IV. BIOLOGIC EFFECTS OF EXPOSURE TO ETO

A. Effects in Animals and Lower Biological Systems

1. Acute Toxicity

Acute lethality studies in animals have been performed in four species and by five different routes of exposure. A wide array of responses follow acute exposure to ETO. These include: nausea, salivation, vomiting, diarrhea, lacrimation, nasal discharge, edema of the lungs, gasping, labored breathing, paralysis (particularly of the hind quarters), convulsion, and death. Deaths which occurred shortly after exposure to ETO were attributed primarily to lung edema, whereas delayed deaths often resulted from secondary infection of the lungs along with general systemic intoxication (Patty, 1963).

a. Inhalation

Of the acute lethal studies, the inhalation studies of Jacobson et al (1956) are the most pertinent to occupational exposure. In those experiments, exposures to various ETO concentrations for 4-hour periods resulted in LC50's of 835 ppm for female mice, 1,460 ppm for male rats, and 960 ppm for male dogs. Hine and Rowe (1973) compiled data on inhalation exposures to illustrate the variable lethal response by species, concentration, and duration of exposure. (Table 1). In general, no deaths were reported at ETO exposure levels of 250-280 ppm for rats, guinea pigs, rabbits, cats, and dogs.

b. Oral and Parenteral

LD50's resulting from oral and parenteral exposures ranged between 141 and 631 mg/kg, and are listed in Table 2. Additional support for this range is provided by Patty (1963) who reported LD50's (following intragastric administration of 1 per cent aqueous solutions) of 330 mg/kg for rats, and 270 mg/kg for guinea pigs. Weil et al (1963) reported the single oral LD50 for rats of 330 (range 290-360) mg/kg.
In another study reported by Patty (1963), a single 200 mg/kg dose of ethylene oxide given intragastrically (as a 10 per cent solution in olive oil), killed all 5 rats in the group, whereas all animals survived a dose of 100 mg/kg.

c. Tissue (including eye) Irritation

The results of studies to determine the acute eye and tissue irritant properties of ETO (in aqueous solution) are summarized in Table 3 (Bruch, 1973). The Draize (1965) system of evaluation, consisting of estimates of the highest doses having no overt actions at various sites after various methods of administration, was used in these studies.

Thickening of skin and ecchymoses followed subcutaneous injection in the guinea pig, while mild irritation occurred after intradermal injection and dermal application in the rabbit. No local reactions were observed after intramuscular injections. As illustrated in Table 3, the irritant properties varied with both concentration and total dose.

Ocular Effects:

The cornea and conjunctivae appear to be less sensitive to ETO than the skin of the rabbit. The reactions to the dermal and ocular applications most closely resemble those seen in actual occupational exposures.

Woodard and Woodard (1971) reported slight irritation in the rabbit eye, with lacrimation and conjunctival erythema. In a series of investigations by McDonald et al (1973), ETO in a balanced salt solution was administered by both ocular instillation and injection into the anterior chamber of the rabbit eye. The maximal concentration which did not produce substantial ocular pathology varied with the specific ocular tissue examined and the route of administration, ranging from 0.1% to greater than 20%. (Table 4). Irritant effects observed after acute ocular instillation were discharge, iritis, corneal cloudiness and damage as evidenced by fluorescein staining. Conjunctival congestion, flare, iritis, corneal opacity, and fluorescein staining were observed after administration into the anterior chamber. Injection into the anterior chamber had more effect on the iris, the lens, and the retina than instillation into the conjunctival sac. Conversely, the latter procedure affected the cornea and the conjunctivae more markedly than the former. Neither differential effect is exceptional. (Alcon Submission, 1973)

The irritation potential of a commercial ophthalmic ointment, the components of which were sterilized with ETO, was compared with that of the product prepared from non-sterilized components. The materials were applied to the eye of the rabbit in a 6-hour heavy dosing regimen (0.5 mg/dose at 20-minute intervals for 6 hours), and in a 5-day dosing regimen more comparable to clinical use (0.1 mg/dose, 5 applications/day for 5 days). Minimal conjunctival congestion was seen after the applications of ointment, but no difference was seen between the sterilized and non-sterilized products. (Alcon Submission, 1973)

2. Sub-Chronic Toxicity

a. Oral and Parenteral

ETO was administered to rats by gavage, 5 days/week, for 3 or 4 weeks, (Hollingsworth et al, 1956), and to rats and dogs by daily subcutaneous injections for 30 days (Woodard and Woodard, 1971). The results of these studies are summarized in Table 5. The no-effect levels for the rat were 30 mg/kg by the oral route, and 18 mg/kg by subcutaneous injection.

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b. Inhalation

Hollingsworth et al (1956) and Jacobson et al (1956) conducted studies in which various animal species were exposed repeatedly to ETO vapor at concentrations ranging from 100 to 841 ppm. The studies are summarized in Table 6. At 100 ppm there was anemia in 1 of the 3 dogs, and 8 of 30 mice and 3 of 20 rats died during 130 exposures of 6-hour duration. At 113 ppm, no rats (of 40), guinea pigs (of 16), rabbits (of 4), or monkeys (of 2) died during 122 to 157 exposures of 7-hour duration. The male rats did have some depression of their growth. Rats of both sexes exhibited increased lung weight. However, at 204 ppm growth depression was noted, an appreciable number of rats died of secondary respiratory infection, and rabbits and monkeys developed posterior paresis. Rats and guinea pigs exhibited increased lung weights also. At higher concentrations more serious conditions developed, including severe nervous and respiratory effects and degeneration of testicular tubules.

On the basis of these studies, Hine and Rowe (1973) proposed permissible exposure limits of 100 ppm for repeated exposures of 4 hours/day for a 2-week period, and 50 ppm for 7-hour exposures on a continuing basis.

3. Chronic Toxicity

No chronic test data could be found.

NIOSH has been informed that a 2-year vapor inhalation study on rats began on April 27, 1977, at the Carnegie-Mellon Institute of Research, Pittsburgh, Pennsylvania. That study is supported by industry and will include cytogenic, mutagenic, and teratogenic evaluations, and a one-generation reproduction study. A similar 2-year study on mice will also be performed. The National Cancer Institute, National Institutes of Health (NIH) is also supporting long term studies on ETO. The NIH studies are underway at Industrial Bio-Test Laboratories, Northbrook, Illinois.

4. Carcinogenic, Mutagenic, Reproductive, Teratogenic, and Metabolic Studies

a. Carcinogenicity

No standard long-term carcinogenicity bioassays have been reported, although two screening experiments have been conducted. Walpole (1957) subjected 12 "stock" rats to repeated subcutaneous injections of 1 g/kg (bw) ETO in arachis oil. The exact dosing schedule was not reported, although the period of injection was 94 days. The animals were maintained for their lifetimes, during which no local sarcomas or other tumors were observed. In other studies, Van Duuren et al (1965) applied (by brush) 0.1 ml of a 10% solution of ETO in acetone three times each week onto the clipped dorsal skins of 30 female ICR/Ha Swiss mice. The animals were 8 weeks of age at the start of the skin painting, which was continued for

their lifetimes. The median survival time was 493 days; no skin tumors were observed.

Two other studies related to carcinogenicity have been conducted. Jacobson et al (1956) exposed rats, mice, and dogs to 100 ppm of ETO (6 hours/day, 5 days/week) for 6 months. There were no significant pathologic changes suggestive of a carcinogenic response. The only observed effects were decreases in red blood cell count, hemoglobin, and hematocrit in dogs. It is felt by scientists at the National Cancer Institute and the International Agency for Research on Cancer that this study was not conducted for a sufficiently long period to test the possibility that ETO causes cancer. In the other "study" (actually, a retrospective analysis of events), 86 female Swiss-Webster germ-free mice were inadvertently maintained on ETO-treated ground-corncob bedding for 150 days, and were then moved to untreated bedding for the remainder of their lives (maximum lifespan = 900 days). Tumors were observed at various sites in 63 mice. In contrast, no tumors were reported in 83 female mice that had not been exposed to the ETO-treated bedding, but were observed for 100- 160 days. (Reyniers et al, 1964). The most common tumors in the exposed group were ovarian, lymphoid (malignant lymphoma), and pulmonary. While the effect is noteworthy, it is not known whether it was due to ethylene oxide, ethylene glycol or some other unknown chemical (or factor).

b. Mutagenicity

Experimental attempts to induce mutations in at least 14 different species through exposure to ETO have been reported. An increased frequency of mutations was observed in 13 of the test species, the exception being a bacteriophage of Escherichia coli [Loveless, 1959].

The data for the species, loci/locus, mutation type, exposure time, exposure route, exposure concentration, and the significant results are summarized in Table 7. The data indicate that several different types of genetic damage may be induced following exposure to ethylene oxide. The induction of point mutations has occurred in both procaryotic and eucaryotic organisms [Table 7]. While the point mutations probably were of the base-pair substitution type in Salmonella typhimurium, the mutational spectra have not been well characterized at the molecular level in other species [Embree, 1975]. In Drosophila melanogaster, the classes of induced mutations included sex-linked lethals, visibles, and "minutes" [Bird, 1952; Fahmy and Fahmy, 1956; Fahmy and Fahmy, 1970; Nakao and Auerbach, 1961]. In plants, the classes of heritable changes include mutations at the chlorophyll and waxy loci and chromosomal abnormalities [Faberge, 1955; Ehrenberg et al, 1956; Ehrenberg et al, 1959; MacKey, 1968; Sulovska et al, 1969; Lindgren and Sulovska, 1969; Roy et al, 1969; Roy and Jana, 1973; Jana and Roy, 1975].

Some evidence suggests that ETO may induce heritable changes in mammals, although no direct observations of such changes, comparable to those observed in lower organisms, have been reported in mammalian systems. Following three 7-hour/day exposures to 250 ppm of ETO, isochromatid and chromatid gaps and breaks were observed in bone-marrow cells sampled 24 hours after the last exposure of the rats [Embree, 1975]. The frequency of gaps and breaks as a function of total exposure time or

total dose was not reported. Following a single oral dose of 9 mg/kg, a significant number (P less than 0.007) of chromosomal abnormalities was observed in the red marrow cells of the femurs of rats. Neither dose-response data nor the rate of return to a normal chromosomal picture was reported [Embree, 1975].

The dose-response relationship for the induction of micronucleated cells in femoral marrow of Long-Evans rats as a function of the concentration of inhaled ETO was studied by Embree (1975), using groups of 5 rats exposed to 0, 10, 25, 50, 250, or 1,000 ppm of ethylene oxide for 4 hours. A statistically significant increase (P less than 0.05) of micronuclei were induced at the 50, 250, and 1,000 ppm levels. While several alkylating agents which are known to induce mutations are also known to induce the formation of micronuclei in bone marrow cells, the biological significance of this adverse effect is poorly defined. These results suggest that the current federal standard of a time-weighted average (TWA) concentration of 50 ppm parts of air for an 8-hour day may not protect exposed employees from all adverse effects resulting from ETO exposure.

The most plausible molecular basis for the induction of heritable changes and other genetic effects following exposure to ethylene oxide is the alkylation of cellular constituents, including deoxyribonucleic acid (DNA). The highly-strained 3-membered ring of the epoxide is broadly reactive toward all classes of cellular nucleophiles. Reactions of ETO with protein [Frankel-Conrat, 1944], and with nucleic acid [Frankel-Conrat, 1961; Ehrenberg et al, 1974] have been measured. The primary identifiable product of the reaction of ETO and DNA was 7-hydroxyethylguanine, although a number of other minor products would also be anticipated. Since the occurrence of these chemical reactions is a consequence of the intrinsic characteristics of the chemical structures, it seems probable that analogous reactions may occur in humans who are occupationally exposed to ETO. These spontaneous reactions result in the formation of covalently altered biomolecules which may possess abnormal functional properties.

In addition to the spontaneous reactions, ETO may also be consumed via detoxifing enzyme-catalysed reactions such as epoxide hydratase and glutathione-S-alkyl tranferase [Arais and Jakoby, 1976]. The rate of consumption of ETO by such enzyme reactions would be a non-linear function of the local concentrations of the substrate. Since the non-catalyzed half-time of the reaction of ETO with water is about 4,200 minutes, whereas the in vivo half-life in the mouse is only about 9 minutes [Ehrenberg et al, 1974], it appears that such enzymatic reactions may rapidly detoxify a large fraction of the absorbed doses in mammals. At a minimum, the greatly reduced half-life of ETO in vivo suggests a non-linear dose-response relationship for induction of genetic effects in mammals due to the existence of saturable detoxifing mechanisms. Further research is required to establish the relative magnitudes of the competing reactions as functions of the doses and of the exposure times.

The observations of: (a) heritable alterations in at least 13 different species, (b) alterations in the structure of the genetic material in somatic cells of the rat, and (c) a covalent chemical reaction between ETO and DNA, support the conclusion that continuous occupational

exposure to significant concentrations of ETO may induce an increase in the frequency of mutations in human populations.

Embree [1975] and Ehrenberg et al [1974] have attempted to estimate the degree of genetic risk to human populations exposed to ETO by comparing the results with the effects of ionizing radiation, and expressing the result in terms of the "rad-equivalent." Embree's estimate (100 mrad-equivalences/ppm-hr) was based on the frequency of micronuclei in bone marrow following exposure to graded doses of ethylene oxide. At present, the mechanism of generation of micronucleated cells in bone marrow is not known, although it is possible that these cells may arise from either the non-disjunction of the chromosomes during mitosis, or malformation of the spindle. While it is conceivable that somewhat related processes may occur in functionally distinct tissue, such as spermatogonia, neither quantitative nor qualitative evidence is available to indicate the magnitude of the effect in the genetically important cell-types following exposure to ethylene oxide. Consequently, Embree's estimate appears to be highly speculative and with minimal theoretical or experimental basis.

The estimate by Ehrenberg et al [1974] of the risk to humans (reported as 20 mrad-equivalences/ppm-hr) is based on the assumption that the rate and mechanism of induction of mutations affecting chlorophyll in metabolically dormant barley seeds were equivalent to the rate of induction of specific locus mutations in the metabolically active tissues of the testes of mammals. The reliability of this estimate as a quantitative measure of genetic risk to human populations exposed to ETO is open to question. In addition to the obvious physiologic differences between plants and mammals, it should be noted that forward chlorophyll mutations in barley seeds may occur at several hundred loci while the specific locus test is limited to one locus or a small number of loci [Ehrenberg et al, 1974]. Consequently, the relative risk estimated by Ehrenberg et al may be in error by several orders of magnitude. Thus, the estimates of genetic risk by Embree [1975] and Ehrenberg et al [1974] probably do not, by themselves, provide a satisfactory basis for the development of occupational exposure standards. Nevertheless, the observations of induction of heritable changes in a broad spectrum of biological organisms, and of the intrinsic chemical reactivity of ETO with cellular constituents, suggest that ETO may have a significant influence on the spontaneous mutation rates of human populations.

Kalling [1967] briefly reported data, obtained from an experiment performed by Ehrenberg and Hallstrom, on the frequency of pathological mitoses observed in phytohaemaglutinin-stimulated lymphocytes of 7 workers who had been "strongly affected" by ETO following an industrial accident 18 months earlier. Analysis of from 6 to 26 metaphases from each individual suggested an increased frequency of pathological mitoses relative to the frequency in 10 non-exposed workers. Kalling's brief description of the results are insufficient to draw substantive conclusions. However, this report of induction of chromosomal abnormalities in humans is consistant with the observations of Embree [1975] in the rat.

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c. Reproductive Effects/Teratogenicity

No data concerning reproductive or teratogenetic effects were found for ETO. Ethylene chlorohydrin (2-chloroethonol), a reaction product of ETO, was shown to cause pronounced teratogenic effects (i.e., anterior hydrophthalmos, a form of buphthalmia) when administered to the developing chicken embryo. The compound was administered in water at pre-incubation (0 hours) or at 96 hours of incubation, at a number of different doses. It should be noted at this point that the limited animal test data on the carcinogenic potential of ethylene chlorohydrin suggests no increased incidence (over controls) when the compound was fed (Ambrose, 1950), or injected subcutaneously (Mason et al, 1971) into rats. Ethylene chlorohydrin is to be tested for effects on reproductive processes by inhalation and by skin painting, under NCI contract.

Studies have shown chromosome aberrations in rat bone marrow cells following exposure to ethylene chlorohydrin (Isakova et al, 1971, and Semenova et al, 1971), and base-pair substitutions (but not frameshift mutations) in Salmonella (Rosenkranz and Wlodkowski, 1974). Ethylene chlorohydrin also induced mutations in bacterium _Klebsiella_ (Voogd and Van Der Vet, 1969).

d. Metabolism.

A comprehensive study designed to isolate and characterize the metabolites of ETO in either lower or higher life forms has not been identified in the literature. However, Frankel-Conrat (1961, 1944) has characterized the spontaneous chemical alkylation of a protein (egg albumin) and viral ribonucleic acid. Brookes and Lawley (1961) characterized the reaction of ETO with guanine. More recently, Ehrenberg et al (1974) have reported the results from several experiments on the fate of inhaled tritium-labeled ETO in mice. The biological half-life was found to be about 9 minutes in the mouse, indicating a rapid metabolic breakdown of the molecule. The second-order rate constants for the reactions of ETO with DNA, and with liver "interphase" protein were found to be 0.9 and 3.3 1/g-hr, respectively. The hydroxyethylated DNA was observed to undergo a slow depurination reaction, a possible pre-mutation lesion, with a half-life of 340 hours. Following inhalation of tritiated ETO, radioactivity (in unidentified chemical form) was found to be associated with proteins isolated from lung, liver, kidney, brain, spleen and testes. Seventy-eight per cent of the absorbed dose was excreted into the urine within 48 hours of the inhalation exposure. Other than the possible isolation of small amounts of 7-hydroxyethyl guanine from the urine, evidence for the alkylation of DNA _in vivo_ was not reported. Since urinary 7-hydroxyethyl guanine may also arise from the alkylation of RNA, the fraction of total absorbed dose which alkylates the genetic material remains to be determined.

The study confirms that ETO is readily absorbed via the respiratory tract and is widely distributed throughout, and reacts with, components of body tissues.

B. Effects in Humans

    1. Acute Toxicity:

Zernik (1933) extrapolated results of animal tests to approximate lethal dosages of ETO for man. He estimated that an exposure to 0.5 mg/l (278 ppm) for 1 hour would be objectionable, but that the same concentration for a day would be dangerous. He further speculated that 1.0 mg/l (556 ppm) would be sufficient to cause sickness and death after a number of hours of inhalation. Systemic poisoning due to inhalation exposure to ETO is rare, but 3 cases have been reported in which headache, vomiting, dyspnea, diarrhea, and lymphocytosis occurred (Hine and Rowe, 1963). Hess and Tilton (1950) observed a similar case. The nausea and vomiting may persist for several hours if untreated. There is also evidence that high vapor concentrations can be sufficiently irritating to cause pulmonary edema in man [Thiess, 1963]. Inhalation of high concentrations of ETO for even short exposure periods can produce a general anesthetic effect in addition to coughing, vomiting, and irritation of the eyes and respiratory passages. Early symptoms are irritation of the eyes, nose, and throat and a "peculiar taste"; effects which may be delayed are headache, nausea, vomiting, dyspnea, cyanosis, pulmonary edema, drowsiness, weakness, incoordination, electrocardiographic abnormalities and urinary excretion of bile pigments.

Thiess (1963) reported on his observations in 41 cases of excessive industrial exposure to ETO, mainly due to accidents. The principal effect after excessive exposure to the vapor was vomiting, recurring periodically for hours, accompanied by nausea and headache. Short exposure to high concentrations of ETO resulted in irritation of the respiratory passages leading to bronchitis, pulmonary edema, and emphysema.

At least two additional cases of accidental poisoning in man by ETO appear in the literature. Blackwood and Erskine (1938) reported cases in 6 men who became ill while working in a ship compartment adjacent to one being fumigated with a commercial mixture of 10% ETO/90% CO_2 [Carboxide]. Ten female employees were reported (Anon, 1947) to have been "overcome" by ETO used as a disinfectant in a California food plant. The Department of Public Health of the State of California issued a report on this incident. Attempts at locating the report have been unsuccessful to date. In neither of these events was the concentration of ethylene oxide known. In both, the gross symptoms of the persons affected included headaches, nausea, vomiting, and respiratory irritation. No permanent ill effects were reported.

A number of dermatologic conditions due to exposure to ETO have been reported. These include:

Skin burns occurred in workers after prolonged contact (duration unspecified) with a 1% solution of ETO in water (Sexton and Henson, 1949). Skin contact with solutions of ETO caused characteristic burns on human volunteers. After a latent period of 1 to 5 hours, edema and erythema, progressing to vesiculation with a tendency to coalesce into blebs, and desquamation was followed by complete healing within 21 days, usually without treatment. In some cases, residual brown pigmentation occurred. Application of the pure liquid to the skin caused frostbite (due to rapid

evaporation of the ETO); 3 of the 8 volunteers became sensitized to ETO solutions.

Human experience indicates that extensive blister formation occurs following even brief contact with 40-80% aqueous solutions of ETO. Lesser or greater concentrations produced blisters only after more prolonged contact. ETO has been reported to be retained in rubber and leather for long periods of time, and not to be removable by washing. Wearing contaminated footwear has resulted in serious foot burns (Phillips and Kaye, 1949). Rubber shoes were donned by laboratory workers immediately after the shoes had been sterilized with ETO. Apparently the ETO had dissolved in the rubber and then diffused out to contact the skin. No such incidents occurred when similar articles of clothing were aired until free of ETO before being worn.

Contact of liquid ETO with exposed skin does not cause skin irritation immediately, but blistering of the skin develops after a time if shoes or clothing wet with ETO are not removed promptly. Contact of liquid ETO with the eyes can cause severe burns. A workman exposed to ETO in an unstated manner was reported by McLaughlin (1946) to have suffered a corneal burn, which was said to have healed promptly.

In 1963, Thiess reported that he had observed the effects of an accidental forceful blast of gaseous ETO that struck a nurse in the eye, nose and mouth, from a gas sterilizer that employed a cartridge of ethylene oxide. This caused no immediate discomfort, but within 2 hours one eye became mildly uncomfortable, and one nostril felt sore and obstructed. The only abnormality in the eye at that time was keratitis epithelialis consisting of fine gray dots in the corneal epithelium in the palpebral fissure. These dots stained with fluorescein. In 3 hours the foreign-body type irritation in the affected eye and soreness in one nostril were enough to interfere with working. The foreign-body type of discomfort reached the maximum 9 to 10 hours after the exposure, but by 15 hours pain began to subside, and in 24 hours the eye was entirely normal, both subjectively and by slit-lamp examination. Soreness in the nose persisted a little longer, but at no time was there respiratory distress or alteration of taste or sensation in the mouth.

Another report by Thiess (1963) described an instance of a squirt of liquid ETO into one eye of a patient, treated immediately with copious irrigation with water; this resulted only in irritation of the conjunctivae lasting for 1 day. Thiess quoted Hollingsworth et al (1956) as having observed intense conjunctivitis in rabbit eyes after application of only one drop of ETO. This condition cleared in 4 days.

2. Chronic Effects

a. Case Histories

Exposure to low concentrations of gaseous ETO may cause delayed nausea and vomiting [Hess and Tilton, 1950]. Continuous exposure to even low concentrations will result in a numbing of the sense of smell [Jacobson et al, 1954].

Protracted contact of the gas or the liquid with the skin was observed (Thiess, 1963) to cause toxic dermatitis with large bullae. There appeared to be little or no tendency toward development of allergic dermatitis. Evidence of irritation was seldom seen in the respiratory tract, contrary to what others had reported, and cough was not a warning symptom. The eyes of workers excessively exposed to ETO vapor occasionally showed slight irritation of the conjunctivae, but there were no injuries of the cornea, and no special treatment was felt to be needed. Thiess (1963) observed no lacrimatory effect that could be considered a warning symptom.

Workers have developed severe skin irritation from wearing rubber gloves which had absorbed ETO; redness, edema, blisters and ulceration were observed (Royce and Moore, 1955). There is considerable evidence [Sexton and Henson, 1949, 1950] which suggests that repeated skin contact with dilute or concentrated aqueous solution of ETO results in the development of skin sensitivity of long duration.

The conclusions of Sexton and Henson (1950) are listed:

(1) The magnitude of the skin injury from ETO seems to be influenced and determined by the length of time of contact and the concentration. The most hazardous aqueous dilutions seem to be those in the 50% range.

(2) Pure anhydrous liquid ETO does not cause primary injury to the dry skin of man.

(3) Rapid vaporization of pure liquid ETO sprayed on the skin results in a freezing reaction of the skin.

(4) Repeated skin injury can result in such delayed sensitivity manifestations as the "flare-up" and "spontaneous flare-up" reactions.

Aqueous solutions of ETO in prolonged contact with the skin are well known to be vesicant (owing presumably to the reactivity of ETO with tissue proteins). The volatility of ETO, and the fact that there are rarely circumstances in which considerable concentrations are held in protracted contact with the eye may account in part for the mildness of ocular injuries which have been observed so far. The comparative insensitivity of the cornea to damage by ETO, shown in Tables 3 and 4, undoubtedly plays a part here also. The highly crosslinked structure of collagen quite possibly contributes to this relative insensitivity of the cornea; the high concentration of glutathione present in the cornea may be a factor also in this insensitivity to injury by ETO.

Delayed sensitization reactions have been reported [Shupak, unpublished] in humans, as has one case of anaphylaxis resulting from residues of ETO in renal dialysis equipment (Poothullil et al, 1974).

b. Epidemiologic Studies

Few studies are known which relate directly to long-term exposure to ETO in medical facilities. Correspondence from the Veterans Administration (VA) (Cobis, 1977) indicates a very low incidence of reports of health incidents relating to use of ETO in VA medical facilities. These consist of "1 patient with minor face irritation, and 12 employee incidents, including watering eyes, nausea, and skin irritation." Cobis reported that the "incidents are now being evaluated by the medical staff to see if they can be verified or if there was any sequela." The VA experience is based on an estimated 400,000 sterilization cycles in which ETO was used in 162 hospitals and 7 outpatient clinics over an average of 8.2 years (total of accumulated years is 1,334). The VA estimates that their facilities are processing 5 million packages of sterile supplies in 79,000 sterilization cycles per year (an average of 63.3 packages per cycle).

A small retrospective morbidity study was conducted on a group of 37 employees who were exposed to ETO levels of approximately 5-10 ppm for a period of 5-16 years in an ETO production plant. (Joyner, 1964). The "exposed" group consisted of males between the ages of 29 and 56. Mean exposure to ETO was 10 2/3 years, with only 3 workers exposed for less than 5 years, and 2 workers exposed for more than 15 years. The "control" group was selected from among volunteers who were participants in the periodic physical examination program, with no attempt at selection except to match ages with those of the study group. Mean length of service (in production units) of the control group was 11 2/3 years. No attempt was made to categorize the specific types of chemical exposures (to agents encountered in the petrochemical industry) of the "control" group. The study included a review of medical records (with scoring of such factors as number of visits to the dispensary, days "lost" from work, and number and type of injury reported),and a "problem-oriented" tabulation of specific illnesses diagnosed by the physicians, and comparison of the morbidity rates. In general, the ETO workers had a better health record than the "control" group. While such a study might have detected major toxic effects, the sample size, "control" group, dose levels, duration of exposure and observation period were not sufficient to assess carcinogenic potential or more subtle toxic effects.

A study by Ehrenberg (1967) involved hematologic and chromosomal investigations of a group of personnel of a factory manufacturing and using ETO. Early in 1960, a preliminary hematological investigation was performed. Twenty-eight persons from the part of the factory where leakage of ETO from tube joints, pumps, etc, was possible (and occurred at least occasionally) were investigated, and 26 persons from other departments working with ETO were used as controls. In addition, 3 persons accidentally exposed to high concentrations of ETO were studied; these were included in the "exposed" group. The subjects were males of about the same age (i.e., 45 years). The "exposed" persons had been active in the ETO department for periods of time varying from 2 to 20 years (avg. 15 years).

Since certain differences were found between exposed and control persons, the ventilation of the factory was improved. A second

analysis was undertaken in the following winter (termed "1961 investigation") comprising all male employees of the factory, now classified as follows:

(1) 66 persons not working in contact with ETO (including the 1960 "control group").

(2) 86 persons intermittently working in ETO premises.

(3) 54 persons who had worked in contact with ETO for some period of time.

(4) 37 persons permanently working in the ETO area (including the 1960 "exposed group").

(5) 8 persons exposed to high concentrations of ETO during repair and clean-up operations following a tube break in a factory. Two of the group required immediate hospitalization for respiratory difficulties.

A comparison of exposed and control persons suggested that:

(1) Persons intermittently exposed to ETO during several years exhibited higher absolute lymphocyte counts than did the control group in the initial study population. Following improvement of ventilation this difference became smaller, although it was still significant 1 year later in the same persons. No significant difference between the exposed and the control groups was found after the study population was enlarged, either because the difference found between the groups selected for the first analysis was accidental, or because younger persons with higher lymphocyte counts were introduced into the control group in larger numbers than into the exposed group.

(2) Certain cell abnormalities were found in the exposed group (3 cases of anisocytosis, 1 of leukemia), that did not appear in the control group.

(3) Lower hemoglobin values were found in the exposed group, in addition to a few cases of slight anemia.

(4) Persons exposed accidentally (group 5 of the later study population) for short periods of time to high concentrations of ETO exhibited higher numbers of chromosomal aberrations than a comparable control group.

NIOSH has been informed that Ehrenberg currently has plans to "follow up" members of the study group.

NIOSH is currently initiating a study of approximately 2,500 workers potentially exposed to ETO during its manufacture and/or use. The study will include mortality, morbidity, and reproductive data if possible.

3. Sensitization Response.

Allergic-type reactions have been reported in workers accidently sprayed with ETO in solution (Sexton and Henson, 1949), and in patients exposed to improperly aerated dressings (Hanifin, 1971). ETO (1% solution) was not a contact sensitizer in the occlusive patch test in guinea pigs, nor did a 0.1% ETO solution produce sensitization by the intracutaneous route in this species (Woodard and Woodard, 1971).

In recent skin irritation studies, delayed sensitization of human subjects developed in response to ETO contained in polyvinyl chloride blocks and films, and in vaseline [Shupak, unpublished data]. This finding supports an earlier report of anaphylaxis from products of ETO sterilization of renal dialysis equipment (Poothullil et al, 1974, and Avashia, 1975).

4. Hemolytic Effects.

Hemolysis has been reported (Hirose et al, 1963 and Stanley et al, 1971) with ETO sterilized devices used for blood perfusion, and with devices used for IV administration in patients. Anemia was reported (Woodard and Woodard, 1971) to have developed in dogs in a 30-day subcutaneous toxicity study with ETO in saline solution. Dogs injected with 6-36 mg/kg daily developed anemia; the severity was dose-related. Examinations were performed prior to, and at the end of, 30 days of dosing. Hence, effects occurring earlier would not have been detected. Therefore, a re-examination of the hematologic effects appeared to be in order. Beagle dogs were dosed intravenously with ETO/glucose solution daily for 3 weeks. Doses were increased from 3-60 mg/kg at intervals. Three controls received only glucose. No evidence of anemia was detected (Balazs, 1976). Further clarification of these apparently conflicting results is needed.

Ehrenberg (1967) observed lymphocytosis in workers exposed to ETO.

C. Tabulation of Biologic Effects of ETO

The following tables summarize the bio-effects data described in the earlier section of this report:

1. Summary of Acute Response of Animals to Inhalation Exposure to Ethylene Oxide
2. Acute Toxicity of Ethylene Oxide (Administered in Aqueous Solution) to Mice, Rats, and Rabbits
3. Summary of Tissue Irritation Studies With Ethylene Oxide
4. Maximum Concentration of Ethylene Oxide Not Producing Substantial Ocular Pathology in Rabbits
5. Summary of Subchronic Toxicity of Ethylene Oxide
6. Results of Repeated Inhalation Exposure of Animals to Vapors of Ethylene Oxide
7. Genetic and Allied Effects of Ethylene Oxide

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Table 1. Summary of Acute Response of Animals to Inhalation Exposure to Ethylene Oxide

ppm by vol. in air	Exposure Time (hr)	Animal	Results
250-280	8	Guinea pig	Slight respiratory changes; no deaths.
	48	Guinea pig	An occasional death.
560-600	7	Guinea pig, cat, and dog	No deaths
	8	Guinea pig	An occasional death.
	22	Guinea pig and cat	Death during (or following) exposure.
	22	Rabbit and dog	No deaths.
710	4	Dog	0/3 died in 14 d.
1,100	5	Rat, guinea pig, rabbit	Moderate injury, no deaths.
	8	Guinea pig, dog, and rabbit	Slight injury, no deaths.
	8	Rat and cat	Death within 24 hr.
1,300-1,400	8	Guinea pig	Majority died in 1-8 d.
	4	Dog	3/3 died first day.
2,200	1.5	Cat	Injurious, no deaths.
	3	Cat	Death within 24 hr.
	4	Guinea pig	Injurious, few deaths.
	4	Rabbit	Injurious, no deaths.
	4	Dog	Death within 24 hr.
3,000	1	Guinea pig	No deaths.
	3	Guinea pig	Death of majority within 1 to 8 d.
	8	Guinea pig	Death of majority within 24 hr.
4,000*	4	Rat	No deaths (of 6).

Table 1 (Continued). Summary of Acute Response of Animals to Inhalation Exposure
to Ethylene Oxide

7,000	20 min	Guinea pig	No evidence of injury.
	1	Guinea pig	Death of majority within 1 to 8 d.
	2.5	Guinea pig	Death within 24 hr.
8,000*	4	Rat	6/6 died.
14,000	10 min	Guinea pig	No evidence of injury.
	20 min	Guinea pig	Majority died in 1 to 8 d.
	1	Guinea pig	Death within 24 hr.
51,000-64,000	5 min	Guinea pig	Majority died in 1 to 8 d.
	10 min	Guinea pig	Death in 24 hr.

Notes: Data compiled by Hine and Rowe (1963) from the studies of Jacobson
et al, (1956), Hollingsworth et al, (1956), Waite et al, (1930),
and Flury and Zernik, (1931). *Data of Weil et al (1963).

Table 2. Acute Toxicity of Ethylene Oxide (Administered in Aqueous Solution) to Mice, Rats, and Rabbits

| Animal | Sex | LD50 (mg/kg) Route of administration | | | |
		Oral	IV	IP	SC
Mouse	M	365	261	178	192
	F	282	261	178	261
Rat	M	242	355	178	141
	F	282	383	153	127
Rabbit	M	631	178	251	200
	F	631	178	251	200

Notes:
(a) Death occurred within 24 hr. in most instances. Toxic signs included ataxia, prostration, labored respiration, and occasional tonic convulsions.
(b) Mouse and rat studies as reported by Bruch (1973). Five animals of each sex were tested at each of six dose levels.
(c) Woodard and Woodard (1971) conducted the study with rabbits, in which one rabbit of each sex was exposed at each of six dose levels.
(d) Injection Route: IV = intravenous, IP = intraperitoneal, SC = Subcutaneous

Table 3. Summary of Tissue Irritation Studies with Ethylene Oxide

Animal Species	Route of Exposure	Maximal concentration tested, % (aq. soln.)	Highest no-effect concentration, %	Total dose of ETO at no-effect level (mg except as noted)
Rabbit	Intramuscular	2	2	10
	Intradermal	2	0.2	0.6%
	Dermal	5	1	5
	Eye	10	2.1	2
Guinea pig	Subcutaneous	5	0.1	0.05%

Notes:
(a) Reference: Woodard and Woodard (1971)
(b) Reactions were scored following a single injection.

Table 4. Maximum Concentration of Ethylene Oxide Not Producing Substantial
Ocular Pathology in Rabbits (ETO Administered in Balanced
Salt Solution).

Examination type	Concentration (%)	
	6 hr acute ocular instillation (b)	Anterior chamber administration
Macroscopic		
Conjunctiva	0.1	1.0
Biomicroscopic		
Cornea	1.0	1.0
Anterior chamber	5.0	0.1
Iris	1.0	0.1
Lens	5.0	0.1
Microscopic		
Conjunctiva	0.1	1.0
Cornea	1.0	1.0
Iris/Ciliary body	1.0	0.1
Lens	more than 20.0	5.0
Retina	more than 20.0	5.0

Notes:
(a) Based on McDonald et al (1973).
(b) 1 drop of solution applied every 10 min for total of 36 applications
in 6 hrs.

Table 5. Summary of Subchronic Toxicity Studies of Ethylene Oxide.

Species	Route of Administration	Highest Level Administered	Effects	No-effect Level
Rat (a)	Oral (gavage) 5 d/wk	100 mg/kg (3 wk)	Loss of wt., gastric irritation, slight liver damage.	30 mg/kg (4 wk)
Rat (b)	Subcutaneous Injection (Daily 30 d)	54 mg/kg	Loss of wt., necrosis, hemorrhage and inflamation at injection site.	18 mg/kg
Dog (b)	Subcutaneous Injection (Daily, 30 d)	54 mg/kg (c)	Severe local inflammatory effects, increased mortality at high level, reduced hemoglobin and hematocrit values at all levels.	not established (d)

Notes:
(a) Hollingsworth et al, (1956)
(b) Woodard and Woodard (1971)
(c) Reduced to 36 mg/kg after 7 days of treatment.
(d) Lowest dose administered was 6 mg/kg.

Table 6. Results of Repeated Inhalation Exposure of Animals to Vapors of Ethylene Oxide

Exposure Regimen				Mortality (c)	Species	Pathologic Findings
Conc. ppm (mg/l)	Hrs./ day	No. Exposures (b)	Over No. Days			
841 (1.51)	7	6	10	10/10	Rat	Gross irritation of the respiratory
	7	8	10	8/8	Guinea pig	tract; mice seemed most susceptible.
	7	4	10	1/1	Rabbit	All animals died.
	7	1	10	5/5 F	Mouse	
	7	7	10	1/1 F	Monkey	
357 (0.64)	7	7	9	2/20	Rat	Moderate loss of body weight and severe
	7	7	9	4/10 F	Mouse	lung injury in rodents. Secondary
	7	33	48 to 85	10/10	Mouse	respiratory infection the primary cause
	7	38	48 to 85	10/10 F	Rat	of death in rodents. Impaired nervous
	7	38	48 to 85	8/10 M	Rat	function at lumbar and sacral level,
	7	48	48 to 85	1/2	Rabbit	reversible in rat, rabbit, and monkey.
	7	---	---	0/1 F	Monkey	Normal blood urea nitrogen (BUN) in all
	7	123	176	0/16	Guinea Pig	species. Normal hematological values in
	7	38 to 41	60	0/2	Monkey	rats, rabbits, and monkeys. Growth depression
	7	94	140	0/2 M	Monkey	slight increase in lung weight in male

guinea pigs, and degeneration of testicular tubules; slight fatty degeneration in cortex of adrenals of females; no nervous system signs. Growth depression. Impairment of function of nervous system: paralysis and muscular atrophy of hind limbs, knee jerk reflexes poor (or non-existent), pain perception poor. Normal pathology upon sacrifice.

290 (0.52)	6	over 6 wk	?	0/3	Dog	Vomiting, occasional tremors, transient paraplegia, anemia.
204 (0.37)	7	122-157	176-226	22/40	Rat	Appreciable number of rats died of secondary
	7	122-157	176-226	1/16	Guinea pig	respiratory infection; growth depression in
	7	122-157	176-226	0/4	Rabbit	rat; posterior paresis in monkey and rabbit;
	7	122-157	176-226	2/10 F	Mouse	increased lung weight in rat and guinea pig.
	7	122-157	176-226	0/2 F	Monkey	Neurologic and muscular atrophy in monkey.

Increased liver and kidney weight in F rats. Slight testicular tubal degeneration in rat.

Table 6 (Continued). Results of Repeated Inhalation Exposure of Animals to Vapors of Ethylene Oxide

113 (0.20)	7	122-157	176-226	0/40	Rat	No findings except growth depression in male rats and increased lung weight of the rats.
	7	122-157	176-226	0/16	Guinea pig	
	7	122-157	176-226	0/4	Rabbit	
	7	122-157	176-226	0/2	Monkey	
100 (0.18)	6	130	?	3/20	Rat	No clinical signs and no significant findings except anemia in one dog.
	6	130	?	8/30	Mouse	
	6	130	?	0/3	Dog	
49 (0.09)	7	127-131	180-184	0/20	Rat	No effect on any of the animals (as judged by general appearance and behavior, mortality, growth, final body and organ weight, and gross and microscopic examination of the tissues).
	7	127-131	180-184	0/8	Guinea pig	
	7	127-131	180-184	0/2	Rabbit	
	7	127-131	180-184	0/10 F	Mice	
	7	127-131	54	0/1	Monkey	

Notes:
a) Based on Hollingsworth et al (1956), and Jacobson et al (1956),
b) Exposures usually conducted on a 5 day/week basis.
c) First figure indicates number of animals which died. Second figure indicates number of animals in exposure group. Unless noted otherwise, the second figure is number of animals of each sex present.

Table 7. Genetic and Allied Effects of Ethylene Oxide

Species or Common Name	Locus/Loci (mutation type)	Time & Route	ETO Conc. (Vol. or Dose)	Results and Reference
Tobacco mosaic virus	---	50 hr, gaseous	160 mm pressure pH 5.0 buffer	Approximately 100 ETO residues per single strand RNA molecule. Main product was the 7-hydroxyethyl-guanine adduct (Frankel-Conrat, 1961)
Egg albumin	---	2 day	---	Up to 80 residues of ETO per protein molecule; reacts with carboxy, amino and phenolic residues of proteins; changes isoelectric point and solubility of protein. (Frankel-Conrat, 1944)
T4-Phage of Escherichia coli	m and r (forward)	10 min, aqueous	0.10 M	No loss of infective units; no increase in mutation frequency (Loveless, 1959)
Phage lambda in Escherichia coli	(prophage induction)	30 min	15mM	lambda-prophage was induced in 1.7% of the treated bacteria (Hussain, et al, 1975)
Salmonella typhimurium TA 1535	histidine (back)	hr (not specified)	---	Increased frequency of base substitution mutations (Embree, 1975)
Escherichia coli	Streptomycin-resistance (forward)	2mM-hr	(2mM-hr)	2 mutants/mM-hr/10(exp. 8) survivors; Swain-Scott s-value reported as 0.96; reaction half-time with H20 about 100 minutes (Hussain, and Osterman-Golkar, 1976)
Neurospora crassa (macroconidia) mold	ad-3A (back)	0.1 to 10 min. aqueous	0.0015 to 0.15M	Under these experimental conditions, the number of mutations per 10(exp. minus 6) survivors could be expressed as 2.61 [(conc)(time)] (exp. 2.69), (Kolmark et al, 1968)
Neurospora crassa	ad-3A (back)	20 min, aqueous suspension	0.14M	Approx. 200-fold increase over spontaneous frequency under optimal conditions; unique "after effect" from ETO (Kilbey et al, 1968)

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Table 7 (Continued). Genetic and Allied Effects of Ethylene Oxide

Barley	chlorophyll (forward)	6 d Gas	80% ETO 20% Air	33-fold increased frequency decreased fertility (Ehrenberg, 1956)
Barley	chlorophyll (forward)	?	0.15% Solution	54-fold increased non-monotonic dose-response curve (Ehrenberg, 1959)
Barley	waxy (forward)	24 hr, Gas exposure of pollen	12.5 ppm	Increased mutation rate not given at 12.5 ppm. Statistically significant at 50 ppm (Lindgren et al, 1969)
Barley	chromosomal abnormalities (forward)	10 hr, aqueous	0.20%	Chromosomal aberrations observed in up to 16% of the germinated kernals (Moutschen-Dahmen et al, 1968)
Barley (pollen)	"waxy" (forward)	24 hr, gas	100 ppm	Greater than 10-fold increase in mutational frequency (Sulovska, 1969)
maize, corn	chromosome rearrangement	2 min gas	5% ETO 95% Air	Inherited chromosomal aberrations observed in 1% of the kernels (Faberge, 1955)
Herb (Pterotheca falconeri)	chromosomal abnormalities (forward)	2 hr, aqueous	45 mM.	Chromosomal aberrations observed in up to 65% of the cells (Mehra et al, 1974)
Rice	chlorophyll loci (forward)	2-12 hr, aqueous	0.1-0.6%	Up to 2% chlorophyll mutations at 12 different phenotypes (Roy et al, 1973)
Rice	chlorophyll (forward)	8 hr, aqueous	0.6% (W/V)	Mutations observed up to 14.6% of F-2 generation (Jana et al, 1975)
Rice	chlorophyll (forward)	10 hr, gaseous	0.15% (V/V)	7.2 fold increased frequency; also decrease germination and fertility (Ando, 1968)
T. aestivum wheat	visibles (forward)	aqueous 5 hr,	0.2%	Approximately 10-fold increase in mutation frequency (MacKey, 1968)
Tradescantia	chromosomal abnormalities	gaseous 5 min,	approx. 1%	Up to 5.1% chromatid breaks, "erosions," and gaps (Smith et al, 1954)

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Table 7 (Continued). Genetic and Allied Effects of Ethylene Oxide

Drosophila melanogaster Fruit fly	all sex-linked recessive lethals (forward)	Injection (Volume not given)	0.8% solution	Muller-5 Test; 9 lethals in chromosomes tested vs. 0 lethals in 494 control chromosomes (Bird, 1952)
Drosophila melanogaster Fruit fly	all sex-linked recessive-lethals; (forward)	Injection	0.10 M. 0.3-0.4 (u)	2 sex-linked recessive lethals per 1,000 chromosomes (Fahmy et al, 1956)
Drosophila melanogaster Fruit fly	"minutes" (small autosomal deletions)	Injection	0.113 M 0.25 ul/animal	Approximate 5-fold increase in frequency of "minutes" (Fahmy et al 1956)
Drosophila melanogaster Fruit fly	sex-linked lethals; translocations (forward)	Injection	0.09 M	Lethals and translocations induced in all stages of spermatogenesis (Nakao et al, 1961)
Rat	chromosomal abnormalities (forward)	Oral	9 mg/kg	Statistically significant increase (P less than .001) in aberrations in femur bone marrow cells (Strekalova, 1971)
Rat	Putative "dominant lethal"	4 hr, Inhalation	1,000 ppm	Significant increase in dead implantations in females mated to treated males at weeks 1, 2, 3 and 5 post-exposure (Embree, 1975)
Rat	---	3 d Inhalation 7 hr/d	250 ppm (450 mg/cu m)	Isochromatid and chromatid gaps and breaks in bone marrow cells sampled 24 hours after last exposure (Embree, 1975)
Human	Chromosomal abnormalities (forward)	approx. 2 hr, gaseous (inhalation & dermal?)	unknown	Exposure from an industrial spill; significant increase (p less than 0.05) in chromosomal aberrations in 7 workers 6 wk after exposure (Ehrenberg et al, 1967)