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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

gestational period. No treatment-related effects were noted in dams exposed to 33 or 10 ppm EO, or in their litters.

Lynch (1983) reported that when adult male cynomolgus monkeys were exposed to 50 or 100 ppm EO (1 h/d, 5 d/wk, 24 months), sperm counts and sperm motility decreased in the exposed monkeys as compared with the controls. The absence of more detailed data does not allow a further assessment of this finding.

2.4.2. Conclusion

These new data reinforce the conclusion reached in the first report (ECETOC, 1982) that EO does not exhibit teratogenic potential under exposure conditions relevant to man. However, embryotoxic effects, dominant lethal effects and toxic effects on sperm cells indicating an effect on reproduction have been recorded.

2.5. Biochemical toxicology

2.5.1. Uptake, distribution and excretion

i) Pulmonary uptake. Adventitious exposures to EO occur mainly via inhalation. Pulmonary uptake into the blood is very efficient, being primarily determined by the alveolar ventilation rate and the concentration of EO in the inspired air (Ehrenberg et al., 1974; Darby et al., 1980). Darby et al.(1980) concluded that the total absorbed dose would be a linear function of the concentration of EO in the inspired air at exposures of up to 1,000 ppm (v/v). For example, a total body uptake of 1.8 µg/min/kgbw can be calculated for a man with an alveolar ventilation rate of 100 ml/min/kgbw exposed to an EO concentration of 10 ppm.

ii) Disposition in tissues. Following absorption into the blood, EO is rapidly distributed throughout the tissues. Determination of the amounts of N_7-(2-hydroxyethyl)guanine residues in DNA showed a generally uniform level of alkylation of the DNA in a wide range of tissues from rats receiving (^{14}C)EO by inhalation exposure (6 hours) at each of the atmospheric concentrations of 1, 10 and 33 ppm (Wright, 1983). The alkylation frequency measured in testicular DNA was approximately half that observed in other tissues. For all tissues,

the alkylation frequency was an approximately linear function of the dose. The results of this study are consistent with rapid equilibration of EO throughout the tissues, with the possible exception of the testes. Results obtained in mice dosed with (^{14}C)EO by intraperitoneal injection (Segerbäck, 1983), although somewhat more limited in scope, were generally consistent with this view. However, in these latter experiments, the frequency of alkylation at N_7 of guanine residues in testicular DNA was similar to that in spleen DNA and approximately half of that for liver DNA. Cumming et al. (1981) reported much larger variations in DNA alkylation in various tissues of the mouse, following inhalation exposure to EO. However, these data are considered to be unreliable for the estimation of either tissue- or DNA-doses of EO because the degree of alkylation was measured as total radioactivity associated with DNA and not as specific reaction products (for discussion see Segerbäck, 1983).

iii) Clearance from tissues and excretion. EO is rapidly cleared from the tissues. On the assumption that the clearance of absorbed EO follows first-order kinetics, biological half-lives of 9 and 10 minutes have been determined for EO in the mouse (Ehrenberg et al., 1974, Osterman-Golkar et al., 1976) and rat (Osterman-Golkar et al., 1983) respectively. A similar value has been deduced for man (Calleman et al., 1978) although a mean half-life of approximately 33 minutes for the clearance of intravenously-administered EO from blood plasma has been measured in dogs (Martis et al., 1982). Inhalation experiments with radiolabelled EO have shown that the urinary route is the main excretory pathway of EO metabolites in both rats (Tyler and McKelvey, 1980 a and b) and mice (Ehrenberg et al., 1974).

Urinary excretion is also a quantitatively significant route for the elimination of EO metabolites in dogs. Thus, on the basis of the selective determination of ethan-1,2-diol in the blood and urine of dogs dosed intravenously with unlabelled EO (25 and 75mg/kgbw), Martis et al. (1982) concluded that this diol was a major metabolite. Clearance of ethan-1,2-diol from the blood was much slower than that of EO. Between 7 and 24% of the administered dose of EO was excreted as ethan-1,2-diol in the urine within 24 hours. 2-Chloroethanol was also detected in the blood of these dogs, but analysis of the batch of

EO used in these experiments revealed the presence of 0.5% of this chemical as a contaminant.

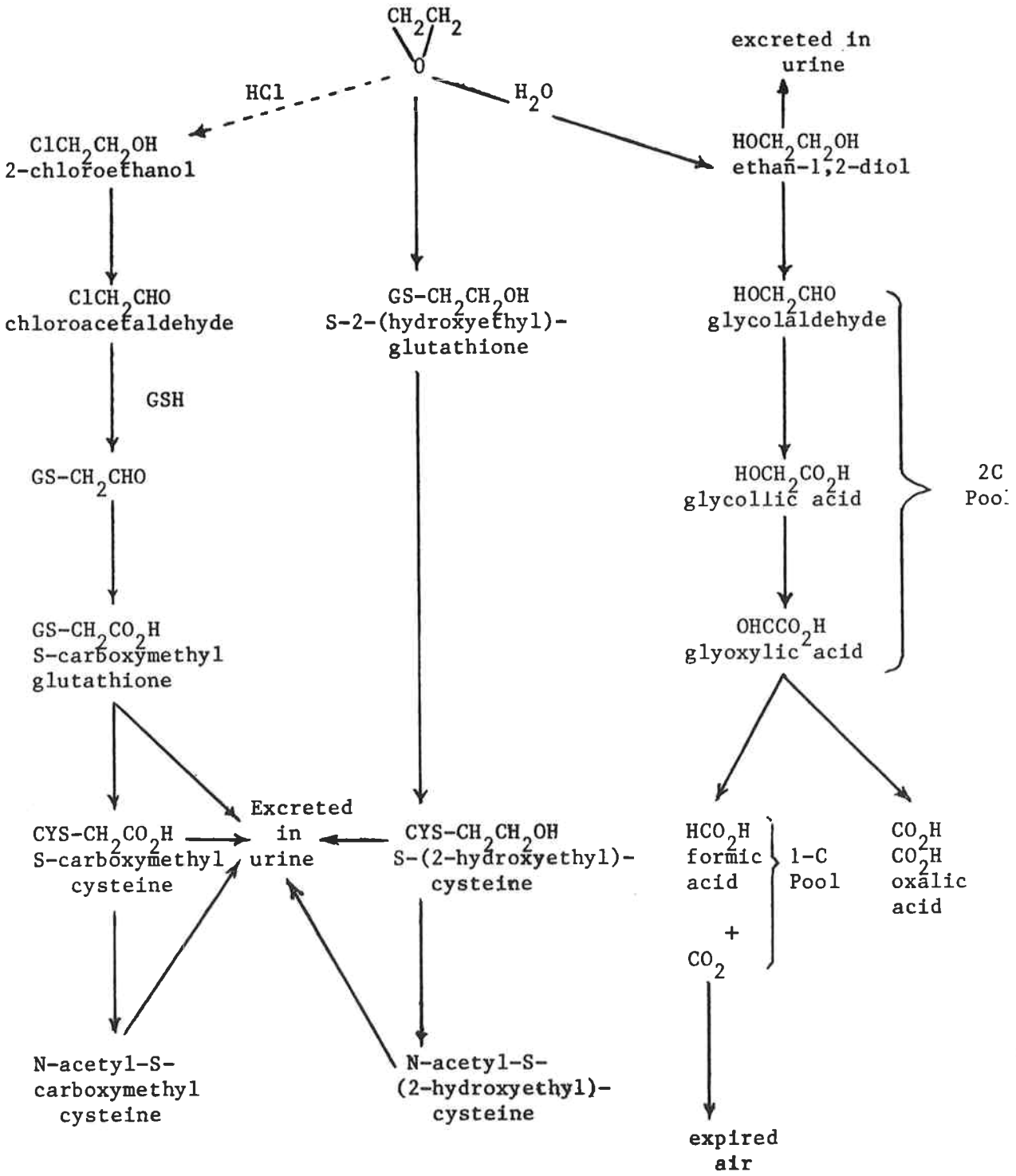
2.5.2. Metabolism (see Fig.1)

The carbon atoms of ethylene oxide possess a residual positive charge which confers electrophilic character on these centres. This electrophilic reactivity is enhanced by the strain imposed by the oxirane ring system, and leads to reaction with a variety of centres including amine, thiol and hydroxyl functions. Such reactions at critical centres in important cellular components are regarded as key events in the toxicity of EO. However, mammalian tissues contain two enzyme systems, the S-glutathione transferases and the epoxide hydrolases, which are especially efficient in scavenging electrophiles (the latter specifically scavenges epoxides), thereby protecting cellular nucleophiles against attack by such agents (Wright, 1980).

The epoxide hydrolases catalyse the hydration of epoxides to the corresponding diols. This reaction invariably results in the loss of the electrophilic character of the oxirane carbon atoms and, in this sense, is regarded as an important detoxification step. The results of in vitro studies reported by Oesch (1973, 1974) indicated that EO is not a substrate for the membrane-bound (microsomal) epoxide hydrolase(s) of rodent liver. However, this subject may merit reinvestigation in the light of the subsequent discovery of another class of epoxide (cytosolic) hydrolases (Mumby et al., 1978).

In view of the susceptibility of EO to spontaneous hydrolysis, it is not surprising that the product of such reaction, ethan-1,2-diol, has been detected as a metabolite in dogs dosed intravenously with 25 and 75 mg/kgbw of unlabelled EO (Martis et al.,1982). However, the rates of formation of ethan-1,2-diol, based on GC analysis of blood and urine, exceeded the spontaneous rate (a "half-life" of 76 hours in water at 37°C has been reported by Osterman-Golkar et al., 1983) and suggested the operation of a catalytic mechanism in vivo. The subsequent metabolism of ethan-1,2-diol to oxalate, formate and CO_2 has been described by Gessner et al. (1961), McChesnay et al. (1971) and Clay and Murphy (1977).

FIG.1 BIOTRANSFORMATION PATHWAYS OF ETHYLENE OXIDE



GSH = glutathione
CYS = cysteine

---------- Possible Route
_____ Established Route

As in the case of hydrolysis, the reaction between the nucleophilic sulphur atom of glutathione and one of the electrophilic carbon atoms of EO, leading to the production of S-(2-hydroxyethyl)glutathione, is an effective detoxification step. A detailed study implicating the glutathione pathway in the detoxification of EO has been reported by Jones and Wells (1981). Rats were dosed intraperitoneally with (1,2-^{14}C)EO (2mg/kgbw). Two urinary metabolites were identified as S-(2-hydroxyethyl)cysteine (9% of administered radioactivity) and its N-acetyl derivative (33% of administered radioactivity), accounting for almost all of the radioactivity excreted in the urine within 50 hours of dosing (43% of the administered radioactivity). These cysteinyl derivatives are typical terminal excretion products of the glutathione pathway. As suggested by Fjellstedt et al.(1983) it seems probable that the initial step in this sequence, i.e. conjugation of EO with glutathione, is catalysed by an S-glutathione epoxide transferase. This enzyme-catalysed reaction would be expected to have general relevance to mammals since glutathione-transferase activity has been detected in all species investigated to date, including man. Although the liver is the richest source of these enzymes, activity has been detected in most other tissues (Jacoby, 1978).

The principal biotransformation reactions undergone by EO, i.e. hydrolysis and conjugation with glutathione, are effective detoxification steps leading to the immediate loss of electrophilic character. However, it has been suggested that an alternative pathway, leading to the activation of EO, may operate in vivo (EOIC, 1983). This suggestion is based on the fact that EO combines with chloride ions (HCl) to yield 2-chloroethanol, a substrate of alcohol dehydrogenase (Blair and Valee, 1966) which catalyses its conversion into the bifunctional mutagen, 2-chloroacetaldehyde. Although this possibility cannot be excluded there are no direct experimental data to support the operation of this pathway in vivo. Although 2-chloroethanol has been detected in the circulating blood of dogs dosed intravenously with large quantities of EO (Martis et al., 1982) such findings cannot be used as evidence for the conversion of EO into 2-chloroethanol in vivo. Thus, subsequent analysis of the batch of EO used in these experiments revealed the presence of a substantial proportion (0.5%) of 2-chloroethanol. It seems unlikely that

2-chloroacetaldehyde would be a quantitatively significant product of the metabolism of EO *in vivo* (Guengerich et al., 1980). It is, perhaps, noteworthy that no product of reaction between 2-chloroacetaldehyde and DNA has been reported to occur in studies designed to investigate the reactions between EO and mammalian DNA *in vivo* (Bolt et al., 1980).

2.5.3. Interactions with cellular components

EO possesses centres of electrophilic reactivity permitting reaction with nucleophilic centres in cellular components *in vitro* (Lawley and Jarman, 1972). Despite the protection afforded by cellular detoxification processes, EO also undergoes reaction with such centres in proteins and nucleic acids *in vivo* (Ehrenberg et al., 1974; Segerbäck, 1983; Wright, 1983). Such reactions with critical nucleophilic centres in DNA provide the fundamental basis for the genotoxic action of this chemical.

The mechanism of DNA alkylation by EO is principally S_N2 (Lawley and Jarman, 1972) and it has been suggested that the pattern of alkylation would resemble that of methyl methanesulphonate (MMS) (Ehrenberg et al., 1974). The N_7-atom of guanine residues is the principal centre for attack in DNA by both MMS and EO, although EO is approximately 8 times less reactive than MMS (Ehrenberg et al., 1974). In the case of EO, reaction at this site, leading to N_7-(2-hydroxyethyl)guanine, accounts for about 90% of the products formed with isolated preparations of DNA. N_7-(2-Hydroxyethyl)guanine is also the predominant product of the reaction of EO with DNA *in vivo* (Potter et al., in preparation). The susceptibility of other centres within DNA to attack by EO has not been investigated to date.

The significance of the N_7-alkylation of guanine residues in DNA by simple monofunctional alkylating agents such as EO is uncertain in respect of the induction of heritable genetic effects (Wright et al., 1979). Nevertheless, it is clear that the measurement of the amounts of such a reaction product (adduct) provides a basis for determining the dose of the alkylating species that penetrates to DNA. This approach has been employed in recent experiments to determine the

target (tissue DNA) dose of EO in male Fischer 344 rats (Wright, 1983). The results of this study showed that the frequency of alkylation of DNA, based on the measurement of N_7-(2-hydroxyethyl) guanine, was an approximately linear function of dose for all tissues investigated. Taking lung DNA as an example, the respective alkylation frequencies were 1 hydroxyethyl group per 3.2×10^7, 3.3×10^6 and 1.02×10^6 nucleotide units at inhalation concentrations of 1, 10 and 33 ppm.

2.5.4. Conclusions

Measurements of DNA adducts in animals exposed to radiolabelled EO have shown that EO is rapidly and homogeneously distributed throughout all tissues with the possible exception of the testes. The frequency of alkylation of DNA in tissue is an approximately linear function of exposure in the range from 1 to 33 ppm.

C. EXPOSURE ASSESSMENT

1. Exposure Levels

1.1. New Data

As already emphasized in the former ECETOC (1982) report there are large differences between the exposure conditions at production, transformation and sterilisation sites. Van Sittert et al. (1983) determined the exposures of workers engaged in the manufacture of EO during the period 1974-1981. Most exposures were below the limit of detection (0.05 ppm), but approximately 10% were higher than 0.1 ppm, with a maximum of 8 ppm in 1980. During 1981, 50 % of the measurements exceeded 0.1 ppm. The geometric mean exposure was below 0.01 ppm in 1974, 1978, and 1980 but a level of 0.12 ppm was recorded in 1981.

In response to a CEFIC questionnaire, data on exposure levels were obtained from various European producers. In the German Federal Republic, exposure levels measured in 1982 by personal air sampling and area monitoring around production units in the open were below 1 ppm. In those chemical industry units where EO was used as an intermediate, the exposure levels were below 5 ppm, with a mean around 2 ppm. At production sites in the Netherlands and the UK, mean 4 or 8 h personal TWA values of less than 0.1 ppm were found

in 1980 and 1981. A single peak value of 8.2 ppm was detected. At sites where EO was used as an intermediate, mean 4 or 8h personal TWAs were less than 0.06 ppm, and the maximum peak level was 0.3 ppm. At road tank filling sites, mean personal TWAs of 3.0 and 4.2 ppm, and peak values of 5.6 and 19 ppm, were found for 1981 and 1980 respectively.

Högstedt et al.(1983) recently reported on exposure levels recorded during studies on the peripheral blood lymphocytes and bone-marrow cells in 28 people exposed to EO at two Swedish sites for sterilising medical equipment. At the first, in June 1975 the sterilising personnel were exposed to TWA levels (area sampling during total shifts) of 28 ppm with the sterilizer open, and 6.4 ppm with it closed, while the packing personnel were exposed to a TWA of 4.4 ppm and the stockroom workers to 12 ppm. New working regimes were then introduced, and by December 1975 TWA area-sampling levels were reduced to 5.0 and 0.6 ppm in the steriliser room (with the steriliser open or closed, respectively), to 0.1-1 ppm for the 12 packers, and to 1.8 ppm in the stockroom. Taking into account the fact that sterilising personnel were exposed for only 1-2 h/d, it was concluded that the 8h-TWA for the 18 exposed subjects at this factory was below 1 ppm at the time of biological sampling in October 1978. At the second site, the 8 h-TWA during biological sampling was estimated at 1-2 ppm for two subjects (one of whom had until 2 years earlier been exposed to considerably higher levels) and below 0.5 ppm for the other eight.

In a study by Johnson and Johnson (1983) three worksites were selected, representing plants with relatively low, intermediate or moderate, and high (presumed) exposure levels to EO, based upon area and personal samples taken between 1977 and 1980. In Worksite 1, personal short-term samples were collected in 1980 and concentrations between non-detectable and 2.7 ppm were found. For steriliser operators, the TWAs estimated on the basis of these short-term samples were about 0.5 ppm. Worksite II contained two adjacent facilities. In the first, the roughly-extrapolated 8h-TWA exposure prior to the implementation of exposure controls was 5-10 ppm with 15 min. peaks of over 100 ppm. Following the implementation of new exposure guidelines (May 1980) the exposures of steriliser-operators were generally less than 1 ppm. In the second facility of Worksite II, with the exception of one sample, 8h-TWAs remained below 5 ppm during 1980, 1981 and 1982. At worksite III, steriliser operators and moulders had roughly-extrapolated

8h-TWA exposures of about 5 to 20 ppm according to area samples taken in 1977 and 1978. In 1980 it was estimated from 2h samples that the 8h-TWA for steriliser operators had increased to 50-200 ppm. After a first corrective action aimed at reducing exposure levels to the original values, sterilisation was terminated at this plant.

The exposure of 32 workers in Belgium to EO was monitored by personal sampling. Three were employed in a sterilising gas distributor unit, twelve were working in the sterilising unit of 3 different producers of medical material, and 17 were in charge of the sterilisation in 5 hospitals. The TWA exposures to EO ranged from 0.3 to 52 ppm with an overall mean of 4.2 ppm. The highest values were found in two hospitals, one still using batch sterilisation with EO vials, the other using leaky sterilising equipment (Wolfs et al., 1983).

At 11 factories operated by Travenol Inc. in which EO was used to sterilise hospital disposables, exposure levels were below 5 ppm. Special safety features ensured that autoclaves could not be opened before flushing. The only workers likely to be exposed were those in quality control, since there may have been some residual EO in the sterilized products (about 2 ppm, together with 5-10 % of ethylene glycol and 2-chloroethanol).(personal communication by Travenol Inc.).

A survey of 65 sterilising units in UK hospitals, at which gases containing from 10 to 100% EO were used, showed that in all cases the 8h-TWAs were less than 5 ppm and a substantial proportion was below 1 ppm. Assessments of a 10-minute TWA were also made, the great majority being below 50 ppm and over half below 10 ppm (Atherton, 1983).

Mouilleseaux et al.(1983) systematically measured atmospheric concentrations of EO in four sterilisation and disinfection facilities in France, three of them in hospitals. The measurements were made over periods of time varying from a few minutes (peak values) to six or eight hours. Atmospheric concentrations of EO increased with the capacity of the incubator. For short periods, concentrations increased mainly on the unloading side, especially when the treated material was discharged. Peak concentrations were between 0.5 and 230 ppm. The 8h-TWAs were between 0.05 and 0.07 ppm in

small facilities and between 0.05 and 5 ppm in large ones. The highest values were observed in the desorption rooms.

Sarto et al.(1983, a and b) defined two groups of exposure levels in sterilisation processes: one of high exposure with TWA levels of 15.8 - 9.8 ppm per sterilisation cycle, and one of low exposure with corresponding TWA exposures of 1.1 - 1.0 ppm.

1.2. Exposure limits

Since the publication of the former ECETOC Report, OSHA (1983) has published a proposed rule which would lower the permitted exposure level of EO to an 8h-TWA of 1 ppm. EPA (1984) also "believes that this 1 ppm TWA level will reduce significantly not only the cancer risk from worker exposure to EO but will also substantially reduce other health hazards including neurotoxic and adverse reproductive effects". In a circular of February 1, 1983, the permitted exposure level in Italy was reduced from 50 to 3 ppm. A lowering of permissible exposure levels is being discussed in other Western European countries.

1.3. Conclusion

Present information indicates that the level of exposure to EO has continued to decrease at production and conversion sites of the chemical industry. In sterilising facilities where there is a greater likelihood of exposure to higher levels it is essential that the measures available to reduce exposure are used (Korpela et al., 1983).

2. Biological Monitoring

2.1. Chromosome and sperm analysis

No new information is available about the significance of chromosome and sperm analyses for routine monitoring (AIHC, 1983).

2.2. Estimation of target dose

Considerable attention has been given to the possibility of monitoring exposure to genotoxic agents by chemical analysis of biological samples (Ehrenberg et al., 1974; Ehrenberg, 1980,a-b; Wright, 1981; ECETOC, 1982). Whereas doses of electrophilic agents delivered to tissue DNA can be readily determined in experimental species, indirect methods are needed in

humans. Ehrenberg and coworkers (loc.cit.) have proposed that the assay of adducts formed with blood proteins, e.g. Hb, may provide a suitable approach. The problem with such an indirect approach is one of validation. The correlation between the Hb dose and the corresponding tissue DNA dose(s) of a given genotoxic agent cannot normally be determined in humans. Accordingly, reliance must be placed on correlation coefficients determined in experimental species. To develop a satisfactory level of confidence in the application of such experimentally-determined coefficients to humans, it is necessary to demonstrate that the coefficients are not subject to significant variation between species.

The results of recent studies reported by Wright (1983) support the view that Hb is likely to provide a reliable DNA dose-monitor for humans exposed to EO. Thus, rats exposed by inhalation to 1, 10 and 33 ppm of (^{14}C)EO demonstrated a relatively uniform level of alkylation of DNA in a wide range of tissues, at each exposure level. Such homogeneous distribution in tissues ostensibly possessing different capacities to metabolise EO suggests that it is unlikely that there will be any significant variation in the correlation between the Hb dose of EO and the tissue DNA dose of EO in mammalian species. Studies by Segerbäck (1983) also support the view that the measurement of hydroxyethyl groups in amino-acid residues in Hb gives a good estimate of the dose of EO delivered to the tissues and to tissue-DNA, in both experimental animals and humans. However, studies in various laboratories revealed a background level of hydroxyethyl groups (and other alkyl residues) in the Hb of humans and experimental species which had not knowingly been exposed to an exogenous source of EO or any obvious precursor. These hydroxyethyl groups are probably introduced into Hb by reaction with endogenously-generated EO, although the origin of this is not yet known. EO has recently been reported in the exhaled air of an unexposed population of rats (Filser and Bolt, 1983). Depending upon individual coefficients of variation, this background hydroxyethylation may complicate the assessment of the contribution of low exogenous exposures to the total target dose of EO in an individual. Nevertheless, it is the total target dose of EO that is the important dose factor when considering risk to the individual.

Several authors (Ehrenberg et al.,1974; Calleman et al.,1978; Wright, 1981, 1983) have proposed that the concept of target dose may be applied to

improve the quality of current risk models that rely on estimates of exposure dose or received dose. Ehrenberg and his colleagues have further developed this approach in an intregrated risk strategy in which risks posed by defined target doses of genotoxic chemicals are expressed in terms of radiation equivalents. Direct experimental evidence to validate this rad-equivalence approach to risk assessment is limited. Nevertheless, results obtained with a range of low molecular weight, monofunctional alkylating agents show promise for the estimation of mutational risks and the method has been applied to assess the risks of heritable damage in humans exposed to EO (Calleman et al.,1978). This risk model is discussed in more detail in Appendix 2.

.3. Conclusions

There is a direct correlation between the reaction of EO with haemoglobin and DNA. The measurement of haemoglobin alkylation in exposed workers provides the basis of a very sensitive procedure for determining the target dose but the existence of background hydroxyethylation may complicate the assessment of the contribution to the target dose of low exogenous sources of EO.

D. GENERAL CONCLUSIONS

. The results of long-term animal studies, particularly the recent observations of an increased incidence of brain tumours in rats, lead to the view that EO is a "putative human chemical carcinogen"(ECETOC, 1980).

. Since the last report, no new epidemiological data have become available. The evidence from the former epidemiological studies on humans is limited and does not permit a conclusion to be drawn about a causal relationship between exposure to EO and the observed tumours. Nevertheless, the observation of an excess incidence of tumours in some of these studies should be treated with concern.

. EO is a potent mutagen in a number of biological systems and an inducer of damage to chromosomes in vitro and in man.

Animal studies do not show teratogenic effects but reveal embryotoxic effects. The results of a recent study on employees engaged in sterilisation

units do not enable a causal relationship to be established between exposure to EO and adverse reproductive effects.

E. RECOMMENDATIONS

1. In view of the evidence regarding the carcinogenicity of EO, its mutagenicity, and the uncertainty about its reproductive toxic effects, care should be taken to reduce exposure to a minimum.

2. There is clearly a need for further epidemiological investigations on the reproductive toxicity, neurotoxicity and carcinogenicity of EO.

3. There is a need for improving current procedures for estimating risk at low levels of exposure to EO.

4. Work needs to be done for the further development and validation of the technique of measuring haemoglobin alkylation as a means of hazard assessment.

F. APPENDICES

1. EXPERIMENTAL STUDIES RELEVANT TO ASSESSING NEUROTOXIC EFFECTS AFTER REPEATED EXPOSURE

Reference	Species	Exposure conditions	Signs noted
Hollings-worth et al. (1956)	Rat Mouse Rabbit Monkey	357 ppm: 7 h/d, 33 to 54 exposures in 48 to 85 days.	High mortality (lung injury found); survivors had paralysis and atrophy of hind-limb muscles ; apparently complete recovery 100-132 days after final exposure.
	Guinea pig	357 ppm: 7 h/d, 123 exposures in 176 days.	Decreased growth ; no neurological effects.
	Monkey	357 ppm: 7 h/d, 38 to 41 exposures in 60 days, or 94 exposures in 140 days	Paralysis with atrophy of hind-limb muscles; poor pain perception in genitalia and hind-limb; decreased knee jerks.
	Rats Guinea pig Mouse Rabbit Monkey	204 ppm: 7 h/d, 122 to 157 exposures in 176 to 226 days.	Decreased growth rate; monkey had decreased hind-limb reflexes, positive Babinski effect and partial paralysis of hind-limbs; rabbits had slight to moderate paralysis; rats died; guinea pigs showed no neurological effects; mice apparently showed no effects.
	Rat Guinea pig Rabbit Monkey	113 ppm: 7 h/d, 122 to 157 exposures in 176 to 226 days.	Decreased growth rate in rats: no neurological effects noted.
	Rat Guinea pig Mouse Monkey	49 ppm: 7 h/d, 127 to 131 exposures in 180 to 184 days.	No adverse effects noted.

Reference	Species	Exposure conditions	Signs noted
Jacobson et al. (1956)	Rat	400 ppm: 6 h/d, 5 d/wk, 6 wks.	Nasal discharge; diarrhoea; hind-limb weakness (recovered after 6 months).
	Mouse	400 ppm: 6 h/d 5 d/wk, 6 wks.	Weight loss; death.
	Dog	2830 ppm: 4 h	Vomiting; transient hind-limb weakness.
		710 ppm: 4h	No effect.
	Rat Mouse Dog	100 ppm: 6 h/d 5 d. 6 months.	Decrease in weight gain of rat; no abnormal clinical signs.
Sprinz et al. (1982)	Monkey	50 and 100 ppm: 5 h/d, 5 d/wk, 24 months.	No treatment-related lesions on microscopic examination of spinal cord, sciatic nerve or ulnar nerve. Axonal dystrophy in distal portion of nucleus gracilis and demyelination in distal portion of fasciculus gracilis.
Snellings (1982-b)	Mouse	236 ppm: 6 h/d 5 d/wk, 10 or 11 wks.	Irwin screen showed abnormalities in tail-reflex, righting reflex, gait, locomotor activity and toe-pinch reflex.
		104 ppm: 6 h/d, 5 d/wk, 10 or 11 wks.	Abnormalities of righting reflex, gait and locomotor activity.
		48 ppm: 5 h/d, 5 d/wk, 10 or 11 wks.	Abnormal gait and locomotor activity.
		10 ppm: 6 h/d, 5 d/wk, 10 or 11 wks.	No abnormalities noted.
		0 ppm (unexposed controls).	No abnormalities noted.

2. THE RAD-EQUIVALENCE APPROACH FOR ASSESSING HUMAN GENETIC RISK

Several authors have suggested that penetrating ionising radiation may be used as a reference standard to estimate genetic risks posed by exposure to genotoxic agents (Crow, 1973; Bridges, 1973 and 1974; Ehrenberg, 1974; Ehrenberg et al., 1974). Ehrenberg and his colleagues have refined this concept, particularly with a view to developing a method for the prospective estimation of risks associated with low exposures to genotoxic agents. The resultant risk model for humans has been applied to EO (Ehrenberg et al, 1974; Calleman et al., 1978).

Prospective risk models are usually based on estimates of human exposure which are interpreted by reference to correlations between exposure (administered dose) and response determined in an experimental species. Ehrenberg and co-workers sought to improve such models by developing procedures to compensate for differences between the experimental species and man in the host-dependent factors that determine the correlation between exposure dose and biological response. They proposed that introducing the concept of tissue dose or target (DNA) dose into such models would significantly improve earlier procedures based on estimates of exposure or received dose. Thus, the target-dose approach is designed to take account of all differences between experimental species and humans with respect to metabolic and other toxicokinetic and toxicodynamic factors that determine the doses of genotoxic agents delivered to their cellular targets and, therefore, the rates of formation of key chemical lesions at the target (DNA in the case of genotoxic agents) (Wright, 1981). To further improve the risk model it would be necessary to compensate for any difference between the experimental species and man with regard to host-dependent factors determining the progression of the key chemical lesions in DNA into the overt biological effect, e.g. mutation or cancer. Ehrenberg has argued that comparative radiation risk data may be useful for this purpose.

2.1. The rad-equivalence model

The prospective risk model proposed by Ehrenberg and his colleagues is based on the quantitative determination of the capacity of low doses (defined in terms of target dose) of the chemical and penetrating ionising radiation (usually acute γ-radiation) to induce defined genetic damage, e.g. a specific malignant neoplasm or specific gene mutation, in the same

experimental species. In this way the capacity of a defined target dose of the genotoxic chemical to induce genetic damage can be expressed in terms of rad-equivalents, i.e. the number of rads giving the same effect.

The significance of experimentally-determined rad-equivalent doses hinges upon their possible value for extrapolation. Thus, Ehrenberg and co-workers have suggested that the rad-equivalent value of a particular genotoxic chemical in a given species may have a similar numerical value in other species, including man. Provided that this key proposition can be substantiated then experimentally-determined rad-equivalence values may be used in conjunction with human radiation-risk coefficients to assess the capacity of genotoxic agents to induce heritable damage in humans.

2.2. The value of experimentally-determined rad-equivalence values for extrapolation

2.2.1. Of mutational risks. Direct experimental evidence to support this concept is scanty. Nevertheless, the extrapolative value of rad-equivalence has been investigated for the induction of mutation by a range of intrinsically reactive, low molecular weight, monofunctional alkylating agents. Each chemical had approximately the same rad-equivalence value for the induction of mutations in a range of biological systems including bacteria, plants and experimental mammals (Ehrenberg et al, 1974; Ehrenberg 1980, a and b). On the basis of this evidence Calleman et al. (1978) concluded that there was no reason for presuming that the value for rad-equivalence established in these very different test systems would differ from that in man. The results indicate that the key damage inflicted at the target (DNA) is a linear function of the target doses of these monofunctional alkylating agents and is the prime determinant of mutation induced by either radiation or the alkylating agents employed. Also, since DNA repair processes are important determinants in the expression of mutation, it may be inferred that the ratio of the effectiveness of the repair of key lesions induced in DNA by a given chemical and by radiation did not vary significantly between the species employed in these studies.

On the basis of this limited evidence, it would appear that experimentally determined rad-equivalence values for the induction of

mutation by EO may be applied tentatively to assess the risk of mutation associated with exposure to EO, i.e. from the human risk coefficients for radiation and an estimate of tissue (target) dose of EO in exposed individuals. Whether the concept of rad-equivalence holds for mutational risks posed by other classes of genotoxic agents, such as bifunctional alkylating agents, remains to be determined.

2.2.2. Of carcinogenic risks

As noted by Ehrenberg, the critical experiments needed to determine the utility of experimentally-determined rad-equivalence values for the quantitative assessment of human cancer risk have not yet been performed (Calleman et al., 1978). In considering the application of the rad-equivalence approach to this risk assessment it would appear to be important to use experimental rad-equivalence values determined for the induction of cancer rather than mutation. Thus it may be argued that a mutation assay would give similar values for two chemicals possessing the same mutagenic potency but differing markedly in tumour-promoting activity. Based on the precept that carcinogenic potency is a composite of initiating (mutagenic) and promoting activities, it follows that the application of the rad-equivalence approach would lead to an erroneous estimate of the cancer risk in the case of at least one of these chemicals. Clearly, the determination of the initiating and promoting activities of the test chemical is an essential requirement of prospective methods for assessing carcinogenic risks. These properties, particularly promoting activity, have not yet been investigated in the case of EO.

To a degree, the need to employ a rad-equivalence value determined in respect of cancer rather than mutation would depend upon whether mutagenic activity is the prime quantitative determinant of the carcinogenicity of the chemical (genotoxic agent). It is to be expected that any intrinsic promoting activity of a genotoxic agent such as EO would be very low at low doses, i.e. initiating activity would be expected to predominate. It would therefore seem reasonable to suggest that at low doses, cells that had been "initiated" by exposure to either genotoxic agents or radiation would be subject to the same general promoting and modulating influences acting within an individual. It follows that an objection to the use of rad-equivalence values

(determined for mutation) to assess cancer risks based on a failure to take due account of the differences between the promoting activities of the chemical and radiation may not be valid at low doses.

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