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# Toxicity of Ethylene Oxide and its Relevance to Man

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European Chemical Industry
Ecology & Toxicology Centre

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

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## 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

included only operators currently working in the plant. Unfortunately, plans on a follow-up study have never been realised.

In 31 persons occupationally exposed to EO (chlorohydrin process) for periods varying from 2 to 20 years (average, 15 years), Ehrenberg and Hällström (1967) observed a slight but significant lymphocytosis ("lymphocyte stimulation"). One case with a chronic lymphatic leukaemia was also found in the exposed group. In a follow-up of the study one year later, when the sample was also enlarged (176 persons with various histories of EO exposure), no signiticant difference in lymphocyte counts could be observed between the exposed persons and the controls (Ehrenberg and Hallström, 1967). If the repeated analysis were restricted to persons who had also participated in the first study, there was however still a difference, though less marked. Lower haemoglobin values and a few cases of slight anaemia were also found in the exposed group.

In 1978 an investigation was initiated by the American Hospital Supply Corporation on 75 employees with varying exposures to EO used as a sterilant for medical equipment (Abrahams,1980). Exposure levels were said to comply with a TLV of 50 ppm, although some short-term exposures exceeded 75 ppm. The results of physical examinations, and a number of laboratory tests, revealed no unusual findings. Blood counts were found to be within normal limits except for 6 persons with slight anaemia and 1 with a slight increase in lymphocyte count.

Stocker et al.(1979) examined 279 persons (time of employment 0.2 to 41.4 years) occupationally exposed to alkylene oxides (EO, propylene oxide) in several production and processing areas. They had also been exposed to other products, for many years in some cases. Twenty-one had been involved in accidents with EO during their time of employment. The EO exposure levels measured during the time of the investigations were below 5 ppm. However, considerably higher values had occurred during disturbances of production, and in the past. The investigation did not demonstrate any influence of EO on the current state of health of the examined workers. An increase of haemoglobin value and a slight lymphocytosis could be correlated with the smoking habits and age of the workers examined.

2.2. Mortality and related studies

A retrospective cohort-study on workers employed in a Swedish EO-producing plant has been reported (Hogstedt et al.,1979 a). The study was a follow-up of a group of EO workers previously examined and in which certain blood abnormalities were indicated (Ehrenberg and Hällström, 1967). Mortality and cancer incidence from 1961 up to 1977 were examined. In a group of 89 full-time exposed workers with at least 1 year of exposure and 10 years or more of induction-latency time, 23 deaths were observed against 13.5 expected ($p<0.05$, based on national statistics). The excess mortality derived mainly from an increased mortality in tumours (9 cases observed against 3.4 cases expected, $p<0.01$) but also in diseases of the circulatory system (12 cases against 6.3 expected, $p<0.05$). The excess mortality with tumours resulted from 3 cases of stomach cancer(0.4 expected, $p<0.01$) and leukaemia (2 cases against 0.14 expected, $p<0.01$; 1 chronic lymphatic leukaemia, 1 acute myeloid leukaemia)*. No significant increase in mortality was observed among 86 maintenance workers intermittently exposed to EO, or among 66 unexposed controls. Since the EO was produced by the chlorohydrin process there might have been significant exposure to other chemicals, e.g., the exposure during the period 1941-47 was estimated as approximately 2.7ppm of ethylene chlorohydrin, 55ppm of ethylene dichloride, 0.03ppm of bis(2-chloroethyl)ether and 330ppm of ethylene. Of these, ethylene chlorohydrin, ethylene dichloride and bis-(2-chloroethyl)ether are reported to have carcinogenic potential. Occasionally, much higher concentrations may have occurred. The exposure to EO has been calculated as probably less than 14ppm during the 1940's (occasionally with levels above the odour threshold, i.e. 715ppm). During the beginning of the 1960's exposures had been on average 5-27ppm and in the 1970's there were indications that they may have been in the 0.5-5ppm range.

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* 1 case of chronic lymphatic leukaemia was also observed in a group of maintenance workers with occasional exposure to EO.

Hogstedt et al.(1979 a-b) reported another Swedish study concerning the incidence of leukaemia in a cohort of 230 persons exposed to EO mixed with methyl formate, used since 1968 for the sterilisation of medical equipment. Between 1972 and 1977, 3 cases of leukaemia were reported : one woman with chronic myeloid leukaemia and early signs of blood dysfunction (increased bruising) from 1968 and onward; one woman with acute myelogenous leukaemia ; and one man with primary macroglobulinaemia. According to national statistics only 0,07 cases would have been expected (Ehrenberg and Hussain,1981). The 8-hour time-weighted average exposure of the two women was estimated to have been about 20 $\pm$ 10 ppm. The man with macroglobulinaemia also had some occupational contact with benzene in laboratory work. Classification of macroglobulinaemia (Morbus Waldenström,) as a leukaemia is questionable. This is a blood disorder with initially localised plasma-cell and IgM producing myeloma in the bone marrow and is normally classified as malignant lymphoma (non-Hodgkin-type, Kiel classification).

A mortality study by Thiess et al.(1981) on 602 persons (8484 person-years of observation) occupationally exposed to, among others, ethylene and propylene oxides (exposure times 0.5 - 42 years), and on 351 persons (3779 person-years) with a 10-year minimum observation period, showed no significant increase of mortality. In this study there was also exposure to benzene, ethylene chlorohydrin, ethylene dichloride, etc. The EO exposure data reflect the present level of technology i.e. they were about 5 ppm. There is evidence, however, that maximum permissible concentrations might occasionally have been exceeded in the past . Total mortality of the cohort was less than expected (48 deaths observed against 53.0 expected, based on the age-adjusted mortality of the population of Rhinehessia-Palatinate, Ludwigshafen and Federal Republic of Germany). The number of malignancies, excluding leukaemia, was 10 against 11.8 expected. One case of acute myeloid leukaemia was observed against 0.15 expected. Differences between observed and expected numbers of deaths in the alkylene oxide-exposed cohort were tested by the Poisson distribution method, and 4 periods of exposure time were studied to seek any eventual trend in terms of a gradient between the period of exposure and disease. No such trend was found. The statistical analysis shows, according to the authors, that none of the observed cancer deaths diverges significantly from the expected number. A risk rate of 1.48 in an internal comparison of the alkylene

oxide cohort with a cohort of styrene-exposed workers seems to indicate the possibility of an increased relative risk of cancer death, but the range of confidence in the 95% level, from 0.88 - 2.50, shows that the result may be by chance.

A fourth study, involving 767 persons with potential exposure to EO at a production plant, has been coordinated by Morgan et al.(1981). The cohort members were employed for at least 5 years between the years 1955 and 1977. Forty-six deaths occurred in this cohort, with 80 expected. No deaths from leukaemia were found, nor were there any statistically-significant excesses from any specific causes of death. Eleven malignant neoplasms were observed, with 15.2 expected. Exposure measurements performed in 1977 generally indicated a relatively low level of exposure (less than 10 ppm, i.e. well below a time-weighted average of 50 ppm).

## 2.3. Conclusions

It can be concluded from the available morbidity data on workers in production, processing and sterilisation plants that under conditions and levels of exposure as mentioned in the studies, the general state of health of the examined persons, as determined by routine physical and laboratory examinations, showed no significant differences from that of the controls, with the exception of chromosome aberrations.

Studies by Hogstedt (1979 a,b) led him to the hypothesis that EO has leukaemogenic, and other oncogenic properties. However, the results from studies by Thiess et al. (1981) and Morgan et al. (1981) do not support the suggestion that EO is carcinogenic to man.

The significance of the epidemiological findings is limited by the small number of observed deaths and the uncertainty of former exposure information. It is therefore not possible to confirm or exclude a cause-effect relationship between EO exposure and the observed excess mortality rate. Nevertheless, the claim of excess incidence of tumours in workers exposed to EO should be treated with some concern. There is clearly a need for further epidemiological investigations.

## 3. Experimental Data

### 3.1. Short- and medium-term effects

3.1.1. Oral and parenteral exposure. The $LD_{50}$ was estimated from studies in which EO was administered in aqueous solution by the oral or parenteral route, to rats, mice and rabbits. The lowest $LD_{50}$'s (127 mg/kg) were observed in rats treated subcutaneously with the EO solution. Higher values were obtained when animals were treated orally. In rabbits, the $LD_{50}$ for EO in aqueous solution was found to be 631 mg/kgbw by this route. Clinical signs of acute toxicity consisted mainly of ataxia, prostration and difficult respiration, with, occasionally, tonic convulsions. Most of the animals that died did so within 24 hours (Woodard and Woodard,1971).

Groups of 5 rats given repeated oral doses of 3, 10, 30 or 100 mg/kgbw EO (dissolved in olive oil) by gavage 5 times a week, suffered marked loss of weight, severe gastric irritation and liver damage at the highest concentration of EO, and the animals in this group were killed at the end of 3 weeks (Hollingworth et al.,1956). No clinical or pathological effects were observed in rats treated with the lower dose-level for 30 days.

The toxicity of parenterally-administered EO, dissolved in saline, was investigated in dogs and rats by the daily subcutaneous injection of 6, 18 or 54 mg/kgbw for a period of 30 days. The 4 dogs treated with the highest concentration became ill after the second injection. Muscle spasm, lowered body temperature and prostration subsequently developed and became more severe, and the dose was reduced from 54 to 36 mg/kgbw. The general condition of the animals improved but they were sluggish, with ataxia and tremors, and their conjunctiva was severely congested. The injection site showed a moderate to a marked inflammatory reaction. Two dogs died during treatment. A reduction in haemoglobin value and an increase in liver weight were observed in all animals at the lowest level of treatment, and increased progressively in severity with the higher doses. Jaundice was observed in the two surviving dogs at the highest level and in two out of four dogs treated with 18 mg/kgbw EO. In a later study, 3 beagles were treated with EO in glucose solution, daily for 3 weeks. Initially, doses were 3 mg/kgbw and were gradually increased to 60 mg/kgbw. No evidence of haemolysis was observed.

All rats survived the duration of the study without any apparent adverse effect, excepting a severe inflammatory reaction at the injection site (Woodard and Woodard,1971).

3.1.2. Inhalation exposure. Several workers (cited by Hire and Rowe, 1981) have studied the response of various species of laboratory animals (including guinea pigs, rabbits, rats, mice and dogs) following a single exposure, for periods of a few minutes to 48 hours, to concentrations of EO in respired air varying from 250 to 64,000 ppm. At concentrations of 14,000 ppm and above, EO was lethal to guinea pigs after 10-60 minutes exposure. Death occurred within 24 hours with the longer exposure but was delayed up to 8 days with the shorter exposure. Delayed deaths (up to 8 days) also occurred when guinea pigs were exposed to 1400 ppm for 8 hours. Exposure to 560-600 ppm for 22 hours caused rapid death in guinea pigs but no deaths were observed when exposure was limited to 7 hours. At 280 ppm and below, no deaths were observed after 7 hours exposure but an occasional death was reported if exposure was continued for 48 hours. All dogs died when exposed to 1400 and 2200 ppm EO for 4 hours, but no deaths were reported when concentrations of 710 ppm or less were employed. Dogs survived a single exposure to 600 ppm EO for 22 hours. Rats and cats succumbed to a single exposure to EO of 1100 ppm for 8 hours; cats survived exposure to 500-600 ppm EO in air for 22 hours. Rabbits survived exposure to 2200 ppm for 4 hours, 1100ppm for 8 hours and 600 ppm for 22 hours. In general, vapour concentrations of EO greater than 1000 ppm were found to irritate the mucous membranes and depress the central nervous system. Early signs of irritation in a number of species included lachrymation, nasal discharge and salivation followed by gasping and laboured breathing. Corneal opacity was observed in certain species, particularly guinea pigs. In animals that survived the exposure period, recovery appeared to be complete until delayed effects including nausea, vomiting, diarrhoea, paralysis of the hind quarters, convulsions and death occurred. At post mortem the lungs were found to be congested, hyperaemic and emphysematous.

Hollingsworth et al.(1956) carried out a series of investigations in which guinea pigs, rabbits, rats, mice and monkeys were exposed to EO in air at concentrations varying from 49-841 ppm for 7 hours daily, 5 days a week, for up to 7 months. Repeated exposure to 204, 357 or 841 ppm EO in air caused irritation of the respiratory passages and injury to the lungs in

all species. None of the animals of the different species survived exposure to 841 ppm EO in respired air for more than 10 days. At 357 ppm, 18 of 20 rats and all of 10 mice died after 38 and 33 days, respectively, with 7 hrs exposure per day ; monkeys survived 94, and guinea pigs 123, such exposures. A secondary respiratory infection was said to have caused the deaths of an "appreciable" number of the rodents during the course of these investigations. There was liver, kidney, adrenal and testes damage in rats and guinea pigs. Delayed but reversible effects were observed in the nervous system of rats, rabbits and monkeys. These were characterised by impairment of functions (both motor and sensory) at the level of the lumbar and sacral region, and paralysis and muscular atrophy of the hind limbs. Monkeys exposed to 204 ppm developed less active knee-jerk reflexes and there was no withdrawal from superficial pain stimuli in the hind feet and the skin of the legs and back. There was a positive Babinski's reflex; deep pain reflexes were elicited in the feet and toe pads. No central nervous system effects occurred when rats, guinea pigs, rabbits or monkeys were exposed to 113 ppm EO in air, 7 hours daily for up to 157 times, but there was growth depression and increased lung weight in rats. Repeated 7-hour exposures to 49 ppm of EO vapour had no adverse effect on any of the species treated as judged by general appearance, behaviour, mortality, growth, body weight gain, organ weights, and gross and microscopic examination of tissues.

In another series of investigations, Jacobson et al.(1956) found that repeated inhalation by rats, mice and dogs of 100 ppm EO in air for six months produced "little" evidence of treatment-related effects, ie. only increased susceptibility of mice to subsequent exposure to higher levels of EO and anaemia in a dog. In 2 of 3 dogs exposed to 290 ppm EO for 6 weeks there was vomiting, occasional slight tremors and transient weakness in the hindlegs. Two of the dogs showed a mild normochromic anaemia while development of a slight hypochromic anaemia was suggested in the third. Rats and mice exposed for the same period to 290 ppm EO in air showed no significant pathological changes apart from marked haemosiderosis in the spleen of a "few" rats. Rats repeatedly exposed to 400 ppm EO in air over a 6-week period developed nasal discharge, diarrhoea, a "tendancy towards the side position" (said to be probably due to weakness of hind quarters), reversible loss of use of hindlegs and laboured breathing. These rats lost weight, as did mice similarly exposed to EO. Thirteen of 20 rats died during the 6-week period.

3.1.3. Skin and eye irritation

Hyperaemia and oedema developed when small pads of cotton wool moistened with 10 or 50% aqueous solutions of EO were applied to the intact, shaved abdominal skin of rabbits for 1-60 minutes. The intensity of response was roughly proportional to the length of exposure time and concentration (Hollingworth et al.,1956).

Ocular instillation of a 2.0% aqueous solution of EO into rabbits' eyes produced no effect in the standard test, but conjunctival irritation was observed after repeated ocular instillation of 0.1% EO dissolved in a balanced salt solution over a 6-hour period. When injected into the anterior chamber of the eye, this concentration of EO did not damage the iris and lens (McDonald et al.,1973).

3.1.4. Conclusions

There are considerable differences between species in their response to acute toxic doses of EO when given by inhalation, parenterally or orally. By the inhalation route, the guinea pig was the most sensitive and the dog was least sensitive, the rat being intermediate in response. Clinical observation of treated animals revealed that acute effects were mainly confined to the CNS and the respiratory system. The former were manifested as depression by high doses of EO, while in the latter lung oedema developed at such doses. Effects on the CNS (motor and sensory) were observed in the lumbar region of rats, rabbits and monkeys. However, they occurred at levels of 204 ppm or above and could not be detected after cessation of treatment. This complete recovery suggests that no permanent damage occurred.

At lower levels, EO seemed to be well tolerated. None of the animal species suffered any ill effects when exposed to levels of 113 ppm by inhalation, for several hours a day for several days. The deaths from pulmonary infection may have been due to the prevalence of pulmonary disease in rats in the animal house where the study was being conducted, but could also have resulted from an immunosuppressive effect of EO.

The species variability in response reported in the above-mentioned investigations appears to be related to the total body dose and peak

blood levels from exposure to EO. The basis for this hypothesis is explained in Appendix 4.

An interesting and important finding is the seemingly innocuous nature of EO in water when instilled into the eye of the rabbit. The solution would be expected to be highly irritant in view of the reactive nature of EO. Perhaps the proteins present in the eye secretions are sufficient to reduce the amount of EO that comes into contact with the cells of the corneal or conjunctival epithelium. The irritancy of EO when applied in a physiological saline solution is explainable on the basis that under these conditions a substantial proportion of EO is converted into ethylene chlorohydrin, which is irritant to tissues (Bruch,1973). Limited data suggest that EO is likely to be irritant in contact with skin but the results are too fragmentary to permit an assessment of irritancy to man.

## 3.2. Reproductive effects

### 3.2.1. Effects on reproductive tissues

a)Testes. After injection or inhalation of EO it can reach the testes, as demonstrated in a number of studies. Hollingworth et al.(1956) report testicular degeneration and fibrosis of the tubules in guinea pigs after 123 exposures to 357 ppm for 7 hr. per day. Other toxic effects (decreased growth, higher incidence of fatal secondary respiratory infections, lung injury and a fatty degeneration of the adrenals) were also observed at this dose level. In rats, 204 ppm caused slight testicular tubal degeneration, and a decrease in testicular size was found after 127 to 133 exposures of 7 hr. per day. There were no abnormal histological findings in the testicular reproductive tissues of rats exposed to 113 ppm for the same period.

Various studies (Bateman, 1973; Appelgren et al.,1977; Ehrenberg et al.,1974,1979) have shown that after intravenous injection of $C^{14}$-EO in experimental animals, radioactivity was detected in numerous organs including the testes. An interpretation of these findings is given in section 3.5 of this chapter.

Following a 4 hours inhalation of 1000 ppm (Embree et al.,1977) a positive dominant lethal effect was observed in the rat. A significant increase in postimplantational foetal deaths was noted during the first

5 weeks of the experiment, but not in the following 5 weeks. This may mean that the germinal cells are affected only after their meiotic division. This is not in accordance with the patterns of activity of potent alkylating agents (Epstein et al., 1970).

Cumming et al.(1981) reported unscheduled DNA synthesis in the testes of mice after 4 hours inhalation of 600 and 800 ppm, and after daily 8-hour exposure to 300 ppm (Cumming et al.,1979) reflecting repair activities of damaged testicular DNA. Under normal conditions no DNA synthesis occurs in the testes during the sperm maturation.

An 8-week pilot study (Snellings, 1978), conducted to determine the dose levels for a long-term inhalation study with CF1 and CD-1 mice and Fisher rats, showed some degree of testicular degeneration in the rat at the highest dose level (436 ppm; 6 hrs/d, 5d/wk) within 3 weeks. However, the high incidence of mortality in this dose group led to termination of the experiment. Thus the testicular effects may to some extent be due to general toxic effects and malnutrition. No testicular degeneration was observed in rats in the 142, 95 and 48 ppm dose groups after 8 weeks of exposure, nor in CD-1 male mice at any dose level.

A subsequent, long-term rat inhalation study (10, 33 and 100 ppm) revealed no testicular effects. The report concludes that the histological examinations show no treatment-related changes of the reproductive tissues. Thus the no-effect level for any testicular damage should clearly be above 100 ppm (Appelgren et al.,1977).

b) Ovaries. No histological effects of EO on ovaries were observed in female rats exposed to 100 ppm in the 2-year inhalation study by Snellings et al. (1979) and Snellings (1979).

### 3.2.2. Effects on Reproductive Function

The effect of EO on fertility was investigated (Snellings et al., 1982) in a one-generation reproductive study with 30 male and 30 female weanling rats per dose group ; 10, 33 and 100ppm of EO in air ; and 2 control groups. For 12 weeks prior to mating the exposure was 6hr/day, 5days/wk. During and after the 2-week mating period the exposure was 7 days/wk. After 1 week of mating the males were rotated to a new female

for which no positive vaginal plug had been established. The 7-day exposure regime was then continued until day 21 post partum.
In the 100ppm group there was a significant reduction in the median number of offspring pups per litter (4 versus 9 or 10 in all other groups, including control groups), and in the median number of implantation sites per mother (6 versus 10 or 11). The ratio of the number of newborn animals to the number of implantation sites per pregnant animal also decreased (to 0.57 versus 0.9-1.0 for controls). The differences between all EO-exposed, and either control, groups were not statistically significant. Pre- and post-implantational loss of eggs, mainly indicative of a mutagenic effect, may have masked effects on male fertility. The authors concluded that the above effects may be a consequence of a decidual reaction, or an indication of embryo or foetal death.

After the second mating period there were more infertile females in the 100 ppm group than in any other group. Also, fewer females, mated in the second week with a male that had successfully impregnated a female in the first week, became pregnant in the 100 ppm group. The male rats showed a significant decrease in fertility in the 100 ppm group. There was no significant difference when all dose groups were compared to the control groups. The duration of the gestation period in the 100 ppm exposure group increased significantly in 7 out of 14 animals for periods longer than 22 days when compared to either control group. Four of these 7 animals had a gestation period of 23 days, and for 2 of them it was 25 or 26 days ; one was undeterminable. All gestation periods in the 33 and 10 ppm groups were of 22 days. Since the normal range is 21 to 23 days, the biological significance of this observation remains questionable. There was no treatment-related adverse effect in the F1a generation during the lactation period even when the dams were exposed during this period. Although all animals, including the controls, had a virus infection, it is assumed that this had not influenced the results of the study. From the results on one generation, it is concluded that the no-adverse effect level should be between 33 and 100 ppm.

3.2.3. Embryotoxicity and teratogenicity

The teratogenic potential of EO has been investigated in CD-1 mice after intravenous application of EO dissolved in aqueous dextrose, at 0, 75 and 150 mg/kgbw during 4-6, 6-8, 8-10 and 10-12 days of gestation (La Borde and Kimmel,1980). The mean foetal body weights decreased significantly in all four groups at 150 mg/kgbw. Signs of maternal toxicity (decreased body weight gain) were recorded in the first, third and fourth gestation-time group, but not during days 6-8 of gestation. On the other hand, in these groups a high resorption rate was also observed, which may explain the reduced body weight gain in the dams. A significant increase of minor abnormalities, such as fused or missing arches and fused or branched ribs in the cervical and thoracic skeleton, was observed in the 150 mg/kgbw group treated at gestation days 6-8 and 10-12 which represent periods of organogenesis. In the group to which 150 mg/kgbw EO were administered at gestation days 6-8, about 19% of the foetuses in each litter showed the above abnormalities. 9.5% malformed foetuses were seen in the day 10-12 group, but here only 5 foetuses were alive. No excess abnormalities occurred in the 75 mg/kgbw dose group when compared to the control group. It should be noted that a dose of 75 mg/kgbw is still in the toxic range. In the same study a teratogenic effect was observed when EO was administered intravenously to the CD-1 mouse, at 150 mg/kgbw on day 6-8 of the gestation period. The signs of maternal toxicity in the high dose groups are doubtful because the decreased body weight gain in the dams may be due to the high resorption rate. On the other hand only the body weights were investigated, and 150 mg/kgbw is an extremely high dose. Appelgren (1977) found that 200 mg/kgbw EO killed 2 of 5 NMRI-mice when given intravenously.

In a teratogenicity study (Snellings, 1979) pregnant Fisher 344 rats were exposed to an atmosphere containing 10, 33 and 100 ppm of EO from days 6 to 15 of the gestation period. Two positive control groups (acetyl salicylic acid by gavage, 500 mg/kgbw on day 9, and 625 mg/kgbw on day 10, of gestation) and two negative control groups were used. The only treatment-related effect was a significant depression of male and female foetal body weights. Body weight gains of the treated and control mothers were not reported. Thus it is possible that the foetal body weight depression is a consequence of a decreased body weight of

the dams. In the acetyl salicylic acid-treated groups significant differences in malformation rates (in relation to the control groups) were observed, such as an increased crown-to-rump length, gross abnormalities, dead foetuses, and visceral and skeletal abnormalities.

3.2.4. Conclusions

EO in high doses caused degenerations in the tubular epithelium of the testes and damage to the male germ cells of small rodents. Potential effects on male fertility at 100 ppm exposure may be masked by presumptive dominant lethal effects. Thus there is no conclusive evidence that a 5 days per week exposure to 100 ppm (for 7 hours per day) from 12 weeks prior to mating, and a following 7 days per week exposure from mating up to the end of lactation, lead to reduced fertility in male or female rats. This is not inconsistent with the dominant lethal effects of EO. An effect on the maternal reproductive function cannot be excluded, however, because the females were also exposed.

The intravenous administration of 150 mg/kgbw (a dose which is 1/3 to 1/2 of the $LD_{50}$) to CD-1 mice during the gestational state of organogenesis leads to a higher-than-normal incidence of commonly occurring abnormalities such as those of the cervical and thoracic skeleton. These effects are assumed to be due to the general systemic toxicity of EO at high doses but a direct effect on the embryos at this dose level cannot be excluded. No embryotoxic effects were observed at 75 mg/kgbw i.v. or with daily inhalation of 100 ppm EO for periods of 6 hr.

3.3. Carcinogenicity

3.3.1. Experimental results

The carcinogenic potential of EO has been investigated by skin application in mice, by gavage and inhalation in rats, and by repeated subcutaneous injection in both rats and mice.

a)Skin application. A group of 30 eight-week old female ICR/Ha Swiss mice were painted on the clipped dorsal skin, three times weekly for life with approximately 0.1ml of a 10% solution of EO in acetone (Van Duuren et al.,1965). The average survival was 493 days. No skin tumours were observed, and no tumour incidences at other sites were reported .

b) Subcutaneous injection. No sarcomas were found when a total dose of 1 g/kgbw of EO was administered to each of 12 rats by repeated subcutaneous injection. Although the dosage schedule was not given, it was stated in the report that the period of treatment was 94 days and that the animals were then observed for their lifetime (Walpole, 1958).

In another experiment, EO dissolved in tricaprylin was administered subcutaneously to mice for a much longer period. Groups of 100 male NMRI mice were treated once weekly for a maximum of 95 weeks with 0.1 ml tricaprylin containing 0.1, 0.3 or 1.0 mg EO. A control group of 200 mice was included. At the termination of the experiment after 106 weeks, 12 local sarcomas and 2 malignant lymphomas were observed in the group that received 1.0 mg EO. Eight local sarcomas, 2 basal cell carcinomas and 4 malignant lymphomas were found in the group treated with 0.3 mg EO. Five local fibrosarcomas and 2 malignant lymphomas were observed in the group treated with 0.1 mg of EO. Four fibrosarcomas and one lymphoma were observed in the controls. Statistically there was a difference between the EO-treated and control animals regarding the number of tumours induced at the injection site, indicating that EO was probably the cause of these tumours (Dunkelberg, 1981).

c) Inhalation exposure. Two inhalation studies have been conducted. In one of these, groups of 120 Fischer 344 rats of each sex were treated with concentrations of 10, 33 and 100 ppm EO vapour 6 hr/day, 5 days per week for about 26 months. Two other groups of 120 Fischer rats (Groups I and II) served as untreated controls (Snellings et al.,1981). Test and control rats of both sexes became infected with a sialodacryoadenitis virus which was thought to account for a higher mortality in the exposed and unexposed rats between the 15th and 17th month. The groups exposed to 100 and 33 ppm were more severely affected than the rest. Mortality rate decreased after the 17th month but increased once more at approximately the 20th month in all groups, and was significantly greater in the 100 ppm group than in controls. Mortality in the group exposed to 33 ppm was greater than in the controls, but the difference was not statistically significant.

A variety of tumours was found in both the treated and untreated groups, but only two types were found to occur with greater frequency in the treated than in the control animals, namely a mononuclear cell

leukaemia and a peritoneal mesothelioma. Pituitary adenomas were found to occur earlier in treated than in control animals. The incidence of mononuclear cell leukaemia increased in both male and female rats. In the males, the cumulative percentage incidence at the end of the experiment was 61, 55 and 41% in groups of rats treated with 100, 33 and 10 ppm, respectively, of EO in inhaled air. The incidence of leukaemia in control groups I and II was 36% and 40% respectively. In the females, the cumulative percentage incidence was 79, 50 and 36% in the 100, 33 and 10 ppm treated groups respectively, while that in groups I and II controls was 21 and 28% respectively.

A statistical analysis of the figures revealed that there was a positive trend, associating a higher incidence or earlier appearance of leukaemia in treated rats compared with controls. This trend was greater in males than in females (Snellings et al.,1981).The mesothelioma found in the peritoneum was seen only in males of treatment and control groups and was thought to develop from the epithelium of the tunica vaginalis. The incidence in the control groups I and II was 5%. In the treated groups the incidence was 47, 25 and 8% for the high, medium and low dose respectively. Pituitary adenomas occured in a high proportion of the rats in both treated and control groups. At the termination of the study the cumulative percentage incidence in males was 64, 48 and 55% for the 100, 33 and 10 ppm groups respectively, and 56 and 57% for control groups I and II. That in females was 75, 74, 71% for the 100, 33 and 10 ppm groups respectively,and 71 and 70% for control groups I and II. Pituitary tumours appeared earlier in the treated than in control animals. According to the authors, this showed a significant positive trend ($P<0.01$) which indicated that the prevalence of pituitary adenomas in male rats of the 100 ppm group was the same, but that the latency period was reduced, if it was assumed that the adenomas were the cause of death. In the females there was no significant elevation of pituitary adenomas in the 33 ppm group, but tumours appeared earlier in the 100 ppm group than in the controls and analysis showed that there was a trend similar to that found in the males, but that the significance was greater ($P<0.0001$) than in the males.

In a second inhalation experiment, the results of which have been presented in a preliminary form, groups of male Fischer 344 rats were

exposed to EO of 99.7% purity in a concentration of 0, 50 or 100 ppm, 7hr/day, 5 days/wk for 24 months. During the course of the experiment rats exposed to 100 ppm showed a depressed weight gain and higher mortality compared with the controls or those exposed to 50 ppm. Only liver and spleen had been examined histologically at the time of the report. The overall incidence (i.e. taking into account leukaemia found in animals that died in the course of the experiment as well as in those sacrificed terminally) was 30, 47 and 40% respectively in controls and the intermediate and high dose groups (Lynch, Lewis and Moorman, 1982). The data in this preliminary report were insufficient to determine whether there was an early appearance of leukaemia as a result of the treatment.

d)Intragastric administration. Dunkelberg (1982) administered EO to groups of 50 female Sprague-Dawley rats by gavage, as a solution in salad (Livio) oil free from polycyclic hydrocarbons, at doses of 30 and 7.5 mg/kgbw, twice weekly for 107 weeks. The amounts administered were contained in about 1ml of solvent. Animals were starved for 16 to 18 hr before treatment. β-propiolactone as a positive control, was administered as a suspension in the same vehicle at 30 mg/kgbw, twice weekly for 50 weeks to a group of 50 female rats of the same strain. A similar group of 50/50 received the vehicle oil alone and served as a negative control. The experiment was terminated after 150 weeks. In the course of the experiment the rats developed pneumonia and were treated with chloramphenicol and tylosin tartrate. Squamous cell carcinomas were observed in the fore-stomach of 29 rats treated with the higher dose of EO, the first appearing after 79 weeks. Two other rats developed fibrosarcoma in the same organ. Four other animals developed carcinoma in situ and 11 developed hyperkeratosis, hyperplasia or papilloma. Eight animals treated with the lower dose developed squamous cell carcinoma, 4 developed carcinoma in situ and 9 others developed hyperkeratosis, hyperplasia or papilloma. In the group treated with β-propiolactone, 46 out of 50 rats developed squamous cell carcinoma of the fore-stomach. The first tumour appeared after the 32nd week of treatment. In most cases these tumours metastasised to the diaphragm, mesentery and liver. No tumours of the stomach were reported in the controls. The incidence of tumours in other organs was of the same order in treated and control rats.

e)Dietary administration.In a 2-year study, Bär and Griepentrog (1969) administered to young rats (100-150 g) a standard diet (Altromin R) which was fumigated at weekly intervals by air containing EO (500-715ppm). The residues of EO in the diet were found to be between 500 and 1400 ppm on the first day of feeding and between 53 and 400 ppm after 6 days. No increase in tumour incidence was observed in test animals compared with controls.

f) Miscellaneous exposure. An increase in uterine tumours was reported in 63 out of a group of 86 female Swiss-Webster, inbred and germ-free mice maintained on EO-treated ground-corncob bedding for 150 days and then on untreated bedding for their lifespan (maximum 900 days). No tumours were reported in 83 female mice, 100-600 days old, which were not exposed to treated bedding (Reyniers et al.,1964). This experiment cannot be evaluated because of the much shorter life-span of the mice maintained on untreated bedding compared with those on treated bedding.

3.3.2. Comments on experimental results

An increased incidence of tumours has been observed in rats treated with EO by inhalation or gavage, and in mice treated subcutaneously with a solution of EO in oil, but no significant increase in tumours occurred in another inhalation experiment with the same strain of rats and exposure concentrations closely similar to those in the previous experiments. Furthermore, a negative result was obtained when a high concentration of EO dissolved in acetone was tested by topical application to mice, or when fumigated food containing high concentrations of EO was fed to rats.

a)Inhalation exposure. The increased incidence of leukaemia and mesothelioma in rats treated with EO by inhalation is questionable as evidence of carcinogenic activity. The leukaemia has a high spontaneous incidence in Fischer 344 rats and is of a type (mononuclear cell leukaemia) which does not occur in other strains of rats. Taking these points into account, some workers suggest that viral and genetic factors play an important role in the development of leukaemia (Davey and Moloney, 1970; Moloney, Boschetti and King, 1970). A similar situation exists in the case of some commonly-occurring tumours in the mouse. Pulmonary adenomas, mammary carcinomas and lymphomas are common in some strains of mice, and viral and genetic factors are known to be

responsible for this high incidence. These tumours are readily induced by a variety of chemical agents but are not regarded as sufficient evidence for concluding that the compound is likely to present a carcinogenic hazard to man, (Grasso,et al., 1977; IARC, 1981).

The same argument would also apply to the increased incidence of testicular mesotheliomas. This type of tumour is relatively common in Fischer 344 rats, approximately 3% of which develop this tumour normally (Goodman,et al., 1970). It is rare in other strains of rats where the normal incidence is about 0.05%. Even when these strains are exposed for prolonged periods to high levels of known carcinogens, the incidence of testicular mesothelioma is not increased.Only nitrosopyrrolidine has been reported to induce this type of tumour in a non-Fisher strain of rat (Greenblatt and Lijinski, 1972). Thus the increased incidence of mesothelioma after exposure of Fisher rats to EO is likely to reflect a genetic tendency of this strain to develop this type of tumour, rather than a true carcinogenic response.

The incidence of pituary adenomas in control and experimental groups in the experiment was very high. Although the tumours were observed to occur earlier in test rats than in controls, it is uncertain whether this is an earlier occurrence or an earlier discovery due to deaths from other causes (e.g. sialodacryoadenitis or leukaemia) so that one cannot conclude from the trend analysis that a direct causal relationship exists between administration of the test compound and the early occurrence of pituitary tumours.

It is noteworthy that the UK Expert Committee on Carcinogenesis (1980) believes that an increase in the number of tumours which have a high spontaneous incidence should not automatically be regarded as proof of carcinogenicity, but only that the problem needs further study. This would suggest that the data of Snellings et al.(1981) provide inconclusive evidence of the carcinogenicity of EO. There was no significant increase in the total incidence of tumours in the second inhalation experiment in rats exposed to EO, although the strain of rats and dose levels employed were identical with those used in the first experiment. Despite the fact that this experiment was incomplete, it seems unlikely that it would confirm the result of the first

experiment, thus throwing further doubt on its validity as an indication of the carcinogenic activity of EO.

b) Subcutaneous administration. The production of local sarcomas by repeated subcutaneous injection of EO dissolved in oil cannot be regarded as a reliable index of carcinogenicity since physical factors which produce chronic tissue damage are known to lead to the production of this type of tumour (Grasso and Golberg,1966). The induction of sarcomas by the injected oil, free of EO, is an illustration of this phenomenon since vegetable oil is removed with difficulty from the injection site, (Grasso, Gangolli and Hooson,1969) and the granuloma it produces has been shown to lead to sarcoma production (Grasso and Golberg, 1966). The higher incidence of tumours in the treated group may merely indicate that the presence of EO intensifies the granulomatous reaction. On the other hand, since EO is a potent mutagen (cf.C.3.4) one cannot entirely exclude the possibility that the higher incidence of tumours reflects the presence of genetic damage in a proliferative lesion.

The negative result obtained with a limited series of subcutaneous injections is important since it indicates that EO does not behave in the same way as proximate carcinogens. Under the same conditions of treatment such agents produce both mammary and connective tissue tumours (Hooson, et al., 1973).

c) Intragastric exposure. Although a dose-related, fairly high incidence of squamous cell carcinomas was obtained when EO was administered by gavage, the tumours occurred much later, and were much less malignant, than those induced by $\beta$-propiolactone. One suspects that the carcinogenic potency of EO is relatively weak, especially in view of the high doses administered.

A negative result was obtained when food fumigated with a high concentration of EO was administered to rats, although the total dose in this experiment appeared to be of the same order as that administered by gavage.

d) Other comments. The absence of tumour production in the skin

of mice after the application of EO in acetone, and in rats fed on a diet fumigated by EO, is difficult to reconcile with the potent mutagenic activity exhibited by the compound. It would seem plausible to suggest that in these experiments EO behaved as a "pure initiator", and that in the absence of tissue hyperplasia, which is thought to possess "promotional" activity, there was little opportunity for tumours to develop. Presumably the high concentration of EO in the gavage experiment must have exerted a serious irritant effect with a considerable degree of hyperplasia, thus accounting for the high incidence of tumours. It must be pointed out that no systemic tumours were induced when EO was administered subcutaneously or by gavage. Equally, no tumours were produced in the respiratory tract of rats when EO was given by inhalation although its genotoxic activity would lead one to expect them.

#### 3.3.3. Conclusions

The experiments reviewed indicate that EO, despite its potent mutagenic properties (cf. 3.4), produces a carcinogenic effect only in situations where a marked degree of tissue injury occurs, or where a pronounced tendency to spontaneous tumour production exists. In those experiments where there was no overt injury, or predisposition to a high spontaneous incidence of tumour production, no carcinogenic effect was observed. For these reasons it is questionable whether this evidence is sufficient to conclude that EO presents a significant carcinogenic hazard to man at current recommended limits of exposure.

The evidence from human epidemiological studies is limited and does not allow a conclusion about cause-effect relationships between exposure to EO and the observed tumours.

### 3.4. Mutagenicity

#### 3.4.1. Evidence of mutagenic effects

The abundant evidence for EO mutagenicity has been reviewed in detail in several reports (IARC working group, 1976; Glaser, 1977; US EPA Rebuttable Presumption, 1978; Fishbein, 1979; Cumming et al.,1981; NIOSH Current Intelligence Bulletin XX,1981; and Ehrenberg and Hussain, 1981) but it is summarised here with special reference to mutagenic potency, and dose-response relationships in prokaryotic and eukaryotic test systems.

a) Micro-organisms. Studies with Salmonella typhimurium (Rannug et al.,1976) showed that EO is a direct-acting mutagen to strain TA1535 when employed as a 9.5 mM ethanolic solution for 1 hour at 25°C. Turtoczky and Ehrenberg (1969) demonstrated mutagenic activity of EO to Escherichia coli at millimolar concentrations, and reverse mutations have been induced in Neurospora crassa at the adenine locus by exposure of macroconidia to aqueous solutions of EO at concentrations as low as 1.5mM for several minutes (Kolmark and Kilbey, 1968; Kilbey and Kolmark, 1968), confirming the earlier work of Kolmark and Westergaard (1953). EO is also reported to be mutagenic to Aspergillus nidulans (Morpurgo,1963).

b) Plants. Studies in barley (seeds exposed to 0.15% EO in water for 10 hours at room temperature) suggested that EO can induce chlorophyll mutations at a rate similar to that of ionising radiations (Ehrenberg et al.,1959). Similar results have been observed in wheat (MacKey, 1968) and in rice (Roy and Jana, 1973; Jana and Roy, 1975). Chromosome aberrations in cultured pollen tubes of Tradescantia paludosa, following exposure of pollen to EO gas, have been observed by Smith and Lotfy (1954).

c) Insects. Exposure of Drosophila melanogaster larvae to yeast-agar food containing EO dissolved in saline, and exposure of adult flies to gasous EO or injections of an EO solution ( 0.5% in saline) induced sex-linked recessive lethal effects in these insects (Bird,1952). Intra-abdominal injections of an EO solution into adult flies were also shown to induce aberrations in salivary gland chromosomes (Fahmy and Fahmy, 1956), and lethal mutations and translocations have been identified in spermatozoa (Watson, 1966).

d) Rodents. Exposure of male Long-Evans rats to 1000 ppm EO for 4 hours was sufficient to induce a significant increase in post-implantational foetal deaths in mated females during the first five weeks post-exposure (Embree et al.,1977). This dominant lethal effect was consistent with previously observed EO-induced chromosome damage in the bone-marrow cells of rats exposed by inhalation (Embree et al.,1977) or by a single i.p. injection of EO in water (Strekalova, 1971).

Generoso et al.(1980) studied the effect on mice of EO dissolved in water and injected intraperitoneally. A dominant lethal study was conducted using a single injection of 150 mg/kgbw (maximum tolerated dose), and a heritable translocation study in males was performed with daily injections on week-days, for 5 weeks, with 60 or 30 mg/kg doses per day. The results clearly showed that EO is effective in inducing dominant lethal mutations and heritable translocations at both dose-levels.

Tissue distribution studies in mice exposed to air containing 1.15 ppm of the labelled agent confirmed that EO would reach the major organs, including the testes (Ehrenberg et al.,1974), and the heritable translocation effects of EO reported by Generoso et al.(1980) were confirmed by cytological investigation of sperm cells.

The effects of EO on the bone marrow of rats and mice was investigated by Appelgren et al.(1978) using the micronucleus test. EO dissolved in water was given to NMRI mice in two intravenous injections of 0.05-0.30g/kgbw, and to Sprague-Dawley rats at 0.1-0.2g/kgbw. An increase in the number of micronuclei in the bone marrow of mice was produced when 1/3 of the $LD_{50}$ for EO (0.29g/kgbw) was given twice. A dose-dependant increase in micronuclei was produced with increasing doses of EO. In rats, doses of EO close to the $LD_{50}$ also produced an increase in micronuclei. Studies with radio-labelled EO in mice confirmed the retention of radio-activity in the testes, bone-marrow and other organs (Appelgren et al.,1977 and 1978).

e) Primates. In a preliminary communication to the Society of Toxicology(USA), Lynch et al.(1982) claimed that after 24 months exposure to 50 and 100 ppm EO, the incidence of chromosome damage and the sister chromatid exchange frequency in cynomologus monkeys were both elevated in a statistically-significant dose-related way. In addition, the phytohaemaglutinin-stimulated division of lymphocytes was inhibited in the treated animals, particularly at the higher dose level.

Star (1980) cultured human fibroplasts which were exposed in vitro to 3.6-3600 ppm EO, and showed a significant increase in sister chromatid exchanges at 36 ppm. Higher levels were cytotoxic.

3.4.2 Conclusions

There is now substantial evidence from tests with both prokaryotic and eukaryotic organisms that EO is a point mutagen (interacting with DNA to induce gene mutations in somatic and reproductive cells), and a chromosome-damaging agent (producing visible clastogenic effects in the somatic cells of higher organisms, including man). It also produces dominant lethal effects in rodents resulting, it is assumed, from chromosome damage to the sperm cells of animals exposed to EO prior to mating.

3.5. Biochemical toxicology

The greatest potential human hazard from EO appears to be associated with frequent and long-term inhalation exposure and hence most of the biochemical experiments were conducted in animals exposed by this route.

3.5.1. Toxicokinetics

The total dose absorbed by inhalation will depend on many factors e.g. the solubility of EO in body fluids, permeability of the lungs, volume of inhalation, volume of blood in the lungs and alveolar concentration of EO. At a constant inspired concentration, the alveolar concentration tends to approach that inspired until body equilibrium is reached. Because of the high solubility of EO in blood, the alveolar concentration, and thus the alveolar tension, is expected to be low at most inspired air concentrations. Total body uptake of EO depends on the alveolar ventilation rate (approximately 60ml/min/kgbw for man) and the air concentration of EO (Darby et al.,1980). Using this model, these workers calculated the total exposure dose for a range of air concentrations (Appendix 4). For example, a 3hr exposure to an EO concentration of 10 ppm would correspond to a dose of 0.195mg/kgbw.

Detailed information on the biochemical toxicology of EO is scant despite its widespread use. In the mouse it appears to be readily detoxified and excreted, the biological half-life being 0.15 hr. This compares with a half-life in water of 76 hr at 37°C, and 6 months at 4°C (Ehrenberg et al.,1974).

Data on the distribution and deposition of radioactivity originally associated with EO has been derived from two studies. In the first,

Ehrenberg et al.(1974) observed that five mice exposed by inhalation to $H^3$-labelled EO excreted an average of 78% of the estimated dose within 48 hours. In this experiment two mice were exposed for 75 minutes to an atmosphere containing 1.15 ppm EO; two mice to an atmosphere containing 7.4 ppm for 60 minutes; and one mouse to an atmosphere containing 33 ppm for 75 minutes. The experiment involved whole-body exposure to a static atmosphere of EO in sealed glass vessels. The EO concentrations given were those at the beginning of exposure. Urine was collected from the animals but only one excretion product, 7-hydroxyethylguanine, was characterised. A trace, 0.007%, of this material appeared in the urine of the two mice exposed to 7.4 ppm. In the second study, Appelgren et al.(1977) injected $C^{14}$-EO into mice (dose level not reported) and followed the distribution using whole— body autoradiography. Two minutes after injection very high concentrations were found in the liver, kidney and pancreas. Between 20 minutes and 4 hours, levels higher than those in blood were found in the liver, kidney, pancreas, intestinal mucosa and contents, lung, epididymis, testicles and cerebellum. Twenty-four hours after injection, radio-activity was still present in the liver, intestinal mucosa, epididymis, cerebellum, bronchi and bone marrow.

Superficially, both studies served to demonstrate that EO (an epoxide of low molecular weight, soluble in water and lipid) is rapidly distributed to all parts of the body irrespective of the route of administration. However, little or no attempt was made to characterise the detected radio-activity. We cannot, therefore, distinguish between radio-activity distributed as EO and "fixed" by alkylation of tissue molecules e.g. proteins, and radio-activity distributed as metabolites and incorporated into tissue molecules by natural biochemical pathways(fig.1). Both of these studies are therefore of limited value.

3.5.2. Metabolism

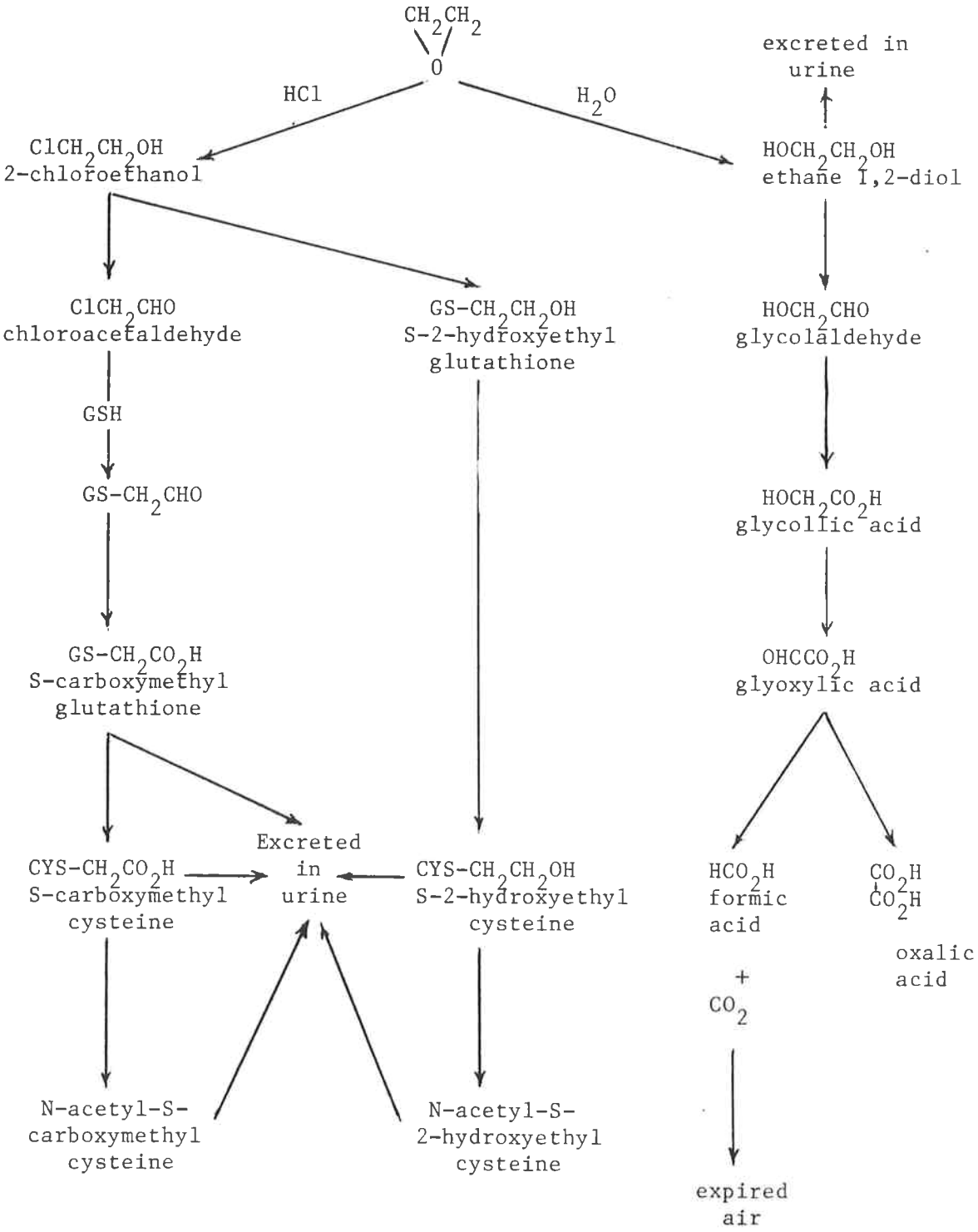
No studies on the detailed metabolic fate of EO were found in the literature. However, one could reasonably expect that the in vivo metabolic reactions would be related to its high chemical reactivity, i.e. that it will react spontaneously with nucleophilic centres such as amine, thiol, chloride and hydroxyl groups (fig.2). Thus the expected metabolites would be ethane 1,2-diol formed by reaction of EO with water, 2-chloro-ethanol formed by reaction with chloride ions (e.g. in

the stomach and blood), and various cysteine-containing conjugates formed by reaction with glutathione. Biotransformation of ethane 1,2-diol would then proceed as described by Gessner et al.(1961) and Chesnay et al.(1971) (fig.1). By analogy with ethane 1,2-diol exposure, the pathway leading to oxalic acid would operate at high EO exposures.

Metabolic transformation of 2-chloroethanol would be expected to proceed either by the pathway described by Johnson (1967) with S-carboxymethyl cysteine and N-acetyl-S-carboxymethyl cysteine as the end products, or, by analogy with 2-bromoethanol (Jones and Wells, 1981), via the conversion of EO which then undergoes conjugation with glutathione and subsequent elimination as S-(2-hydroxyethyl)cysteine and N-acetyl-S-(2-hydroxyethyl)cysteine.

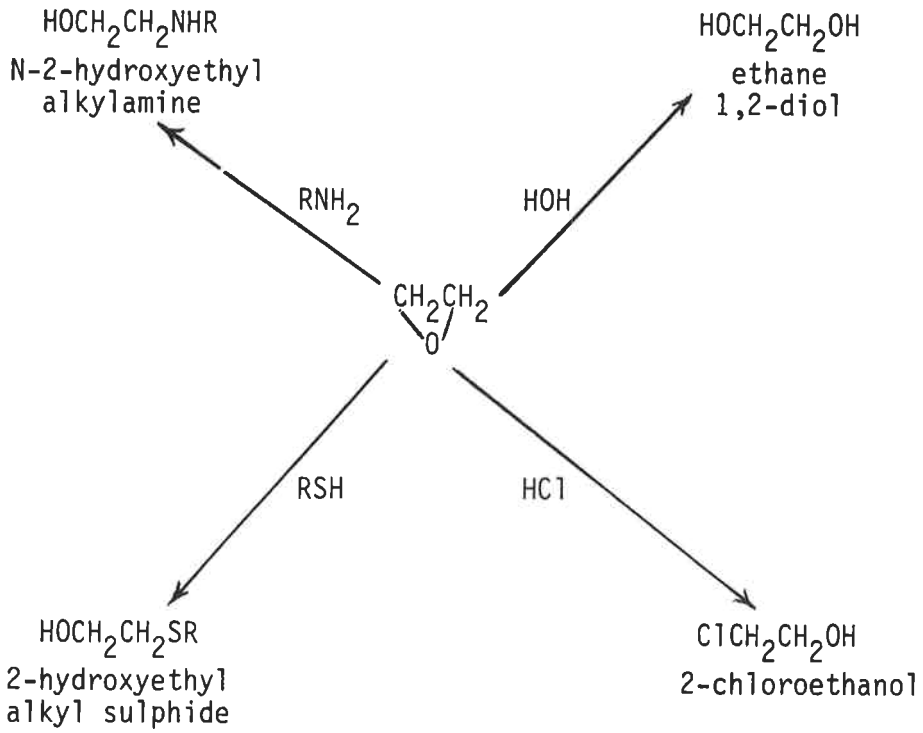
A detailed study of the involvement of glutathione in the detoxification of EO has been reported by Jones and Wells (1981). Rats were dosed intraperitoneally with 2mg/kgbw (1,2-$C^{14}$) EO. Respired air was monitored for total radioactivity over the first 6 hr of dosing and urine was collected for up to 50 hr after dosing. During the first 6 hr after dosing, 1.5% of applied radioactivity was excreted as $C^{14}O_2$ and 1.0% as unchanged EO. Two urinary metabolites were identified: S-(2-hydroxyethyl)cysteine (9% of administered radioactivity) and N-acetyl-S-(2-hydroxyethyl)cysteine (33% of administered radioactivity). Approximately 43% of the administered radioactive dose was excreted in the urine within 50 hr of dosing. The involvement of a glutathione epoxide transferase in these reactions was not studied but has been suggested by Fjellstedt et al.(1973).

FIG.1 PROPOSED BIOTRANSFORMATION OF ETHYLENE OXIDE




GSH = glutathione
CYS = cysteine

FIG.2 SOME IMPORTANT CHEMICAL REACTIONS OF ETHYLENE OXIDE



The involvement of enzyme systems, such as epoxide hydratase, in protecting the cell from small aliphatic epoxides such as EO is uncertain. Oesch (1973,1974) has indicated that such small, highly reactive molecules are not *in vitro* substrates for epoxide hydratase. Studies on ethylene metabolism (Conolly et al.,1978) have shown that in rats pretreated with Aroclor 1254, exposure to 10,000-50,000 ppm ethylene caused a dose-related haemorrhagic liver necrosis. However, trichloropropene oxide, which inhibits hepatic epoxide hydratase as well as depleting hepatic glutathione, did not increase the hepatoxicity of ethylene over the inhaled concentration range of 11,000-51,000 ppm. This study suggests that epoxide hydratase is not involved in EO metabolism.

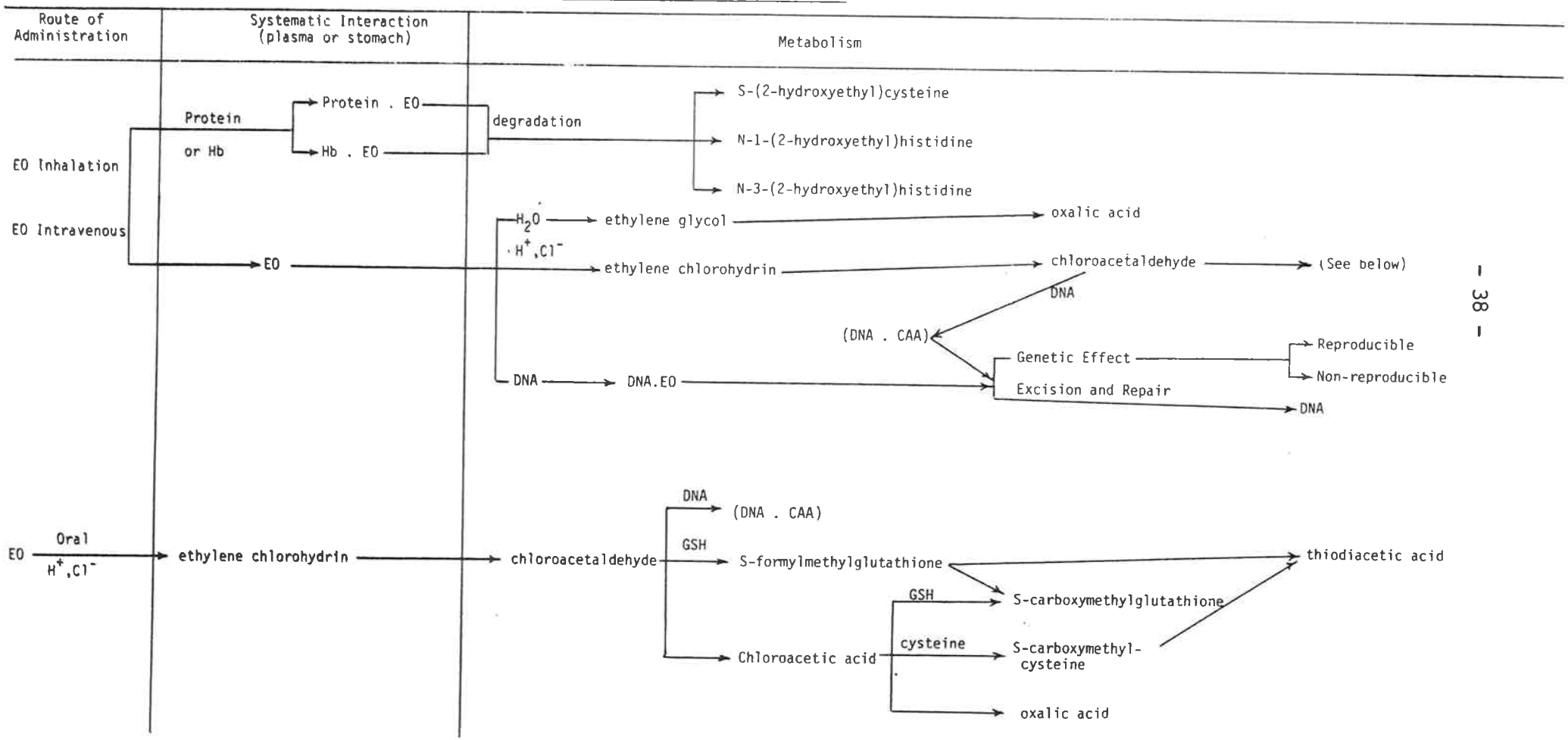
### 3.5.3. Conclusions

The biological toxicology of EO is summarised in fig. 3. Clearly, EO has the potential to react with nucleophilic centres in such cellular macromolecules as nucleic acids and proteins. The apparent lack of reactivity *in vivo* must be related to the existence of effective protective mechanisms. In the absence of adequate toxicokinetic data we can only speculate as to the nature of these mechanisms, although there is some evidence that cellular glutathione plays a role.

## 3.6. Biochemical interactions

If epoxides in the cell are not effectively detoxified, then because of their electrophilic nature they will react with nucleophilic centres in cellular macromolecules. Despite the absence of a systematic study of such products, there is no reason to doubt that, depending on nucleophilicity, substrate constants and steric factors, the same centres in DNA will be alkylated as with methylating agents. (Lawley,1966,1974; Lawley et al.,1971-1972; Shooter,1975; Shooter et al.,1974). Indeed, *in vitro* studies have shown that 7-(2 hydroxyethyl)-guanine is the major reaction product with guanosine, deoxyguanosine and guanilic acid (Brookes and Lawley,1961). Ehrenberg et al.(1974) estimate that hydroxyethylation at the N-7 atom of guanine accounts for 90% of the reaction of EO with isolated DNA. Other sites of *in vitro* interaction include the N-1 atom of adenosine (Windmueller and Kaplan,1962) and the N-3 atom of uridine (Ukita et al.,1963).

FIG.3 TOXICOKINETICS OF EO METABOLISM



In vivo studies with tritiated EO (Ehrenberg et al.,1974) demonstrated, despite some difficulties with an unidentified impurity of high specific activity, a degree of hydroxyethylation in kidney DNA equivalent to 1.7μCi EO per gram DNA (this is equivalent to 1.34 mg EO per gram DNA at a specific radioactivity of EO of 56μCi $mmol^{-1}$). No data are currently available describing interaction at the O-6 atom of guanine although in mechanistic terms this may represent a critical target for genotoxicity.

Interaction of EO with nucleophilic centres in proteins, although of questionable genotoxicological significance, is quantitatively greater than with such centres in DNA. By analogy with other alkylating agents, interactions under physiological conditions could be predicted at sterically-accessible cysteine thiol groups, histidine ring (N-1 and N-3) nitrogens, methionine thiol groups and possibly terminal amino groups of valine (reviewed by Ehrenberg and Hussain,1981). Preliminary in vivo experiments with tritiated EO have demonstrated transfer of radioactivity to tissue protein fractions from liver, spleen, brain and testes. This transfer appears to be dose dependent up to an inhalation dose of 40 ppm.hr. (Ehrenberg et al.,1974). Their data led these authors to suggest that the degree of tissue-protein alkylation could be used to monitor tissue doses of alkylating agents. One feature highlighted by these results was the presence of detectable levels of N-3-(hydroxyethyl)--histidine (up to 2.8 mmol/gm haemoglobin) in the unexposed group, confirming the finding of Ehrenberg et al.(1977). The origin of this background level is thought to be EO generated during the metabolism of endogenously produced ethylene (Wright, 1981), or from ethylene in the atmosphere (Ehrenberg et al.,1977).

According to some current concepts of risk assessment (Ehrenberg et al., 1974; Wright, 1981) the tissue (target) dose is described as the concentration-time integral for exposure of the tissue (target) to the ultimate genotoxic reactant. Thus, the rate of formation of the key lesions(s) is a function of the physicochemical properties of the reactants and the concentration of the ultimate toxic form of the chemical in the micro-environment of the target. Further, the amount of key lesions present at any time is a function of their rate of formation, their rate of repair (or rate of turnover of the target) and the duration of the exposure of the target. Estimation of target dose, particularly human target dose, poses major theoretical and practical problems. For example,

it is essential that the ultimate genotoxic reactant(s) are identical in the experimental species and in the human. This correlation between species is, of course, a pre-requisite for the selection of any model used to evaluate toxicological risk. However, the identity of the ultimate toxicant is often assumed rather than experimentally established (Wright,1981). In experimental species, radiotracer techniques can be used to determine the nature and quantities of specific DNA adducts.

There is at present no direct proof that the haemoglobin alkylation approach indicates genotoxic carcinogenic effects. What is lacking in all of the assessment studies is a detailed correlation relating exposure dose, haemoglobin adduct formation (blood tissue dose) and DNA adduct formation in target organs (DNA dose). Without such data no meaningful extrapolation can be made from haemoglobin alkylation to genetic risk. Such direct methods cannot, however, be applied in humans. Thus in our quest for the assessment of risk to man, the haemoglobin-alkylation approach may provide a suitable indirect method for determining the nature of the ultimate genotoxic reactants and for estimating DNA (target) dose.

Alternative attempts have been made to assess risk, based on the rad-equivalent concept (Ehrenberg et al., 1974) (cf Appendix 5).

## D. CONCLUSIONS: INTERPRETATION OF EXPERIMENTAL TOXICOLOGICAL DATA IN TERMS OF HUMAN RISK

Detailed conclusions about the specific toxicological effects of EO are given in the previous chapters. In this chapter an attempt is made to correlate all available data, and to extrapolate the effects observed in animals and micro-organisms to those which might occur in man when exposed under conditions similar to those recorded in the animal experiments.

1. The most likely form of human exposure to EO is by inhalation of the gas during its manufacture, or its use in the synthesis of chemicals and as a fumigant or sterilising agent. Exposure is known to be generally below the present recommended exposure control limits in the chemical manufacturing industry. However, these values are sometimes exceeded when EO is used as a sterilising or fumigating agent, or as a result of inappropriate handling.

2. The results of relevant short- and medium-term animal studies suggest that exposure to 1000 ppm EO vapour for a brief period would lead to eye and nose irritation in man. If exposure at this level is prolonged, or if the concentration is higher than 1000 ppm, some delayed adverse effects attributable to CNS depression and respiratory difficulty may occur.

   On short- and medium-term exposure of experimental animals to EO vapour the lowest level at which adverse symptoms (irritation of the eyes and upper respiratory tract) occurred was about 200 ppm, so that some adverse effects may be expected in man at around this concentration if exposure is prolonged and is repeated several times during the working week. No irreversible injury to workers is expected to result from such exposures, since complete recovery occurred in all the animal species exposed to high, but non-lethal, EO vapour concentrations.

3. No adverse effects on skin were reported in experimental animals exposed by inhalation, possibly because the animal body was protected either as part of the experimental design, by its hair, or by the absence of skin-perspiration. In contrast, severe skin irritation and blister formation has occurred in humans under conditions of perspiration and occlusion.

   Human exposure to liquid EO, or to a concentrated solution in an organic liquid or water, is unlikely. No information is available about the sensitisation

potential of EO in experimental animals. One clinical case draws attention to the possibility that EO may be a human sensitiser.

4. High doses of EO have been shown to cause adverse effects on the reproductive tissues of the testes in experimental animals. Also, toxic effects on the developing embryo have been observed in mice after intravenous administration of maternally toxic doses. In the absence of a knowledge of species-specific biotransformation mechanisms, one may speculate that these effects could also occur in similarly-exposed humans. On the other hand, rat inhalation studies at low doses showed no embryotoxic effects, and only minor effects on fertility when 100 ppm were inhaled daily for 6 hours. At 33 ppm there was no influence on the fertility of male or female rats. Consideration of the exposure level at which reproductive effects are produced in animal experiments, and of present industrial exposure levels, indicates a safety factor which suggests that current occupational exposure is unlikely to be associated with adverse effects on reproduction in man.

5. An increased incidence of tumours was observed in rats treated with EO by inhalation or by gavage, and in mice treated subcutaneously with a solution of EO in oil, but no significant increase in tumours occurred in another inhalation experiment using the same strain of rats and exposure concentrations closely similar to those in the previous experiments. Furthermore, a negative result was obtained when a high concentration of EO dissolved in acetone was tested by topical application in mice, or when fumigated food containing high concentrations of EO was fed to rats.

   The experiments reviewed indicate that EO, despite its potent mutagenic properties, produces a carcinogenic effect only in situations where a marked degree of tissue injury occurs, or where a pronounced tendency to spontaneous tumour production exists. In those experiments where there was no overt tissue injury, or predisposition to a high spontaneous incidence of tumour production, no carcinogenic effect was observed. For these reasons it is questionable whether this evidence is sufficient to allow the conclusion that EO presents a significant carcinogenic hazard to man at current recommended limits of exposure.

The evidence from human epidemiological studies is limited and does not allow a conclusion to be drawn about cause-effect relationships between exposure to EO and the observed tumours.

6. EO is a potent mutagen and clastogen which has been shown to affect both somatic and germ cells in various species. It binds covalently and irreversibly to mammalian protein, human protein, and mammalian DNA.

## E. FURTHER INVESTIGATIONS TO BE CONSIDERED

The Working Group identified some areas in which further investigations should be considered, over and above the studies in progress as listed in Appendix 6. The first two of the suggested investigations merit strong consideration.

1. The feasibility of carrying out retrospective and prospective epidemiological studies on well-defined occupational cohorts exposed to EO should be examined. These studies could include mortality, morbidity and reproductive effects.

2. Reproductive toxicology studies paying particular attention to effects on the sperm, e.g. induction of unscheduled DNA repair, abnormality, mortality and fertilising potential, should be considered.

The following investigations would be desirable in that they would improve our knowledge of the mechanisms of EO toxicity or would aid in the possible development of techniques for monitoring human exposure.

- Further studies of the immunosuppressive action of EO if real evidence arises that it is an immunosuppressive agent.

- Further studies of the alkylation of haemoglobin and DNA by EO.

- A detailed metabolic/toxicokinetic study in which the protocol should be sufficiently broad to include investigations on the stability, and degradation products, of EO in the vehicles used in the toxicology studies, and to explain the apparent low reactivity of EO in selected mammalian systems.

- Considerable attention has been paid to the possibility of monitoring exposure to EO by biological means. A number of biological tests (alkylation of haemoglobin, chromosome aberrations and sperm examinations) have been suggested. More work is, however, necessary to investigate the sensitivity and suitability of these tests as screens for human exposure to EO.

- Short-term experiments to study the tissue reaction and its relevance to an understanding of tumours induced by EO in the stomach and subcutaneous tissues of rats, since non-carcinogenic but irritant chemicals are known to

produce tumours in these systems.

This report and its conclusions should be reviewed when the results of current and proposed toxicological studies are available.

# F. APPENDICES

## APPENDIX I: PRODUCTION, PROPERTIES AND USE OF ETHYLENE OXIDE..

Synonyms : dimethyleneoxide: 1,2-epoxyethane; EO; ETO; oxirane; oxidoethane; β-oxidoethane; etheneoxide.

Ethylene oxide is synthesised commercially from ethylene via the intermediate ethylene chlorohydrin, or by direct oxidation of ethylene with oxygen or air. Current production capacities are about 2.8 million tonnes/year in the USA, 0.7 million tonnes/year in Japan and 1.2 million tonnes/year in Western Europe.

EO is very reactive and the exothermic nature of its reactions present a number of problems concerning its storage, handling and use. In table 1 are summarised the most important properties of EO.

---

TABLE 1 : Properties of EO

$CH_2$-$CH_2$ ; $C_2H_4O$
O

| | |
|---|---|
| CAS N° 75-21-8 | |
| Molecular Weight: | 44.05 |
| Density (20/4°) : | 0.869 |
| Freezing point °C : | -112.5° |
| Boiling point °C : | + 10.4°C (760 mm Hg) |
| Vapour Density (40°C) : | 1.49 |
| Vapour Pressure (20°C): | 1095 mm Hg |
| Soluble in : | water, acetone, methanol, diethylether, benzene, carbon tetrachloride, most organic solvents, blood. |
| Flashpoint : | 20°C |
| Odour threshold : | lower, 320 ppm<br>higher, 700 ppm |
| Odour: | ether-like |

TABLE 2 : Principal Uses of EO (%)

| | US | Japan (JETOC;1982) |
|---|---|---|
| 1. For production of : | | |
| antifreeze<br>polyester fibres | 48 | 73<br>(includes some other uses) |
| surfactive agents<br>(industrial, home laundry, dish-washing) | 15 | 13 |
| ethanolamines<br>(for soaps, detergents, textile chemicals). | 7 | 7 |
| glycol ethers | 7 | 4 |
| 2. For production of poly-ethylene glycols ; as fumigating and sterilising agents ; other uses | 23 | 3 |

Ethylene oxide is used as a sterilant for health care products, as a fumigant for spices and seasonings, in libraries as a book preservative, in dairy packaging, and in animal and plant quarantine services at ports of entry.

## APPENDIX 2. EXPOSURE LIMITS IN THE WORKING ENVIRONMENT (1)

| | ppm (2) | mg/m$^3$ (2) |
|---|---|---|
| **EUROPE** | | |
| Belgium, Holland, Italy, UK | 50 | 90 |
| FRG (Germany) | 10 | 18 |
| Denmark | 10 | 18 |
| | 10-L | 18-L |
| | (absolute ceiling) | |
| Finland | 10 | 18 |
| | ceiling 20(15min) | 36(15min.) |
| France | 5 | 9 |
| Norway | 20 | 36 |
| | (proposal, 5) | (proposal, 9) |
| Sweden | 5(8hr) | 9(8hr) |
| | 10(15min) | 18(15min) |
| USSR | 0.5 | 0.9 |
| **OUTSIDE EUROPE** | | |
| Japan, USA (OSHA Fed. Standard) | 50 | 90 |
| ACGIH (USA) | 10 | 18 |

(1) Values refer to TLV's, MAK's etc. They are currently under discussion in some countries. No direct comparison between these exposure limits is possible where different criteria are used as the basis for them. Important differences relate to the choice of critical health effect, safety factors, technological and economic feasibility, and the decision-making process.

(2) 1ppm = 1.80 mg/m$^3$ 1mg/m$^3$ = 0.55 ppm

APPENDIX 3 : EO MONITORING

1. Exposure Measurement: in air

The measurement of airborne ethylene oxide for comparison with the recommended occupational exposure limit is preferably conducted by personal monitoring in the breathing zone, although a measure of workplace air quality may also be gathered from location monitoring.

1.1. Personal monitoring and location monitoring techniques

In current practice, TWA (Time Weighted Average) measurements for EO usually involve a small sampling pump to draw a known volume of air through an adsorption tube containing charcoal. The adsorbed ethylene oxide is then desorbed by solvent (NIOSH,1977) or by heating (Charlton,-1982), and is estimated by GLC. Adsorption onto charcoal in a special design of tube, followed by carbon disulphide extraction and GLC analysis of the extract, is favoured by NIOSH (1977). An elegant technique is the development of the passive dosimeter where no pump is used but a diffusion or permeation mechanism operates to permit EO retention on the charcoal adsorbant (Charlton,1982). This is followed by solvent (or preferably thermal) desorption and estimation of the EO by GLC. The above sampling methods involve compact equipment and are suitable for both personal and location (TWA) monitoring. They are sensitive down to 0.1 ppm depending on sample size, GLC equipment, etc.

Other methods, usually involving more cumbersome equipment, are available and may find application for location monitoring:

a)Into a 10 l. Teflon gas bag, air is pumped at a rate of 20cc. per minute if an entire 8-hour shift is to be monitored (HIMA, 1981). Alternatively, grab samples may be taken in evacuated vessels or a suitable syringe. In both cases the gas mixture is analysed by GLC.

b)In an impinger method (HIMA, 1981) the sampled air is bubbled through 0.1 N sulphuric acid to convert the EO into ethylene glycol which is then determined by GLC. It is suitable for EO in the 1 - 8 ppm range.

c)Short-term or grab sample measurements at a location may be made

with commercially available colourimetric indicator tubes through which air is drawn in a specified manner . Tubes are available from many commercial suppliers including Dräger, Gastec, Kitagawa and Siebetec.

d)In the US, the American Society of Testing Materials is also developing a charcoal tube method for the determination of EO in workplace atmospheres.

1.2. Equipment safety

Care must be taken to ensure that procedures for sampling EO in workplace air are intrinsically safe because of risk of fire or explosion with the equipment, arising from electrically or mechanically generated sparks, etc.

1.3. Recommendation for measurement strategy

In surveying the TWA concentrations, it is recommended to measure EO concentrations down to at least 1 ppm, and preferably 0.1 ppm. At least five measurements should be made for any single occupational group, however small. Further guidance on the number of measurements needed for the reliable assessment of average exposure for an occupational group is given in Table 3 (Liedel,1977).

TABLE 3 - Sample Size for Initial Testing of Occupational Groups

| Size of Occupational Group | No. of Measurements Suggested |
|---|---|
| 1- 5 | 5 |
| 6 | 6 |
| 7- 8 | 7 |
| 9 | 8 |
| 10 | 9 |
| 11-12 | 10 |
| 13-14 | 11 |
| 15-17 | 12 |
| 18-20 | 13 |
| 21-24 | 14 |
| 25-29 | 15 |
| 30-37 | 16 |
| 38-49 | 17 |
| 50 | 18 |
| Over 100 | 22 |

Once average exposures for occupational groups are thus established, routine personal monitoring should be conducted in such a way that those groups whose average exposure comes closest to the Occupational Exposure Limit are most frequently tested (Roach, 1977).

## 2. Exposure Assessment by Biological Methods

Considerable attention has been paid to the possibility of monitoring exposure to EO by biological means. Although a number of biological tests (alkylation of haemoglobin; examination of chromosome aberrations and sperm) have been suggested, none of them are specific for EO. Furthermore, the sensitivity of these tests has yet to be fully established. At present there is no biological test which can be used for routine monitoring of EO-exposed personnel.

APPENDIX 4 : INHALATION EXPOSURE

quoted verbatim from T.D. Darby (1981)

Rate of accumulation and total dose varies with species and conditions of exposure, and determination of total body dose and peak blood level values resulting from inhalation exposure are important to the dose-response relations among various animal studies and to the assessment of human risk associated with exposure.

Since EO is very soluble in blood, only dead space EO would be exhaled. Total body uptake of EO is primarily dependent upon the alveolar ventilation rate. This value is given as 60 ml/min/kg of body weight for man (Guyton, 1976). While lipid-soluble molecules diffuse rapidly from the blood to tissues of the body water, soluble agents diffuse more slowly into select organs. EO distributes poorly into body fat (Tyler et al.,1980). Goldstein et al.(1974) describe the multiple factors that effect uptake and distribution of inhaled gases. These authors use a value of 0.3 liters per breath for alveolar ventilation rate in a 70 kg. human. With a normal inspiration the total effective lung volume is equal to the functional capacity and end-expiratory volume, and represents about 2.8 liters total. With gaseous agents that have high solubility like EO (until air concentrations greater than 100 ppm are reached), little if any EO remains in the alveoli just before the next inspiration (Tyler et al.,1980; Goldstein et al.,1974). Naturally, the time required to equilibrate the body water with the inspired air will be very much longer. The concentration of EO in alveolar air ($ETO_c$) that passes into blood with each breath in a 70 kg. man would be 0.3 liters times $ETO_c$. Blood level plateau, then, is directly related to clearance of $ETO_c$ (Goldstein, A.L. et al.,1974). Thus, short periods of exposure to high concentrations of EO would not be expected to produce the same dose of EO as longer periods of exposure to low concentrations. Tables 4 and 5 illustrate the kinetics of inhalation exposure to ethylene oxide for 15 minutes and for 3 hours respectively (Martis et al.,1979). The area under the blood level curve is proportional to the total dose resulting from the exposure. Of course, time period for exposure, and air concentration, affect tissue concentrations of EO and its metabolites.

Tables 4 and 5 compare calculated total dose exposure for man and the rat at two air concentrations of EO. While alveolar ventilation rates vary for man under work conditions and for different strains of rats, it is generally agreed that on an average the alveolar ventilation rate for the rat is four times greater than for

man (Altman et al.,1974). Naturally, alveolar ventilation rate is determined by oxygen consumption since oxygen consumption is directly related to metabolic rate.

TABLE 4 : Calculated Total Dose in Man and Rat Based on Six Hours of Exposure

| | Rat | Air Concentration ppm | Man |
|---|---|---|---|
| Total body uptake in μg/min/kgbw | 20 | 50 | 5 |
| | 40 | 100 | 10 |
| Total dose (6 hours exposure) in mg/kgbw | 8 | 50 | 2 |
| | 16 | 100 | 4 |

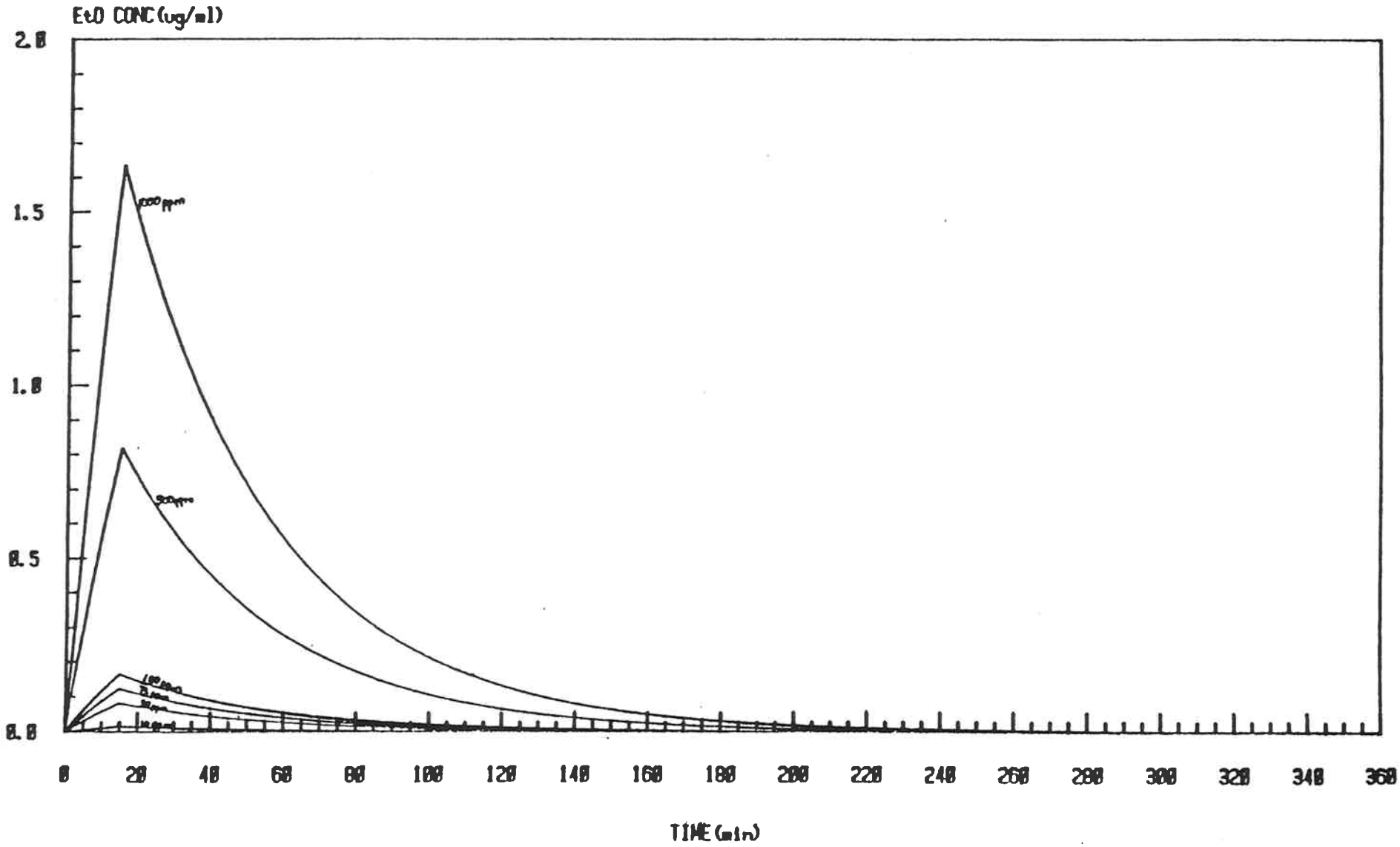
TABLE 5 : Calculated Exposure Dose vs Air Concentration for Ethylene Oxide

An exposure to 10 ppm for 15 minutes would provide only one-fourth the dose that a six-hour exposure to 1 ppm would allow. Given all data available, a TWA exposure level of 10 ppm for eight hours would provide a total body dose of near 0.4 mg/kg of body weight. This value would be 40 times less than the dose in a rat receiving 100 ppm for six hours.

| Air Conc, in ppm | Total Body Uptake, in μg/min/kgbw | Total Dose with 15 min. Exposure, in mg/kgbw | Total Dose with 6 Hours Exposure, in mg/kgbw |
|---|---|---|---|
| 1 | 0.11 | 0.002 | 0.040 |
| 10 | 1.08 | 0.016 | 0.390 |
| 50 | 5.40 | 0.082 | 1.944 |
| 75 | 8.10 | 0.121 | 2.916 |
| 100 | 10.80 | 0.162 | 3.888 |
| 500 | 54.00 | 0.820 | 19.440 |
| 1000 | 108.00 | 1.620 | 38.880 |

Tyler and McKelvey (1980) reported the total body dose of EO resulting from exposure of male Fischer 344 rats to air concentrations of 10, 100, or 1000 ppm for 6 hours. These investigators used $C^{14}$-labeled EO and the inhalation exposure was similar to that used in the bioassay carcinogenesis studies carried out by Snellings, W.M. et al.,(1981). The mean dose level for the rats exposed to 100 ppm of EO for 6 hours was 20.24 mg/kg, a value that is five times greater than the calculated dose for man exposed to 100 ppm for 6 hours (4 mg/kgbw). Since the dose per kilogram of body weight resulting from this exposure in the rat is significantly greater than that expected with a similar exposure in man, certainly carcinogenesis risk assessment should take the exposure dose into consideration rather than just air exposure concentration.

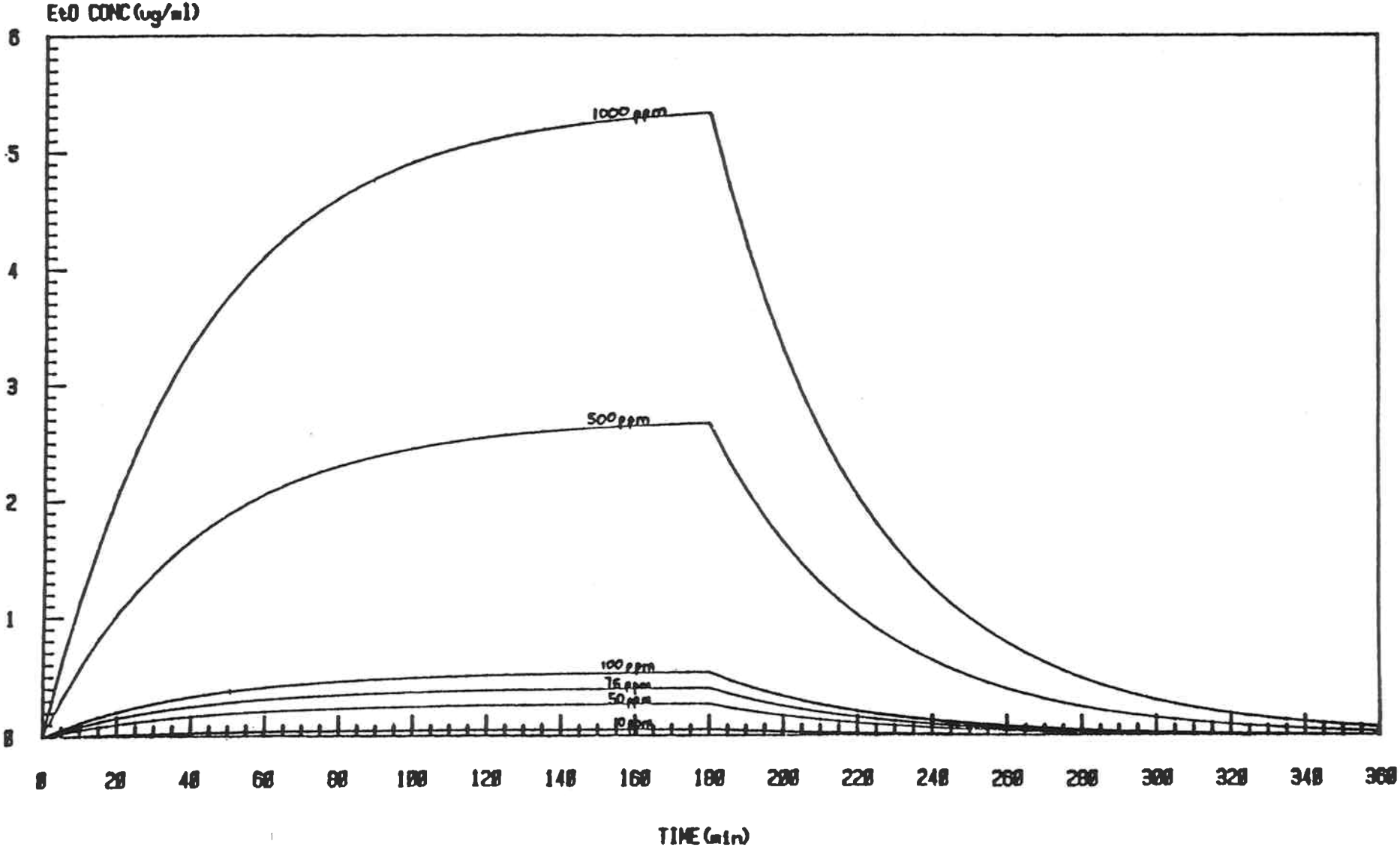
FIG 4 ETHYLENE OXIDE INHALATION KINETICS - Darby (1980)




Using equations for pharmacokinetic parameters determined for the dog, estimated blood concentrations of ethylene oxide with exposure of 15 minutes to air concentrations of 10, 50, 75, 100, 500 and 1000 ppm. These values were determined using an alveolar ventilation rate of 60 ml/min/kg.

FIG 5

# ETHYLENE OXIDE INHALATION KINETICS - Darby (1980)




This figure illustrates calculated blood concentrations for ethylene oxide resulting from 3 hours of exposure to air concentrations of 10, 50, 75, 100, 500, and 1000 ppm. The equations used for the calculations were derived from intravenous injection studies in dogs. These blood level values were determined using an alveolar ventilation rate of 60 ml/min/kg.

## APPENDIX 5. RAD-EQUIVALENTS AND RISK ASSESSMENT.

Various models have been proposed for the assessment of genetic risk to man by indirect methods. These include estimating the degree of DNA alkylation by monitoring blood protein alkylation (Ehrenberg et al., 1977; Wright, 1981); assessment of the degree of chromosome damage in circulating lymphocytes stimulated to divide in vitro (Thiess, 1981); and comparison of the expected genetic damage by monofunctional alkylating agents with that observed in experimental systems using radiation as the standard mutagen (Ehrenberg et al., 1974; Calleman et al., 1978). In the last case, the degree of risk is expressed in terms of rad-equivalent dose, ie. the number of rads of acute radiation that would give the same genotoxic effect as a unit dose of the test chemical.

In experiments with mice (Ehrenberg et al., 1974) the rad-equivalent exposure levels were determined from the tissue dose of EO and the dose-response data from EO- and X-ray-induced chlorophyll mutations in barley. The mice were exposed to 1-35 ppm EO for 1-2 hr (ie. 0.3-2% of the $LD_{50}$)and the tissue dose was determined chemically in order to assess the degree of alkylation. The results were consistent with the assumption that all the EO inhaled was absorbed, and that it was rapidly distributed through the body and rapidly detoxified. The tissue dose was found to be proportional to the exposure dose, and in the testes the former was 0.05μM per ppm per hour exposed. The degree of alkylation of DNA also agreed with that expected from the tissue dose determined.

In estimates of rad-equivalent doses for man, it is assumed that monofunctional alkylating agents would give similar rad-equivalents for mutation in different biological systems. Tissue doses were estimated from measurements of the alkylation of haemoglobin. With these assumptions, a tissue dose of 1μM per hour is equal to 80 rads per year of X-radiations. According to Ehrenberg et al. (1974) this would mean that human exposure to 5 ppm EO for 40 hours per week is equivalent to a gonad dose of 4 rads per year (one rad X-radiation = $2 \times 10^{-4}$ cancer risk ; UNSCEAR, 1977).

The theory assumes, almost certainly erroneously, that all DNA alkylation sites are equally important with respect to genetic risk, and also that the dose of the chemical mutagen arriving at the target tissue can always be equated directly with the original exposure of the whole animal. The error incurred by ignoring pharmacokinetics in this model increases with increasing animal size. In other words,

the concept that the dose of chemical genotoxic agent could be equated directly with a dose of nuclear radiation is approximated only in unicellular microorganisms. In the case of multicellular organisms, the path-length and energy of the damaging radiation particle is still sufficient for it to be assumed that all the radiation reaches its target, whereas various protective mechanisms are most likely to neutralise the action of a potentially genotoxic chemical before it can reach its genetic target.

APPENDIX 6 : CURRENT STUDIES

| TYPE OF TESTING | PRINCIPAL INVESTIGATOR | SPONSOR (CONTRACT No) | LAB. | TITLE | SPECIES | ROUTE, DOSE | STATUS | SOURCE |
|---|---|---|---|---|---|---|---|---|
| 1. Carcinogenic | | NCI (NCI C50088) | Battelle, Richland, WA | Carcinogenesis Bioassay of Ethylene Oxide | Mice (B6C3F1) Rat (F344) | Inhalation, two-dose | Prechronic began 2/80 - Chronic treatment to begin 7/81(mouse) | Tox-Tips 52-41; NTP, FY80 p.107 |
| 2. Carcinogenic/ Mutagenic | Yang, N.C. | NCI | University of Chicago | Molecular Mechanisms of Mutagenesis and Carcinogenesis | | | Unknown, funded to 3/80 | |
| 3. Mutagenic | Niemeier, R. | NIOSH | NIOSH | Mutagenic Potential of Industrial Compounds | | | Unknown, funded to 9/80 | |
| 4. Mutagenic | Fine, L.J. | NIOSH | Harvard Univ. | Occupational Exposures and Rates of DNA Damage | Human | | Unknown, funded to 8/80. Objective to investigate relationship between DNA damage and exposure to carcinogens or mutagens by determining chromosome aberrations and sister chromatid exchanges. | Cancer Projects |
| 5. Mutagenic | Chang, C. | NCI | Purdue | Interactions of Alkylating Agents and Nucleic Acids | | | Unknown, funded to 1/81. Objective to study mechanism of alkylating agents with RNA and DNA. | Cancer Projects |

APPENDIX 6(2) : CURRENT STUDIES

| TYPE OF TESTING | PRINCIPAL INVESTIGATOR | SPONSOR (CONTRACT No) | LAB. | TITLE | SPECIES | ROUTE, DOSE | STATUS | SOURCE |
|---|---|---|---|---|---|---|---|---|
| 6. Mutagenic | Niemeier,R. | NIOSH | NIOSH | Mutagenic Potential of EO | Rat | Inhalation | Unknown, funded to 1978. Tests include dominant lethal and Drosophila melanogaster recessive. | Cancer Projects; Tox-Tips 27-9 |
| 7. Mutagenic | | FDA | FDA | | | | On test in FY80 | NTP,FY80, p.159 |
| 8. Mutagenic/ Reproduc- | Niemeier, R. | NIOSH | NIOSH | Reproductive/Mutagenic Protential of Industrial Compounds | | | Unknown, funded to 9/80. | |
| 9. Teratogenic | Niemeier, R. | NIOSH | NIOSH | Teratogenic Risks of Workplace Contaminants | | | Unknown, funded to 9/80 | |
| 10. Teratogenic | Hardin, B. | NIOSH | NIOSH | Teratogenic Risks of Workplace Contaminants | | | In process, funded to 6/82 | |
| 11. Teratogenic | Niemeier, R. | NIOSH | NIOSH | Teratogenic Effects of EO in Rats and Rabbits | Sprague-Dawley rat, New Zealand white rabbit | Inhalation; MTD and 50 ppm | Unknown, funded to 1978. | Cancer Projects; Tox-Tips 27-11 |
| 12. Teratogenic | | FDA | FDA | | Mouse, rabbit | Intravenous | Unknown, Report scheduled in FY80. | NTP, FY80, p.161 NTP, FY80, p. 73 |
| 13. Chronic | Khan, A. | NIOSH | NIOSH | Chronic Inhalation Toxicity of Organic Oxides | | | In process, funded to 5/82 | |

APPENDIX 6(3) : CURRENT STUDIES

| TYPE OF TESTING | PRINCIPAL INVESTIGATOR | SPONSOR (CONTRACT No) | LAB. | TITLE | SPECIES | ROUTE, DOSE | STATUS | SOURCE |
|---|---|---|---|---|---|---|---|---|
| 14. Chronic | Lewis, T. | NIOSH, CDC | NIOSH | Chronic Inhalation Toxicity of Organic Oxides | Monkey, rat | Inhalation; 2 dose levels 18 months | Unknown, objective includes determination of cardiopulmonary response in monkey | Toxicology Research Projects Directory; Vol.4 No.2, 1979 Tox-Tips 25-31 |
| Acute | | | | | Rabbit | Inhalation | Unknown,objective includes acute pulmonary response | |
| 15. Chronic | | NIOSH | NIOSH | | | | On test in FY80 | NTP, FY80, p.166 |
| 16. Chronic/ Neuro-toxic/ Behavioural | Moorman, W. | NIOSH | NIOSH | Chronic Inhalation Toxicity of Organic Oxides | | Inhalation | In process, funded to 9/81 | |
| 17. Neuro-toxic | Setzer, J. | NIOSH | NIOSH | Neurotoxicity of Ethylene and Propylene Oxides | | | In process, funded to 9/81. | |
| 18. Neuro-toxic | Johnson, B. | NIOSH | NIOSH | Neurotoxicity of Ethylene and Propylene Oxide | | | Unknown, funded to 9/80. | |
| 19. Epidemi-ology | Rinsky, R. | NIOSH,NCI,UCC | NIOSH | Mortality Study of Chemical Plants in the Kanawha Valley in West Virginia | Human | | In process, funded to 3/82 | Tox-Tips 44-9 |

APPENDIX 6(4) : CURRENT STUDIES

| TYPE OF TESTING | PRINCIPAL INVESTIGATOR | SPONSOR (CONTRACT No) | LAB. | TITLE | SPECIES | ROUTE, DOSE | STATUS | SOURCE |
|---|---|---|---|---|---|---|---|---|
| 20. Epidemiology | Rinsky, R. | NIOSH, NCI (C21-588) | NIOSH | Mortality and Industrial Hygiene Study of Workers Exposed to EO | Human | | Unknown, funded to 12/80. Results to be used in development of exposure standard. | Cancer Projects; Tox-Tips 36-37; IARC Epidemiology Directory 1978, # 769 |
| 21. | Charles,J.M. | EPA | EPA | Study of the Bronchiopulmonary Toxicity of Pesticides | | | In process, funded to 9/82. | |
| 22. | Oser, J. | NIOSH | NIOSH | Industrial Hygiene Study of New Agents | | | Unknown, funded to 9/79. | |

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## H. MEMBERS OF THE WORKING GROUPS

### WG TOXICITY

| | |
|---|---|
| E. Löser (Chairman) | Institute of Toxicology<br>BAYER AG<br>Wuppertal |
| P. Grasso | Occupational Health Unit Division<br>BP RESEARCH CENTER<br>Sunbury-on-Thames |
| R. Jäckh | Department of Toxicology<br>BASF AG<br>Ludwigshafen |
| E. Longstaff | Central Toxicology Laboratory<br>IMPERIAL CHEMICAL INDUSTRIES PLC<br>Alderley Park |
| R.J. Millischer | Service de Médecine et de Toxicologie<br>P.C.U.K.<br>Paris |
| M. Wooder | Toxicology Division<br>SHELL INTERNATIONAL CHEMICAL COMPANY Ltd,<br>London |

### WG HUMAN EXPOSURE

| | |
|---|---|
| A.P. Wright (Chairman) | Medical Department,<br>IMPERIAL CHEMICAL INDUSTRIES PLC<br>Wilton, Middlesbrough |
| J.C. Aubrun | Service de Médecine et de Toxicologie<br>P.C.U.K.<br>Paris |
| J. Bäckström | Consultant Toxicologist<br>ASSOCIATION OF SWEDISH CHEMICAL INDUSTRIES<br>Stockholm |
| H. Engelhard | Medical Department<br>BAYER AG<br>Dormagen |
| W.H. Stocker | Occupational Medicine and Health Protection<br>BASF AG<br>Ludwigshafen |
| N.J. Westerman-van der Horst | Group Toxicology Division<br>SHELL INTERNATIONALE RESEARCH MAATSCHAPPIJ B.V.<br>Den Haag |

## I. MEMBERS OF ECETOC SCIENTIFIC COMMITTEE

| | |
|---|---|
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| - H. ZELLER (Vice-Chairman) | BASF<br>Ludwigshafen |
| - M. SHARRATT (Vice-Chairman), Head of Toxicology | BP Group<br>Sunbury |
| - J. BÄCKSTRÖM, Consultant Toxicologist | ASSOCIATION OF SWEDISH CHEMICAL INDUSTRIES<br>Stockholm |
| - B. BROECKER, Coordinator, Product-related Environmental Problems | HOECHST<br>Frankfurt |
| - L. CAILLARD, Directorate of Industrial Toxicological and Ecotoxicological Service | RHONE-POULENC<br>Paris |
| - H.O. ESSER, Vice-director,Agrochemical Division | CIBA-GEIGY<br>Basel |
| - S.E. JAGGERS, Manager, Industrial and Regulatory Toxicology | I.C.I. Central Toxicology Laboratories<br>Alderley Park |
| - U. KORALLUS, Medical Director | BAYER<br>Leverkusen |
| - R. MATTIUSSI,Coordinator, Department of Occupational Health, Safety, Industrial Toxicology and Environment | MONTEDISON<br>Milan |
| - H.G. NOSLER, Head of Coordination Centre for Consumer Safety and Environmental Protection | HENKEL<br>Düsseldorf |
| - J.F. NEWMAN, Consultant | I.C.I.<br>Jealotts Hill |
| - J.P. TASSIGNON, Counsellor, Industrial Medicine and Toxicology | SOLVAY<br>Brussels |
| - C. de TORREGROSA NAVARO, Director, Medical Services | UNION EXPLOSIVOS RIO TINTO<br>Madrid |
| - H. VERSCHUUREN, Toxicology and Registration Department | DOW CHEMICAL<br>Rotterdam |
| - J. VAN DER HARST, Environmental Toxicology and Risk Assessment | SHELL<br>Den Haag |