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Ethylene Oxide Toxicology and its Relevance to Man: An up-dating of ECETOC Technical Report No 5

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CONTENTS

A. INTRODUCTION

In 1982 ECETOC reviewed and published its comments on the available toxicological information on ethylene oxide (EO) and attempted to assess its relevance for health effects in man (ECETOC, 1982). Since that review was published, further results from a number of biological and epidemiological studies have become available which indicated a need for a reassessment of the conclusions. In addition it was considered appropriate to conduct a more detailed consideration of the biochemical toxicology and the rad-equivalent theory. The authors have also discussed their views on the toxicity of EO with some outside experts in the field whose suggestions and comments have been taken into account in preparing the current document (see section H).

B. TOXICOLOGICAL DATA

1. Human Data

1.1. Skin and eye effects

Shupack et al.(1981) have provided additional evidence that EO produces irritation of the skin in a non-sensitised subject at 1000 ppm (0.1 %) under occluded conditions.

Delayed effects on the internal eye have been described for the first time in three of a group of four male EO workers in the sterilising industry (Jay et al., 1982). The effects consisted of both anterior vacuolar and posterior subcapsular cataracts, and vacuoles in the anterior lens. The age of the three workers was 31, 34 and 35 years. The authors suggest that the observed effects may be related to an accidental high exposure some two years earlier due to a leaking steriliser.

1.2. Epidemiology

1.2.1. Reproductive effects. In a retrospective study, Hemminki et al.(1982) analysed the incidence of spontaneous abortions among all female sterilising staff employed during 1980 in 80 Finnish hospitals in which ethylene oxide, glutaraldehyde and formaldehyde were used to sterilise instruments. Data obtained by questionnaire showed that the frequency of spontaneous abortions was 15.1 % for the exposed and 4.6 % for the

non-exposed pregnant subjects. This study draws attention to toxic effects on reproduction in employees engaged in sterilising processes in which EO was used alone as well as in mixtures with formaldehyde and glutaraldehyde. The lack of comprehensive exposure data and the problems inherent in this type of investigation indicate a need for cautious interpretation. In a later paper Hemminki et al.(1983) stated that the number of persons in the cohorts was "not large enough to compare abortion rates and known ethylene oxide concentrations".

1.2.2. Current epidemiological studies. The initiation of new epidemiological studies has been announced. In Sweden, the National Board of Occupational Safety and Health is performing follow-up studies of the two previously reported cohort studies (Hogstedt, 1979 a and b). A new cohort study of about 350 persons occupationally exposed to EO and/or propylene oxide since 1962-63 or later is in progress. Exposure to other chemicals is also being considered in this study (Hogstedt, 1983, personal communication). The results of these studies are expected by mid-1984. In the Federal Republic of Germany a mortality study has been started by the VCI (Association of the Chemical Industry). Five companies which produce or process ethylene oxide are collaborating in this, but the results will not be available before the end of 1984.

1.2.3. Human cytogenetic data. These are considered in section 2.1.5.

2. Experimental Data

2.1. Mutagenicity and clastogenicity

2.1.1. Micro-organisms. Spore preparations of Bacillus subtilis (variant niger) were 97.7% colony type 1 before EO treatment and 50% atypical type amongst survivors after treatment. Various characteristics of cell morphology and nutrient requirements suggested that EO had induced mutations in the surviving spores (Jones and Adams, 1981).

EO was found to be mutagenic in Schizosaccharomyces pombe. The addition of a mouse liver S9 fraction lowered the response (Migliore et al.,1982).

2.1.2. Rats and mice. In a study by Generoso et al.(1982) a single

intraperitoneal injection (150 mg/kgbw) of EO in male mice produced dominant-lethal mutations. In a heritable translocation study by the same authors in which male mice were injected daily for 5 weeks with 60 or 30 mg/kgbw/day, increases in the frequencies of heritable translocations were observed at both dose levels. The exposure of male mice to EO by inhalation (225 ppm, 6h/d, 5 d/wk) also caused dominant-lethal mutations. Higher frequencies were produced in those mice exposed for 11 weeks than in those exposed for only 2 weeks, but there was a progressive reduction in dominant lethal effects with time of mating after exposure. This was more pronounced in the short-term exposure group (Generoso et al.,1983).

The mutagenic effects of EO after parenteral administration were reflected by an increased micronucleus frequency in the bone marrow of mice, dominant-lethal mutations in the germ cells of male rats, and in "the ana- and telophase division stages" of the liver cells in rats (Lyarskii et al.,1983). The minimum effective dose of EO, established by "the ana- and telophase method" in the liver cells of rats (subcutaneous administration for three months) was 5 mg/kgbw.

Cytogenetic analysis of the peripheral blood lymphocytes showed significant increases in the frequency of sister chromatid exchange (SCE) in rats exposed to EO for 6 h/d for 1 or 3 days at concentrations of 50, 150 or 450 ppm. No significant concentration-dependent increase in chromosome breakage was observed (Kligerman et al.,1983-a; 1983-b).

2.1.3. Rabbits. Groups of rabbits were exposed, by inhalation, to EO concentrations of 10, 50 and 250 ppm for 6 h/d, 5 d/wk for 12 weeks. Blood samples were taken before, during and after the exposure. At 10 ppm there was no detectable increase in sister chromatid exchange rates. Significant increases were observed at 50 and 250 ppm. These enhanced rates declined after the exposure but were still higher than the base line 15 weeks afterwards. There was no effect on standard haematological parameters (erythrocyte and leucocyte counts, haematocrit, haemoglobin concentration) or on levels of blood and liver glutathione (GSH), either during or after exposure (Yager and Benz, 1982).

2.1.4. Mammalian cells in culture. Exposure of a human amniotic cell line to EO

at concentrations of from 5 to 10 mM for 1 hour gave a dose-dependent increase in the number of chromatid aberrations. The incidence of gaps, breaks, exchanges and complexes all increased. At the highest dose (9% survival) the number of chromatid exchanges increased 20-fold, and the number of induced breaks increased 50-fold (Poirier and Papadopoulo, 1982).

Human lymphocytes exposed to EO in vitro showed dose-dependent increases (maximum 4-fold) in SCEs at doses ranging from 10 to 35 micrograms.ml^{-1} (in media) during a 20 min. exposure period (Garry et al., 1982).

2.1.5. Cytogenetic effects in humans. Cytogenetic effects in circulating blood lymphocytes, and in bone marrow cells, of twenty-eight individuals occupationally exposed to EO were compared with those in 20 controls matched for smoking and age. At the time of sampling the exposure levels were below 1 ppm, but exposures up to 52 ppm had been recorded in previous years. A statistically-significant increase in the levels of total chromosome abnormalities (breaks and gaps) was observed in the exposed group. However, the difference was not marked and no significant increase in SCEs or micronuclei in lymphocytes were observed (Högstedt et al.,1983). Inspection of the data revealed that almost all of the increase in chromosome aberrations could be ascribed to gaps, which are mostly considered as artefacts; see Report of the Ad Hoc Ctee, 1972. When these are excluded no significant difference is evident between exposed and control groups.

The frequency of SCEs in peripheral lymphocytes in hospital steriliser workers exposed to EO increased with the estimated cumulative dose during the six months preceding SCE analysis (Yager et al.,1983). The mean frequency of SCEs was statistically significantly higher in subjects exposed to a mean cumulative dose of 501 mg than in those exposed to a mean cumulative dose of 13 mg (exposure to 0.01 ppm for 2 h/d).

Laurent et al. (1982; 1983-a; 1983-b) reported that 25 Belgian hospital workers engaged in the sterilisation of medical equipment with EO showed a significant increase in the rate of SCEs in peripheral lymphocytes as compared to 22 unexposed workers, and that the effect was cumulative,

dose-related, and possibly persistent. The cumulative dose of EO inhaled in the two-year period by the exposed workers was estimated to lie within the range of 500 to 5800 mg.

In a preliminary study in three US sterilisation plants, chromosome changes were followed for up to 2 years in workers who had been exposed to EO at levels ranging from 1 to 200 ppm. With relatively high exposures to EO an increase of SCEs was observed, the values tending to decline slowly with time but persisting for at least 24 months after cessation of exposure. No increase in SCEs was observed in workers exposed to low levels of EO (Johnson and Johnson, 1983). Because of the small numbers involved and the uncertainty of the exposure levels, the interpretation of these findings is difficult and the persistence of the observed changes in SCE frequency, which is very unusual, would have to be verified.

Sarto et al.(1983, a and b) reported their findings of SCEs in hospital sterilisation operators exposed to high (15.8 ± 9.8 ppm) and low (1.1 ± 1.0 ppm) levels of EO for several years. In both cases there appeared to be statistically significant increases in the levels of observed SCEs in circulating lymphocytes. However, the biological significance of the small reported increases (SCEs were 13.0 ± 1.8 and 11.0 ± 1.6 for high and low exposure levels respectively, and 10.2 ± 1.2 and 9.8 ± 1.4 for the controls) is clearly debatable. Further analysis of the data, which are not given in detail in the paper, would be needed before the genotoxic risk to man could be adequately assessed.

Hansen et al.(1984) determined the levels of SCE formation in 14 steriliser workers in comparison with 14 matched controls. The steriliser workers were exposed to less than 5 ppm EO (TWA). There were no statistically significant differences in SCE levels between the exposed workers and the controls. Increased SCE levels were associated with smoking.

2.1.6. Conclusions. New information is consistent with the previous conclusion (ECETOC, 1982) that EO is a mutagen and a clastogen. The apparent persistence of induced SCEs in the Laurent, and Johnson and Johnson, studies merits further investigation. When considered with the previously

reported studies (ECETOC, 1982) the new data support the view that the effects on DNA can be detected at exposure levels of 5 to 10 ppm. At concentrations below this, neither induced chromosomal aberrations nor sister chromatid exchanges have been clearly or consistently demonstrated. Thus it is concluded that currently-available chromosomal monitoring techniques do not detect damage due to low levels of EO and that the effect, if any, cannot be distinguished with confidence from background incidence.

2.2. Carcinogenicity

2.2.1. Results. Preliminary information from the National Institute of Occupational Safety and Health (NIOSH) indicates that tumours of the central nervous system developed in Fischer 344 rats exposed to EO vapour. The tumours were described as cerebral gliomas and the incidence was reported to be 5/79(6.3%), 2/77(2.6%) and 0/38(0%)in male rats exposed to 100,50 and 0 ppm of EO vapour, respectively (Anon., 1982).

This report prompted a histological examination of brain tissue from the Bushy Run study in which the same strain of rats were exposed by inhalation to 0, 0 (2 control groups), 10, 33 and 100 ppm of EO vapour (Snellings et al., 1981). Brain tissues obtained at the 12 and 18 month interim kills, and from animals which had died during the course of the experiment, were included in the examination. No tumours were observed in any of the groups killed after 6 or 12 months exposure. In those killed after 18 months exposure, three tumours were found, two in females (one from a control and one from the 100 ppm group) and one in a male exposed to 33 ppm. At the terminal kill 2 years from the start of the experiment the following incidence of brain tumours was observed in males : 3/30 in the 100 ppm group; 1/39 in the 33 ppm group; 0/51 in the 10 ppm group; and 1/48 and 0/49 in the two control groups. Of the females, 2/26 and 2/48 in the 100 and 33 ppm groups, respectively, were found to have brain tumours. None were found in the 10 ppm group or in the two female control groups.

The combined incidence of brain tumours in male rats that died or were killed at 18 and 24 months was 0, 1.0, 1.0, 5.1 and 7.1 percent, and for female rats 0, 1.0, 1.0, 3.0 and 4.0 percent, corresponding to exposure

levels of 0, 0, 10, 33 and 100 ppm, respectively. These tumours consisted of gliomas, malignant reticulosis and granular cell tumours. Since the latter are of meningeal origin, they were not considered relevant as an index of the brain tumour induction. When these were subtracted from the total tumour incidence, the percentage incidence in males was 0, 1.0, 0, 4.1 and 6.1, and in females it was 0, 0, 1.0, 2.0 and 3.0, for the 0, 0, 10, 33 and 100 ppm groups respectively. The dose-related increase in brain tumours found in this study confirms the similar findings in the NIOSH study which was also conducted on F344 rats.

No further comment can be made on the NIOSH study until more information is available. The details provided on the Bushy Run study indicate that the increase in tumour incidence was statistically significant and shows a positive trend when the incidence in the treated group is compared with that of the controls. Most of these tumours, however, are of microscopic dimension and therefore escaped detection at necropsy. Of the three tumours observed macroscopically in the 100 ppm group, two were found at the final sacrifice and the other was observed in a rat that died prior to sacrifice. There was no indication of an early appearance of these tumours in the animals treated with the highest dose.

2.2.2. Assessment of carcinogenic activity of EO in animals

In a previous report (ECETOC, 1982) evidence was presented of carcinogenic effects in rodents exposed to EO by subcutaneous injection, gavage and inhalation, but certain reservations were expressed regarding the interpretation of the experimental findings with respect to human risk.

Dunkelberg (1981) reported dose-dependent increases in the number of tumours at the injection site in mice dosed subcutaneously with EO dissolved in tricaprylin, but such an effect is generally regarded to be an unreliable index of carcinogenicity. On the other hand, the induction of stomach tumours in rats treated by gavage with a solution of EO in vegetable oil (Dunkelberg, 1982) is indicative of carcinogenic activity. The relevance of this finding to man is questionable because occupational exposure occurs by inhalation rather than ingestion (NCI, 1970; DHSS,

1982). The results of an inhalation study (Snellings et al., 1981) showed dose-dependent increases in mononuclear leukaemia (lymphoma) and peritoneal mesothelioma in Fischer 344 rats exposed to EO. The interpretation of these findings is complicated by the occurrence of substantial incidences of such tumours in the control animals (leukaemia 40% in males, 25% in females; mesotheliomas 5% in males). The incidences of leukaemia (lymphomas) observed in the controls of this study were typical for the Fischer 344 rat. (Goodman, 1979; DHSS, 1982).

Following the publication of the previous ECETOC report (1982), brain tumours have been reported to occur in rats exposed to EO in the inhalation studies conducted by NIOSH and at the Bushy Run Centre. Such tumours are rare in rats (Goodman, 1979) and an increased incidence would normally be regarded as unequivocal evidence of carcinogenicity. However, the tumours displayed some unusual features which complicate the interpretation of the results. The microscopic size of the CNS tumours and their late appearance is an unusual phenomenon in chemically-induced carcinogenesis (Janish and Schreiber, 1977). Normally, in experiments of this sort a proportion of the tumours appear at an early stage, grow to a considerable size, and often cause the death of the animal. In conclusion, there are features of each of these carcinogenicity studies which complicate the interpretation of the results of each individual study but, viewed overall, the results are consistent with the conclusion that EO is a weak animal carcinogen.

2.3. Neurotoxicity

2.3.1. Experimental results

Neurotoxicity, treated very briefly in the previous ECETOC (1982) report, is dealt with more comprehensively here (see Appendix 1).

According to Jacobson et al. (1956) short exposure of dogs to EO at concentrations of 2830 ppm caused convulsions and vomiting. At 1383 ppm, vomiting was observed in the absence of convulsions. This effect was not observed at /10 ppm. More recently Northup et al. (1981) have described ataxia, jerky movements, irritability and tremor following the intravenous injection of sublethal doses of EO into rats.

A characteristic neurotoxic effect, predominantly peripheral and affecting the lumbosacral nerves in parallel with paralysis and subsequent atrophy of the muscles of the hind limbs, decrease in pain perception and retardation of reflexes was observed in several species, including monkeys (Hollingworth et al.,1956; Sprinz et al., 1982). In those studies where post-exposure observations have been sufficiently prolonged, a slow but apparently full recovery within 3 to 6 months was generally observed. The species responding in this way were rat, mouse, rabbit, monkey and dog. The guinea pig is either insensitive to the neurotoxic potential of EO, or the effect occurs only at concentrations where other severe toxic effects prevent diagnosis. On the basis of the clinical observations on animals outlined above, the lowest concentration of EO likely to produce clinical evidence of hind-limb paralysis would lie above 200 ppm, with a no-effect concentration in the range of 100 ppm. Similar conclusions were drawn on the basis of clinical observations in a long-term bioassay with Fischer 344 rats exposed to EO vapour at concentrations of 10, 33 and 100 ppm (6h/d, 5d/wk, 24 months) (Snellings et al., 1981).

Cynomolgus monkeys (12 animals per group) exposed to 50 or 100 ppm of EO (6h/d, 5d/wk, 24 months) showed no clinical evidence of neurotoxicity nor any effect on peripheral nerve conduction. Light-microscopic investigations were performed with pairs of animals from each group. These revealed no differences between the ulnar and sciatic nerves from EO-exposed and control monkeys (Sprinz et al.,1982). However, demyelination was observed in the distal portion of the fasciculus gracilis in 1 of 2 monkeys of both the 100 and 50 ppm EO groups. An axonal dystrophy was also noted in the nucleus gracilis. There were no clinical findings which could account for the histological changes. The pathogenesis and biological significance of these findings in this very small population of exposed primates are still uncertain.

Hind-limb paralysis was not observed in a recent study involving the repeated exposure of mice to EO vapour at various concentrations in the range 10-236 ppm (Snellings, 1982-b). However, dose-related trends were recorded in the Irwin neuro-behavioural screening test (Irwin, 1966). The threshold for the induction of behavioural effects was 50 ppm, and the no-effect concentration was 10 ppm (cf. Appendix F). However,

no abnormalities were revealed by light-microscopic examination of the sciatic nerve or gastrocnemius muscle removed from mice exposed at any level. The no-effect level for the neuro-behavioural effects is therefore somewhat lower than that observed for obvious peripheral neurotoxic effects.

2.3.2 Conclusions

Acute exposure of common laboratory mammals to EO vapour may produce effects on the central nervous system such as vomiting, narcosis, convulsions and respiratory depression. These effects appear at concentrations above 200 ppm, depending on the species, and are reversible. Similar effects have been recorded in man as a consequence of acute exposure to high levels of EO (ECETOC, 1982). Repeated exposures may result in peripheral neuropathy characterised by paralysis of the hind-limb muscles and impairment of sensation and reflexes, such effects being only gradually reversible. The lowest effect-concentration resulting from the use of a neuromuscular screen in mice is 50 ppm. The effects do not occur at 10 ppm.

2.4. Reproductive toxicity

2.4.1. Experimental results

Hackett et al.(1982) exposed groups of rats, by inhalation, to 150 ppm of EO, 7 h/d : group 1 from day 7 through 16 of gestation (dg 7 through 16), group 2 from dg 1 through 16, and group 3 for 5d/wk for 3 wks prior to mating, and daily from dg 1 through 16. Unexposed males were used in mating. Foetal weight and crown-rump length were reduced in litters from all exposed groups of rats. Foetal morphological changes included reduced ossification of the skull and sternebrae in litters from all exposed groups, and an increased incidence of hydroureter (not statistically significant) in litters of group 1 in the absence of any significant adverse effect on maternal body-weight gain or food consumption. Reduction in food consumption and body weight were significant in the parent animals of groups 2 and 3. The incidence of resorptions increased significantly only in litters from rats of group 3.

Results from an inhalation teratology study in rats have been reported (Snellings et al.,1982-a). Pregnant Fischer 344 rats were exposed 6 hours daily to 10, 33 or 100 ppm of ethylene oxide on dg 6 through 15. No treatment-related effects were noted in the dams. Foetal weights of both males and females were significantly depressed, and a higher incidence (not statistically significant) of delayed ossification, a sign of retardation, was noted in the 100 ppm group. No effects from exposure were noted in the dams or foetuses in the 33 and 10 ppm groups.

Intravenous studies were carried out by Kimmel et al.(1982) in rabbits, at doses of 0, 9, 18, and 36 mg/kgbw administered daily on dg 6 through 14, or at doses of 0, 18, and 36 mg/kgbw, administered daily on dg 6 through 9. Preliminary studies had indicated that the maximum tolerated dose (MTD) was approximately 40 mg/kgbw. A statistically significant trend toward decreased maternal weight gain with increasing dose was seen during treatment, and throughout gestation after treatment either on dg 6 through 9, or 6 through 14. No significant effects were seen in the foetal parameters examined after treatment on dg 6 through 9. However, significant increases in mean number and percent resorptions/litter were noted in the 36 mg/kgbw dose group treated on dg 6 through 14. Thus, EO administered intravenously to pregnant rabbits causes some embryotoxic effects after treatment throughout organogenesis at a dose that also produces maternal toxicity. No structural malformations were detected in the study on rabbits, in contrast to the higher incidence of the commonly-occurring abnormalities resulting from the intravenous administration of EO to the mouse (Laborde and Kimmel, 1980).

The results of an inhalation teratology study in New Zealand White rabbits have also been reported (Hackett et al., 1982). The rabbits were artificially inseminated and placed on one of the following exposure regimens at 150 ppm for 7 h/d: (1) exposure from dg 7 through 19; and (2) exposure from dg 1 through 19. There was no evidence of maternal toxicity, adverse effects on development, or structural malformations.

In a one-generation reproductive study in rats (Snellings et al.,1982-b) male and female rats were exposed to 10, 33 or 100 ppm of EO. In the 100 ppm group statistically significant observations were made regarding decreased implantations, smaller litters and increased length of