well as reticulocytosis (Fujishiro et al., 1990; Mori et al., 1990). They also showed a decrease in glutathione reductase and of activation of creatine kinase in blood and several organs (Fujishiro et al., 1991; Katoh et al., 1988, 1989; Matsuoka et al., 1990; Mori et al., 1990), abnormal porphyrin/heme metabolism (Fujishiro et al., 1990), and an increase in liver lipid peroxidation (Kato et al., 1988, 1989).

In rabbits, as the result of experiments in which they were exposed to 250 ppm (458

mg/m³) for 12 weeks, there was no change in the hematological parameters (Yager and Benz, 1982). However in dogs, as the result of experiments in which they were exposed to 100 ppm (183 mg/m³) for 6 months, a decrease in RBCs, Hb, and Ht were observed in 2/3 of the samples (Jacobson et al., 1956).

b-3 Other toxicity

In mice, as the result of experiments in which they were exposed for 14 weeks to 100 ppm (183 mg/m³), alteration of kidney tubules was observed, and in those exposed to 600 ppm (1,098 mg/m³), necrosis of kidney tubules was observed (U.S. NTP, 1987).

In F344 rats, as the result of experiments in which they were exposed to ethylene oxide for about 2 years, in those exposed to 33 ppm and 101 ppm (60.4 mg/m³ and 183 mg/m³), inhibition of weight increase was observed, and in those exposed to 101 ppm (60.4 mg/m³), an increase in mortality was observed (Snellings et al., 1984b).

Other nonneoplastic effects observed included, by exposure to 50 ppm (92 mg/m³) or more, an increase in respective frequency of serum aspartate aminotransferases (AST), of increase in the absolute weight of the kidneys and adrenal glands, inflammatory lesions in the lungs, nasal cavity, trachea, and inner ears, proliferative changes and degenerative changes in the adrenal cortex, multiple mineralization in choroid and sclera of the eye (Lynch et al., 1984a, b).

c. Subcutaneous administration

In rats, as the result of experiments in which 18 mg/kg and 54 mg/kg ethylene oxide were administered for 30 days by subcutaneous administration, a decrease in weight, bleeding and inflammation at the sites of administration were observed in the group administered 54 mg/kg (Hollingsworth et al., 1956).

In dogs, as the result of experiments in which 36 mg/kg ethylene oxide was administered for 30 days by subcutaneous administration, anemia with bone marrow hyperplasia and extramedullary hematopoiesis were observed (Woodward and Woodward, 1971), but no effect was observed in the experiment using the same amount administered for 21 days by subcutaneous administration (Bolaz, 1976).

From the above data, regarding the repeat dose toxicity of ethylene oxide, major symptoms were observed in the nervous system and blood/lymphatic systems, and the effect on the nervous system was observed with minimum dose. The NOEL for rats and mice is 10 ppm (18.3 mg/m³) from the results of an experiment where mice were administered 0 ppm, 10 ppm, 48 ppm, 104 ppm, and 236 ppm ethylene oxide for 10 - 11 weeks by subcutaneous administration, and neurotoxicity and malformed spermatozoa were observed in those administered 48 ppm or more, and in the F344

rats that were administered 10 ppm, 33 ppm, and 100 ppm for 2 years by subcutaneous administration, inhibition of weight increase was observed in those administered 33 ppm (60.4 mg/m³).

Table 7-4 The results of the repeat dose toxicity test on ethylene oxide

Animals	Method of administration	Period of administration	Volume of administration	Results	Reference
Rat 5 females	Forced oral administration	21 days (5 days/week) 30 days (5 days/week)	100 mg/kg/day 3 mg/kg/day, 10 mg/kg/day, 30 mg/kg/day	Weight decrease, stimulus in stomach, slight hepatopathy No effect	Hollingsworth et al., 1956
B6C3F ₁ mouse (male, female) 30 mice/group	Inhalation exposure	10 - 11 weeks, (6 hours/day, 5 days/week)	0, 10, 48, 104, or 236 ppm	48 ppm or more: Male: inhibition of locomotor activity Female: kyphotic gait 104 ppm or more: Male: kyphotic gait Female: inhibition of locomotor activity 236 ppm: Male and female: decrease in righting reflex, RBCs, and Hb	Snellings et al., 1984a
Mouse 30 females (white)	Inhalation exposure	26 weeks, (6 hours/day, 5 days/week)	100 ppm (183 mg/m ³ /day)	No effect	Jacobson et al., 1956
Mouse	Inhalation exposure	10 - 13 weeks (6 hours/day, 5 days/week)	255, 425, or 600 ppm (232, 467, or 1,100 mg/m ³ /day)	All groups: Decrease in RBCs, Hb, Ht, bone marrow cell density, and the number of lymphocytes 600 ppm: Lymphocyte necrosis in thymus and spleen, necrosis of lymphoid tissue, aplastic anemia	Popp et al., 1986
Mouse	Inhalation exposure	14 weeks (6 hours/day, 5 days/week)	100-600 ppm (183-1,098 mg/m ³ /day)	100 - 400 ppm: Alteration of kidney tubules (male and female) 600 ppm:	U.S. NTP, 1977

Rat	Inhalation exposure	13 weeks (6 hours/day, 3 days/week)	500 ppm (915 mg/m ³ /day)	Necrosis of kidney tubules (male and female) Necrosis of thymus lymphocytes (male and female) Necrosis of spleen lymphocytes (male) 200 ppm or more: Rhinitis (male and female) Hindlimb ataxia, axonopathy of myelinated fiber in hindlimb nerve	Hollingsworth et al., 1956; Matuoka et al., 1990; Mori et al., 1990; Ohnishi et al., 1985, 1986
Rat	Inhalation exposure	26 weeks (6 hours/day, 3 days/week)	100-500 ppm (183-915 mg/m ³ /day)	All groups: Anemia, glutathione reductase and activation of creatine kinase in blood and in several organs, abnormal porphyrin/heme metabolism, increase in liver lipid peroxidation	Fujisiro et al., 1990, 1991; Katoh et al., 1988, 1989; Matuoka et al., 1990; Mori et al., 1990
F344 rat (male and female) 120 rats/group	Inhalation exposure	About 2 years (6 hours/day, 5 days/week)	0, 10, 33, or 101 ppm (18.3, 60.4, or 183 mg/m ³ /day)	Groups administered 33 ppm and 101 ppm: Inhibition of weight increase Group administered 101 ppm: Increase in mortality	Snellings et al., 1984b
Rabbit	Inhalation exposure	12 weeks	250 ppm (458 mg/m ³ /day)	No change in hematological parameters	Yager & Benz, 1982
Rabbit 2 males and females/group,	Inhalation exposure	176 - 226 days, (7 hours/day,	49, 113, or 204 ppm	113 ppm: Growth inhibition and increase in lung weight	Hollingsworth et al., 1956

Guinea pig 8 pigs/group		5 days/week)	(89.7, 207, or 373 mg/m ³ /day)	204 ppm: Paralysis and muscle atrophy, slight congestive edema in the lung	
Beagle 3 dogs/group	Inhalation exposure	130 days (6 hours/day, 5 days/week)	100, or 290 ppm (183, or 531 mg/m ³ /day)	100 ppm: Decrease in RBCs, Hb, and Ht in 2 out of 3 examples 290 ppm: Vomiting, tremor, weakness of hindlimb, lung congestion, muscle atrophy	Jacobson et al., 1956
Cynomolgus monkey	Inhalation exposure	Inhalation exposure for 2 years intermittently	50, or 100 ppm	Both groups: Delay in nerve conduction velocity, axonal dystrophy of the gracile nucleus in medulla and demyelination of the axon terminal in fasciculus gracilis, atrophy of skeletal muscles	Lynch et al., 1984a, b; Sprinz et al., 1982
Monkeys 2 females/group, one male and one female/group	Inhalation exposure	176 - 226 days, (7 hours/day, 5 days/week)	113, or 204 ppm (207, or 373 mg/m ³ /day)	113 ppm: Growth inhibition and increase in lung weight	Hollingsworth et al., 1956
		60 days	357 ppm (653 mg/m ³ /day)	204 ppm: Paralysis and muscle atrophy, slight congestive edema in the lung 357 ppm: Growth inhibition, neurological damage including hindlimb paralysis and muscle atrophy	
2 males Rat	Subcutaneous	140 days 30 days	357 ppm 18, or 54 mg/kg/day	54 mg/kg/day: Weight decrease, bleeding and inflammation at sites of administration	
Dog	Subcutaneous	30 days	36 mg/kg/day	Anemia, bone marrow hyperplasia, extramedullary hematopoiesis	Woodward & Woodward, 1971
Dog	Subcutaneous	21 days	36 mg/kg/day	No effect	Bolaz, 1976

7.3.5 Reproductive and developmental toxicity

The results of reproductive and developmental toxicity experiments on ethylene oxide are shown in Table 7-5.

a. Reproductive toxicity

In the results of experiments on Swiss-Webster mice in which 200 ppm (366 mg/m³) was administered by inhalation exposure for 5 days, an increase in malformed spermatozoa was observed (Ribeiro et al., 1987). In addition, in the results of experiments on female crossbred mice in which 200 ppm and 1200 ppm (549 mg/m³ and 2,196 mg/m³) were administered before mating, an increase in fetus absorption and a decrease in implantation and the number of live fetus were observed (Generoso et al., 1987).

The effect on reproduction in rats is the same as that in mice. As the results of experiment on Wistar rats in which 0 ppm, 50 ppm, 100 ppm, 250 ppm ethylene oxide were administered by inhalation exposure for 6 hours/day, 5 days/week, for 13 weeks, a decrease in epididymis weight and of the number of spermatozoa, and an increase in malformed sperm heads (deformity) were observed in those administered 250 ppm (458 mg/m³), and malformed sperm heads (deformity) increased in those administered 50 ppm (92 mg/m³) and 100 ppm (183 mg/m³) (Mori et al., 1991). The LOAEL based on the deformity of sperm heads was judged as 50 ppm (92 mg/m³).

Developmental or fetal toxicity was observed in the reproductive experiment in which 100 - 150 ppm (183 - 275 mg/m³) ethylene oxide was administered by inhalation exposure to the rats before mating and during pregnancy. Under these exposure conditions, toxic symptoms were not observed in mothers, but a decrease in the number of implantations, an increase in embryo absorption, and a decrease in the number of babies born were observed (Hackett et al., 1982; Hardin et al., 1983; Snellings et al., 1982a, b) and obvious fetal toxicity was observed (Environment Canada and Health Canada, 2001).

In the results of experiments on cynomolgus monkeys in which 0 ppm, 50 ppm, 100 ppm ethylene oxide were administered by inhalation exposure for 7 hours/day, 5 days/week, for 24 months, a decrease in the number and mobility of spermatozoa were observed (Lynch et al., 1984b).

b. Developmental toxicity

b-1 Inhalation exposure

In the results of experiments on female mice in which 1,200 ppm (2,196 mg/m³) was administered by short-term inhalation exposure in various frequencies after mating, congenital malformations were observed in the babies born, including omphalocele, eyeball defect (anophthalmia), schistothorax, acardia, uranoschisis, and defect in the tail or leg (Generoso et al., 1987; Rutledge and Generoso, 1989). In addition, an increase in the number of fetal deaths during

the middle and later stages of pregnancy and difficulty in weaning babies were observed (Generoso et al., 1987; Rutledge and Generoso, 1989; Rutledge et al., 1992).

In the results of experiments on female SD rats in which 150 ppm (275 mg/m³) ethylene oxide which is a toxic concentration for mothers was administered by inhalation exposure before pregnancy, around conception, and during pregnancy, a decrease in fetal weight and crown-rump length and a delay in ossification occurred (Hackett et al., 1982; Hardin et al., 1983). These changes were observed for every exposure period and, therefore, the exposure before pregnancy is considered to be an important factor (IARC, 1985).

In the results of experiments on F344 rats in which 100 ppm (183 mg/m³) ethylene oxide, which is not a toxic concentration for mothers, was administered by inhalation exposure during organogenesis stage, fetal weight decreased (Snellings et al., 1982a).

In the results of experiments on pregnant SD rats in which 0 ppm, 800 ppm, 1,200 ppm (0 mg/m³, 1,464 mg/m³, 2,196 mg/m³) were administered by short-term repetitive inhalation exposure for 30 minutes, 3 times/day during 6 - 15 days pregnancy, the group administered 800 ppm or more showed fetal toxicity indicated by a decrease in fetal weight and the group administered 1,200 ppm or more showed toxicity to the mother, causing inhibition of weight increase in the mothers, but there was no proof of teratogenicity (Saillenfait et al., 1996).

In the results of experiments on F344 rats in which 0 ppm, 10 ppm, 33 ppm, 100 ppm (0 mg/m³, 18.3 mg/m³, 60 mg/m³, 183 mg/m³) ethylene oxide were administered to 30 males and 30 females per group by inhalation exposure for 6 hours/day, 5 days/week, for 12 weeks, and where female rats became pregnant after male and female rats were housed together for 2 weeks, and where they were administered ethylene oxide again during 1 to 19 days pregnancy before delivery and also administered 5 hours/day, 7 days/week from the 5th day to the 21st day after the delivery, the group administered 100 ppm (183 mg/m³) showed significant extension of pregnancy compared to the other groups. Though the number of babies born and implantations per rat decreased in the concentration of 100 ppm, there was no effect on survival or delivery and there was no effect on the weight and organs of the parent generation (Snellings et al., 1982b).

In rabbits, as the result of experiments in which 150 ppm (275 mg/m³) ethylene oxide (99.7% purity) was administered by inhalation exposure for 7 hours/day during 7 - 19 days pregnancy or 1 - 19 days pregnancy, no toxicity in either mothers or the fetus and no teratogenicity was observed (Hackett et al., 1982).

b-2 Intravenous administration

In mice, as a result of experiments in which 0 mg/kg/day, 75 mg/kg/day, 150 mg/kg/day of ethylene oxide were administered with 5% extrose solution by intravenous administration for 4 - 6 days, 6 - 8 days, 8 - 10 days, 10 - 12 days pregnancy, significant increases were observed in the

incidence rates of frontonasal dysplasia and vertebral synostosis with the groups administered high doses, i.e. 19.3% in 6 - 8 days pregnancy and 9.5% in 10 - 12 days pregnancy compared to 0 - 2.3% of the control group. In high dosage groups, mothers died after the administrations of 4 - 6 days, 8 - 10 days, 10 - 12 days pregnancy (LaBorde & Kimmel, 1980).

In the results of experiments on NZW rabbits in which 0 mg/kg/day, 9 mg/kg/day, 18 mg/kg/day, 36 mg/kg/day of ethylene oxide were administered in 6 - 14 days pregnancy, and 0 mg/kg/day, 18 mg/kg/day, 36 mg/kg/day were administered in 6 - 9 days pregnancy, significant inhibition of weight increase in mothers was observed in the groups administered 18 and 36 mg/kg. In the group exposed during 6 - 9 days pregnancy, no effect of fetal toxicity was observed, but a decrease in the number of live fetuses and an increase in the number of absorbed embryos per rabbit depending on the dose was observed in the group exposed during 6 - 14 days pregnancy (Jones-Price et al., 1982).

b-3 Intraperitoneal administration

In the results of experiments on pregnant mice in which 125 mg/kg ethylene oxide was administered once by intraperitoneal administration either during the zygotic period or embryonic period, a decrease in the number of live fetuses and an increase in frequency of skeletal defects after implantation were observed (there is no description about the toxicity to mothers) (Polifka et al., 1966).

From the above data on reproductive and developmental toxicity, toxicities on testis and on fetus, developmental toxicity, and teratogenicity of ethylene oxide were observed in various administration routes. Regarding the inhalation route that exposure is expected in the general environment, the minimum toxic level is LOAEL 50 ppm (92 mg/m³) of rats based on spermatogenesis.

Table 7-5 The results of reproductive and developmental toxicity experiment on ethylene oxide

Animals	Method of administration	Period of administration	Volume of administration	Results	Reference
Reproductive toxicity					
Swiss-Webster mouse	Inhalation exposure	5 days	200 ppm (366 mg/m ³)	Increase in the rate of abnormal spermatozoa	Ribeiro et al., 1987
Mouse (female)	Inhalation exposure	Exposure before mating	300, or 1,200 ppm (549, or 2,196 mg/m ³)	Increase in fetal absorption, decrease in the numbers of implantation and of live fetuses	Generoso et al., 1987
Wistar rat	Inhalation exposure	13 weeks (6 hours/day, 5 days/week)	0, 50, 100, or 250 ppm	50 ppm, 100 ppm: Increase in malformed sperm heads (deformity) 250 ppm (458 mg/m ³): Decrease in epididymis weight, decrease in the number of spermatozoa, increase in malformed sperm heads (deformity) LOAEL 50 ppm (92 mg/m ³) (Judgment of this assessment report)	Mori et al., 1991
Rat	Inhalation exposure	Before mating and during pregnancy	100-150 ppm (183 - 275 mg/m ³)	No toxicity symptom in mothers	Hackett et al., 1982; Hardin et al., 1983; Snellings et al., 1982a, b
Cynomolgus monkey (mature male) 12 monkeys/group	Inhalation exposure	24 months (7 hours/day, 5 days/week)	0, 50, or 100 ppm	50 ppm (92 mg/m ³) or more; Decrease in the number and mobility of spermatozoa Malformed sperm heads did not increase.	Lynch et al., 1984b
Developmental toxicity					
Mouse (female)	Inhalation exposure	After mating, various	1,200 ppm (2,196 mg/m ³)	Babies born had congenital malformations including	Generoso et al., 1987;

		frequencies		omphalocele, eyeball defect (anophthalmia), schistothorax, acardia, uranoschisis, and defects in the tail or leg.	Rutledge and Generoso, 1989
Mouse (female)	Inhalation exposure	Middle and later days of pregnancy	ND	Increase in the number of fetal deaths, difficulty weaning babies	Generoso et al., 1987; Rutledge and Generoso, 1989; Rutledge et al., 1992
SD rat (female)	Inhalation exposure	Before pregnancy, around conception, and during pregnancy	150 ppm (275 mg/m ³) (toxic concentration for mothers)	Decrease in fetal weight and crown-rump length, delay in ossification	Hackett et al., 1982; Hardin et al., 1983
F344 rat	Inhalation exposure	During organogenesis stage	100 ppm (183 mg/m ³) (non-toxic concentration for mothers)	Decrease in fetal weight	Snellings et al., 1982a
SD rat (female)	Inhalation exposure	6-15 days pregnancy (30 minutes, 3 times/day)	0, 800, or 1,200 ppm (0, 1,464, or 2,196 mg/m ³)	800 ppm or more: Decrease in fetal weight 1,200 ppm: Inhibition of weight increase in mothers, no teratogenicity	Saillenfait et al., 1982b
F344 rat (male and female) 30 rats/group	Inhalation exposure	As written in the column on the right	0, 10, 33, or 100 ppm (0, 18.3, 60, or 183 mg/m ³) 6 hours/day, 5 days/week, for 12 weeks, having male and female rats live together for 2 weeks	100 ppm (183 mg/m ³) group: Female: extension of pregnancy 100 ppm group: Decrease in the numbers of babies born and implantation No effect on survival or delivery Parents: no effect on weight and organs	Snellings et al., 1982b

				to get the female rats pregnant, ethylene oxide again administered during 1 to 19 days pregnancy, then delivery. Further administered 5 hours/day, 7 days/week from 5th to 21st day after the delivery		
Rabbit	Inhalation exposure	7-19 days pregnancy or 1-19days pregnancy (7 hours/day)	150 ppm (275 mg/m ³)	No toxicity in mothers, no fetal toxicity, no teratogenicity	Hackett et al., 1982	
Mouse	Intravenous	4-6, 6-8, or 8-10 days, 10-12 days pregnancy	0, 75, or 150 mg/kg/day (5% extrose solution)	Incidence rates of frontonasal dysplasia and vertebral synostosis 0 mg/kg/day group: 0 - 2.3% increase 150 mg/kg/day: 6 - 8 days: 19.3% increase 10 - 12 days: 9.5% increase Mothers died after the administrations of 4 - 6 days, 8 - 10 days, 10 - 12 days pregnancy.	LaBoude & Kimmel, 1980	
NZW rabbit	Intravenous	6-14 days pregnancy	0, 9, 18, or 36 mg/kg/day	18 mg/kg or more: Inhibition of weight increase in mother animals Decrease in the number of live fetus and increase in the number of absorbed embryo per rabbit	Jones-Price et al., 1982	
NZW rabbit	Intravenous	6-9 days pregnancy	0, 18, or 36 mg/kg/day	18 mg/kg or more: Inhibition of weight increase in mother animals No fetal toxicity	Jones-Price et al., 1982	

Mouse	Intraperitoneal, once	Pregnancy, zygotic period or embryonic period	125 mg/kg	Decrease in the number of live fetus and increase in frequency of skeletal defects No description about the toxicity to mothers	Polifka et al., 1996
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ND: No data available

7.3.6 Genetic Toxicity

Table 7-6 shows the result of genetic toxicity tests for ethylene oxide, and Table 7-7 shows the summary of the test results.

Ethylene oxide is a strong alkylating agent, and shows genetic toxicity (Environment Canada and Health Canada, 2001) by irreversibly binding covalently to protein (Fraenkel-Conrat, 1944) and DNA (Ehrenberg et al., 1974) of mammals including humans (Calleman et al., 1978). In vitro reaction part is N7 of guanine (Brookes and Lawley, 1961), N1 of adenosine (Windmueller and Kaplan, 1962), and N3 of uridine (Ukita et al., 1963) (U.S. NTP, 1987).

Ethylene oxide interacts with protein's nucleophilic center (Ehrenberg et al., 1974; Segerback, 1983). Ethylene oxide mutagenicity in bacteria and plant systems has been reported in viruses (Hussain and Ehrenberg, 1975; Jordy et al., 1975), Salmonella typhimurium (Salmonella typhimurium) (Embree and Hine, 1975), coli bacteria (Hussain and Osterman-Golkar, 1976), bread mold (*Neurospora crassa*) (Kilbey and Kolmark, 1968), fission yeast (*Schizosaccharomyces pombe*) (Migliore et al., 1982), barley (Ehrenberg, 1959; Ehrenberg et al., 1956; Lindgren and Sulovska, 1969; Sulovska, 1969), rice (Jana and Roy, 1975), wheat (Mackey, 1968), and spiderwort family (*Tradescantia paludosa*) (Smith and Lofty, 1954).

a. in vitro

DNA damage and genetic mutation occurred in bacteria, yeast, and fungus. Genetic conversion was induced in yeast. Observed effects in mammal cells were genetic mutation, small cell formation, abnormal chromosome cell genetic transformation, irregular DNA generation, sister chromatid exchange, and DNA strand breakage.

In the Ames test, mutagenicity was found in *Salmonella typhimurium* TA1535 and TA100 (Pfeiffer and Dunkelberg, 1980). However, it was not found in T2 bacteriophage (Cookson et al., 1971).

Ethylene oxide induced mutation in HGPRT position of Chinese Hamster Ovary (CHO). This activity was not affected by S9 which is aroclor-induced rat liver (Tan et al., 1981).

Ethylene oxide also induced genetic mutation in plant cells such as barley, rice, and peas (Environment Canada and Health Canada, 2001). Damage in chromosome and sister chromatid exchange were found in barley, wheat, and in pollen of spiderwort (*Tradescantia paludosa*) (Ehrenberg et al., 1956, 1959; Mackey, 1968; Moutschen-Dahmen et al., 1968; Smith and Lofty, 1954). In a test that exposed barley seeds to ethylene oxide gas of 1.5×10^6 mg/m³ (80%) density for 6 days, reproduction dysfunction which was caused by abnormal chromosomes increased by 500%, and genetic mutation of the second generation of chlorophyll increased by 330% by the same treatment. When barley seeds were soaked in 3,084 mg/L and 11,894 mg/L solution for 2 hours, genetic mutation of the second generation chlorophyll increased to 3.7, which is a 1380% increase

(Ehrenberg et al., 1956). Rice has two gene types, and its mutagenic property decreases as the density of ethylene oxide increases. The density range was 888~6,167 mg/L in 8-hour exposure (Jana and Roy, 1975).

b. in vivo

The study result of genetic toxicity of in-vivo ethylene oxide was positive for all administration routes of oral, inhalation, and injection (IARC, 1994).

Drosophila (*Drosophila melanogaster*) which was exposed to ethylene oxide showed dose-related, sex-linked, recessive, lethal mutation (Bird, 1952) and autosomal chromosome-deleted mutation (Fahmy and Fahmy, 1956). Lethal mutation and translocation were also induced in all stages of antheridia of *drosophila* (Nakao and Auerbach, 1961).

As for mammals, genetic mutation in the lungs (IaI position) and spleen T-lymph cells (HPRT position) were found in transgenic mice which were exposed to ethylene oxide by inhalation (Sisk et al., 1997; U.S. NTP, 1987; Walker et al., 1997a). The average frequency of mutation with HPRT position increased by 500%- 560% (Walker et al., 1997b) when we compared the male F344 rats which were exposed to 200 ppm (366 mg/m³) ethylene oxide by inhalation with the B6C3F1 mouse which were not exposed. In the test of exposing 200ppm ethylene oxide to Big Blue (IaI transgenic) B6C3F1 mice for 6 hours/day, 5 days/week, and for 48 hours, the average frequency of mutation with HPRT position increased by 500% (Recio et al., 1999). However, it did not increase with lower density or shorter exposure (Recio et al., 1999).

Abnormal chromosome was found in the bone marrow cells of the rats which were exposed to 0.6~63 ppm (1~112 mg/m³) of ethylene oxide by inhalation (Fomenko and Strekalova, 1973; Strekalova et al., 1975), male Long-Evans rats which were exposed to 250 ppm (450 mg/m³) of ethylene oxide by inhalation for 7 hours/day for 3 days (Embree and Hine, 1977), monkeys which were exposed to 50 and 100 ppm (90 and 180 mg/m³) of ethylene oxide by inhalation, and rats for which 9 mg/kg of ethylene oxide water solution was given orally (Strekalova, 1971). In addition, the increase of the number of small cells was found in the bone marrow cells of mice and rats which 10~200 mg/kg of ethylene oxide was given intraperitoneally (Conan et al., 1979), or intravenously injected (Appelgren et al., 1978).

c. Genetic Toxicity In Reproduction Cells

Ethylene oxide induced dominant fatal effect in mice and rats. In tests where 0, 165, 204, 250, 300 ppm (0, 302, 373, 458, 549 mg/m³) of ethylene oxide was given to male (C3H×101) F1 mice for 6 hours/day, 5 days/week, for 6 weeks, the dominant fatal rate increased depending on the doses, and a significant difference was shown when the density was 373 mg/m³ or more (P<0.01) (Generoso et al., 1990). In tests where mice were given 300 ppm (549 mg/m³) ethylene

oxide inhalation for 6 hours/day for 4 days, 600 ppm of ethylene oxide for 3 hours/day for 4 days, and 1,200 ppm (2,200 mg/m³) of ethylene oxide for 1.5 hours/day for 4 days, the fatal rates for fetuses were 11, 32 and 64% respectively. This indicated that the dominant fatal reaction increases as the density increases even if the amount of the total exposure amount (1,800 ppm/day [3,300 mg/m³/day) is the same (Generoso et al., 1986).

The summary of the results is shown in Table 7-7.

The summary of the observations above is as follows. Ethylene oxide caused DNA damage in bacteria, and genetic toxicity was found in plants, fungus, and insects. In addition, ethylene oxide caused abnormal chromosomes in plants and genetic translocation in insects. It also caused genetic toxicity in cultured cells of mammals, and induced DNA damage, abnormal chromosome, and sister chromatid exchange. In vivo, ethylene oxide alkylated DNA in mice and rats, and caused sister chromatid exchange, abnormal chromosome, small cells, dominant fatal mutation, and genetic translocation.

Table 7-6 The result of genotoxicity tests for ethylene oxide

	Test name	Test material	Test condition	Dose		Result		Reference
				Min.	Max	-S9	+S9	
<i>in vitro</i>	Reversion test	Salmonella typhimurium TA1535, TA100	ND	ND		+	+	Pfeiffer & Dunkelberg, 1980
		T2 bacteriophage	ND	ND		-	-	Cookson et al., 1971
	Mutation test	CHO cell	S9 fraction derived from rat liver induced aroclor	ND		+	-	Tan et al., 1981
	Gene mutation test	Barley seed	6 days	1.5×10 ⁶ mg/m ³ (80%) Ethylene oxide gas		+	+	Ehrenberg et al., 1956
		Barley seed	2 hours	3,084, 11,894 mg/L		+	+	Ehrenberg et al., 1956
	Chromosome breakage test	Barley/wheat/spiderwort (<i>Tradescantia paludosa</i>) pollen	ND	ND		+	+	Ehrenberg et al., 1956, 1959; Mackey, 1968; Moutschen-Dahmen et al., 1968; Smith & Lotfy, 1954
	Chromosome aberration test	Barley seed	6 days	1.5×10 ⁶ mg/m ³ (80%) Ethylene oxide gas		+	+	Ehrenberg et al., 1956
	Sister chromosome exchanges (SCEs)	Barley/wheat/spiderwort (<i>Tradescantia paludosa</i>) pollen	ND	ND		+	+	Ehrenberg et al., 1956, 1959; Mackey, 1968; Moutschen-Dahmen et al., 1968; Smith & Lotfy, 1954

	Mutagenicity test	Rice (<i>Oryza sativa</i>) had two genotypes	8 hours	888-6, 167 mg/L	+ +	Jana & Roy, 1975
<i>in vivo</i>	Sex-linked recessive lethal mutation test	<i>Drosophila (Drosophila melanogaster)</i>	ND	ND	+	Bird, 1952
	Autosomal deletion mutation test	<i>Drosophila</i>	ND	ND	+	Fahmy & Fahmy, 1956
	Lethal mutation test	<i>Drosophila</i>	ND	ND	+	Nakao & Auerbach, 1961
	Gene mutation test	Transgenic mouse	ND	ND	+	Sisk et al., 1997; U.S. NTP, 1987; Walker et al., 1997a
	Mutation test	F344 Rat (Male)	ND	200 ppm (366 mg/m ³)	+	Walker et al., 1997b
		B6C3F1 Mouse			+	
		B6C3F1 Big Blue Mouse (<i>lacI</i> transgenic mouse)	6 hours/day, 5 days/week, 48 weeks	200 ppm	+	Recio et al., 1999
			6 hours/day 5 days/week < 48 weeks	< 200 ppm	-	Recio et al., 1999
	Chromosome aberration test	Rat bone marrow cell	ND	0.6-63 ppm (1-112 mg/m ³)	+	Fomenko & Strekalova, 1973; Strekalova et al., 1975
		Rat bone marrow cell Long-Evans (Male)	7 hours/day 3 days, inhalation exposure	250 ppm (450 mg/m ³)	+	Embree & Hine, 1977
Ape bone marrow cell		Inhalation exposure	50, 100 ppm (90, 180 mg/m ³)	+	Strekalova, 1971	
Rat		Oral administration	9 mg/kg	+		

Micronuclei test	Mouse bone marrow cell	Intraperitoneal	10-200 mg/kg	+	Conan et al., 1979
	Rat bone marrow cell		10-200 mg/kg	+	
	Mouse bone marrow cell	Intravenous injection	ND	+	Appelgren et al., 1978
	Rat bone marrow cell		ND	+	
Dominant lethal effect	Mouse (C3H×101) F1 (Male)	Exposure for 6 weeks (6 hours/day, 5 days/week)	0, 165, 204, 250, 300 ppm (0, 302, 373, 458, 549 mg/m ³)	+	Generoso et al., 1990
	Mouse	4-day Treatment: 300 ppm (549 mg/m ³) (6 hours/day), 600 ppm (3 hours/day), 1,200 ppm (2,200 mg/m ³) (1.5 hours/day)	300 ppm (549 mg/m ³)	+	Generoso et al., 1986
Unscheduled DNA synthesis test	Human lymphocyte	ND	0.5 mg/kg	w+	Pero et al. 1981

+: Positive, -: Negative, w+: Weak positive, ND: no data

Table 7-7 The result of genotoxicity tests of ethylene oxide (summary)

	DNA damage property	Mutagenic property	Chromosome aberration	Other
Protozoa	+	+	ND	ND
Fungi/Chlorophyta	ND	+	+	ND
Insect	ND	+	+	ND
Mammal cell (<i>in vitro</i>)	+	+	+	+
Mammal cell (<i>in vivo</i>)	+	ND	+	+
Human (<i>in vivo</i>)	+ ¹⁾	ND	+	+

ND: No data.

1) Only one example.

7.3.7 Carcinogenicity

The results of a carcinogenicity study of ethylene oxide are shown in Table 7-8 and carcinogenicity evaluations of ethylene oxide conducted by international organizations are shown in Table 7-9.

a. oral administration

Diet fumigated by ethylene oxide was administered to rats (strain unknown, weight 100-150g, 25 males and 25 females/group) for two years. Diet was fumigated in the air including ethylene oxide at 490-710 ppm (900-1,300mg/m³) every week. Ethylene oxide of 500-1,400mg/kg in the diet after the fumigation decreased to 53-400mg/kg in six days. Ethylene oxide changed into ethylene glycol and ethylene chlorohydrin in six days. At the end of the study of a period of two years, 13 of 50 cases survived in the control group and 16 of 50 cases survived in the treated group. An increase in tumors was not found in the treated group. (Bar and Griepentrog, 1969)

Female SD rats (about 90 days age, 50/group) were studied in an untreated group, a solvent control group and groups administered by gavage 7.5 and 30mg/kg of ethylene oxide (purity 99.7%) twice a week for about three years. A dose-related increase of tumors (mainly squamous cell carcinomas) was found in the forestomach. In the high dose group, malignant tumors (29 cases of squamous cell carcinomas in the forestomach and two fibrosarcomas) were found in 31 of 50 cases (62%). In the low dose group, tumors appeared in 8 of 50 cases (16%) while in the control group, tumors were not found in the stomach. (Dunkelberg, 1982)

b. inhalation exposure

In the experiment where 0, 50, 100 ppm (0, 92,183 mg/m³) of ethylene oxide was exposed to female and male B6C3F1 mice (female and male each 50/group) for six hours a day, five days a week for 102 weeks, dose-related significant increase of bronchiolar and alveolar lung cancer was

found. (U.S. NTP, 1987) The incidence of bronchiolar and alveolar lung cancer was six of 50 cases, 10 of 50 cases and 16 of 50 cases ($p=0.019$) in males in the control group, the low dose group and the high dose group respectively, while in females, 0 of 49 cases, 1 of 48 cases and 7 of 49 cases ($p=0.017$) respectively. Papillary cystic adenoma of the Harderian gland was found in 1 of 43 cases, 9 of 44 cases ($p=0.012$) and 8 of 42 cases ($p=0.012$) respectively in males while in females 1 of 46 cases, 6 of 46 cases and 8 of 47 cases ($p=0.033$) respectively. In addition, the incidence of hematopoietic malignant lymphomas was 9 of 49 cases, 6 of 48 cases and 22 of 49 cases ($p=0.005$) respectively; the incidence of uterine adenocarcinomas was 0 of 49 cases, 1 of 47 cases and 5 of 49 cases ($p=0.051$) respectively. Incidences of each disease increased depending on the dosage. (U.S. NTP, 1987)

In the study where 70 and 200 ppm (128, 336 mg/m³) of ethylene oxide was exposed to female A/J mice for six hours a day, five days a week for six months, dose-related increase of pulmonary adenomatosis was found. (Adkins et al., 1986)

In the study where 0, 10, 33, 100 ppm (0, 18.3, 60.4, 183mg/m³) of ethylene oxide was exposed to female and male F344 rats (120 female and 120 male /group) for six hours a day, five days a week for two years, ethylene oxide-related tumors (mononuclear-cell leukemias, peritoneal mesotheliomas, brain tumors, subcutaneous fibromas and spleen adenomas) were observed. (Garman and Snellings; 1986; Garman et al., 1985; Snellings et al., 1984b). After two-years exposure, the incidence of mononuclear-cell leukemias was 11 of 116 cases, 11 of 54cases, 14 of 48 cases and 15 of 26 cases in the control group, the low dose group, a medium dose group and the high dose group ($p=0.001$ at the highest dose group) respectively, which increased depending on the dosage. (Snelling et al., 1984b) A dose-related increase of peritoneal mesothelioma was found in male rats which were examined in death or moribund state. A dose-related increase of primary brain tumors (neurogliocytomas, malignant reticulosises and granular cytomas) was found in both sexes of the rats (Garman and Snellings, 1986; Garman et al., 1985 ; Snellings et al., 1984b). The incidence of subcutaneous fibromas (15 of 58 cases) significantly increased in the male rats of the highest exposure group. The increase of the incidence of mononuclear-cell leukemias, mesotheliomas and brain tumors appeared in the late period of this study (after about 20-24 months). (Snellings et al., 1984b; Golberg, 1986)

The same result was obtained in the experiment which 0, 50, 100 ppm (0, 92, 183 mg/m³) of ethylene oxide was exposed to male F344 rats (80 /group) for seven hours a day, five days a week for 104 weeks (Lynch et al., 1984a,b). The incidence of mononuclear-cell leukemias was 24 of 77 cases, 38 of 79 cases and 30 of 76 cases respectively. A significant increase was noted in the 50 ppm group ($p=0.03$). As the mortality rate increased in the 100 ppm group, a dose-response relationship was not obtained. The peritoneal mesotheliomas (3 of 78 cases, 9 of 79 cases and 21 of 79 cases) and mixed neurogliocytoma of the brain (0 of 76 cases, 2 of 77 cases and 5 of 79 cases)

increased depending on the dosage. The result was statistically significant in the 100 ppm group.

c. percutaneous and subcutaneous administration

One hundred female NMRI mice per group (six to eight weeks age) were studied in an untreated group, a solvent control group, groups where 0.1, 0.3, 1.0mg/mouse of ethylene oxide (purity, 99.7 %) (tricaprylin solvent) was subcutaneously administered once a week for 95 weeks. In this experiment, tumors at the administration site, mainly fibrosarcoma increased depending on the dosage. The first tumor appeared at 50 weeks. The incidence of subcutaneous sarcoma was 0 of 200 cases, 4 of 200 cases, 5 of 100 cases, 8 of 100 cases and 11 of 100 cases in the untreated group, the solvent control group and the groups administered 0.1, 0.3, 1.0mg of ethylene oxide per mouse, respectively. (Cochran Armitage test for trend: $p < 0.001$). Dose-dependent relationship was noted at tumor incidence rate statistically corrected to 600 days. (Dunkelberg, 1981) In the experiment where 0.1 mL of 10 % ethylene oxide (acetone solvent) was applied to the back skin for life, the 50 % survival period was 493 days and skin tumor was not found. (Van Duuren et al., 1965)

As shown in the above, in the inhalation exposure study of mice, ethylene oxide induced an increase of lung cancers and adenomas of the Harderian gland in mice of each sex and malignant lymphomas, uterine adenomas and adenomas of mammary gland or squamous-cell carcinoma in female mice. In the experiment of rats, ethylene oxide induced an increase of mononuclear-cell leukemia, neurogliocytoma of brain and peritoneal mesothelioma in male rats. In the study of gavage, it induced an increase of squamous-cell carcinoma in the forestomach in female rats.

Regarding carcinogenicity of ethylene oxide, IARC (2002), Japan Society of Occupational Health (2002) and U.S. NTP (2002) evaluates that this agent is carcinogenic to humans while ACGIH (2002) evaluates that this agent is predicted to be carcinogenic to humans. IARC (1994) lists the reasons why the agent was classified exceptionally into group 1 (carcinogenic agent to humans) though there is insufficient evidence of carcinogenicity in humans while there is sufficient evidence of carcinogenicity in experimental animals. It states that ethylene oxide induced (i) a sensitive persistent dose-related increase in the frequency of chromosome aberrations and sister chromatid exchange (SCE) in peripheral lymphocytes and micronuclei in bone-marrow cells of exposed workers, (ii) malignant tumors of lymphatic and hematopoietic system in both humans and experimental animals, (iii) dose-related increase of frequency of hemoglobin adducts in exposed workers, dose-related increase of DNA and hemoglobin adducts in exposed rodents, (iv) appearance of gene mutation and heritable translocation of germ cells in exposed rodents, (v) directly acting alkylating agent with a powerful mutagen and clastogen at all phylogenetic levels.

Table 7-8 Result of carcinogenicity of ethylene oxide

Animal	Administration method	Administration period	Dose	Tumor	Frequency of expression	Reference
Rat (Male/female) 25 rats/group	Oral administration (Feeding)	2 years	500-1,400mg/kg smoked feed, (decreased to 53-400mg/kg after 6 days) ^{Note)}	No increase in tumors survival rate: Control group: 13/50 Treated group: 16/50		Bar & Griepentr og, 1969
SD rat (Female) Approx. 100 days old 50 rats/group	Forced oral administration	Approx. 3 years 2 times/week	No treatment, Solvent control group, 7.5, 30 mg/kg (Purity: 99.7%)	Squamous carcinoma of the rumen Fibrosarcoma of the rumen	0 7.5 30 mg/kg	Dunkelberg, 1982
					0 8/50 29/50	
B6C3F1 Mouse (Male/female)	Inhalation exposure	102 weeks (6 hours/day 5 days/week)	0, 50, 100 ppm (0, 92, 183 mg/m ³)	lung cancer (Male)* lung cancer (Female)* Malignant lymphoma (Female)* Uterine adenocarcinoma* Breast adenocarcinoma/ gland squamous carcinoma* Harderian gland carcinoma/ cystadenoma (Male)* Harderian gland carcinoma/ cystadenoma (Female)* * Dose correlation in Test of Trend	0 50 100 ppm	U.S. NTP, 1987
					6/50 10/50 16/50 0/49 1/48 7/49 9/49 6/48 22/49 0/49 1/47 5/49 1/49 8/48 6/49 1/43 9/44 8/42 1/46 6/46 8/47	
A/J Mouse (Female)	Inhalation exposure	6 months (6 hours/day 5 days/week)	70, 200 ppm (128, 366 mg/m ³)	Number of cancer bearing animals (%) Lung adenoma/mouse	0 70 200 ppm 28 56 87 0.46 0.86* 2.14*	Adkins et al., 1986
					* Significant differences	
F344 Rat (Male/female)	Inhalation exposure	2 years (6 hours/day 5 days/week)	0, 10, 33, 100 ppm (0, 18.3, 60.4, 183 mg/m ³)	Monocytic leukemia (Male)	0 10 33 100 ppm 13/97 9/51 12/39 9/30	Garman & Snellings, 1986;

				Monocytic leukemia (Female) Peritoneal mesothelioma (Male) Primary brain tumor(Male) Primary brain tumor(Female)	11/116 11/54 14/48 15/26* 2/97 2/51 4/39 4/30 1/181 1/92 5/85* 7/87* 1/188 1/94 3/92 4/80*	Garman et al., 1985; Snellings et al., 1984b
F344 Rat (Male)	Inhalation exposure	104 weeks (7 hours/day 5 days/week)	0, 50, 100 ppm (0, 92, 183 mg/m3)	Monocytic leukemia Peritoneal mesothelioma Mixed type brain glioma	0 50 100 ppm 24/77 38/79* 30/76 3/78 9/79 21/79* 0/76 2/77 5/79* * Significant differences	Lynch et al., 1984a,b
NMRI Mouse (Female) 6-8 weeks old	Subcutaneous injection	95 weeks (1 time/week)	No treatment, Solvent control group, 0.1,.3, 1.0 mg /mouse (Purity: 99.7%)	Increase in tumors in administered site, mainly fibrosarcomas, dependant on the dose First expression of tumors: 50 weeks Frequency of subcutaneous sarcoma: The dose-dependency was confirmed.	Untreated, solvent control group, 0.1, 0.3, 1.0 mg/mouse 0/200 , 4/200 , 5/100 , 8/100 , 11/100	Dunkelberg, 1981
ICR /Ha Swiss Mouse (Female) 8 weeks old	Lifelong application on dorsal skin	3 times/week	10% solution 0.1mL (Solvent: acetone)	50% survival time: 493 days Skin tumor: No		Van Duuren et al., 1965

Note) Ethylene Oxide changes to ethylene glycol and ethylene chlorohydrin for 6 days.

Table 7-9 Evaluations of carcinogenicity of ethylene oxide in the international organizations, etc.

Organization/Source	Classification	Classification criteria
IARC (2002)	Group 1	Carcinogenic to humans.
ACGIH (2002)	A2	Suspected human carcinogen.
The Japan Society for Occupational Health (2002)	Group 1	Substances are carcinogenic to humans.
U.S. EPA (2003)	-	This substance has not been evaluated for human carcinogenicity as of 2002.
U.S. NTP (2002)	K	Substances known to be a human carcinogen

7.4 Effects to Human Health (Summary)

Most of the reports for ethylene oxide were made for inhalation due to the physicochemical characteristics of ethylene oxide. Metabolic pathway and excretion of other metabolites were either hydrolysis or glutathioneconjugation. Tested animals' metabolism of ethylene oxide was qualitatively similar to human's, but it is quantitatively various. This variety is the cause of the large metabolic difference in species and also individuals.

Ethylene oxide is irritating to humans, and it is also a sensitizing material. The main effect of long exposure to ethylene oxide is disorders of the nervous system, mainly in frequent movement disorders of sensorimotor. The increase of risk of miscarriage was suggested by exposure during pregnancy.

As for the toxicity of repetitive administration of ethylene oxide, changes are found mainly in the nervous system and blood lymph system, and the effect on the nervous system was observed with the lowest dose. In the test that 0, 10, 48, 104, and 236 ppm was exposed to a mouse for inhalation for 10 or 11 weeks, we found out that changes were found in nervous toxicity and sperm formation by administering 48ppm or more ethylene oxide. In the test where a F344 rat was exposed to 10, 33, and 100 ppm by inhalation for 2 years, weight increase was inhibited from 33ppm (60.4 mg/m³). Therefore, NOEL for rats and mice is 10 ppm (18.3 mg/m³).

As for toxicity for reproductive and developmental toxicity, it was found that ethylene oxide causes testis toxicity, fetus toxicity, developmental toxicity, and teratogenicity by all methods of administration. The inhalation route is considered as the method of exposure in the general environment, and the minimum dose for toxicity was LOAEL 50 ppm (92 mg/m³) for a rat which was targeted for sperm formation. Increase of frequency in abnormal chromosome of peripheral blood cell and micronucleus/sister chromatid exchange etc. were reported in a horizontal study for a group of people who were exposed to ethylene oxide at work. There is no confirmed evidence for causing a clastogenic effect, but the increase of frequency of cytogenetic change tends to occur in the exposure of 5 ppm (9.2 mg/m³) or more of ethylene oxide and a relationship of dose and reaction was found. In addition, although there were not many participants in this test, some reports indicated that positive genetic toxicity was observed as a result of exposure to high-density ethylene oxide.

Ethylene oxide is DNA cytotoxic for bacteria, and genetic toxicity was found for plants, fungus, and insects. Abnormal chromosome was also found in plants, and genetic translocation was found in insects. Genetic toxicity was also found in cultured cells in mammals, and it caused damage to DNA, abnormal chromosomes, and sister chromatid exchange. Sister chromatid exchange, abnormal chromosomes, small nucleus, dominant lethal mutation, and genetic translocation were caused in mammals.

It is suggested that there is a correlation between exposure to ethylene oxide at work and cancer in the

lymphoid and the hematopoietic systems, and there is a possibility that ethylene oxide causes cancer in humans. When animals were exposed to ethylene oxide by inhalation as a cancer test, the frequency of expression of naturally-occurring and frequent leukemia and brain cancer increased in F344 rats, and the frequency of lymphoma increased in mice. In addition, ethylene oxide is a strong alkylating agent which causes genetic toxicity in experiment animals. IARC categorizes ethylene oxide as Group 1 (materials which have a potential to cause cancer to humans).

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¹⁾The database search was conducted in April 2004, and the bibliography has been updated with new data from source information, etc.

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