Technical Report

No 5

Toxicity of Ethylene Oxide and its Relevance to Man

September 1982

ECETOC

September 20, 1982

Technical Report

N° 5

TOXICITY OF ETHYLENE OXIDE AND ITS RELEVANCE TO MAN

European Chemical Industry
Ecology & Toxicology Centre

Avenue Louise 250, B. 63
B - 1050 Brussels, Belgium

CONTENTS

	page
SUMMARY	
A. INTRODUCTION	1
B. CONDITIONS OF HUMAN EXPOSURE	2
1. Routes of Exposure	2
2. Exposure Levels and Number of People Exposed	2
3. EO Monitoring	3
C. TOXICOLOGICAL DATA	4
1. Human Data - Local and Systemic Effects	4
1.1. Local effects	4
1.1.1. On the skin	4
1.1.2. On the eyes	4
1.2. Systemic effects	5
1.2.1. Neurotoxicity	5
1.2.2. Reproductive toxicity	6
1.2.3. Clastogenicity	7
1.2.4. Others	8
1.3. Conclusions	9
2. Epidemiological Data	9
2.1. Morbidity studies	9
2.2. Mortality and related studies	11
2.3. Conclusions	13
3. Experimental Data	14
3.1. Short- and medium-term effects	14
3.1.1. Oral and parenteral exposure	14
3.1.2. Inhalation exposure	15
3.1.3. Skin and eye irritation	17
3.1.4. Conclusions	17
3.2. Reproductive effects	18
3.2.1. Effects on reproductive tissues	18
a) Testes	18
b) Ovaries	19
3.2.2. Effects on reproductive function	19
3.2.3. Embryotoxicity and teratogenicity	21

3.2.4. Conclusions 22
3.3. Carcinogenicity 22
3.3.1. Experimental results 22
a) Skin application 22
b) Subcutaneous injection 23
c) Inhalation exposure 23
d) Intragastric administration 25
e) Dietary administration 26
f) Miscellaneous exposure 26
3.3.2. Comments on experimental results 26
a) Inhalation exposure 26
b) Subcutaneous administration 28
c) Intragastric exposure 28
d) Other comments 28
3.3.3. Conclusions 29
3.4. Mutagenicity 29
3.4.1. Evidence of mutagenic effects 29
a) Micro-organisms 30
b) Plants 30
c) Insects 30
d) Rodents 30
e) Primates 31
3.4.2. Conclusions 32
3.5. Biochemical toxicology 32
3.5.1. Toxicokinetics 32
3.5.2. Metabolism 33
3.5.3. Conclusions 37
3.6. Biochemical interactions 37
D. CONCLUSIONS : INTERPRETATION OF ANIMAL DATA IN TERMS OF HUMAN RISK 41

E. FURTHER INVESTIGATIONS TO BE CONSIDERED 44

F. APPENDICES 46
1. Production, Properties and Use of EO 46
2. Exposure Limits in the Working Environment 48
3. EO Monitoring 49
4. Inhalation Exposure 52
5. Rad-Equivalents and Risk Assessment 57
6. Current Studies 59

G. BIBLIOGRAPHY 63

H. MEMBERS OF THE WORKING GROUPS 73

I. MEMBERS OF ECETOC SCIENTIFIC COMMITTEE 74

SUMMARY

Ethylene oxide (EO) is a major industrial chemical used widely in a variety of applications. The current recommended exposure limits are based mainly on the results of animal studies published in 1956. Results from recent animal and epidemiological studies have prompted a re-evaluation of its toxic hazard to man. ECETOC therefore set up a Working Group to evaluate the numerous, relevant animal toxicity studies so as to assess the significance of the results for human exposure and to advise on the necessity for any further studies; to determine what studies have been made relating to human toxicity as a result of exposure to EO ; and to make recommendations for further studies which may be necessary to assess more fully the significance of human exposure to EO.

Inhalation of EO is the most likely form of human exposure. In the chemical manufacturing industry using continuous processes the exposure levels have for some time been generally well below the recommended exposure limits. These values may, however, have been exceeded in the past when EO was used as a chemical intermediate and may be exceeded even now when it is used as a sterilising or fumigating agent, or as a result of inappropriate handling.

Evidence from studies on humans have indicated that EO is capable of causing acute dermal and eye irritation, but there is no evidence of sensitising effects. Chronic exposure to low levels of EO during manufacture produced no proven clinical symptomology, but high exposure levels e.g. as reported to occur in hospital sterilising plant, have been shown to induce chromosome aberrations in circulating lymphocytes. The significance of this effect is, however, unclear. The suggested association between chronic exposure and induced leukaemia is not substantiated by the evidence available.

Animal experiments show that EO does not cause teratogenic effects, reduced fertility or permanent damage to nervous tissue. It is acutely toxic when injected in aqueous solution into rodents and causes chromosome damage and dominant lethal and heritable translocation effects at high dose levels. These data and those obtained from lower organisms, confirm that EO is a potent mutagen. Several carcinogenicity studies in rats and mice by inhalation, dermal and gastric routes have yielded equivocal or irreproducible results, and while in some cases tumours have undoubtedly been induced by EO, the relevance for man

of these animal model systems is questionable. EO emerges from this review as an irritant gas possessing mutagenic properties in higher organisms but with no obvious carcinogenic properties, at least in those model systems not confounded by chronic irritancy factors. EO clearly has the potential to react with nucleophilic centres in such macromolecular molecules as nucleic acids and proteins, and the apparent lack of carcinogenicity in man is presumably related to the existence of effective protective mechanisms.

A number of studies in progress on various aspects of the toxicology of EO are listed. Bearing these in mind, the Working Group identified several areas worthy of further investigation in order to clarify some of the remaining uncertainties.

A. INTRODUCTION

Ethylene oxide (CAS No.75-21-8) is a major industrial intermediate with current world production capacity in excess of 4 million tonnes per annum (see Appendix 1).

Since a number of workers may be exposed to this chemical under various production and use conditions, recommended control limits for occupational exposure have been established, mostly based on the 50 ppm value adopted by the American Conference of Governmental Industrial Hygienists (cf. Appendix 2). This level was in turn mainly based on the results of studies by i) Hollingworth et al.(1956) who found growth depression and injury to the liver, kidneys, adrenals and testes of rats and guinea pigs, after exposure to ethylene oxide (EO) at levels ranging from 204 to 841 ppm; and ii) Jacobson et al.(1956) who found that dogs exposed to 290 ppm EO, showed muscular atrophy, weakness and anaemia.

Concern about EO and its present toxicological status has been raised primarily as a result of evidence from mutagenicity, carcinogenicity and epidemiological studies indicating that EO may be a human mutagen and perhaps a human carcinogen. Consequently ECETOC set out firstly to evaluate the numerous relevant animal toxicity studies so as to assess the significance of the results for human exposure and to advise on the necessity for any further studies; secondly to determine what studies have been made relating to human toxicity as a result of exposure to EO; and finally to make recommendations for further studies which may be necessary to assess more fully the significance and consequences of human exposure to EO.

In this report are summarised findings concerning the effects of EO on animals and humans, and their significance for man under current working practices.

B. CONDITIONS OF HUMAN EXPOSURE

A knowledge of the conditions of exposure together with an assessment of the toxicological properties of a chemical are the major features necessary for risk assessment (ECETOC, 1982). The number of people exposed, the levels to which they are exposed, and the different possible routes of exposure must be considered. Analytical and statistical techniques are necessary to permit a meaningful assessment of exposure levels.

1. Routes of Exposure.

Human exposure to EO may occur via several routes. The gas may be absorbed by inhalation. It will contact the skin if it dissolves in natural perspiration, which may be trapped by occlusive clothing. Less usually, skin and eye exposure may result from contact with the liquid. Residual traces in sterilised materials (plastic matrix of haemodialysis tubing, plastic prostheses, skin dressings) and in food that has been fumigated with EO (Darby et al., 1980) can also lead to exposure.

Of the different exposure routes, the greatest potential human exposure appears to be that associated with inhalation, particularly as a result of exposure during the sterilising of medical equipment in hospitals.

2. Exposure Levels and Number of People Exposed

Large differences exist between the exposure conditions in the producer and user industries. The equipment in chemical manufacturing plants is often located outdoors and consists of closed and highly automated systems. The exposure levels are usually very low (Koketsu, 1978). A limited survey of exposure conditions in Western European production, and associated chemical transformation, units revealed time-weighted averages well below 5 ppm, in general below 1 ppm. This does not however exclude that a peak exposure may arise, especially in cases of accidental leakage (cf.Appendix 2).

In contrast to such manufacturing plants, those industries and activities in which discontinuous processes are used, although using only a small proportion of the total EO, are associated with relatively high occupational exposure. In the sterilisation industry the time-weighted average for area and personal sampling is generally below 50 ppm, but occasional peaks of one

hundred or even five thousand ppm may be found in cases of accidents and improper functioning of the sterilisation equipment (NIOSH, 1977; Glaser, 1980).

NIOSH (1977) estimated that in the US, 75,000 health care workers were employed in sterilisation areas where EO was used, and that 25,000 others may have been incidentally exposed. It should be noted that this specific use refers to only 0.02 % of the total US production of EO. We may expect that in Europe a large number of workers are exposed in this specific branch of EO use. Whilst the largest quantity of EO is found in chemical manufacturing plants (cf. Appendix 1.), the number of workers at these sites is limited. In Europe about 900 workers produce and handle 1,150,000 T/y EO,and in Japan about 700 workers are responsible for the production and handling of 647,000 T/y EO.

3. EO Monitoring.

Various methods are available for measuring EO levels in the work environment. Area sampling is distinguished from personal sampling, the latter being the best way to monitor employee exposure. Both methods, which are described in Appendix 3, give the EO level in air as short-term or eight-hour time-weighted averages. While, at present, good physico-chemical monitoring techniques for exposure are known, no reliable biological monitoring method is available and attempts are being made to develop them. (cf. Appendix 3).

C. TOXICOLOGICAL DATA

1. Human Data - Local and Systemic Effects

Until recently, evaluation of the effect of EO on humans was related only to acute effects of a non-specific nature. During the past 5 years evidence has arisen which suggests that exposure to EO at high levels may produce effects on the nervous and reproductive systems, and on chromosomes.

1.1. Local effects

1.1.1. On the skin. EO in liquid form applied to the skin of volunteers rapidly evaporated without leaving any mark or irritation. A 15 min. contact with cotton wool soaked in undiluted EO produced no effect in 4 volunteers (Greaves, Walker and Greeson, 1932). However, extensive skin burns with blister formation have been described as the result of exposure to aqueous solutions of EO (Sexton and Henson, 1949; Philipps and Kaye, 1944) and this effect has been repeatedly observed over many years by physicians working in the industry.

Later work clearly established that the extent and progress of the skin reaction was related to prolonged, intimate skin contact and impeded evaporation. The magnitude of the skin injury appeared to be determined by the length of time of contact and the concentration of the offending solution. The most hazardous concentration in water seems to be in the 50% range, but irritation was also caused by exposure to 1% solutions (Sexton and Henson, 1950). Repeated experiments on the skin with various concentrations of EO in water and with undiluted liquid EO resulted in "flare up" reactions in 3 out of 8 volunteers. The duration of the "sensitisation" has not been established (Sexton and Henson, 1950).

Hanifin (1971) described delayed skin reactions in patients exposed to EO which was inadvertently retained in certain batches of prepared skin dressings.

1.1.2. On the eyes. Severe corneal burns were reported in a workman accidentally exposed to liquid EO (McLaughin, 1946). Thiess (1963) reported an incident in which liquid EO was accidentally squirted into one eye. This was treated immediately by copious irrigation with water, after which a mild irritation of the conjunctiva lasted only 1 day.

1.2. Systemic effects

1.2.1. Neurotoxicity. Joyner (1964) examined 37 workers who had been occupationally exposed to a presumed level of 5-10 ppm EO for an average of 10.7 years. There were no statistically-significant increases in the incidence of the several nervous system diseases looked for in the exposed group compared to the control group.

Following misuse of EO in a sterilisation process, three workers developed neuropathy of the lower limbs (Jenson,1978). Clinical observations and follow-up indicated that these effects were reversible.

Gross et al.(1978,1979) reported four cases of neurological disorder in operators working in a medical sterilisation plant which was found to have leaked during the first two months of operation. The level of EO was not monitored and it was presumed by the authors that as all operators had smelled EO the levels were greater than 700 ppm. Three of the operators who had worked on the plant for two years developed peripheral neuropathy. The fourth operator, who had an acute cerebral episode with convulsions but no peripheral nerve involvement, had worked on the plant for only three weeks ("70 hours per week") during the period when the steriliser leaked. He recovered fully and did not develop neuropathy. Within 2 weeks of removal from EO exposure the remaining 3 operators showed marked subjective improvement, although an improvement in nerve conduction occurred in only 1 of the 3 cases.

Garry et al.(1979), in their study of sister chromatid exchanges in lymphocytes cultured from individuals exposed to EO, noted four chronically-exposed persons who reported non-specific upper respiratory and neurologic symptoms and symptoms indicating a possible effect on the nervous system. A case of polyneuropathy is currently under investigation by NIOSH (1982).A case of delayed aphonia due to occupational EO exposure was reported to be of nervous system, rather than functional, origin (Troisi, 1965).

From an examination of 76 workers, aged between 20 and 49 years, who had worked for 3 to 6 years in an EO-production plant, Ostrovskaya (1971, 1973) noted that EO might cause dysfunction of the autonomic nervous system combined with vascular changes and hypertension, as well as early changes in the myocardium, and liver dysfunction.Spazovski et

al.(1980) observed neurasthenia and a statistically-significant increase in vegetative deviations (vasospastic and vasodystonic) in exposed female and male workers.

1.2.2. Reproductive toxicity. In an epidemiological study, Joyner (1964) examined 37 workers with an average of 10.7 years of continuous occupational exposure to 5-10 ppm EO in air. Compared to a control group consisting of 41 non-exposed operators, there was no statistically-greater incidence of such disorders as benign prostatic hypertrophy, acute prostatitis, spermatoceles or seminomas of the testicle, but no measure of reproductive capacity was undertaken. More recently, in a NIOSH Current Intelligence Bulletin (1981), it was reported that in 1978 a company manufacturing and distributing health-care products began to investigate the possible adverse effects of EO on its workers. The investigations included sperm analysis, but the results from this were inconclusive.

Russian authors have drawn attention to effects on reproduction in female workers engaged in the manufacture of EO. A proneness to immature and premature termination of pregnancies, after exposure to levels reportedly not exceeding 0.55 ppm, were noted by Yakubova (1970). Disturbances in menstrual cycles have also been reported by this author, but no attempts seem to have been made to age-match the controls.

Disturbances in the menstrual cycle have also been reported to occur in 17.5% of the females workforce in ethylene and EO plants as compared with 6.3% in controls of the same age. Concomitant exposure to high levels of unsaturated hydrocarbons might however be a confounding factor (Spasovski, 1980)

In a study of children born to mothers exposed to organic solvents during pregnancy, Holberg (1979) described one case of multiple congenital defects in a stillborn child which could be attributed to an exposure to various chemicals including EO. The attribution of the effects to EO exposure seems very doubtful.

1.2.3. Clastogenicity. Chromosomal aberrations have been reported to occur in human lymphocytes cultured after both accidental high exposure and protracted low-level exposure. Significantly high frequencies of chromosome breaks and translocations were observed in a group of 8 persons, 18 months after an accidental exposure to EO (Ehrenberg and Hallstrom,1967).

A comparison was made between the chromosomal status of a population of 75 workers at American Hospital Supply Corporation, exposed to EO within an environment complying with the TWA of 50 ppm, and a population of 37 unexposed workers. There was a statistically- significant increase in the number of chromosomal aberrations and sister-chromatid exchanges in the peripheral lymphocytes of the exposed workers (exposed mean = 8.84, non-exposed mean = 3.58; $p<0.0001$) (Abrahams, 1980). In a similar study, Garry et al.(1979) compared 12 exposed and 12 non-exposed workers and demonstrated a moderate excess of chromosome damage (exposed mean = 9.35, non-exposed mean = 6.37).

Thiess et al.(1981) examined the lymphocytes of workers exposed to EO and other alkylene oxides at estimated exposure levels below 100 ppm (12 hours, alternating shifts). Four exposure groups were studied and compared with an appropriate non-exposed population :

a) long-term exposure (greater than 20 years).....11 persons
b) exposure for less that 20 years.....6 persons
c) long-term exposure plus an accidental, very high exposure.....21 persons
d) accident (short-term exposure to high concentrations > 2000 ppm).....5 persons

Chromosome aberrations (excluding gaps) apparently increased significantly (3.5% vs 1%)only in those workers exposed for more than 20 years No other statistically-significant effects were recorded although some increase in abberration frequency was observed in the groups b and c. A possible drawback of this work is, however, that a 72hr incubation period was used for the phytohaemagglutinin-stimulated lymphocytes which could reduce the frequency of observable chromosome damage (due to spontaneous selection of "viable cells" only). The more

usual 48hr incubation period allows all chromosome aberrations to be visualised whether they are viable or not.

A reciprocal argument can be applied to the work by Pero et al.(1981) who examined factory workers exposed to EO (0.5-1.0 ppm) by cytogenetic analysis and unscheduled DNA synthesis (UDS) in circulating lymphocytes. They concluded that the total chromatid gaps plus breaks were significantly elevated, and that the UDS was significantly reduced in the EO-exposed group compared with controls. However, because a 72hr incubation period was used following phytohemagglutinin stimulation, it is not possible to conclude with confidence that the observed chromatid damage was EO-induced, and did not simply arise spontaneously during the culture process, especially as the reported spontaneous control levels were unusually high in chromatid aberration frequency.

A pilot study of chromosome changes in workers in a sterilising plant in the USA is in progress and preliminary results indicate that there may be a dose-response relationship between exposure level and chromosome changes, especially sister chromatid exchange (Johnson and Johnson Co.,1982). A pilot study of workers in a sterilising plant in the U.S.A. (Litton Bionetics,1982) has revealed sister chromatid exchange after exposure to levels well above the current TLV. The clinical significance of this has not yet been established.

1.2.4. Others. The earliest reports of systemic poisoning by EO involved exposure to the gas in combination with CO_2 (9:1), a commercial mixture (Cartox, T-gas) used for sterilisation and fumigation (Lundberg, 1938; Blackwood and Erskine, 1938; Metz, 1939). The main symptoms consisted of nausea and repeated vomiting, whereas diarrhoea, headache, giddiness, excitement and confusion occurred much less frequently.

In 1963, Thiess reported 41 cases of excessive exposure to undiluted EO gas during industrial use and manufacture. The exposure time varied but was believed to have been relatively short. In contrast to experience from animal exposure, irritation of the mucous membranes was not the first or the most important symptom. The principal effects appeared to have been severe vomiting, recurring periodically for several hours, and sometimes headaches. In one case a narcotic effect was induced.

Hine and Rowe (1981) refer to headache, dyspnoe, vomiting and diarrhoea in three cases of exposure to high levels of EO. These symptoms were also observed in the case reported by Hess and Tilton (1950) who warned that nausea and vomiting may persist for several hours.

Anaphylaxis has been described in one patient receiving haemodialysis treatment with equipment which had been sterilised with EO (Poothullil et al., 1975). Subsequently IgE and IgG antibodies and EO hapten specificity have been demonstrated in this patient (Dolovich and Bell, 1978).

1.3. Conclusions

Accidental overexposure of man to gaseous EO results predominantly in nausea and repeated vomiting. Irritation of the respiratory tract and eyes, headache, diarrhoea, and effects on the central nervous system have been less frequently described. After exceptionally high exposure to the gas, some drowsiness, weakness and loss of consciousness occur. Liquid EO and EO solutions irritate the eyes, skin and mucous membranes. There is limited evidence to indicate that EO has a sensitising potential. In contrast to the effects on animals, gaseous EO does not apparently irritate the human mucous membranes. Any effects on the nervous system resulted from exposure to EO at high levels. Both peripheral neuropathy and encephalopathy were found to be reversible. The present data do not suggest that any reprotoxic effect is induced in humans as a result of exposure to EO. Evidence from human studies indicates that EO is a clastogen.

2. Epidemiological Data

2.1. Morbidity studies

Joyner (1964) studied 37 operators at a plant producing EO by direct oxidation of ethylene. They had been exposed for between 5 and 16 yr. (average, 10 yr) at a level presumed to be of the order of 5 to 10 ppm.The examined cohort showed a better state of health than an age-matched control group from the same plant. There was no increased incidence of tumours. The two groups participated in a periodical physical examination programme and a number of clinical laboratory tests was carried out. A re-evaluation of the data might be taken to indicate lymphocytosis amongst the exposed operators. It should be noted that the study group