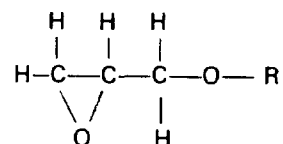


III. BIOLOGIC EFFECTS OF EXPOSURE

Although many glycidyl ethers could theoretically be synthesized, relatively few are used in industry today. The current toxicologic data on most of these ethers are incomplete, and it is therefore necessary to draw inferences based on their physical, chemical, and toxicologic characteristics to assess their potential health hazards.

All glycidyl ethers are characterized by the presence of the 2,3-epoxypropyl group and an ether linkage to another organic group. They have the generalized formula:



The monoglycidyl ethers discussed in this document can be represented by the formula B-O-R, and the diglycidyl ethers by B-(O-R)_n-O-B, where B is the 2,3-epoxypropyl group, O is oxygen, R can range from a simple alkyl to a complex hydrocarbon group, and n = 0 to 3. No polymerized forms, such as occur in cured epoxy resins, are included. Glycidyl ethers on which toxicologic data have been found are listed in Table XIV-1, with names conforming to the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC), other synonyms, and structural formulas. Physical and chemical properties of some glycidyl ethers are presented in Table XIV-2 [1-5].

The three-membered ring that oxygen forms by bridging between two adjacent carbon atoms makes up the epoxide or oxirane ring. Because this ring is highly strained, epoxide-containing compounds will react with

almost all nucleophilic (electron-donating) substances [6,7]. Ring opening will occur when the compounds are treated with halogen acids, sulfonic acids, bisulfite, thiosulfate, carboxylic acids and anhydrides, hydrogen cyanide, water, alcohols, amines, aldehydes, and the like [7]. These reactions are described in more detail in Appendix V.

Glycidyl ethers have become important because of their high reactivity. In organic synthesis, epoxides are used as chemical reagents in the manufacture of a wide variety of materials [7]. The glycidyl ethers most commonly used today include allyl glycidyl ether (AGE), n-butyl glycidyl ether (BGE), o-cresyl glycidyl ether (CGE), isopropyl glycidyl ether (IGE), phenyl glycidyl ether (PGE), resorcinol diglycidyl ether, 1,4-butanediol diglycidyl ether, alkyl or aliphatic glycidyl ethers and diphenylol propane diglycidyl ether. The last compound is the oligomer with the lowest molecular weight of the diglycidyl ether of bisphenol A, probably the most common component of uncured epoxy resins. Resorcinol diglycidyl ether is a solid; the other glycidyl ethers listed are liquids, but most have low vapor pressures at ambient temperatures. However, most reactions with glycidyl ethers occur at higher than ambient temperatures, so that the vapor pressures become appreciable.

Because of its toxicity, di(2,3-epoxypropyl) ether (also called diglycidyl ether or DGE) does not appear to be generally used outside of experimental laboratories. It is included in this document, however, because it is the simplest of the diglycidyl ethers and is therefore representative of that group of compounds.

Triethylene glycol diglycidyl ether has been used as an antineoplastic agent [8-16]. Some data concerning this glycidyl ether have

been included in this chapter to aid in relating the structures and toxicities of the various glycidyl ethers.

Extent of Exposure

The major use of the glycidyl ethers is as reactive diluents in epoxy resin systems. After all the components of an epoxy resin system have been mixed, the epoxide groups react to form cross-linkages within the resin. In a completely cured epoxy resin, glycidyl ethers no longer exist [17(p 79)]. However, because epoxy resins have such a wide range of applications, workers often must handle glycidyl ethers and the uncured resins containing them in processes like tooling and molding, manufacturing and using adhesives, roof and floor construction, and applying protective coatings [17(pp 25,153),18]. Uncured resins used in protective coatings are often applied by spraying, so that the applicators could be exposed to large quantities of vapors and mists containing glycidyl ethers. Work practices appropriate for handling glycidyl ethers should be adhered to in processes involving an uncured epoxy resin system.

Glycidyl ethers such as PGE and BGE are synthesized by adding the appropriate alcohol to epichlorohydrin in the presence of a catalyst. The intermediate chlorohydrin is not isolated and undergoes dehydrochlorination to yield a glycidyl ether [17(p 152)]. Commercial manufacture of glycidyl ethers takes place within an enclosed system, but workers may be exposed to glycidyl ethers during drumming operations at the end of the process [17(p 154)]. Very small quantities of glycidyl ethers are used for other purposes, most of which are proprietary in nature [17(pp 25,153)]; in these instances, identification of exposed workers and estimation of their extent of exposure become difficult.

NIOSH estimates that 118,000 workers in the United States are exposed to glycidyl ethers and that an additional 1,000,000 workers are exposed to epoxy resins. Occupations involving potential exposure to glycidyl ethers are listed in Table XIV-3 [17(pp 11,25,153),19-22].

Effects on Humans

The only studies found describing biologic effects on humans of the glycidyl ethers used commercially in the United States concern dermatitis, sensitization, irritation, and allergic reactions following skin contact. No studies of the effects of inhalation of any of the glycidyl ethers by humans have been found.

In 1956, Hine et al [23] reviewed the medical records of workers exposed to glycidyl ethers and of all workers requiring first-aid treatment at one plant between 1947 and 1956. Exposure to PGE involved approximately 20 workers for about 2 months each year. No worker had more than 600 hours of cumulative exposure. Exposure to AGE was at about half this rate; exposure to DGE and IGE had been limited to a few man-months, and exposure to BGE had involved about eight men for 3 months. Ten cases of occupational dermatitis resulting from exposure to AGE and 13 resulting from exposure to PGE were reported in this group of workers. No cases of dermatitis from IGE, BGE, or DGE were reported.

The symptoms and signs of dermatitis resulting from exposure to AGE were tenderness, reddening, itching, swelling, blister formation, and whitish macules [23]. In one instance, there was eye irritation from AGE vapor. The signs of dermatitis resulting from exposure to PGE were more severe, consisting of second-degree burns, blister formation, brownish

lesions, diffuse erythematous rash, erythematous vesicular rash, dry and defatted areas, watery discharge from the affected area, macular rash and papules, swelling of connective tissues, and edema.

The 10 patients with dermatitis from exposure to AGE were treated by the first-aid nurses a total of 26 times and were referred to physicians a total of 7 times [23]. The duration of treatment ranged from 1 to 8 days. The 13 episodes of dermatitis from exposure to PGE required 118 first-aid visits and were referred to physicians a total of 36 times. The duration of treatment for these complaints ranged from 1 to 56 days. Three cases of dermatitis did not respond readily to treatment, and these workers were referred to dermatologists. In most cases, the absence of immediate pain or burning resulted in a delay in initial treatment, and, in one case, the worker's failure to remove socks contaminated with PGE for several days increased the severity of the burn [23]. Four of the 23 workers with occupational dermatitis developed sensitivity reactions to AGE or PGE.

Both AGE and PGE caused irritation and sensitization, but the data presented by Hine et al [23] indicated that the effects of PGE were more persistent and less responsive to treatment. The authors stated that repeated contact with any of the compounds would probably give rise to dermatitis, although no human effects have been reported from exposure to DGE, IGE, or BGE. The severity of injury from PGE was increased when the compound was not immediately removed from contact with the skin. The authors also pointed out that the vapor of AGE was irritating to the eyes. The information presented indicates that these glycidyl ethers are potentially irritating to the eyes and skin after minimal contact and that they are probably irritating to the respiratory tract as well.

Hine and colleagues [23] noticed that they suffered from irritation of the eyes, nose, and respiratory tract when exposed to the glycidyl ethers during experiments with animals. These exposures occurred at room temperature. The investigators used AGE, BGE, IGE, PGE, and DGE in their studies, and they did not indicate which glycidyl ethers were the most irritating.

In 1965, Zschunke and Behrbohm [24] observed 15 cases of occupational dermatitis in workers exposed to PGE, which was being added to "chloroparaffins" and polyvinyl chloride as a stabilizer, in two cable-manufacturing plants. In one plant, 12 of 18 workers developed eczema. In another plant, only the three persons referred to a physician because of suspected occupational eczema were examined. In these patients, the eczema had developed on areas of the hands, the lower arms, and the right side of the abdomen, which had come into contact with cable-coating material containing PGE. Workers with abdominal skin irritation had carried large pieces of sheathing material pressed tightly against their torsos while they were feeding the cable-sheathing machine. The reddened areas contained papules and papulo-vesicles, and the patients complained of severe itching. Ten of these 15 cases of occupational dermatitis were severe enough to cause the workers to miss 11-68 days of work (mean 30.5 days). Eight of the 15 patients reacted positively to 24-hour patch tests with PGE in concentrations of 1.0-0.001% in 70% ethanol or peanut oil. The authors conducted further tests with both industrial and high-purity grades of PGE. The results of these tests were described as identical, leading the authors to exclude the possibility that impurities in the industrial-grade PGE might have been the cause of the dermatitis. The authors

believed that the concentration of PGE used might have been too low to demonstrate sensitivity in the seven workers whose patch tests were negative, since the concentration at which they were occupationally exposed was about 3%.

Patch tests with 0.01% PGE were also performed on 58 persons considered not to have been exposed previously to the glycidyl ether, and no positive reactions were observed [24]. In seven other patients with eczema who had contact with epoxy resins but no known exposure to PGE, the tests were positive. These data indicate either that the epoxy resins to which the patients were exposed contained PGE as a reactive diluent or that cross-sensitization to PGE from the reactive diluent used in the resins had occurred.

Kligman [25], in 1966, tested the sensitization potential of a number of compounds, including BGE. BGE, 1 ml of a 10% suspension in mineral oil or petrolatum, was applied to the forearms or lower legs of 25 healthy adults on a cloth patch, 1.5 inches square, that was covered with plastic tape for 48 hours. A 24-hour rest period was allowed between each of five exposures. After the final induction exposure, the subjects were challenged with 0.4 ml of 10% BGE in petrolatum on a 1-inch-square patch on the lower back or forearm for 48 hours. The author classified BGE as a strong sensitizer because 19 of 24 subjects became sensitized to it (a strong sensitizer was defined as one that sensitized 14-20 of 25 subjects) [25].

Lea et al [26] have also examined the irritating and sensitizing properties of BGE. Pure BGE was applied to the backs of five persons on cotton patches that were covered with cellophane and held in place with

adhesive tape for 48 hours, unless discomfort caused earlier removal. They developed skin irritation characterized by erythema, edema, multiple vesiculation, and superficial ulceration. When lower concentrations of the ether were tested, 17 of 25 persons (68%) reacted to a 10% suspension of BGE in petrolatum, 8 of 25 (32%) reacted to a 5% suspension, 1 of 25 (4%) reacted to a 2.5% suspension, and none of 25 (0%) reacted to a 1.25% suspension. The reactions to the various dilutions of BGE ranged from mild erythema to the severe reaction described above and demonstrated that the irritation potential was dose dependent. Two weeks after these irritancy studies were completed, sensitization tests were performed with a 1.25% suspension, the concentration previously determined to be nonirritating. The results of the patch tests were checked at 24 hours, 48 hours, and 5 days. Five of 25, or 20%, had become sensitized to BGE. The induction methods reported by Lea et al [26] were less stringent than those reported previously by Kligman [25], and the challenge concentration was lower. This probably accounts for the differences in sensitization rates reported in the two papers. This study [26] provides further evidence that the sensitizing effects as well as the irritative effects of BGE, and possibly of all glycidyl ethers, are dose dependent.

In 1964, Fregert and Rorsman [27] tested the allergenic properties of AGE, BGE, and PGE on people who were known to have contact allergies to epoxy resins of the diglycidyl ethers of bisphenol A. The test compounds were diluted to 0.25% in acetone before being used in the patch tests. The type of patch and the length of time it was left in place were not specified. The authors also performed a study to determine the concentration of PGE that could be used in a patch test without producing

primary irritation in individuals not allergic to epoxy resins, so that sensitization or allergic response could be distinguished from irritation.

Fourteen of 20 persons reacted to PGE, 3 of 20 to BGE, and 2 of 20 to AGE [27]. Four persons who reacted positively to PGE were also tested with CGE to determine whether these two glycidyl ethers, which were very similar in structure, had similar effects. All four reacted positively to CGE. Ten persons not allergic to epoxy resins were patch-tested with 1.0% PGE in acetone (a concentration at which no primary irritation occurred). Two became sensitized. The authors classified PGE as a very strong sensitizing agent. This study shows that persons exposed to epoxy resins, presumably only in the uncured state, may develop sensitivities to glycidyl ethers, and that cross-sensitization between the glycidyl ethers may occur.

Lundin and Fregert [28], in 1977, reported that 34 workers who had developed allergic contact dermatitis were patch-tested with different oligomers of the diglycidyl ether of bisphenol A. No experimental details were given. All of them developed positive reactions to the smallest oligomer (diphenylol propane diglycidyl ether), with a molecular weight of 340, but none reacted to the oligomer with a molecular weight of 624. Lundin and Fregert [28] suggested that the oligomer with a molecular weight of 340 was a stronger allergen and that the workers had been exposed to it more extensively, since it makes up a large proportion of many low-molecular-weight resins. Workers who had become sensitized to low-molecular-weight epoxy resins also had positive reactions to a resin with an average molecular weight of 1,850 [28]. Lundin and Fregert believed that these were reactions to the small amounts of the oligomer with a molecular weight of 340 that were present in the high-molecular-weight

resin. They noted that the amount of the small oligomer in this resin was usually not sufficient to induce sensitization but might be sufficient to produce a reaction in a person who had previously become sensitized.

In 1977, Malten [29] reported that diphenylol propane diglycidyl ether had been used in a standard European patch-test series since 1974. Persons suffering from eczema were patch-tested with this compound, and about 2% had positive skin reactions. Malten said that most of these people were women, but it was not clear whether this referred to the persons with eczema or only to those with positive reactions in the skin tests. In general, the causes of their sensitivity could not be identified.

In a 1976 report from Procter and Gamble Limited [30], data were presented for human sensitization to two alkyl glycidyl ethers. Procter and Gamble Epoxide No. 7 (R group predominantly C8 and C10 alkyl groups) caused sensitization at concentrations of 10% in mineral oil in "several" of 12 persons during a pilot study. Epoxide No. 8 (R group predominantly C12 and C14 alkyl groups) was tested on 57 persons. Each subject received nine induction applications of the glycidyl ether as a 10% solution in diethyl phthalate. A challenge application of the same substance 14 days later produced a questionable response in one individual. No other sensitization was reported, and another challenge on this individual and nine other subjects 6 weeks later produced no evidence of sensitization. Details of the experimental procedures were not reported in this communication. These results indicated that the C8-C10 alkyl glycidyl ether was a human skin sensitizer but the C12-C14 alkyl glycidyl ether was not, under these experimental conditions; however, the report noted that

the latter compound was considered to be a potential skin sensitizer because of positive results obtained in animal studies by Thorgeirsson et al [31].

No reports describing systemic effects in humans occupationally exposed to glycidyl ethers have been found. However, toxic side effects have been described in patients who received triethylene glycol diglycidyl ether as an antitumor agent. This substance has been used in cancer therapy in Europe and Australia since the 1960's. When triethylene glycol diglycidyl ether was administered by intravenous (iv) injection, intraarterial infusion, or bladder infusion in repeated doses totaling 75-800 mg/kg, leukopenia and bone marrow depression have been the most consistent effects noted [11-13,15,16]. In one study [16], a dose-related incidence of leukopenia was observed, with the condition occurring in 11 of 13 patients given weekly iv injections of 100 mg/kg, in 4 of 6 treated with 50 mg/kg, and in none of 6 at 10 mg/kg. Hypotension and loss of consciousness [13], drowsiness and lethargy [16], and nausea and vomiting [13,16] have also been reported. Intraarterial administration has produced edema [12,32] and hair loss [12,13,15,16] in the region of the injection, and dysuria has been reported following bladder infusion [11].

The results of the human studies indicate that the glycidyl ethers are sensitizers and irritants and that these effects are dose dependent. The relative sensitization potentials appear to be PGE and CGE > BGE > AGE. There is insufficient information to include DGE, IGE, or diphenylol propane diglycidyl ether (the diglycidyl ether of bisphenol A) in a series based on relative potencies. Systemic toxicity was also observed after high, repeated doses by intrarterial or iv infusion of triethylene glycol

diglycidyl ether. The systemic effects included nausea and vomiting, cardiovascular and bone marrow depression, hair loss, and irritation and edema. These results suggest that all of the lower-molecular-weight glycidyl ethers are irritants and sensitizers and that they may attack rapidly dividing tissues.

Epidemiologic Studies

No epidemiologic studies of workers occupationally exposed to glycidyl ethers have been found in the literature.

Animal Toxicity

Toxicologic data on only a few glycidyl ethers have been found, and much of this work has been done by the same few investigators. Studies of carcinogenicity, mutagenicity, and effects on reproduction are especially scarce.

(a) General Toxicity

Range-finding studies have provided data on the toxicities of several glycidyl ethers in various animal species. These are summarized in Table XIV-4. Hine et al [23] evaluated the effects of AGE, BGE, IGE, PGE, DGE, and, in a separate study [33], resorcinol diglycidyl ether. Smyth et al [34] and Czajkowska and Stetkiewicz [35] have also reported acute toxicity data on PGE, and Soellner and Irrgang [36] compared the toxicities of PGE and CGE. BGE and butanediol diglycidyl ether were evaluated in a study of uncured epoxy resins by Cornish and Block [32]. Weil et al [37] also tested BGE, and a study by Procter and Gamble Limited [30] compared the toxicities of BGE and of two alkyl glycidyl ethers containing alkyl radicals in the ranges C8-C10 and C12-C14. Hine et al [23,33] used Long-

Evans rats (body weight 89-150 g), Webster mice (16-22 g), and albino rabbits (2.0-3.2 kg); Smyth et al [34] and Weil et al [37] used Carworth-Wistar rats (90-120 g) and albino rabbits; Cornish and Block [32] used Sprague-Dawley rats (150-250 g) and albino rabbits; and Czajkowska and Stetkiewicz [35] used Wistar rats (280-350 g).

Toxicity was evaluated in single-dose oral studies by administering the material by gastric intubation. In dermal studies, test material was kept in contact with the shaved skin under a plastic sleeve for 24 hours [32,34] or 7 hours [23]. Soellner and Irrgang [36] administered a single subcutaneous (sc) injection of the test substances to mice. Acute inhalation hazard was evaluated by determining the longest single exposure at concentrations near saturation that permitted all animals to survive for 14 days [32,34], or by using nominal concentrations and calculating the resulting LC50's for 4-hour or 8-hour exposures [23]. Mortality during a 14-day observation period was the basis for all calculations except those of Hine et al [33] on resorcinol diglycidyl ether, which were based on mortality within 10 days of exposure.

DGE and AGE were the most toxic of the glycidyl ethers tested when administered in a single oral dose (Table XIV-4). LD50 values for other glycidyl ethers with molecular weights of less than 250 were similar; they were generally in the range of 2-4 g/kg in rats, indicating that these compounds are only slightly hazardous by this route [38]. The two alkyl glycidyl ethers (C8-C10 and C12-C14) and diphenylol propane diglycidyl ether, which have molecular weights of more than 300, were much less toxic than the other compounds.

Hine et al [23,33] also administered two of the glycidyl ethers intraperitoneally (ip). BGE administered ip to groups of five rats (121-161 g) and five mice (21-29 g) gave LD50's of 1.14 and 0.70 g/kg, respectively. These LD50 values showed a relatively small decrease (2.4 and 2.0 times, respectively) by this route, which, according to the authors, suggested ready absorption from the gastrointestinal tract [23]. However, ip administration of resorcinol diglycidyl ether gave LD50's of 0.178 g/kg in rats and 0.243 g/kg in mice, a decrease of approximately 14.5-fold in rats and 4-fold in mice, indicating that this aromatic glycidyl ether was less readily absorbed when administered orally than when injected ip [33]. Unfortunately, Hine et al [33] did not present data on the LD50 of resorcinol diglycidyl ether by percutaneous absorption. The LD50's obtained by painting glycidyl ethers on the skin of rabbits were generally similar to the oral LD50's, suggesting that these materials are readily absorbed through the skin.

In acute inhalation exposures (Table XIV-4), DGE was by far the most lethal to mice, with an LC50 of 30 ppm (160 mg/cu m), but it was nonlethal to rats at the highest concentration tested, 200 ppm (1,060 mg/cu m) [23]. BGE was more lethal to rats than to mice, while AGE and IGE showed no marked species differences; LC50's for PGE were not obtained. Hine et al [23] reported that the LC50 for PGE was greater than 100 ppm (600 mg/cu m). However, their calculations were based on a vapor pressure of 0.1 mmHg for PGE; other investigators have reported that this is an erroneous figure, the actual vapor pressure being estimated to be 0.01 mmHg at 25 C [39,40]. The latter figure yields a theoretical saturated air concentration of 13 ppm (80 mg/cu m) at 25 C. Hence, throughout this document, the

concentration of PGE vapor obtained by Hine et al is corrected to "about 10 ppm" (60 mg/cu m).

Smyth et al [34] determined that the maximum period for which rats could tolerate exposure to "concentrated" PGE vapor with no mortality was 8 hours; Cornish and Block [32] reported values for butanediol diglycidyl ether and BGE of 8 and 2 hours, respectively, and Weil et al [37] also reported 2 hours for BGE. It should be noted that the theoretical saturated air concentration for BGE at 25 C is about 4,000 ppm (21,300 mg/cu m) while that for PGE is only 13 ppm (80 mg/cu m).

(b) Dermal Effects

The irritant effects of several of the glycidyl ethers have been studied in single- and repeated-application experiments [23,30,32-35,37,41,42]. In the single-application studies, 0.5 ml of the undiluted compounds was applied to the clipped skin of albino rabbits on two abraded and two intact sites, according to the method described by Draize [43]. The test compounds were left in contact with the skin for 24 hours. In repeated-application studies, the undiluted test compounds were applied to the clipped skin of rabbits for 1 [23] or 7 hours [33]. Applications were repeated 5 days/week until maximum eschar formation or signs of systemic toxicity were noted.

Results of these skin application studies are summarized in Table XIV-5. All the glycidyl ethers tested produced moderate or severe skin irritation under these conditions. DGE was the most irritating of the compounds, and it also produced severe irritation in both rabbits and rats when the duration of skin contact was reduced to 7 hours [23,41]. It was followed in irritant potential by resorcinol diglycidyl ether [33], AGE and

IGE [23], butanediol diglycidyl ether [32], and the C12-C14 and C8-C10 alkyl glycidyl ethers [30]. BGE [23,30,32,37,42] and PGE [23,30,34], produced widely disparate degrees of skin irritation, ranging from very mild to severe, in tests by different investigators using similar methodology.

In a 1977 study designed to determine the effects of repeated exposure to airborne PGE at concentrations close to the 1976 TLV of 10 ppm (60 mg/cu m), Terrill and Lee [39] found hair loss and associated skin damage in exposed rats. Groups of 32 rats of each sex and 6 male beagles were exposed to PGE at 0 (controls), 1, 5, and 12 ppm (6, 30, and 70 mg/cu m) for 6 hours/day, 5 days/week, for 90 days. Chamber concentrations were monitored by ultraviolet analysis of impinger samples taken hourly and were determined as TWA concentrations. Animals were weighed twice weekly, and blood and urine samples were taken for analysis from 20 rats from each group and from all dogs on days 30, 60, and 90 of exposure and 30, 60, and 90 days after exposure ended. Twelve rats from each exposure group were killed at days 30, 60, and 90 of exposure and 28 days after exposure ended, and three dogs from each group were killed at the end of exposure and 90 days later, for examination of all major organs.

The only effect seen in any of the test animals was hair loss in the rats exposed at 5 and 12 ppm (30 and 70 mg/cu m), affecting 10% of the males and 25% of the females by the 90th day of exposure [39]. Microscopic examination of the skin showed inflammatory cellular infiltration of the dermis, damaged hair follicles, and hyperkeratosis. The authors concluded that these conditions were attributable to chemical irritation of the skin and not to systemic toxicity. They concurred with the observations of Hine

et al [23] that dermatitis is the principal hazard associated with exposure to PGE and suggested that a TLV of 1 ppm (6 mg/cu m) might be necessary to protect workers against skin irritation.

Several glycidyl ethers have been evaluated for their allergenic activity in skin sensitization tests. Thorgeirsson et al [31,44] and Lundin and Fregert [28] investigated the allergenicity of several glycidyl ethers using the guinea pig maximization test described by Magnusson and Kligman [45]. Groups of 10-20 guinea pigs were exposed to the glycidyl ethers in a two-stage induction process. In the first stage, test materials were administered by intracutaneous injection in a shaved area on the animal's back. Each guinea pig received three pairs of injections: (1) 0.1 ml of the test substance in propylene glycol at a dilution previously found not to cause severe irritation or serious systemic toxicity; (2) 0.1 ml of the glycidyl ether solution mixed with 0.1 ml of Freund's complete adjuvant; (3) 0.1 ml of Freund's adjuvant blended with 0.1 ml of water. Control animals were inoculated only with Freund's adjuvant, an emulsion of paraffin oil and water containing heat-killed tubercule bacteria [46], which has increased the sensitivity of guinea pigs to allergens so that it approximates that of humans [45]. In the second stage of induction, conducted 1 week later, a 2 x 4-cm piece of filter paper saturated with the 10% glycidyl ether in propylene glycol was placed on the skin of the animals, covering the original injection sites, and occluded for 48 hours. Two weeks later, the animals were challenged by applying 1 drop of the test substances, at a dilution previously found to be nonirritating, to the shaved skin of the flank, with occlusion for 24 hours [31]. Some compounds were also tested for their ability to induce

cross-sensitization by challenging with glycidyl ethers other than the one used in the induction procedure. Twenty-four hours after removal of the patches, the challenge sites were shaved and evaluated for redness and swelling.

All animals exposed to the alkyl glycidyl ether became sensitized to the substance; 75% of the test animals were cross-sensitized to an epoxy resin of bisphenol A, and 33% were sensitized to BGE and CGE [31]. Diphenylol propane diglycidyl ether (the diglycidyl ether of bisphenol A with a molecular weight of 340) produced sensitization in 80-100% of the test animals, but exposure to the oligomer of this glycidyl ether with a molecular weight of 624 produced only 56-60% positive reactions, and oligomers with molecular weights of 908 and 1,192 produced no sensitization. Of the animals sensitized to the oligomer with a molecular weight of 624, 30% reacted to that having a molecular weight of 340, but no reciprocal cross-sensitivity was observed. One animal sensitized with the oligomer having a molecular weight of 908 cross-reacted to that having a molecular weight of 624. Neopentyl glycol diglycidyl ether produced sensitization in 87% of the test animals, CGE in 75%, and butanediol diglycidyl ether in 60% [28]. Only 50% of the animals exposed to BGE had a positive response to the challenge dose of BGE, but all reacted positively to the C12-C14 alkyl glycidyl ether and 67% to CGE; none reacted to an epoxy resin of bisphenol A [31].

Thorgeirsson et al [44] also found that, although a single intradermal injection of the diglycidyl ether of bisphenol A was sufficient to sensitize 30% of the guinea pigs tested, no sensitization was produced by topical application alone. However, when the skin was pretreated with

sodium lauryl sulfate to produce irritation, 47% of the animals were sensitized, indicating that skin irritation enhanced the development of sensitization. None of the oligomers of bisphenol A studied were primary irritants; patch tests with 25% solutions of each of them caused no irritation. These data suggest that workers who come in contact with these oligomers are much more likely to become sensitized if their skins are irritated.

Weil et al [37] reported that BGE had sensitized 16 of 17 guinea pigs tested with the material 3 weeks after being given a series of 8 intracutaneous injections; PGE sensitized 1 of 18 animals in a similar test. Zschunke and Behrbohm [24] reported probable sensitization to PGE in guinea pigs induced by repeated topical applications, but they did not obtain positive reactions to PGE at low challenge concentrations.

Sensitization studies in guinea pigs have shown that all the glycidyl ethers tested that had molecular weights of 624 or less caused some degree of allergic response. The sensitizing capacity of oligomers of the diglycidyl ether of bisphenol A decreased with increasing molecular weight [44]; however, the oligomer of bisphenol A with a molecular weight of 340 (diphenylol propane diglycidyl ether) and the C12-C14 alkyl glycidyl ether were more allergenic than the low-molecular-weight glycidyl ethers tested. Thorgeirsson et al [31] postulated that one factor making the alkyl glycidyl ether a more active sensitizer than BGE was its longer aliphatic chains, which caused it to be more lipid soluble; thus, it could penetrate the skin more readily.

Although the C12-C14 alkyl glycidyl ether and diphenylol propane diglycidyl ether were the most active sensitizers, they are relatively low

in acute toxicity [30,37] and have low irritation potential [30,44]. The very limited animal toxicity data available are not sufficient for an attempted correlation of sensitization and irritation potentials. The most severe irritant, DGE, has not been examined for sensitization potential.

(c) Eye Effects

The abilities of glycidyl ethers to irritate the eyes have been evaluated in direct-application studies on rabbits [23,30,32-35,37,41,42]. The undiluted glycidyl ethers were introduced into the conjunctival sac of one eye of each animal, while the other eye served as a control. Eye irritation was scored at intervals after application by the method described by Draize [43] or by Smyth et al [34].

Results of these studies are presented in Table XIV-5. From these findings, the eye irritant potentials of the glycidyl ethers tested can be ranked in descending order as follows: DGE, AGE, butanediol diglycidyl ether, resorcinol diglycidyl ether, IGE, and the C8-C10 and C12-C14 alkyl glycidyl ethers. Irritation produced by PGE in different tests was reported to range from mild to severe, and that for BGE ranged from mild to moderate. None of the glycidyl ethers used in these tests caused permanent damage to the eye.

In 1962, Mettler et al [47] studied the effects of a 4-hour exposure to DGE at concentrations of 20-27 ppm (106-144 mg/cu m), average 24 ppm (128 mg/cu m), on intact corneal epithelium, on deepithelialized cornea, and on the regeneration of corneal tissue of 3-month-old male white rabbits. Airborne DGE vapor was produced by volatilizing the pure material at a constant rate. The rate of regeneration of corneal epithelium was measured by the time required for regeneration of an area (7-10 mm in

diameter) of cornea denuded of epithelium by trephination. In both untrephined and trephined rabbits exposed to DGE, there was an almost total loss of adhesion between the corneal epithelium and the stroma [47]. This effect was very severe, but it did not seem to increase the regeneration time of the epithelium. The exposure did produce a dense, milky opacification of the corneal stroma, resulting in permanent corneal scarring and new vessel formation. The iritis and keratitis that resulted appeared to be related to exposure, but the trauma of removal of the epithelium may also have been a factor.

Hine et al [23] also noted eye irritation from some glycidyl ethers during exposures to the airborne vapors. Corneal opacity was seen in some rats after a single 8-hour exposure to AGE and IGE at unspecified concentrations, but not in rats exposed for 8 hours to BGE, PGE, or DGE. In rats exposed to AGE for 7 hours/day, 5 days/week, corneal opacity developed in all 10 animals exposed at 900 ppm (4,200 mg/cu m) for 5 weeks, in 6 of 10 exposed at 600 ppm (2,800 mg/cu m) for 5 weeks, and in 1 of 10 exposed at 400 ppm (1,870 mg/cu m) for 10 weeks. No eye damage was reported in rats exposed to AGE at 260 ppm (1,210 mg/cu m) for 10 weeks. Slight eye irritation was also observed in rats exposed to IGE at 400 ppm for 10 weeks, but only "minimal signs" of eye irritation were observed in rats exposed to PGE at a concentration of about 10 ppm (60 mg/cu m). In another study [41], rabbits exposed to DGE for 24 hours at 3, 6, 12, or 24 ppm (16-128 mg/cu m) developed erythema and edema of the conjunctiva at all concentrations. In those exposed at 24 ppm (128 mg/cu m), corneal opacity appeared by the 3rd day.

Corneal opacity has also occurred after cutaneous applications of DGE to the shaved backs of rats at a dose of 125 mg/kg/day, 5 days/week, for 4 weeks [41]. Six such applications at 250 and 500 mg/kg/day also produced corneal opacity. Animals in this study were not caged individually, and the application sites were not covered, permitting the eyes of the animals possibly to touch the application areas on other animals. Thus, the eye effects may have resulted from direct ocular contact with DGE.

All the glycidyl ethers tested produced some degree of primary eye irritation when applied directly to the eyes. DGE, AGE, and IGE have been reported to affect the eyes of animals exposed to their airborne vapors [23,41].

(d) Systemic Effects

The toxic effects resulting from acute exposure to DGE, AGE, BGE, IGE, PGE [23], and resorcinol diglycidyl ether [33] were described by Hine et al. In the former study [23], all the compounds produced labored breathing and CNS depression when administered orally. This was preceded by incoordination, reduced motor activity, and, with BGE, by agitation and excitement. The animals were usually comatose at the time of death. Animals that survived exposure to PGE showed reversal of depression, with increased CNS activity. Watering of the eyes was noted in animals given AGE. With dermal application, signs of toxic activity were described as minimal. Depression was noted only with DGE and PGE. Death usually occurred within 17 hours, but was delayed for up to 5 days in some cases. The most frequent effect produced by inhalation of the glycidyl ethers was irritation of the lungs. Microscopic examination of stained sections showed pneumonitis. Discoloration of the liver and kidneys was frequently

noted in exposed animals, but microscopic examination of sections of these organs did not show consistent tissue changes. Focal inflammatory cells and moderate congestion were seen in the livers of some rats after administration of AGE, BGE, and IGE. Gross examination also showed hyperemia of the adrenal gland and adhesions of the stomach to adjacent tissues after oral administration.

Orally administered resorcinol diglycidyl ether also produced few evident effects [33]. The authors reported moderate CNS depression, slightly labored breathing, and, in surviving animals, loss of weight and diarrhea. Findings from gross examination of organs were described as nonspecific, with local irritation being the principal effect. There were no notable specific differences among rats, mice, and rabbits.

In a study designed to compare effects of different routes of administration of PGE, Czajkowska and Stetkiewicz [35] described the toxic effects occurring in rats as a result of single oral and dermal exposures. The organs of animals that died as a result of exposure or that were killed 6-72 hours or 14 days after exposure were examined for gross changes. Tissue samples for microscopic examination were taken from the cerebrum, cerebellum, lungs, heart, spleen, liver, kidneys, stomach, intestines, bladder, and skin.

In rats given PGE orally, deaths occurred within 6-24 hours, while those exposed dermally died after 12-48 hours [35]. Narcosis was observed in both groups. With both routes of exposure, gross and microscopic examination showed hyperemia of internal organs, especially the liver and kidneys, hemorrhages in the submeningeal and subpleural regions, and darkening of the epithelium in the kidney tubules and in liver tissue.

Rats receiving PGE orally also showed necrotic foci in the mucous membranes of the stomach. The most apparent changes from exposure by this route were in the liver. Rats dying 6-8 hours after oral administration had acute degenerative changes, including necrosis in the subcapsular region of the liver where it contacted the stomach wall; after 20-72 hours, the necrosis in this area of the liver was extensive. After 14 days, adhesion of the liver to the stomach wall was macroscopically evident; microscopically, there were necrotic foci in the subcapsular region separated from the remaining parenchyma by a fibrous band of tissue composed largely of uninuclear cells and offshoot noduli, which indicated that regeneration was occurring. After dermal application, the major changes were in the skin, which showed hyperemia and necrosis involving the subcutaneous layers. In two rats that died after 18 and 20 hours, extravasation within the peritoneal cavity indicated sites of damage to the internal organs. One of these animals had necrosis of the subcapsular region of the liver, and the other had a hyperemic and hemorrhagic loop of the small intestine. After 14 days, no effects were observed in the internal organs of the surviving rats, and the skin showed evidence of regeneration and scar formation.

The authors [35] concluded that PGE had a strong toxic effect at the site of administration, resulting in necrosis of the mucous membranes or skin, and was able to penetrate such barriers and damage underlying or contiguous tissue. They noted that systemic effects with both routes of administration included circulatory disorders resulting in hyperemia, increased permeability of the capillaries, and damage to parenchymatous organs.

These authors [35] also calculated the rate of skin absorption of PGE, using a total of eight rabbits and five rats. The material was placed in contact with the skin for 1-4 hours by one of two methods: (1) Petri dishes filled with cotton saturated with PGE were applied to the abdominal skin of rabbits, and the difference in weight of the petri dish at the beginning and end of exposure was used to calculate the absorption rate; (2) gauze saturated with 900-1,200 mg of PGE was applied to the skin of rats and rabbits, and the amount absorbed was calculated as the difference between the amount applied and the amount determined titrimetrically at the end of the experiment. In both cases, evaporation was prevented by covering the area with foil and an elastic bandage. The calculated absorption rates were 4.2 mg/sq cm/hour for rabbits and 13.6 mg/sq cm/hour for rats. Using the dermal LD50 determined in this study (2.16 g/kg), the authors calculated that a rat weighing 250 g with an exposed surface of 16 sq cm would absorb a lethal dose within about 2 hours. They postulated that, assuming 100% absorption from the lungs, a rat with a pulmonary ventilation rate of 73 ml/minute exposed to airborne PGE vapors at 0.6 mg/liter (600 mg/cu m; 100 ppm) for 8 hours would absorb 0.084 g/kg, about 1/30 of the LD50. However, it is very rare for all of an inhaled substance to be absorbed from the lungs.

Because of its low toxicity and low vapor pressure, the authors [35] concluded that PGE presents little risk from acute inhalation exposure under industrial conditions, although they cautioned that this does not apply where aerosols of PGE are released into the air. They deemed irritative effects and dermal absorption to be the major risks to workers occupationally exposed to PGE.

Effects of repeated exposures to the glycidyl ethers were also evaluated in the studies by Hine et al [23,33]. For the long-term inhalation studies [23], groups of 10 rats were exposed to vapors of AGE or IGE at 400 ppm (1,870 and 1,900 mg/cu m, respectively) for 7 hours/day, 5 days/week, for 10 weeks or to PGE on the same schedule at a concentration approaching saturation, approximately 10 ppm (60 mg/cu m). In another experiment, groups of 10 rats received, on the same schedule, exposures to AGE at 260, 600, and 900 ppm (1,210, 2,800, and 4,200 mg/cu m) [23]. Severe toxic effects made it necessary to terminate the study after 25 exposures to AGE at 600 and 900 ppm, but the group exposed at 260 ppm (1,210 mg/cu m) received 50 exposures. The rats were observed throughout the exposure period and were weighed weekly. Control groups were exposed to uncontaminated air. All survivors were killed at the end of the experiment, and blood samples were collected for hemoglobin determinations. At necropsy, lung, liver, and kidney weights were recorded, and sections of these organs and of the brain, thyroid, thymus, heart, stomach, intestine, pancreas, adrenals, testes, and bladder from alternate animals were prepared for microscopic examination.

Only AGE was lethal in this inhalation test; at 600 and 900 ppm (2,800 and 4,200 mg/cu m), 7 or 8 of 10 animals in each group died between the 7th and 21st exposures and, at 400 ppm (1,870 mg/cu m), one rat died after the 18th exposure. [23]. AGE caused decreased weight gain ($P < 0.01$) at all concentrations. At 260 ppm (1,210 mg/cu m), the only other changes observed were slight eye irritation and respiratory distress persisting throughout the exposure period. The only statistically significant change in organ/body weight ratio was that for the kidneys of the animals exposed

to AGE at 400 ppm ($P < 0.01$). Because only a few animals survived exposure to AGE at 600 and 900 ppm, statistical comparisons could not be made. Animals exposed to IGE showed slight eye irritation and respiratory distress [23]. They also had a significant decrease in mean weekly weight gains ($P < 0.01$). Concentrations of hemoglobin in the blood increased in rats exposed to all compounds except AGE, but there was no evidence that red blood cell production in the bone marrow or extramedullary hemopoietic centers had been affected.

Necropsy of rats exposed to AGE at 400 ppm (1,870 mg/cu m) showed a greater decrease in peritoneal fat than was found in rats exposed to IGE [23]. Necropsy of one rat that died after the 18th exposure revealed severe emphysema, a mottled liver, and enlarged and congested adrenal glands; emphysema, bronchiectasis, and bronchopneumonia were each seen in single rats that survived the entire exposure period. Rats exposed to AGE at 600 and 900 ppm (2,800 and 4,200 mg/cu m) had more severe abnormal changes in the lungs, including bronchopneumonic consolidation, severe emphysema, bronchiectasis, and inflammation. Necrotic spleens were found in two of the rats exposed to AGE at 900 ppm.

Weight gain in rats exposed to PGE was similar to that in controls [23]. The tissues of these rats did not differ in appearance from those of control animals, except that two rats showed peribronchial and perivascular pulmonary infiltration by inflammatory cells and "cloudy swelling" (early stage of necrosis) in their livers.

The chronic toxicity of resorcinol diglycidyl ether was also evaluated by repeated inhalation studies in rats [33]. Ten male Long-Evans rats, 80-104 g, were exposed 7 hours/day, 5 days/week, for 10 weeks to air

described as saturated with resorcinol diglycidyl ether. The authors did not report the concentration of airborne resorcinol diglycidyl ether, but since it is a solid at room temperature, the concentration at saturation in air would probably be low. Ten control rats were exposed to uncontaminated air. The only toxic effect observed in exposed rats was slight encrustation of the eyelids of some animals. No gross or microscopic lesions were found, and exposed animals did not differ significantly from controls in weight gain or organ weight/body weight ratios.

Soellner and Irrgang [36] reported that CGE and PGE had antispasmodic and muscle relaxant effects in animals. These glycidyl ethers were 3-40 times more effective than their corresponding glycerol ethers in relieving spasms induced in guinea pig small intestine with barium chloride, acetylcholine, or histamine. Muscle relaxant effects were investigated in revolving drum tests with mice. By sc injection, the minimum effective doses that caused mice to lose their ability to remain in the drum were 430 mg/kg for PGE and 390 mg/kg for CGE, indicating only slight muscle relaxant effects.

Kodama et al [48] and Hine et al [41] investigated effects on the hemopoietic system in animals exposed to glycidyl ethers. In the first study [48], groups of five male Long-Evans rats weighing 151 ± 32 g received intramuscular (im) injections of BGE, PGE, or AGE at 400 mg/kg/day or DGE at 25 mg/kg/day. Rats that served as negative controls received injections of propylene glycol at 230 mg/kg/day, and positive control animals received a single im injection of a known alkylating agent, either busulfan at 10 mg/kg or mechlorethamine hydrochloride at 0.5 or 5 mg/kg. Blood samples were analyzed, and sections of bone marrow, lungs, liver, kidneys,

adrenals, thymus, spleen, and testes were examined microscopically as well.

Since both BGE and PGE had minimal toxic effects, and the leukocyte counts in the rats rose rather than fell after three injections [48], no further work was done with these compounds. Rats that received four injections of AGE had swelling at the injection site and lost weight [48]. Two rats died, and post-mortem examination showed pulmonary congestion in one and a small spleen and no visible thymus in the other. The three surviving rats had involuted thymuses at necropsy. Microscopic examination showed atrophy or loss of lymphoid tissue, focal necrosis of the pancreas and testes, hemorrhage into the thymus, hemorrhage into the periphery of the liver, and pneumonia. The leukocyte count was significantly reduced in all animals. Bone marrow contained a normal number of nucleated cells, but the myeloid-to-erythroid ratio was low. The rats that received six injections of DGE gained weight normally, and none died. Edema at the injection site was the only grossly observable effect. The leukocyte count was significantly decreased. Bone marrow was not examined in these animals.

Negative controls gained weight normally and showed no signs of intoxication, but their mean leukocyte count rose almost 40% [48]. The positive control animals that received busulfan continued to gain weight, and none died. The mean leukocyte count, the number of nucleated marrow cells, and the ratio of myeloid to erythroid cells were decreased. Animals that received mechlorethamine lost weight, and three died. Nucleated marrow cells and the myeloid-to-erythroid ratio decreased.

The absence of hemopoietic effects with BGE and PGE was considered by the authors [48] to indicate that monofunctional alkylating agents are

considerably less active than polyfunctional alkylating agents. The activity of AGE was attributed to the reactive sites on the double-bonded carbon rather than to the epoxide moiety.

In a more extensive study of the effects of DGE on the hemopoietic system, Hine et al [41] administered the compound by several routes to three species of animals, using both single and repeated exposures. General chronic toxicity of the compound was also evaluated in rats exposed to the vapor at low concentrations. Male Long-Evans rats (115-145 g) received single and repeated cutaneous applications to their shaved backs and repeated vapor exposures. Male New Zealand rabbits (1.9-4.2 kg) were given single applications on their shaved backs, single and repeated iv injections, and single vapor exposures. A total of 14 mongrel dogs were administered the material by im or iv injections. Hematologic examinations were the same as those used by Kodama et al [48].

Single cutaneous applications of DGE at 0.5 and 1 g/kg to groups of five rats each and of 1.13 g/kg to four rabbits produced a reduction in the leukocyte count and weight loss in all three groups [41]. The rabbits showed a decrease in hemoglobin concentration, and one died on day 11. Repeated cutaneous applications of 125 mg/kg, 5 days/week, for 4 weeks killed two of five rats. Six applications of 250 or 500 mg/kg in 11 days killed four rats and produced weight loss, enlarged myeloid cells, reductions in number of leukocytes and increases in percentages of polymorphonuclear cells, hemorrhage of the adrenal medulla, increased myeloid-to-erythroid ratios among the nucleated cells of the bone marrow, corneal opacity, and swollen forepaws. Necrosis was seen in microscopic examination of sections of the skin, proximal convoluted tubules, lymphoid

tissue, and testes of these rats. Focal necrosis of the pancreas and lymphoid atrophy of the thymus were found in rats exposed at 500 mg/kg.

A second 4-week series of cutaneous applications to rats was conducted with DGE, 10% in acetone [41]. This series resulted in focal inflammation of the epithelium in animals given 15, 30, and 60 mg/kg. In animals given 30 and 60 mg/kg, weight gain was retarded, and there was a decrease in the percentage of polymorphonuclear cells but no decrease in total leukocyte counts. According to the authors, 15 mg/kg appeared to be the no-effect level for repeated cutaneous applications.

Groups of three rabbits were exposed by inhalation for a single 24-hour period to DGE at 3, 6, 12, or 24 ppm (16, 32, 64, or 128 mg/cu m) [41]. Body weights, leukocyte counts, and percentages of polymorphonuclear cells were checked weekly for 3 weeks after exposure. Corneal opacity appeared by the 3rd day in rabbits exposed at 24 ppm. Two of these rabbits died on the 5th day, the third died on the 7th day, and all three lost 30-35% of body weight before they died. There were increases in total leukocytes and percentage of polymorphonuclear cells prior to death; thrombocytosis was noted on the 3rd day. Necropsies on the two rabbits that died first showed purulent lungs with pericardial adhesions in one and peribronchiolitis in the other; both had atrophied testes. Microscopic examination revealed bronchopneumonia, serous hepatitis, focal atelectasis, peribronchiolitis, and focal hemorrhages in lungs and kidneys in one animal or the other. Some basophilia at 6 ppm and possibly increased thrombocyte counts at 12 ppm were seen. Conjunctival erythema and edema with respiratory distress and nasal discharge were seen in all groups.

Rats exposed to DGE 3 or 4 times at 20 ppm (106 mg/cu m) for 4 hours lost weight, and 3 of 30 rats died [41]. Lung edema and congestion were seen in two that died and in one of the survivors. Blood changes seen in the rats included intense cytoplasmic basophilia, grossly distorted lymphocytic nuclei with indistinct cellular membranes, and lowered leukocyte and marrow cell counts.

In chronic inhalation experiments, groups of 30 male rats each were exposed to DGE at nominal concentrations of 3 and 0.3 ppm (16 and 1.6 mg/cu m), 4 hours/day, 5 days/week, for 19 exposures in 29 days and 60 exposures in 90 days, respectively [41]. The authors reported that the actual concentrations in the higher-exposure experiments ranged from 1.3 to 2.5 ppm (7-13 mg/cu m); for exposure at 0.3 ppm, the true value was estimated on the basis of "occasional" analysis to vary within ± 0.2 ppm (± 1 mg/cu m).

Five rats died during exposure at 3 ppm (16 mg/cu m) [41]. One had bronchopneumonia and necrosis of the pancreas and spleen and another had pneumonia. After the final exposure, 15 rats were killed and examined; autopsy showed one rat with necrosis of the testicular tubules and one with inflammation of the larynx. Seven of the 15 experimental animals and 4 of 10 unexposed controls had peribronchiolitis. The exposed animals differed significantly from controls ($P < 0.05$) in the following criteria: decreased body weight and organ weight/body weight ratios of thymus and spleen; decreased leukocytes, polymorphonuclear cells, and marrow nucleated cells; increased erythrocytes and myeloid-to-erythroid ratio; and increased mortality. The other 10 rats were killed 1 year after exposure; their weight gain and blood and bone marrow findings were within the expected normal range. One had acute inflammation of the large bronchi and

"atypical epithelium of neoplastic appearance"; three had peribroncholitis; and one had fatty dystrophy of the liver.

None of the rats exposed to DGE at 0.3 ppm died (1.6 mg/cu m) [41]. One case of pneumonia was the only abnormality in 10 rats killed for autopsy after 20 exposures. After 60 exposures, 5 of 10 rats examined had "poorly defined" focal degeneration of the germinal epithelium and 1 had acute periobronchiolitis. Exposed animals had reduced weight gain and lower leukocyte counts than controls, but the differences were not significant. The blood of two animals showed eosinophilia in over half the polymorphonuclear cells. The remaining 10 experimental and 10 control rats were killed 1 year after exposure ended. Two control rats and one experimental rat had bronchopneumonia, and one reticulum-cell sarcoma was reported in an experimental rat. All blood values were normal.

Hine et al [41] concluded that exposure to DGE at 3 ppm (16 mg/cu m) depressed the hemopoietic system in rats, but that exposure at 0.3 ppm (1.6 mg/cu m) did not. They noted that testicular necrosis occurred at both exposure levels. It is difficult to evaluate the significance of the damage to the testes seen at 0.3 ppm from the description provided by the authors, but it is noteworthy that this effect was seen in 5 of 10 animals after 60 exposures at this low concentration. The authors considered that the bronchopneumonia might be related to the regimen forced upon the rats, but they concluded that the "possible neoplasms" were not attributable to exposure to DGE.

Hine et al [41] also administered DGE by iv injection to dogs and rabbits. Two of three dogs receiving weekly injections of 25 mg/kg died of pneumonia; one had loss of bone marrow with fat replacement after three

injections, and the other had massive infarction in the lungs, slight glycogen degeneration of the renal tubules, and hyaline degeneration of the testicular tubules after three injections. All three dogs at this dose showed significant decreases in leukocytes ($P < 0.01$). Three dogs given injections of 12.5 mg/kg showed no gross signs of systemic toxicity, but irritation at the injection site occurred in two. Leukocyte counts decreased in all three but returned to normal after 1-5 weeks. These dogs were killed for autopsy after one to three series of three injections. Their bone marrows were normal, and the only abnormalities noted were hemorrhage into the spleen in one and "possible testicular atrophy" in another.

Rabbits given four weekly iv injections of DGE at 25 mg/kg had slight decreases in leukocyte counts [41]. Higher iv doses, 50-200 mg/kg, caused decreases in leukocyte counts, severe lung congestion, kidney ischemia, ascites, and death.

The most consistent systemic effects reported in animals exposed to glycidyl ethers have been in rapidly dividing tissues, ie, the bone marrow [23,41,48] and the germinal epithelium of the testes [33,41,48,49]. At higher doses, glycidyl ethers have produced more severe tissue damage; irritation, congestion, and necrotic changes were observed in many organ systems, generally appearing first at or near the site of administration [23,35,41,48].

(e) Carcinogenesis, Mutagenesis, Teratogenesis, and Effects
on Reproduction

Investigations of carcinogenic activity have been found only for DGE, resorcinol diglycidyl ether, hydroquinone diglycidyl ether, diphenylol

propane diglycidyl ether, and triethylene glycol diglycidyl ether. Many glycidyl ethers have been assessed for their ability to induce mutations and chromosomal aberrations, but studies of teratogenic and reproductive effects are scarce.

In 1957, McCammon et al [50] tested a number of compounds thought to be present in the air pollutants that result from the oxidation of aliphatic hydrocarbons in gasoline and diesel fuels. Twenty compounds were evaluated for their tumorigenic potentials in C57B1 mice by painting on the interscapular skin three times/week. In addition, Long-Evans rats received three of the compounds by sc injection. The authors reported that resorcinol diglycidyl ether was tumorigenic in both mice and rats. DGE was said to be tumorigenic only in mice, but the authors did not indicate whether it was tested in rats. These compounds also produced sebaceous gland suppression, intense hyperkeratosis, parakeratosis, and epithelial hyperplasia in mice. Because this report was an abstract, details of the study were not given. One of the authors (HL Falk, personal communication, May 1978) has indicated that the data from this study have been lost, but that the tumors produced were benign papillomas.

In a 1963 report on the tumorigenic potential of selected epoxides, Kotin and Falk [51] provided additional information on the tumorigenicity of glycidyl ethers in mice [50]. Twenty C57BL mice were used in each treatment group. In the animals exposed to DGE at a total dose of 0.75 millimole in acetone, the first tumor appeared after 5 months; 4 of the 10 animals (40%) surviving at this time developed skin tumors. One mouse (8%) in the group exposed to DGE at 0.25 millimole and 1 of 14 surviving mice (7%) exposed to resorcinol diglycidyl ether at 0.75 millimole developed

skin tumors. Resorcinol diglycidyl ether at 0.25 millimole and hydroquinone diglycidyl ether at 1 millimole caused no skin tumors in any of the mice. No malignant lymphomas or pulmonary adenomas were produced by any of these diepoxides. In a written communication (January 1978), Falk noted that the skin tumors produced by the glycidyl ethers in this study were all benign papillomas and that controls receiving only acetone did not develop any papillomas.

In 1963, Weil et al [37] tested the effects of diphenylol propane diglycidyl ether on mice in a lifetime carcinogenicity study. The compound was tested in trials on two groups of mice by painting the undiluted compound on the shaved backs of 90-day-old C3H mice three times/week. Up to 40 mice were used in each trial, but the exact number was not specified. The mice were painted for up to 23 months. Positive controls were treated similarly with a 0.2% solution of methyl cholanthrene in acetone. At the end of 12 months, 26 mice from one trial and 36 from the other were still alive. At the end of 17 months, 14 and 26 remained alive, and at the end of 24 months, 1 and 0 were alive. No carcinomas were found in these mice; the only tumor, a papilloma, appeared in one group after 16 months of exposure. The positive control substance produced an unspecified number of tumors in mice, with a mean latent period of 3-5 months.

Shimkin et al [52], in 1966, reported the results of a study designed to show the carcinogenic potential of several alkylating agents, including triethylene glycol diglycidyl ether. Mice of the A strain received 12 ip injections, 3 times/week for 4 weeks, at 5 different total doses ranging from 56 to 7,208 mg/kg. Each group contained 15 mice of each sex. During the experimental period, untreated controls were maintained and killed at

monthly intervals to determine the incidence of spontaneous pulmonary tumors. An additional control group received only the vehicle (water) by ip injection. The duration of the experiment was 39 weeks.

A slight increase in lung tumors over the expected spontaneous incidence was observed (37% or a mean of 0.48 tumors/mouse for males, 27% or 0.29 tumors/mouse for females) [52]. At the highest dose (7,208 mg/kg), the tumor incidence was 70%, with 1.2 tumors/mouse. At total doses estimated by the authors to be below 3,777 mg/kg, the tumor incidence decreased to expected spontaneous levels. The spontaneous incidence was estimated from a mathematical relationship between the logarithm of the number of lung tumors and the logarithm of the dose that best represented the point at which one lung tumor/mouse would be predicted. The authors concluded that triethylene glycol diglycidyl ether was only weakly carcinogenic at the highest doses used. However, this study lasted for only about 9 months, whereas assays of carcinogenic potential with this strain commonly are conducted for 18-20 months. The authors also reported that testicular atrophy with decreased spermatogenic activity was seen in mice 39 weeks after treatment with this compound at high doses.

Cytotoxic effects on mammalian bone marrow cells have been observed with AGE and DGE [41,48]. Studies with other cell types have also shown cytotoxic effects of glycidyl ethers. Loveless [6] found that treating root-tip meristems of the broad bean, Vicia faba, with DGE or resorcinol diglycidyl ether produced radiomimetic effects. He defined a radiomimetic agent as one that acted upon the resting cell to produce chromosomal aberrations apparent in subsequent cell divisions. Other studies have also

demonstrated chromosomal aberrations produced by DGE in the broad bean [53,54] and other plant species [55].

Certain glycidyl ethers have damaged mammalian tumor cells. Triethylene glycol diglycidyl ether has been used therapeutically as an antitumor agent [11-13,15,16]. Hendry et al [56] have shown tumor inhibition and radiomimetic effects of diethylene glycol diglycidyl ether and butanediol diglycidyl ether in an in vivo study. Rats were implanted with Walker tumors, and the compounds to be tested were injected ip during a 10- to 12-day period after tumor implantation. According to the authors, there was a correlation between tumor inhibitory activity of the glycidyl ethers and the ability to induce chromosomal changes of the radiomimetic type in the implanted tumors. Diethylene glycol diglycidyl ether at a total ip dose of 1.5 mg/g inhibited tumor growth by 84% compared with that in controls. At a daily dose of 0.4 mg/g, some inhibition of mitosis was seen in the tumor as well as in the bone marrow. "Exploded" metaphases were seen in the tumor and chromosome fragmentation and pyknotic nuclei were seen in the bone marrow at this dose. At 0.2 mg/g/day, there was almost complete inhibition of mitosis with a few chromosome fragments, but only partial inhibition of mitosis with some pyknotic nuclei was seen in the bone marrow. A dose of 0.1 mg/g/day caused a few pyknotic nuclei in the bone marrow. Tumors in rats exposed to diethylene glycol diglycidyl ether at doses of 0.4 and 0.1 mg/g/day showed an increased number of anaphases (over control values) after 24 hours, indicating that the compound caused specific chromosomal damage in tumor tissue in rats with the Walker tumor.

Butanediol diglycidyl ether at a total dose of 1.2 mg/g caused a 74% inhibition in tumor growth compared with controls [56]. Doses of 0.2 mg/g/day caused "exploded" metaphases in the tumor and true chromosome bridges and chromosome fragmentation in the bone marrow. At a dose of 0.1 mg/g/day, no cytotoxic effects were observed in the tumor. However, chromosome bridges and fragmentation were found in the bone marrow. After 24 hours, an excess number of anaphases with chromosome damage in tumor tissue was noted at both daily dose levels.

In a study from EI du Pont de Nemours and Company [49], Terrill reported no increase in chromosomal aberrations in the bone marrow cells of rats exposed to PGE at concentrations up to 11.2 ppm (68.8 mg/cu m) 6 hours/day for 19 consecutive days.

Wade et al [57] examined the mutagenicity of AGE, BGE, DGE, diphenylol propane diglycidyl ether, and the diglycidyl ether of substituted glycerin with Salmonella typhimurium. They used the histidine-dependent mutant strains TA98, which is reverted to histidine independence by frameshift mutation, and TA100, which is reverted by base-pair substitution. The compounds were tested with and without the addition of liver microsomal extract from rats pretreated with phenobarbital. A substance was considered mutagenic in this test if it produced histidine-independent revertants at two or more times the spontaneous rate.

When 10 mg of the glycidyl ether was applied to the center of agar plates containing bacteria of the TA100 strain, AGE and BGE caused mutations at over 10 times the spontaneous rate, and the diglycidyl ether of substituted glycerin increased the mutation rate about 4 times [57].

Diphenylol propane diglycidyl ether showed no mutagenic activity at this dose. None of these four glycidyl ethers produced an increase in mutations when 50 μg was spotted on the agar plates. DGE was toxic to bacteria even at this low dose, reducing the number of revertant colonies/plate to below spontaneous levels. When DGE was incorporated directly into the medium in quantities of 50-500 $\mu\text{g}/\text{plate}$, it produced a dose-dependent mutagenic effect in strain TA100, with the highest dose inducing mutations at about 10 times the spontaneous rate. Addition of the liver microsomal extract generally produced a decrease in the mutagenic activity of DGE, to about 5 times the spontaneous rate at the highest dose. Liver microsomes had little effect on the mutagenic activity of the other glycidyl ethers tested. Results of these tests are summarized in Table XIV-6.

The glycidyl ethers did not show mutagenic activity in strain TA98, indicating that they act by causing base-pair substitutions [57]. Since diphenylol propane diglycidyl ether and two higher-molecular-weight oligomers of this compound (the diglycidyl ether of bisphenol A) were nonmutagenic, the authors suggested that the size of these molecules may have inhibited uptake by the bacteria or caused decreased rates of reaction with genetic material because of steric hindrance. This view is supported by the fact that glycidyl ether of the next-highest molecular weight, the diglycidyl ether of substituted glycerin (molecular weight 300), induced fewer revertant colonies than AGE or BGE (molecular weights 114 and 130).

In a 1977 report prepared for Dow Chemical USA by Pullin and Legator [58], the mutagenic potential of BGE, CGE, the C12-C14 alkyl glycidyl ether, neopentyl glycol diglycidyl ether, diphenylol propane diglycidyl

ether, and dicyclopentadiene glycidyl ether were examined. The mutagenicity testing program evaluated the compounds in six microbial and mammalian test systems:

(1) The microbial mutagenic assay (Ames test) determined activity in reverting histidine-requiring mutant strains of S. typhimurium to histidine independence. The compounds were tested at 0.5-2.0 μ moles/plate, both with and without a microsomal extract from the livers of rats pretreated with phenobarbital or Aroclor.

(2) In the body-fluid analysis, the urine of mice treated with the glycidyl ethers was tested for mutagenic activity against S. typhimurium both with and without the addition of beta-glucuronidase. The mice received the glycidyl ethers orally in doses of 125-1,000 mg/kg/day for 4 days before urine was collected for testing.

(3) The host-mediated assay is designed to determine the effects of in vivo metabolism of a compound on its mutagenicity. Mutant strains of S. typhimurium were injected into the peritoneal cavity of mice that had been given glycidyl ethers in oral doses of 125-1,000 mg/kg/day for 5 days. Six hours after inoculation, exudate was withdrawn from the peritoneal cavity and plated in serial dilutions to determine the frequency of mutations to histidine independence.

(4) In the micronucleus test, the bone marrow from mice that had received glycidyl ethers orally for 5 days was examined microscopically for the presence of micronuclei.

(5) To study the induction of DNA repair, glycidyl ethers were incubated at 37 C with human mononucleated white blood cells (G-0 phase) and tritiated thymidine. Cells were analyzed for incorporation of tritiated thymidine by liquid scintillation counting and autoradiography.

(6) The dominant lethal assay tested mutagenic effects of glycidyl ethers on the reproductive cells of mice. Male B6D2F1 hybrid mice, 8-10 weeks old, were bred to three virgin females each week for 2 weeks to provide baseline information on the fertility of each male, litter size, and spontaneous fetal deaths. The male mice were then treated topically with undiluted glycidyl ethers on their shaved and chemically depilated backs three times/week for a minimum of 8 weeks. Groups of 10 male mice of proven fertility received BGE, CGE, or neopentyl glycol diglycidyl ether at 1.5 g/kg, the alkyl glycidyl ether or dicyclopentadiene glycidyl ether at 2.0 g/kg, or diphenylol propane diglycidyl ether at 3 g/kg. Two other groups were treated with saline, as a negative control, or with triethylene-melamine, as a positive control. The exposed mice were caged individually with three 8- to 10-week-old virgin females each week for 2 weeks. All females were killed for examination of their uteri 13-14 days after presumptive mating. The percentage of pregnancies, total number of implants, and number of fetal deaths were used as criteria of dominant lethality.

Results of these tests are summarized in Table XIV-6. All of the glycidyl ethers tested showed some activity in the Ames test with S. typhimurium strain TA1535, which is reverted by base-pair substitution, but not with strain TA98 [58]. One glycidyl ether was minimally active in the body-fluid analysis, and three showed some activity in the host-mediated assay, which the authors attributed to decreased growth of microorganisms in the host animals. Three diglycidyl ethers produced an increase in unscheduled DNA synthesis in human white blood cells, but none produced excess micronuclei in the bone marrow cells of mice.

Only BGE was significantly mutagenic to mice in the dominant lethal test, causing a significant increase in the number of fetal deaths ($P=0.04$) [58]. The number of pregnancies was significantly less than in the control group ($P=0.05$), but pretreatment data also showed significantly fewer pregnancies in the test group than in the controls. In the Ames test, BGE produced mutations at 4-13 times spontaneous rates, and its mutagenic activity was markedly decreased by the addition of microsomes. BGE also caused a significant increase ($P<0.05$) in unscheduled DNA synthesis in white cells. The authors classed this compound as mutagenic and suggested that the lack of activity of BGE in the body-fluid analysis, host-mediated assay, and micronucleus test might have resulted from the low doses used in these tests. Since BGE was detoxified by mammalian microsomes in the microbial assay but was apparently not deactivated by metabolism in the dominant lethal test, they concluded that the metabolic properties of the liver homogenate did not "truly reflect the complex and dynamic metabolic processes of an intact animal." The authors emphasized that BGE posed a hazard through percutaneous absorption, a common route of exposure for the worker. However, the dosage of BGE used in the dominant lethal test was very high.

CGE and neopentyl glycol diglycidyl ether were classified as weakly mutagenic on the basis of these test results [58]. CGE was the most mutagenic of the compounds in the Ames test, producing mutations at up to 58 times the spontaneous rates, but it was deactivated to control levels in the presence of microsomes. Neopentyl glycol diglycidyl ether caused mutations in S. typhimurium at up to 7 times the spontaneous rate, and addition of microsomes had no consistent effect on its activity. In the

body-fluid analysis, both compounds had minimal mutagenic effects only in the presence of betaglucuronidase. Both caused significant unscheduled DNA synthesis in human white blood cells ($P < 0.05$).

Dicyclopentadiene glycidyl ether and diphenylol propane diglycidyl ether were mutagenic in bacteria but not in animal systems [58]. In the absence of mammalian microsomes, they produced mutations in S. typhimurium at about 2-4 times the spontaneous rate; effects of adding microsomes were inconsistent, but diphenylol propane diglycidyl ether at 2.0 μ moles/plate was activated by liver microsomes from Aroclor-pretreated rats. Diphenylol propane diglycidyl ether also increased mutation frequencies in the host-mediated assay. The authors described these two glycidyl ethers as minimally mutagenic in humans.

The C12-C14 alkyl glycidyl ether was minimally active in the microbial assay only in the presence of microsomes from Aroclor-pretreated rats [58]. It also showed minimal activity in the host-mediated assay. The authors classified this glycidyl ether as nonmutagenic.

Results of these screening tests [58] suggest that all these glycidyl ethers have some mutagenic potential. Only BGE was reported to be a mammalian mutagen on the basis of the results of the mouse dominant lethal test. However, only a single negative control group of 10 rats was used in this test, and several of the test groups differed significantly from controls in pretreatment data for the criteria used as indicators of dominant lethality. Despite these shortcomings in experimental design, there were significant differences ($p = .04$) between the control groups and the BGE-treated group in the proportion of deaths/pregnancy.

In a 1974 study from EI du Pont de Nemours and Company [49], Barsky reported mutagenicity tests of PGE in S. typhimurium. PGE was tested at concentrations of 25-300 $\mu\text{g}/\text{plate}$ without rat liver homogenate and 500-10,000 $\mu\text{g}/\text{plate}$ in the presence of the homogenate. PGE was mutagenic in strains TA1535 and TA100 both with and without metabolic activation, but it showed some increase in activity in the presence of the liver homogenate (Table XIV-6). At the highest concentration used in the activated assay, PGE produced mutations in strain TA100 at nearly 70 times the spontaneous rate. No mutagenic activity was observed in strains sensitive to frameshift mutation.

In the same laboratory report [49], Terrill described a two-generation reproduction and mutagenesis study in rats exposed to PGE vapor. Three groups of eight male ChR-CD rats (360 g) were exposed to PGE at 1.75, 5.84, or 11.20 ppm (10, 33, or 71 mg/cu m) 6 hours/day for 19 consecutive days. A fourth group of rats served as controls. The male rats were mated for 6 consecutive weeks to three females/week. One of each group of three females was killed on the 18th day of pregnancy and examined for implantations, resorptions, and any abnormalities of the ovaries, uterus, or fetuses. The two remaining females were allowed to raise their pups, which were then paired for mating, and the offspring of these rats were also examined for abnormalities. Exposed males were killed for autopsy after the mating trials, and the testes and epididymides were examined microscopically. Eight first-generation offspring of each sex were also killed for autopsy.

No significant increases in fetal deaths or preimplantation loss were seen in females bred to mice exposed to PGE, indicating that PGE did not

produce dominant lethal mutations [49]. The only abnormality noted in the autopsies was focal degeneration of the seminiferous tubules in 1 of 8 rats exposed at 1.75 ppm (10 mg/cu m), 1 of 8 at 5.84 ppm (33 mg/cu m), and 3 of 8 at 11.20 ppm (71 mg/cu m). Personnel evaluating these slides felt that the evidence of degeneration was inconclusive and might have resulted from improper sectioning. Statistical analysis of the incidence of testicular atrophy using the Fisher exact test showed no significant treatment-related effect in any exposed group. The author concluded that the testicular effects were not treatment-related. However, since testicular degeneration has also been reported in animals exposed to DGE at low concentrations or to AGE, DGE, or triethylene glycol diglycidyl ether at high doses [41,48,52], the effects seen in this study [49] may be related to exposure to PGE.

The teratogenic potential of PGE was also evaluated by Terrill in this study [49]. Four groups of 25 female ChR-CD rats (200 g) were exposed to PGE vapor at 1.7, 5.7, or 11.5 ppm (10, 35, or 71 mg/cu m) for 12 days, beginning on the 4th day of gestation. No abnormal signs were observed in the exposed females. They were killed on the 20th day of gestation, and the corpora lutea and fetuses were enumerated. All the fetuses were examined for visible defects. Two-thirds of the fetuses were fixed and cleared and their skeletons stained in situ to show any variations and anomalies of ossification. The other fetuses were fixed and sectioned for examination. There were no significant differences between the control group and experimental groups in maternal body weight, mortality, early delivery, gross pathology, implantation efficiency, fetal survival, size, sex, ossification variations, or malformations. The author [49] concluded

that, under the test conditions, PGE was not teratogenic.

Of the few glycidyl ethers that have been investigated for their carcinogenicity, only two were demonstrated to produce an increased incidence of tumors in animals. Triethylene glycol diglycidyl ether injected ip at very high doses produced an excess of lung tumors in mice [52]. DGE at a concentration of 0.75 millimole produced papillomas in 4 of 10 mice when painted on the skin 3 times/week in a lifetime study [50]. In similar skin painting tests, resorcinol diglycidyl ether [50] and diphenylol propane diglycidyl ether [37] each produced only one papilloma in 14 and 40 mice, respectively, and hydroquinone diglycidyl ether [50] produced no tumors.

All glycidyl ethers that have been tested have shown mutagenic activity in bacteria [49,57,58]. Data from these studies permit the compounds to be ranked in descending order approximately as follows on the basis of their activity in the Ames assay: CGE and DGE > BGE > PGE > neopentyl glycol diglycidyl ether > dicyclopentadiene glycidyl ether > the diglycidyl ether of substituted glycerin > diphenylol propane diglycidyl ether > the C12-C14 alkyl glycidyl ether. The four most active compounds showed reduced mutagenic activity in the presence of a mammalian liver homogenate, a 10-fold reduction in the case of CGE; the two least mutagenic compounds showed a slight increase in activity when the liver homogenate was added, and the activity of the other glycidyl ethers was generally unaffected.

Only one glycidyl ether, BGE, was mutagenic in mammals in the dominant lethal test [58]. BGE also induced unscheduled DNA synthesis in human white blood cells, as did CGE and neopentyl glycol diglycidyl ether.

(f) Metabolism

Little is known about specific pathways for catabolism of glycidyl ethers. Since glycidyl ethers contain the epoxide ring, it seems reasonable to assume that they have common pathways with other epoxide compounds. Glycidyl ethers are highly reactive in biologic systems. One demonstration of such activity is a short biologic half-life. Duncan and Snow [59] injected rats iv with 300 mg/kg of triethylene glycol diglycidyl ether. After 1 minute, less than 10% of the dose could be found in the blood, and its metabolic half-life was calculated to be about 12 minutes. Only 0.4% of the administered dose was excreted unchanged.

Three types of metabolic reactions have been proposed for epoxide compounds [60]. These are shown in Figure III-1.

Two of these conversions are enzymatic. Oesch et al [60-63] have isolated an enzyme that they called epoxide hydrase from the livers of various species of animals, including rats, guinea pigs, monkeys, and humans. The enzyme reduced epoxides to their corresponding diols. BGE and PGE were among the glycidyl ethers acted upon by epoxide hydrase. Soellner and Irrgang [36] presented evidence that CGE was metabolized to its corresponding diol, which was apparently more neurotoxic than the parent compound.

The second enzymatic reaction is a conjugation of epoxides with glutathione. Glutathione-S-epoxide conjugase has been isolated from the livers of rats and ferrets [64] and of several wild birds [65]. Boyland and Williams [64] reported activity with PGE, substituted PGE's, resorcinol diglycidyl ether, 1-naphthyl glycidyl ether, and 4,4'-bisglycidyl bisphenyl ether. Wit and Snel [65] reported conjugation of glutathione with PGE.

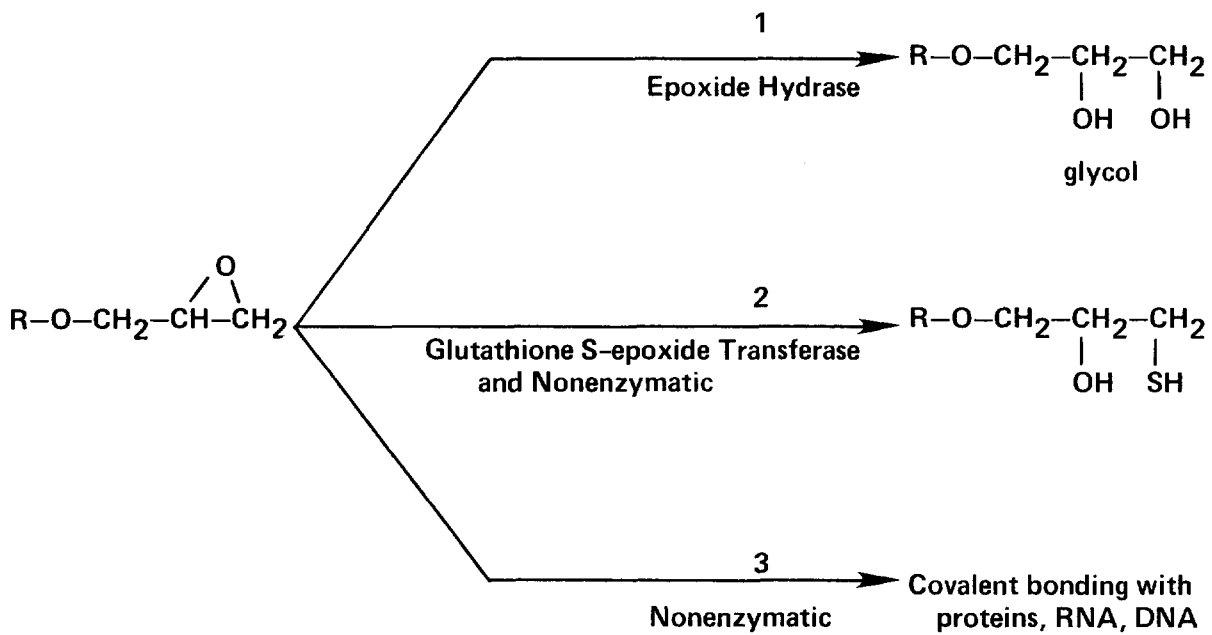


FIGURE III-1

PROPOSED METABOLIC PATHWAYS FOR GLYCIDYL ETHERS

Adapted from reference 60

Mukhtar and Bresnick [66] demonstrated that pretreatment of rats with 3-methylcholanthrene and phenobarbital enhanced glutathione-S-epoxide conjugase activity by 40%-60%.

The nonenzymatic reactions of epoxides are covalent bonding to proteins [60]. Loveless [6] has proposed a mechanism that would explain the high degree of biologic activity demonstrated by epoxides. He suggested that the "strained" character of the epoxide ring caused it to undergo an SN1 type of reaction in which the ring opened under the polarizing influence of a reactant, forming a carbonium ion which then reacted with water, proteins, or such nucleophilic compounds as RNA, DNA, histones, or proteins. The bulk of the available evidence on humans occupationally exposed to glycidyl ethers indicates that these substances reacted with skin proteins, giving rise to a contact skin sensitivity [24-28,30]. In animal experiments, only when large amounts contaminated the skin or were absorbed into the body was there evidence that glycidyl ethers reacted with nuclear elements to induce hemopoietic effects, mutations, or neoplasms [11-13,15,37,54,58,67].

Correlation of Exposure and Effect

Adverse effects reported in humans exposed to glycidyl ethers have generally been limited to irritation and sensitization. PGE [23] and BGE [26] have produced severe skin irritation in humans, causing vesiculation, blistering, burns, and ulceration. The response to BGE was dose-dependent, with no irritation observed at 1.25%. AGE has produced skin irritation and eye irritation in humans [23].

Sensitization tests in humans with glycidyl ethers have been positive for all compounds tested, including PGE [24], BGE [25,26], and the C8-C10 alkyl glycidyl ether [30]. Cross-sensitization to CGE has occurred in humans sensitive to PGE, and sensitivity to AGE, BGE, and PGE has been demonstrated in humans occupationally exposed to epoxy resins of bisphenol A [27].

In patients treated with the antitumorigenic drug triethylene glycol diglycidyl ether, CNS effects, leukopenia, bone marrow depression, and regional edema and hair loss have been reported as side effects of therapy [12,13,16]. These systemic effects occurred following iv or intraarterial injection of repeated doses, and no comparable effects have been reported after occupational exposure to other glycidyl ethers.

Several glycidyl ethers have produced irritation and sensitization in animals. All the glycidyl ethers tested (DGE, AGE, IGE, PGE, BGE, resorcinol diglycidyl ether, butanediol diglycidyl ether, and diphenylol propane diglycidyl ether) were skin irritants in tests on guinea pigs, ranging from mild to very severe [28,31,44]. In addition, all of those tested for skin sensitization (BGE, PGE, CGE, the C12-C14 alkyl glycidyl ether, diphenylol propane diglycidyl ether, neopentyl glycol diglycidyl ether, and butanediol diglycidyl ether) gave positive results [24,28,30,31,37,44]. Eye irritation in animals resulted from exposure to airborne AGE, IGE, and DGE [23,41,47] and from direct instillation of these compounds or of PGE, resorcinol diglycidyl ether, butanediol diglycidyl ether, or the C8-C10 and C12-C14 alkyl glycidyl ethers [23,30,32-35,37].

In animals, glycidyl ethers have produced CNS effects, including muscular incoordination, reduced motor activity, agitation and excitement,

deep depression, narcotic sleep, and coma [23,33,35]. The route of administration plays an important role in the onset, duration, and severity of CNS effects. Each of the following, DGE, AGE, BGE, IGE, PGE, and resorcinol diglycidyl ether, produced CNS depression when administered orally [23,33,35], whereas only DGE and PGE [23,35] produced depression with dermal administration; after inhalation exposures, CNS depression was reported to have occurred immediately before death, appearing earlier only with BGE and AGE [23]. The progression of signs was usually from muscular incoordination and reduced motor activity to moderate depression (and, with BGE, agitation and excitement) to deep depression and coma before death. Animals that survived exposure to PGE showed a reversal of the progression [23]. CGE at very high doses has had antispasmodic and muscle relaxant effects in animals [36].

Many of the glycidyl ethers produced widespread systemic effects, such as necrosis, edema, inflammation, hyperemia, hemorrhaging, and tissue degeneration. The most frequent effect produced by inhalation of DGE, AGE, BGE, IGE, or PGE was lung irritation, specifically pneumonitis [23]. Rats exposed to AGE at 400 ppm (2,000 mg/cu m) for 7 hours/day, 5 days/week, for 10 weeks had abnormal changes in the lungs, such as severe emphysema, bronchiectasis, and bronchopneumonia; those exposed to PGE at about 10 ppm (50 mg/cu m) on the same schedule had peribronchial and perivascular pulmonary inflammatory cell infiltration [23]. Resorcinol diglycidyl ether did not produce any lung anomalies in rats exposed to an airstream saturated with it for 7 hours/day, 5 days/week, for 10 weeks; the concentration of airborne resorcinol diglycidyl ether was not reported, but it would have been very low, since this glycidyl ether is a solid at room

temperatures [33]. No gross changes were noted in the lungs of rabbits exposed to DGE at 3, 6, or 12 ppm (16, 32, or 64 mg/cu m) for 24 hours. At 24 ppm (128 mg/cu m) for 24 hours, DGE caused purulence in the lungs, with pericardial adhesions, peribronchiolitis, bronchopneumonia, focal atelectasis, and focal hemorrhages in rabbits. DGE also caused pneumonia and massive infarction in the lungs of one of three dogs injected iv at a dose of 25 mg/kg [41]. Intramuscular injections of 400 mg/kg of AGE produced pulmonary congestion in one rat after the second daily injection; microscopic examination confirmed pneumonia [48].

The effects of glycidyl ethers in organ systems were primarily irritation and necrosis. Local and widespread inflammation, congestion, and necrosis resulted after exposure of rats to DGE, AGE, IGE, PGE, and BGE by the oral, inhalation, or dermal routes [23,35,41,48]. The organs and tissues affected were the adrenal gland, liver, lungs, stomach, kidneys, brain, skin, peritoneum, small intestine, thymus, spleen, lymph nodes, testes, and pancreas.

Circulatory system disorders were also evident in animals exposed to PGE and included hyperemia and increased permeability of the capillaries [35]. AGE given by im injection to rats produced significantly reduced leukocyte counts and a decreased myeloid-to-erythroid ratio, although the number of nucleated cells in the bone marrow and the percentage of polymorphonuclear cells was normal [48]. Animals given im injections of IGE, PGE, and BGE did not show evidence of hemopoietic changes [23,48].

In rabbits, DGE produced decreases in leukocyte counts and percentages of polymorphonuclear cells at iv doses of 50, 100, or 200 mg/kg [41]. By inhalation, DGE at 24 ppm (128 mg/cu m) caused an increase in

leukocytes and polymorphonuclear cells prior to death; thrombocytosis was also noted. At DGE concentrations of 12 ppm (64 mg/cu m), thrombocyte counts were increased, and at 6 ppm (32 mg/cu m), some basophilia was seen. In rats, three or four exposures to DGE at 20 ppm (110 mg/cu m) for 4 hours produced intense cytoplasmic basophilia, grossly distorted lymphocytic nuclei with indistinct cellular membranes, and lowered leukocyte and marrow cell counts. Long-term exposures of rats for 4 hours/day, 5 days/week to DGE at 3 ppm (16 mg/cu m) for 19 exposures in 29 days caused decreases in leukocyte counts, polymorphonuclear cells, and marrow nucleated cell counts. Blood cell morphology was normal in rats exposed at 0.3 ppm (1.6 mg/cu m) for 20 exposures, but over half the polymorphonuclear cells of two rats contained eosinophilic granules after 60 exposures.

A summary of the effects of dermal contact with glycidyl ethers on humans is presented in Table III-1. A summary of the effects of exposure to glycidyl ethers on animals is presented in Table III-2.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

No reports of the carcinogenic, mutagenic, teratogenic, or reproductive effects of the glycidyl ethers on humans were found in the literature. However, such effects have been investigated in animals for some of the glycidyl ethers.

The carcinogenic potentials of DGE, resorcinol diglycidyl ether, diphenylol propane diglycidyl ether, and hydroquinone diglycidyl ether have been studied in skin painting tests on animals [37,50,51]. No malignant tumors have been observed with any of these compounds. DGE at a total dose of 0.75 millimole produced skin papillomas in 4 of 10 surviving mice

painted with the compound 3 times/week, with the first tumor appearing after 5 months; DGE at 0.25 millimole produced a papilloma in one test animal [51]. Resorcinol diglycidyl ether was said to be carcinogenic in both mice and rats, but no supporting data were provided [50]. A later paper by the same investigators showed that resorcinol diglycidyl ether had induced a benign tumor in 1 of 14 test animals at a dose of 0.75 millimole and produced no tumors at 0.25 millimole [51]. Undiluted diphenylol propane diglycidyl ether painted on the skin three times/week produced 1 papilloma in 40 surviving mice after 16 months [37]. Hydroquinone diglycidyl ether at a dose of 1 millimole caused no skin tumors in mice [51].

Triethylene glycol diglycidyl ether, which has been used as an antitumor agent, has been found to be carcinogenic in mice at very high ip doses [52]. A total dose of 7,208 mg/kg over a 4-week period produced a 70% incidence of lung tumors. The authors calculated that the lowest dose that would raise the incidence of tumors above spontaneous levels was greater than 3.7 g/kg.

Several glycidyl ethers have produced effects described as radiomimetic or cytotoxic. As in the case of triethylene glycol diglycidyl ether, diethylene glycol diglycidyl ether and butanediol diglycidyl ether have had antitumorigenic effects [56]. Diethylene glycol diglycidyl ether injected ip at a total dose of 1.5 mg/g caused an 84% inhibition of implanted Walker tumors in rats. This compound produced chromosomal aberrations and inhibition of mitosis in the tumor cells and bone marrow at doses of 0.1-0.4 mg/g/day. Butanediol diglycidyl ether at a total dose of 1.2 mg/g caused a 74% inhibition in tumor growth. At 0.2 mg/g, this

compound caused chromosomal aberrations in tumor and bone marrow cells, and at 0.1 mg/g, the only cytotoxic effects seen were chromosome bridges and fragmentation in the bone marrow.

Exposure to AGE and DGE caused decreased leukocyte counts attributed to cytotoxic effects on the bone marrow in rats, rabbits, and dogs [41,48]. In the same study [48], BGE and PGE did not produce a decrease in leukocytes when administered to rats by ip injection. Rats exposed to PGE by inhalation at up to 11.2 ppm (68.8 mg/cu m), 6 hours/day for 19 days, had no increase in chromosomal aberrations in the bone marrow [49]. None of six glycidyl ethers tested, including BGE and CGE, produced micronuclei in the bone marrow cells of mice [58]. DGE and resorcinol diglycidyl ether have produced radiomimetic effects in plant cells [6,53-55].

Several glycidyl ethers have been tested for mutagenic activity (see Table XIV-6). AGE, BGE, CGE, DGE, PGE, neopentyl glycol diglycidyl ether, dicyclopentadiene diglycidyl ether, and the diglycidyl ether of substituted glycerine all produced a mutagenic response in Salmonella typhimurium in the Ames assay [49,57,58]. Diphenylol propane diglycidyl ether gave weakly positive results in this test in one study [58] and negative results in another [57]. The C12-C14 alkyl glycidyl ether showed weak mutagenic activity only when activated by the addition of a rat-liver microsomal extract [58]. Metabolism by mammalian microsomes decreased the activity of BGE, CGE, DGE, and PGE and had little or no effect on the mutagenicity of the other compounds tested [49,57,58]. None of the compounds tested showed definite mutagenic activity in the host-mediated assay test [58].

Urinary metabolites of CGE and neopentyl glycol diglycidyl ether caused a weak mutagenic response in Salmonella in the mouse body-fluid analysis, but other glycidyl ethers were not active in this test [58]. These two compounds and BGE also induced unscheduled DNA synthesis in human mononucleated white blood cells [58]. Only BGE has shown mutagenic activity in mice in the dominant lethal test [58]. When this compound was painted on the skin of male mice at 1.5 g/kg, it produced a significant increase in the number of fetal deaths in females to which they were subsequently bred.

Only PGE has been studied for its teratogenic effects, and it produced no teratogenesis in the offspring of female mice exposed at 11.5 (68.8 mg/cu m) ppm during days 4-15 of gestation [49].

Testicular degeneration has been noted in several animal species after exposure to AGE, DGE, PGE, and triethylene glycol diglycidyl ether [41,48,49,52]. Necrosis of the testes was reported in rats that received six dermal applications of DGE at 250 or 500 mg/kg [41] or four im injections of AGE at 400 mg/kg [48]. Testicular atrophy with decreased spermatogenic activity was seen in mice receiving high ip doses of triethylene glycol diglycidyl ether [52]. Atrophied testes were found in two rabbits that died after a single 24-hour exposure to DGE at 24 ppm (128 mg/cu m) and possibly in a dog that received six 12.5 mg/kg iv doses of this compound [41]. In chronic inhalation experiments, 1 of 15 rats exposed to DGE at 3 ppm (16 mg/cu m) had necrosis of testicular tubules after 19 exposures; 5 of 10 rats exposed at 0.3 (1.6 mg/cu m) had "poorly defined" focal degeneration in the testes after 60 exposures [41]. In rats exposed to PGE at 1.75-11.2 ppm (10-71 mg/cu m) for 19 days, focal

degeneration of the seminiferous tubules was observed in 5 of 24, but the investigator considered that this damage was of questionable significance and was probably not treatment related [49]. Only in the PGE study was any attempt made to correlate testicular damage with effects on reproduction, and this study showed no significant mutagenic or reproductive effects [49].

Lack of data on most of the glycidyl ethers makes it difficult to correlate variations in the results of tests of their mutagenicity and carcinogenicity with differences in the structure of particular compounds within the class. Thus, only tentative conclusions can be drawn about the potential of glycidyl ethers to cause cancer or mutations.

Because of the presence of epoxide groups, the glycidyl ethers would be expected to be biologically active; epoxides have been shown to be mutagenic and carcinogenic [37,56], and epoxide intermediates have been identified or postulated as the mutagenic or carcinogenic metabolites of other compounds [68]. However, the limited data available on metabolism of glycidyl ethers indicates that they are rapidly metabolized to less cytotoxic substances [36,59,60]. They conjugate readily with proteins, and are thus active skin sensitizers, but the available evidence indicates that effects that might result from conjugation with nuclear macromolecules occurred only at very high dose levels, when detoxification mechanisms may have been overwhelmed.

Because diglycidyl ethers include twice as many of the hypothetically active epoxide moieties, they might be expected to have greater carcinogenic or mutagenic potential than monoglycidyl ethers. All 10 compounds that have been tested showed mutagenic activity in bacterial

tests [49,57,58]. The most active mutagens, however, were monoglycidyl ethers. The quantitative difference in activity and the varying effects produced by the addition of mammalian liver microsomes suggest differences in metabolic pathways for the glycidyl ethers. Their mutagenic activity was also variously affected by test systems involving in vivo mammalian metabolism. BGE, a monoglycidyl ether, was the only glycidyl ether shown to be a mammalian mutagen in the mouse dominant lethal test, and it was not the most active compound in bacterial tests; BGE was also partially deactivated by mammalian microsomes in vitro and showed no mutagenic activity in the body-fluid test, host-mediated assay, or micronucleus test [58]. Since the only data found on tumorigenicity testing concerned diglycidyl ethers, no direct evidence is available on their activity relative to that of monoglycidyl ethers. However, Weil et al [37] found that 5 of 17 diepoxide compounds tested were tumorigenic to mice, while none of 11 monoepoxides were. They concluded that the "currently prevalent" generalization that diepoxides are carcinogenic was not supported. Because no generalizations about the carcinogenic hazards of working with epoxy compounds could be made from the existing data, they emphasized that each compound must be individually tested for its carcinogenic potential.

Shimkin et al [52] have pointed out certain structural factors that may affect the carcinogenic potential of alkylating agents such as the glycidyl ethers. They suggest that those that are stable enough to survive the transfer to a susceptible organ and that structurally resemble a naturally occurring substrate tend to be the most active.

Such diversity in results after testing various monoglycidyl and diglycidyl ethers only serves to emphasize the necessity to avoid making generalizations regarding the potential of an individual glycidyl ether to be mutagenic or carcinogenic. However, these findings, together with studies indicating that DGE, and possibly resorcinol diglycidyl ether and neopentyl glycol diglycidyl ether, can produce skin tumors [50,51], indicate that gross skin contact with glycidyl ethers may represent an important hazard to worker health. Because of their low vapor pressures, most of these compounds are unlikely to be present in workplace air at concentrations sufficient to permit their reaching the nuclei of somatic or reproductive cells and causing neoplastic or mutagenic effects. However, because of their demonstrated mutagenicity, the glycidyl ethers should, in the absence of adequate carcinogenicity test data on individual compounds, be regarded as potentially serious hazards.

TABLE III-1

EFFECTS OF SKIN CONTACT WITH GLYCIDYL ETHERS ON HUMANS

Compound	Exposure Concen- tration	Exposure Duration	Effects	Reference
AGE	-	300 hr	Dermatitis in 10/20	23
BGE	-	3 mo	Dermatitis in 0/8	23
"	100%	48 hr	Severe irritation in 5/5	26
"	10%	-	Positive patch-tests with 10% BGE in 19/24	25
"	10%	48 hr	Irritation in 17/25; posi- tive patch tests with 1.25% BGE in 5/25	26
"	5%	"	Irritation in 8/25	26
"	2.5%	"	Irritation in 1/25	26
"	1.25%	"	Irritation in 0/25	26
DGE	-	a few mo	No dermatitis reported	23
IGE	-	"	"	23
PGE	-	600 hr	Dermatitis in 13/20	23
"	3%	-	Dermatitis in 12/18; posi- tive patch-tests with 0.001-1% PGE in 8/15 with dermatitis	24

TABLE III-2

EFFECTS OF EXPOSURE TO GLYCIDYL ETHERS ON ANIMALS

Compound	Effect	Species	Dose or Exposure Concentration	Duration	Route of Exposure	Reference
AGE	Death	Rats	2,800 mg/cu m	5 wk	inhalation	23
"	"	Mice	1,260 mg/cu m	4 hr	"	23
"	Decreased weight gain	Rats	1,210 mg/cu m	10 wk	"	23
"	Lung damage	"	1,870 mg/cu m	10 wk	"	23
"	Testicular degeneration	"	400 mg/kg/d	3-4 d	im	48
"	Decreased leukocytes	"	"	"	"	48
"	Skin irritation	Rabbits	Undiluted	-	dermal	23
"	Eye irritation	"	"	-	ocular	23
"	"	Rats	1,200 mg/cu m	10 wk	inhalation	23
BGE	Death	"	5,500 mg/cu m	8 hr	"	23
"	Dominant lethal	Mice	1,500 mg/kg/d	24 d	dermal	58
"	Increased leukocytes	Rats	400 mg/kg/d	3 d	im	48
"	Sensitization	Guinea pigs	10%	8 d	dermal	31
"	Skin irritation	Rabbits	Undiluted	-	dermal	23,30,32,42
"	Eye irritation	"	"	-	ocular	23,32
CGE	Death	Mice	980 mg/kg	1 dose	sc	36
"	Muscle relaxation	Rats	390 mg/kg	"	"	36
"	Sensitization	Guinea pigs	5-25%	-	dermal	28

TABLE III-2 (CONTINUED)

EFFECTS OF EXPOSURE TO GLYCIDYL ETHERS ON ANIMALS

Compound	Effect	Species	Dose or Exposure Concentration	Duration	Route of Exposure	Reference
DGE	Death	Mice	160 mg/cu m	4 hr	inhalation	23
"	"	Rabbits	128 mg/cu m	24 hr	"	41
"	"	Rats	106 mg/cu m	3-4 d	"	41
"	"	"	200 mg/kg/d	6 d	dermal	41
"	Organ damage	Rabbits	128 mg/cu m	24 hr	inhalation	41
"	Lung damage	Rats	106 mg/cu m	3-4 d	"	41
"	Testicular degeneration	Rabbits	128 mg/cu m	24 hr	"	41
"	"	Rats	1.6 mg/cu m	60 d	"	41
"	Weight loss	Rabbits	128 mg/cu m	24 hr	"	41
"	"	Rats	106 mg/cu m	3-4 d	"	41
"	"	"	250 mg/kg/d	6 d	dermal	41
"	Decreased weight gain	"	32 mg/kg/d	6 d	"	41
"	Decreased leukocytes	"	16 mg/cu m	19 d	inhalation	41
"	"	"	500 mg/kg	1 dose	dermal	41
"	"	"	200 mg/kg d	6 d	"	41
"	Skin tumors	Mice	0.25 mM	8 wk	"	41
"	Skin irritation	Rabbits	Undiluted	-	"	41
"	Eye irritation	"	"	-	ocular	23
"	"	Rats	250 mg/kg/d	6 d	dermal	41

TABLE III-2 (CONTINUED)

EFFECTS OF EXPOSURE TO GLYCIDYL ETHERS ON ANIMALS

Compound	Effect	Species	Dose or Exposure Concentration	Duration	Route of Exposure	Reference
IGE	Death	Mice	7,130 mg/cu m	4 hr	inhalation	23
"	"	Rats	5,230 mg/cu m	8 hr	"	23
"	Decreased weight gain	"	1,900 mg/cu m	10 wk	"	23
"	Respiratory distress	"	"	"	"	23
"	Skin irritation	Rabbits	Undiluted	-	dermal	23
"	Eye irritation	"	"	-	ocular	23
"	"	Rats	1,900 mg/cu m	10 wk	inhalation	23
PGE	Death	Rabbits	3,000 mg/kg	1 dose	dermal	23
"	Organ necrosis	Rats	2,200 mg/kg	"	"	35
"	Muscle relaxation	"	430 mg/kg	"	sc	36
"	Lung irritation	"	60 mg/cu m	10 wk	inhalation	23,39
"	Testicular degeneration	"	10 mg/cu m	19 d	"	49
"	Increased leukocytes	"	400 mg/kg/d	3 d	im	24
"	Sensitization	Guinea pigs	Undiluted	7 d	dermal	24
"	Skin irritation	Rats	70 mg/cu m	3 mo	inhalation	39
"	"	Rabbits	Undiluted	-	dermal	23,34, 35
"	Eye irritation	"	"	-	ocular	23,34, 35
"	"	Rats	60 mg/cu m	10 wk	inhalation	23
Alkyl glycidyl ether (C12-C14)	Sensitization	Guinea pigs	5-25%	-	dermal	28

TABLE III-2 (CONTINUED)

EFFECTS OF EXPOSURE TO GLYCIDYL ETHERS ON ANIMALS

Compound	Effect	Species	Dose or Exposure Concentration	Duration	Route of Exposure	Reference
Butanediol diglycidyl ether	Death	Rats	1,130 mg/kg	1 dose	dermal	32
"	Bone marrow cytotoxicity	"	100 mg/kg	"	ip	56
"	Tumor cell inhibition	"	1,200 mg/kg (total dose)	-		56
"	Sensitization	Guinea pigs	10%	-	dermal	28
"	Skin irritation	Rabbits	Undiluted	-	"	32
"	Eye irritation	"	"	-	ocular	32
Diethylene glycol diglycidyl ether	Bone marrow cytotoxicity	Rats	100 mg/kg	1 dose	ip	56
"	Tumor cell inhibition	"	1,500 mg/kg (total dose)	-		56
Diphenylol propane diglycidyl ether	Death	Rabbits	22,000 mg/kg	1 dose	dermal	37
"	Sensitization	Guinea pigs	5%	-	"	7
"	Skin irritation	Rabbits	Undiluted	-	"	37
"	Eye irritation	"	"	-	ocular	37
Resorcinol diglycidyl ether	Death	"	-	7 d	dermal	33
"	Skin tumors	Mice	0.75 mM	8 wk	"	51
"	Skin irritation	Rabbits	Undiluted	-	"	33
Triethylene glycol diglycidyl ether	Lung tumors	Mice	7,200 mg/kg (total dose)	-	ip	52
"	Testicular degeneration	"	-	-	"	52