III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Epichlorohydrin (formula weight 92.53) is a colorless liquid at room temperature with a distinctive odor described as "ethereal," "chloroform-like," and "garlic-like." [1] Its boiling point is 116.4 C and the vapor pressure at 29 C is 20 mmHg. [2] The low latent heat of vaporization of epichlorohydrin (9,060 cal/mole) [3] contributes to the rapid evaporation of spills. Other physical properties are given in Table XIII-1. [2,4,5]

Epichlorohydrin is a reactive molecule which may form covalent bonds under biologic conditions. [6,7] In reactions with alcohols, amines, thiols, and other nucleophilic biochemical constituents of the cell, the epoxide ring opens and forms a new, stable, covalent carbon-hetero atom bond as shown in the reaction in Figure XIII-1. The initial reaction product may undergo a second nucleophilic reaction to form stable, covalent cross-linking bonds between two molecules either by direct displacement of the chlorine atom or through the formation of an unstable, short-lived cyclic intermediate. [8] These cross-linking bonds may have a high degree of chemical stability, such as is typically found in epoxy resins. [4] The chemical characteristic of epichlorohydrin to react as a bifunctional alkylating agent is an intrinsic property of its molecular structure.

Biologic organisms contain many different chemical nucleophiles, such as alcohols, acids, amines, sulfhydryls, carbohydrates, lipids, proteins, ribonucleic acids, and deoxyribonucleic acids. [9] Epichlorohydrin has a greater tendency to react with more readily polarized groups, such as sulfhydryl groups, than with less readily polarized groups, such as

hydroxyls. The half-life of epichlorohydrin in water at a pH of 7 is 36.3 hours. [6] Although the half-life of epichlorohydrin in biologic tissues is not known, the known presence of large numbers of nucleophiles in tissue suggests that it is shorter than the half-life in water. Mammalian enzymes which catalyze the hydrolysis of epichlorohydrin, or otherwise degrade it, would further decrease the biologic half-life. Under hypothetical, constant, nonlethal exposure conditions, a steady-state concentration of epichlorohydrin would eventually be achieved in all tissues, resulting in a steady-state rate of alkylation of tissue constituents. For reviews of the pertinent literature, the reader is referred to Ross [6] and Loveless. [7]

Extent of Exposure

Epichlorohydrin is commercially synthesized from allyl chloride, [12] allyl alcohol, [12] dichlorhydrin-glycerine, [11,12] or propylene. [13] The total US production of epichlorohydrin was about 550 million pounds in 1975. [14] Projected expansion plans, if accomplished, would increase total production to 715 million pounds by 1978. [14]

In 1969, the total US production of crude epichlorohydrin was about 322 million pounds. [12] An estimated 58% of this was used in the manufacture of synthetic glycerine and 42% was processed to refined epichlorohydrin. [12] Refined epichlorohydrin is used in the manufacture of epoxy resins, surface active agents, pharmaceuticals, insecticides, agricultural chemicals, textile chemicals, coatings, adhesives, ion-exchange resins, solvents, plasticizers, [15] glycidyl esters, [16] ethynyl-ethylenic alcohol, [17] and fatty acid derivatives. [16] Most

epoxy resin is synthesized by alkylating bisphenol A with epichlorohydrin. [4]

A number of cases of dermal sensitization have been reported in workers in the epoxy resin-producing and resin-using industries. [18-20] Attempts to identify the causative agents have been only partially successful. [18-20] Traces of free epichlorohydrin have been found in the resin in the USSR [21] and Sweden (S Fregert, written communication, September 1975); however, a few case reports have indicated that epichlorohydrin itself is probably not the responsible chemical. [19,22]

In the United States, a hazard survey was conducted by NIOSH around electronic component-molding operations where several different epoxy resins were used. [23] Most of these resins were made from bisphenol A and epichlorohydrin and had to be heated prior to use. Environmental monitoring using charcoal-tube and gas chromatography indicated that epichlorohydrin was not present in the air in any detectable quantity. The lowest limit of detection was 0.008 mg or approximately 0.02 mg/cu m (0.005 ppm).

NIOSH estimates that 50,000 employees may be exposed to epichlorohydrin in the United States.

Historical Reports

The first report on epichlorohydrin toxicity was made by Von Kossa [24] in 1898. He found that, following dermal contact, epichlorohydrin induced transitory burning and slight irritation in human subjects. One person with sensitive skin developed an eczema lasting 3 weeks following a 1-hour application of 0.5 g of epichlorohydrin. Von Kossa [24] also

investigated the effects of epichlorohydrin on a dog, rabbits, pigeons, and frogs to evaluate its usefulness as an anesthetic agent. A dog was reported to have received a total of 381 g of epichlorohydrin within 7 days by an unspecified route and to have died on the eighth day. A swollen liver and edema of the superficial abdominal tissues were observed. Subcutaneous injection of 1.16 g of epichlorohydrin into a rabbit produced labored breathing, cyanosis, narcosis, and death in about an hour. At autopsy, emphysematous and hyperemic lungs were evident. Daily subcutaneous injections of 0.15 g of epichlorohydrin were administered to another rabbit for 7 days, and the animal died on the eighth day. Hemorrhages in the stomach, especially in the fundus, along with a seared appearance of the adjacent mucous membrane in the stomach were observed at autopsy. An unspecified amount of albumin was detected in the urine. When a cloth soaked in 5 cc of epichlorohydrin was placed next to a pigeon in a jar, sneezing, labored breathing, body trembling, narcosis, and death resulted within an hour. Another pigeon injected intramuscularly with 0.07 g of epichlorohydrin on 2 consecutive days died on the second day. Autopsy revealed edema at the injection site, plethoric meningeal blood vessels, intestinal inflammation, and hemorrhage. The dorsal lymph sacs of two frogs were each injected with 0.15 g of epichlorohydrin. Exophthalmos and irregular respiration were observed. Although apparent recovery occurred within an hour after injection, both frogs died about 5 hours later. A third frog, whose heart had been exposed, was injected similarly with 0.15 g of epichlorohydrin. The cardiac rate decreased from 40 to 34 beats/minute within 20 minutes, and the animal died within 6 hours of epichlorohydrin administration. Von Kossa [24] believed that the severe

irritating action of epichlorohydrin on the gastrointestinal tract would make it unsuitable as an anesthetic. He also indicated that its strong depressant effects on the heart and respiration would make it unsatisfactory for any use in medicine. The narcotic and the persisting dermal effects of epichlorohydrin, as well as the effects on respiration, liver, and gastrointestinal tract reported by Von Kossa [24] in 1898, have been confirmed repeatedly by other investigators. [25-32] These effects are further discussed in Animal Toxicity.

Effects on Humans

In 1964, Schultz [33] reported the case of a 39-year-old temporary worker exposed to a gust of epichlorohydrin from a presumably empty tank. He immediately experienced irritation of the eyes and throat, followed by facial swelling, nausea, vomiting, headache, and dyspnea. The following day, he went to the clinic where his liver was found to be enlarged. Two days after the exposure, a slight jaundice associated with a serum bilirubin of 3.44 mg% was observed. The accepted range of normal values for the concentration of bilirubin in serum is 0.1-1.2 mg%. [34] After 18 days, his jaundice had subsided, but his liver was still slightly enlarged. Five months later, the same hospital conducted a medical evaluation of the man; the major findings were bronchitic alterations in the right lung, elevated blood pressure, and liver damage. Liver function was still abnormal, as indicated by elevated amounts of urobilin and urobilinogen in the urine. Eight months later, the man still had a serum bilirubin of 2.6 mg% and abnormal amounts of urobilin and urobilinogen in the urine. Nearly 2 years after the accident, the author [33] examined this worker. A liver

biopsy indicated fatty changes, while delayed sulfobromophthalein (BSP) elimination, increased galactose excretion, and increased concentrations of urinary bile pigments indicated altered liver function. Hypertension (degree not reported) and chronic asthma-like bronchitis also were present. The author reported that other possible causes of liver damage, such as nutritional defects, alcoholism, diabetes, tuberculosis, and prior liver disease, were explored and ruled out. Although the author concluded that the liver damage and the asthma-like bronchitis were induced by epichlorohydrin poisoning, a possibility remains that the liver damage had some other cause. Hypertension was judged to be independent of the epichlorohydrin exposure. No renal damage was found. It is evident from this report [33] that irritation of eyes and throat, facial swelling, nausea, vomiting, headache, and dyspnea are the immediate effects of acute overexposure to epichlorohydrin on humans. However, it is not possible to draw any general conclusions on chronic effects resulting from an acute epichlorohydrin inhalation from this report on a single subject.

A case of a 53-year-old worker who was exposed for about 30 minutes to fumes of epichlorohydrin from a leaking condensor was briefly reported by Thoburn (written communication, May 1976). Several hours after the exposure, the worker complained of burning of his nose and throat, cough, and chest congestion. Other symptoms included running nose, eye tenderness, and headache followed by nausea. The man was hospitalized briefly. The symptoms faded within 3 to 4 days, without definite sequelae except that the man reported that he had more frequent infections of the upper respiratory tract than he had experienced previously. He also complained that these infections were often followed by productive

after the exposure, measurements indicative of air trapping in the lungs (increase of residual volume by 40% and arterial pO2 of 77 mm of Hg, instead of the normal 95 mm) were made. While the report is indicative of the hazards associated with acute exposures to high but unknown concentrations of epichlorohydrin, quantitative conclusions cannot be drawn from the available information.

In 1970, Ippen and Mathies [35] reported burns resulting from dermal contact with epichlorohydrin in five male workers, aged 19-32 years. On separate occasions, two workers came into contact with mixtures of epichlorohydrin and methanol. Two days after exposure, one worker noticed redness of the hands. By the 3rd day, the symptoms had intensified so that he went to the clinic. He had severe redness, swelling, and red papules on both hands. Under treatment with corticoid ointments and tablets of 3-pyridine methanol, a vasodilator drug, the redness and swelling subsided so gradually that he did not return to work until 22 days after the accident. More than 2 months (73 days) after the accident, he still had a red discoloration of the hands and erythema at the sites of the papules. The second worker wiped up about 1.5 liters of an epichlorohydrin-methanol mixture (2:3) from the floor. Even though he washed his hands with soap and water, he experienced redness and itching of the palms severe enough to require the application of corticoid ointment on the 4th day. He was admitted to the hospital 2 days later because of intensified itching of the palms and edema of the palms, fingers, and backs of his hands. During his 11-day hospital stay, he was treated with iv injections of Reparil (a saponin) and with heparinoid ointment bandages. The redness and swelling gradually diminished, but the skin of his hands

remained rough. The patient did not return for followup examination after leaving the hospital.

A third worker [35] splashed a small amount of epichlorohydrin on his right trouser leg. [32] He experienced a burning sensation on the thigh and observed a slight redness after 10 minutes. He applied a local anesthetic cream. Two days later, because of increased redness and burning, he visited an outpatient clinic. On the anterior side of the thigh, two palm-sized and several small dark-red and extremely dry areas, as though tanned, were observed. The patient was treated with antibacterial ointments as an outpatient. The redness and burning subsided with only moderate residual erythema, so that he was able to return to work after 9 days' absence.

The two remaining incidents involved recurring exposures to epichlorohydrin. [35] A 19-year-old apprentice spilled epichlorohydrin on his shoe and stocking which he removed 6 hours later. Burning, itching, blisters, and skin erosion developed and intensified within the next 2 days. Severe skin erosion (5 cm in diameter) on the dorsum of the foot and painful, enlarged lymph nodes in the groin were evident when he was admitted to a hospital 10 days after the accident. He was effectively treated with penicillin injections, moist bandages, and corticoid ointments and was able to return to work on the 34th day after the accident. Almost 2 years later, he resumed work with epichlorohydrin for 3 days, during which he spilled the liquid on his protective rubber gloves. During the night of the 3rd day after resuming work with epichlorohydrin, he noticed burning, swelling, reddening, and vesiculation on several fingers of both hands. He went to a clinic on the 4th day, was hospitalized for 10 days,

and was treated with metal foil bandages and bland ointments. He returned to work on the 20th day following the reexposure to epichlorohydrin. In a followup examination 8 days later, persistent redness of the affected fingers was evident.

The last of the five case studies dealt with a 32-year-old worker who spilled epichlorohydrin in his right safety shoe. [35] Although he removed the shoe immediately and rinsed his foot with lukewarm water, a spotty redness developed within 2 hours over the ball and the base joint of the large toe. Physiologic saline compresses were applied for 5 days. He returned to work on the 6th day. Eight days later, he again spilled epichlorohydrin into his right shoe. Despite being aware of a slight burning sensation during that afternoon, he returned to work on the following day. On the 3d day after the accident a blister developed, and he went back to the hospital. After treatment with physiologic saline, and later with a salve of unspecified nature for 14 days, he was able to leave the hospital. During his hospital stay, peripheral arteriosclerosis with hyperlipidemia, as evidenced by 1,117 total lipids, 268 total cholesterol, 620 mg% esterified fatty acids, and 368 mg% triglycerides, was diagnosed. Normal values stated in Todd-Sanford Clinical Diagnosis by Laboratory Methods [34] for total lipids, total cholesterol, esterified fatty acids, and triglycerides are 600, 250, 200, and 100 mg%, respectively. The authors concluded that no causative relation between epichlorohydrin exposure and the arteriosclerosis with hyperlipidemia existed, but that the exposures may have had an undesirable effect on the worker's health.

Ippen and Mathies [35] referred to the skin effects from epichlorohydrin as protracted chemical burns and suggested several work

practices to prevent their occurrence. They felt that specific work practices were necessary since epichlorohydrin penetrates rubber and leather. They noted that there was a latent period of several minutes to several hours between contact with epichlorohydrin and its manifestations. The authors drew attention to similar latent periods for burns caused by X-rays or by such alkylating agents as ethylene oxide. The authors [35] recommended that studies of the peripheral vasculature be done as soon as possible after dermal contact with epichlorohydrin to distinguish between sequelae and preexisting vascular alterations. This recommendation was made because of the persistent erythema observed after every accident and the pronounced peripheral arteriosclerosis diagnosed in one patient. No indication of sensitization was reported in the two workers whose contact with epichlorohydrin was repeated. These cases demonstrated that skin exposure to liquid epichlorohydrin can cause severe chemical burns. When skin contact with epichlorohydrin was short, the severity of the burns was much less than when skin contact was prolonged. Therefore, it can be concluded that the intensity of the burns is dependent on the duration and extent of exposure, which control the extent of reaction with cellular constituents.

In 1966, Pet'ko et al [11] investigated the health of workers engaged in the production of epichlorohydrin from dichlorhydrin glycerine (DCG) in Russia. Environmental monitoring for both epichlorohydrin and DCG was done by an unspecified sampling method. In some instances, the epichlorohydrin concentration in air was reported to be 2-14 times greater than its maximum permissible concentration. Neither the measured nor the permissible concentration of epichlorohydrin was reported. In the working zone of the

workers who withdrew samples for analysis from the epichlorohydrin production process, the concentration range in the ambient air was 19-21 mg/cu m or approximately 4.9-5.5 ppm. When workers poured epichlorohydrin into the filling tanks, the concentrations in air reached 12-15 mg/cu m (approximately 3.1-3.9 ppm). During an emergency due to mechanical difficulties, airborne epichlorohydrin concentrations of 210-211 mg/cu m (approximately 54.6-54.9 ppm) occurred. Forty-nine men and 33 women, predominately in the 20-35 age range, who worked in the epichlorohydrin production areas were examined. Medical examinations were directed to assessing the symptoms of the effects on ocular mucous membranes, the skin, and the respiratory, cardiovascular, and nervous systems. Morphologic examinations of peripheral blood, reticulocyte counts, and urinalyses were done. The bilirubin, cholesterol, and protein concentrations in the blood were also determined. Allergic manifestations of unspecified nature were mentioned as being present in some workers with only 2.5-3 years of service in the plant manufacturing epichlorohydrin. Although the authors considered the individual worker's length of service to be unrelated to the nature and frequency of the complaints, they did not report the nature of the complaints. They concluded that no deviations from the normal which could be interpreted in terms of occupational factors, other than two cases of occupational dermatitis, were identifiable. The dermatitis cases were not discussed. Pet'ko et al [11] recommended that further studies be done, and that annual medical examinations by an internist, a dermatologist, and a neuropathologist be given. The authors gave no explanation for including a neuropathologist, nor did they specify the clinical basis for recommending blood and urine examinations.

In 1966, Fomin [28] determined the human olfactory threshold and subthreshold of epichlorohydrin to be 0.3 mg/cu m (approximately 0.08 ppm) and 0.2 mg/cu m (approximately 0.05 ppm), respectively, using an unspecified test method. The effects of epichlorohydrin on the light sensitivity of the eyes were investigated in four volunteer subjects. No significant ocular changes occurred with exposure to epichlorohydrin at a concentration range of 0.2-0.75 mg/cu m (approximately 0.05-0.19 ppm). The concentration of epichlorohydrin in air was determined by using an iodometric method based on oxidation of epichlorohydrin to formaldehyde and further reaction with chromotropic acid. A total of 67 electroencephalographic (EEG) recordings were done in 5 subjects, and the voltage of the spikes of the alpha rhythm was analyzed quantitatively. An epichlorohydrin concentration of 0.3 mg/cu m (approximately 0.08 ppm), the olfactory threshold, caused significant (P value not given) changes in all five subjects. These changes included increased voltage of the spikes of the alpha rhythm in the EEG's of four subjects and decreased voltage in that of the fifth, the latter within 10 minutes after exposure. Epichlorohydrin at a concentration of 0.2 mg/cu m (approximately 0.05 ppm) caused no changes. Unspecified conditioned reflexes were also analyzed but the results were not reported. Although the psychologic and physiologic significances of such changes in the EEG recordings are not clearly defined, the results do indicate that epichlorohydrin is capable of causing changes in the alpha rhythms of the EEG. No other reports of similar findings have been found in the literature.

In 1970, Fregert and Gruvberger [36] studied sensitization and cross-sensitization of epichlorohydrin with propene oxide (propylene oxide) on

skin. With Fregert as the subject, the authors found that, following application for 2 days of patches containing epichlorohydrin diluted to 0.1, 0.5, and 1.0% with ethanol, no immediate effects on the skin were visible, but reactions developed after 8-11 days at all test sites. Retesting with 0.1 and 0.01% epichlorohydrin induced a positive reaction after 2 days to 0.1% epichlorohydrin and resulted in erythema with 0.01% epichlorohydrin. Propene oxide, 0.2% in ethanol, gave a positive reaction when it was applied to skin previously exposed to epichlorohydrin. Negative reactions were obtained with 1-chloropropane (1%), 1-chloro-2-propanol (1%), and ethylene oxide (1%) after application of these compounds to areas where epichlorohydrin had been applied. They concluded that the epoxy group and the three carbon atoms in the chain, but not the chlorine, were necessary for the cross-sensitization capacity. This report lacks experimental details and involved only one subject and, thus, is not a sufficient basis for a general conclusion about sensitization to epichlorohydrin or cross-sensitization to propene oxide.

Jirasek and Kalensky [19] examined 17 workers who developed eczema while working with epoxy resin. To identify the causative agent, they conducted dermal tests using several chemicals including epichlorohydrin. A 1% epichlorohydrin solution in ethanol produced positive reactions in three of the subjects. Experimental details were not reported.

In 1971, a summary sheet drawn by industry [37] stated that 1-hour exposures to epichlorohydrin at a concentration of 20 ppm induced transient burning of the eyes and nasal passages in manufacturing-plant workers, and that a similar exposure at a concentration of 40 ppm caused eye and throat irritation lasting 48 hours. It was further suggested that a concentration

of 100 ppm would be intolerable for even a short period. No details of these exposures were given. In addition, the method by which the epichlorohydrin concentrations were measured or verified was not reported. In the absence of such information, it is reasonable to assume that these observations are derived from accidental occupational exposures to epichlorohydrin.

In 1971, Wexler [38] reported similar information. At a concentration of 20 ppm, exposure to epichlorohydrin resulted in burning of the eyes and nasal mucosa within 1 hour. At a concentration of 40 ppm, the epichlorohydrin-induced throat irritation lasted 48 hours despite immediate medical treatment. Wexler [38] further stated that exposure to epichlorohydrin at concentrations greater than 100 ppm could not be tolerated by humans because of the occurrence of lung edema and kidney lesions. Not enough detail was given to permit adequate evaluation of this paper. The occurrence of severe effects at 100 ppm is supported, however, by the previous report [37] in which it was postulated that exposure at 100 ppm would be intolerable.

A study of 48 employees who had been exposed at least once to epichlorohydrin was reported by Kilian (written communication, June 1976). These people were studied for periods of from 7 days to 13 years after exposure; the mean length of followup was 6.3 years. Blood samples were analyzed for hemoglobin, albumin, globulin, cholesterol, BUN, uric acid, bilirubin and glucose concentrations, and alkaline phosphatase, lactic dehydrogenase, SGOT, and SGPT activities. Hematocrit index and total and differential leukocyte counts were determined also. The various values obtained were compared with those used by the company as normals.

The blood chemistries of two of the employees were all within the normal range. Overall, the most frequently observed changes involved differential leukocyte values. The majority of the percentage of polymorphonuclear leukocytes determinations were below normal for 39 employees and above normal for 4 employees. The majority of the percentage of monocytes determinations were elevated for 35 employees; none had the greater number of the determinations below normal. Because the leukocytic and the monocytic changes were common to 32 of the 48 employees, it is reasonable to assume they are related. However, there is no proof that either was the result of exposure to epichlorohydrin.

A total of 16 employees had the greater number of determinations of percentage of eosinophilic leukocytes that were higher than normal. Total leukocyte counts and hemoglobin concentrations were increased in 15 employees, while fewer total leukocytes and lower hemoglobin concentrations, respectively, were reported for 5 and 8 employees. With respect to enzyme determinations, SGOT activities were decreased in a total of 13 employees and increased in only 1. The other determinations rarely deviated from their respective normal ranges.

In the absence of further details on the extent of epichlorohydrin exposure and individual histories, no general conclusions can be drawn from these data. In the majority of the 48 cases, the deviations from normal ranges were no longer detectable after a few months. In five of the reported cases, however, one or more of the measured values remained outside the normal range from 7 to 13 years after exposure. Reexamination of one of these men about 4.5 months later gave values for the various measurements that were within the normal ranges.

From the available data, it is not possible to attribute these persistent changes solely to epichlorohydrin exposure. The most frequently observed deviations from the normal ranges were a decrease in the percentage of polymorphonuclear leukocytes and an increase in the percentage of monocytes in the total leukocytes count of the blood; even these changes were not consistent in any employee. The data suggest that a detailed study of the medical histories of these individuals and their possible exposure to other chemicals is necessary before the hazards of permanent injury from epichlorohydrin exposure can be evaluated. These results indicate that, despite the finding of mutagenic activity for microorganisms in the urine of people who had been exposed to epichlorohydrin, there is no firm evidence that nonincapacitating exposures to this chemical produce persistant effects on metabolic and homeostatic mechanisms of the human body.

Epidemiologic Study

No published epidemiologic studies on workers exposed to epichlorohydrin were found. Kilian (written communication, April 1976) reported a retrospective morbidity study conducted on 680 DOW Chemical Company employees, the results of which were analyzed by an outside consulting firm. The medical examination data on 507 employees who had been exposed to epichlorohydrin for at least 6 months were presented. The longest term of employment was 16 years, but the majority of the people (exact number not given) were exposed to epichlorohydrin for periods of 5 years or less. Environmental monitoring data were not available, but each employee was classified on the basis of work history and job titles as

having either minimal or moderate exposure. A total of 110 employees were assigned to the moderate-exposure group and 397 to the minimal-exposure group. The measurements of toxic effects were: illness episodes, electrocardiogram (ECG), X-ray examination of the chest, pulmonary function tests, and laboratory measurements such as urinalysis, hemograms, and blood chemistries. Illness, defined as absence of an employee from work for 7 or more days, was classified as either respiratory or nonrespiratory in nature. Urinalysis data included measurements of the presence of protein and erythrocytes in the urine. Hemograms included hematocrit index, white blood cell count, lymphocyte cell count, and eosinophil cell count. Blood chemistry included concentrations of creatinine and BUN, albumin-to-globulin ratio, and lactate dehydrogenase, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) activities. An attempt was made to correlate any medical abnormalities with the degree of exposure.

In the minimal-exposure group, 213 employees had 1,343 episodes of illness (6.3 illnesses/employee), while in the moderate group 193 illness episodes occurred in 49 employees (4.0 illnesses/employee). Employees in the minimal-exposure group had 254 (39%) respiratory illnesses while employed in the epichlorohydrin-exposure area and 231 (33%) such episodes while employed in other areas. In the moderate-exposure group, 57 (55%) of all illnesses during epichlorohydrin exposure were respiratory and only 24 (27%) were respiratory during nonexposure to epichlorohydrin. The consultants concluded that illness during employment in an epichlorohydrin-exposure area is more likely to be respiratory than one during employment elsewhere.

Examination of the ECG revealed that 5.0% of the employees in the minimal-exposure group and 2.7% in the moderate-exposure group had abnormal records. The consultants concluded that epichlorohydrin exposure had not influenced the ECG records.

Study of pulmonary roentgenograms indicated that, in both the moderate- and minimal-exposure groups, 1.8% contained abnormal findings, such as emphysematous blebs, mild pneumonic infiltration, pneumonia, mild chronic pulmonary fibrosis, minor emphysema, and infiltrative lesions. The consultants noted that, in addition to the same frequency of abnormal X-radiographs in both exposure groups, a rate of 1.8% did not appear to be greater than that which might be expected in similar unexposed workers. The urinalysis showed no clear difference between the two exposure groups or any group tendency toward abnormality. Hematocrit index, lymphocyte count, SGOT and SGPT activities, and creatinine and BUN values were within the normal range. White blood cell counts were above the normal limit during the 4th, 8th, and 12th years of employment for the moderate-exposure group. The mean group values for monocyte cell count were within normal limits except for the employees with more than 6 years of moderate exposure, in whom values fluctuated fairly widely around the laboratory normal. The difference between the average values for the two exposure groups was statistically significant (t value was 4.35 at a P value of less than 0.05) at the 3rd year. Eosinophil cell count was slightly elevated for the moderate-exposure group during the 2nd and 5th years of epichlorohydrin exposure. Lactate dehydrogenase activity was elevated in both groups. Examination of pulmonary function data indicated that the mean values for both groups did not differ from their normal values. The

albumin-to-globulin ratio was normal for both groups, but, at the 4th year of exposure, the ratio for the moderate-exposure group was significantly lower (t value 3.88, with a P value of less than 0.05) than that of the minimal-exposure group. Based on all these findings, the consultants concluded that there were no toxic effects on the blood, liver, or kidneys related to the epichlorohydrin exposures.

This study, although helpful, is inadequate to fully estimate the health hazards associated with epichlorohydrin exposure for several reasons. The data base comprises a group of employees occupationally exposed to epichlorohydrin for periods of 6 months-16 years, but no consideration was given to those who dropped out because of illness, retirement, or death. There is no way to evaluate from this study the importance of the group lost from the observation. A major deficiency of this study is the lack of a control group. In the absence of such a group, the "normal" range of the clinical and the biochemical tests is dependent on laboratory values alone. Further, because of the lack of controls, the consultants compared the effects of the moderate-exposure group with those of the minimal-exposure group. The consultants stated that the measure of exposure was "a crude one" and that it was only an estimate by the company of whether a given individual's exposure was minimal or moderate. No quantitative data with regard to exposure were provided, and there is no indication of even what range of concentration was referred to by the classifications "moderate" and "minimal." Therefore, any absence of dose-related effects could be accounted for both by the absence of a clear distinction between the groups and by loss of an affected segment of population. From the biochemical data, it is possible to conclude that

groups of individuals "exposed" to epichlorohydrin do not show variations from the measured norms held to be appropriate by the laboratory. Further, because of the deficiencies discussed in this paragraph, the significance of the finding on respiratory tract illness is dubious. If the number of tests in any year of employment was related to the number of employees, the length of employment in the moderate-exposure group was much shorter than in the minimal-exposure group, ie, less than half the workers were still employed during the 1st year and available for tests. In the minimal-exposure group, this point was reached in the 2nd year. The absence of mortality data further limits the usefulness of the study for detecting any chronic effects such as cancer. Animal studies [39-41] have indicated that epichlorohydrin induces sterility in rats; yet, reproductive histories were not checked. In summary, the blood chemistry and liver and kidney functions in groups of individuals exposed to epichlorohydrin at unknown concentrations were not different from the company laboratory normal values.

Animal Toxicity

Animal studies provide useful models to confirm and to provide guidance in understanding the effects of epichlorohydrin on humans. Animal toxicity studies with epichlorohydrin have used different species of animals exposed at different concentrations for different exposure periods. [25-27,29-31,42-49] To facilitate comparison of the data, the total amount of epichlorohydrin inhaled has been calculated from the concentration of epichlorohydrin in the inhaled air and the duration of exposure by the equation of MacFarland. [50] The calculated amount of inhaled

epichlorohydrin is not equivalent to the total mass of epichlorohydrin absorbed by the experimental animal or subject for two reasons. First, the formula is based solely on respiratory considerations and does not include terms for other routes of exposure, such as oral and dermal. Secondly, experimental evidence with other low-molecular-weight lipophilic molecules indicates that less than 100% of the inhaled material will be adsorbed by the respiratory system. [50-54] Consequently, the amount of inhaled epichlorohydrin may be less than or greater than the true mass of epichlorohydrin absorbed by the entire organism. The minute volumes used in this document are 1.23 liter/minute/kg in mice, 0.61 liter/minute/kg in rats, and 0.25 liter/minute/kg in rabbits. [56,58] If the body weight of animals in the studies considered in this document differed from those used by Guyton [57] or Crosfill and Widdicombe, [56] the minute volume was estimated by linear extrapolation. The absolute values obtained are likely to be underestimates of the minute volume since the animals used by Guyton [57] were either resting or recovering from anesthesia, and the animals used by Crosfill and Widdicombe [56] were all anesthetized. In contrast, the epichlorohydrin-treated animals were generally free to move about and hence may have had increased minute volumes. Where the actual weights of the experimental animals were not stated, it is assumed that the mice weighed 0.02 kg and the rats, 0.25 kg. It is also assumed that the animals were placed in the inhalation chamber after a constant concentration of epichlorohydrin had been reached. Based on these assumptions, these calculations provide a crude estimate of the relative mass of epichlorohydrin inhaled via the pulmonary route by the different species.

In 1941, Freuder and Leake [25] investigated the effects of epichlorohydrin administered by various routes to several species of animals. Acute toxicity was determined by exposing white mice to epichlorohydrin vapor at concentrations of 2,370, 8,300, and 16,600 ppm for 30-60 minutes. The concentrations of epichlorohydrin were estimated from the air flowrate and the total weight of epichlorohydrin lost from the container. Irritation of the nose and eyes were observed, the effects being more severe at higher concentrations. All 30 mice exposed at 16,600 ppm for 30 minutes (approximately 2,315 mg/kg, inhaled amount) and all 20 mice exposed at 8,300 ppm for 30 minutes (equivalent to 1,158 mg/kg, inhaled amount) died. All 30 mice exposed at 2,370 ppm of epichlorohydrin for 60 minutes (approximately 661 mg/kg, inhaled amount) survived.

Ten white mice were exposed to epichlorohydrin vapor at 2,370 ppm for 60 minutes daily until all died. [25] Two animals died on the 3d day of administration, two more died on the 6th day, one on the 7th, two on the 8th, one on the 9th, and the last two on the 16th day. A similar sequence of signs was evident in all mice exposed to epichlorohydrin. Nose and eye irritations were followed by gradual cyanosis, muscular relaxation of the extremities, stiffening of the tail, and a fine body tremor. The respiration decreased markedly several hours before death and ceased completely before cardiac arrest. Terminal clonic convulsions occurred in some animals.

The effect of absorption of pure epichlorohydrin through the skin was investigated on rats and on guinea pigs. [25] A gauze wetted with epichlorohydrin was applied for 1 hour to a shaved abdominal area (about 1 sq cm) of each rat. A double layer of adhesive plastic was applied over

the gauze to hold it in place and to reduce evaporation. Local irritation and discoloration developed, followed by systemic signs of epichlorohydrin poisoning. The doses were estimated to be 2, 1, or 0.5 cc/kg. At 2 cc/kg, 18 of 20 rats died; at 1 cc/kg, 2 of 10 rats died; and, at 0.5 cc/kg, all 10 rats survived. Six of 10 rats survived three daily dermal applications of epichlorohydrin at 1 cc/kg, while four applications of epichlorohydrin at 0.5 cc/kg killed all 10 rats. All of three guinea pigs survived single and repeated dermal applications up to a cumulative dose of 52 millimoles/kg (4 cc/kg) of epichlorohydrin. Evaporation was probably not prevented completely, and the amount of epichlorohydrin absorbed was probably less than implied by the dose figures quoted.

Groups of 15 white mice each were also administered epichlorohydrin in gum arabic solution by stomach tube. [25] Epichlorohydrin at a single dose of 0.5 cc/kg killed all 15 mice, whereas one dose of 0.23 cc/kg induced no fatalities. The effects of daily oral administration of epichlorohydrin also were investigated in groups of 15 mice. A total of 21 repeated administrations at 0.08 cc/kg and 4 at 0.23 cc/kg were required to kill all the mice in the group. The authors stated that the typical signs described previously were again evident. Subcutaneous injections of 0.08, 0.16, or 0.23 cc/kg of epichlorohydrin in gum arabic solution were administered daily to 10 white mice until all the animals died. Daily administration of epichlorohydrin at a dose of 0.08 cc/kg for 21 days or at 0.23 cc/kg for 4 days was sufficient to kill all the mice in the group. Thus, regardless of the route of administration (oral or subcutaneous), similar lethal effects were observed at equal doses.

A transitory fall in blood pressure without any marked effect on respiration was observed in three cats and in two dogs, which had been anesthetized with pentobarbital and given single iv injections of epichlorohydrin at a dose of 0.1 millimole/kg (0.008 cc/kg). [27] The minimum lethal iv dose was determined to be about 1 millimole/kg (0.08 cc/kg) in cats and dogs. Death occurred within 2 hours of epichlorohydrin administration. Freuder and Leake [25] stated that deaths from exposure to epichlorohydrin in the animals were due to the effects on the CNS, especially on the respiratory center. They further stated that muscular paralysis and gradual depression of respiration sometimes preceded the respiratory and cardiac failure. The authors observed no microscopic changes in the lungs, heart, kidneys, spleen, or bowel. This report indicates that epichlorohydrin presents a hazard of systemic poisoning from skin absorption. It is noteworthy that the authors did not examine the liver or observe kidney damage.

In 1949, Carpenter et al [58] reported the effect of inhalation of epichlorohydrin on rats as a part of a toxicity range-finding study in which the results were given only in ranges rather than in specific figures. Inhalation of epichlorohydrin at 250 ppm for 4 hours killed two, three, or four of six rats. The authors rated epichlorohydrin as definitely hazardous.

In 1959, Gage [26] exposed five groups of eight albino Wistar rats each (four males and four females) to epichlorohydrin vapor at 120, 56, 27, 17, and 9 ppm. Use of control animals was not reported. The vapor was generated by atomizing epichlorohydrin in propanol solution into a metered stream of air. The concentration of epichlorohydrin was calculated from

the internal diameter of the feed syringe, the rate of piston movement, and the airflow. The concentration was also determined colorimetrically each day. All animals were exposed for 6 hours/day, 5 days/week, for 11-19 exposures. All rats [26] inhaling epichlorohydrin at a concentration of 120 ppm for 11 exposures (approximately 1,132 mg/kg, total amount inhaled) experienced labored breathing, profuse nasal discharge, a marked loss in weight, and leukocytosis. Urinary protein excretion was "more than double the normal value," which suggests damage especially to the kidneys. Microscopic examination of the kidneys showed areas of leukocytic infiltration in all rats and atrophy of the peripheral cortical tubules in four of the eight rats. Microscopic examination of the liver revealed general congestion which was accompanied in one case by areas of necrosis. Lung congestion, edema, consolidation, and inflamed areas with signs of abscess formation were also observed. Although epichlorohydrin is the most plausible causative agent, the absence of controls exposed to the propanol vehicle alone makes it difficult to attribute these changes solely to epichlorohydrin.

The rats exposed to epichlorohydrin at 56 ppm for 18 periods (approximately 864 mg/kg total amount inhaled) showed respiratory distress, nasal discharge, and weight loss. [28] Urinary protein excretion, hemoglobin concentrations in blood, and differential cell counts were normal. Microscopic examination showed only an abscess in one lung, which the author did not attribute to epichlorohydrin exposure.

Rats exposed to epichlorohydrin vapor at 27 ppm for 18 days (approximately 417 mg/kg total amount inhaled) showed mild nasal irritation. [28] The lung of one rat contained hemorrhagic and

consolidated areas. Rats exposed to epichlorohydrin at 17 ppm for 19 exposure periods showed no apparent effects at necropsy or on histopathologic examination. This exposure most likely resulted in an estimated inhaled amount of about 277 mg/kg. Two of eight rats developed pulmonary infections when exposed to epichlorohydrin at 9 ppm for 18 exposures (approximately 139 mg/kg, total amount inhaled); no effects were observed in the other six animals.

Gage [26] also exposed two groups of two rabbits each to epichlorohydrin vapor. The periods of exposure were not mentioned, but probably, like the rats, the rabbits were exposed 6 hours/day, 5 days/week. The first group was exposed for 20 daily periods at 35 ppm (approximately 439 mg/kg total amount inhaled). The second group was exposed for two periods at 16 ppm (approximately 20 mg/kg total amount inhaled). The latter exposure was reduced to 9 ppm and was continued for 20 more days (approximately 113 mg/kg total amount inhaled). Thus, the second group of rabbits is estimated to have inhaled a total amount of 133 mg/kg. Inhalation of epichlorohydrin at 35 or 16 ppm produced nasal irritation. At 9 ppm, no adverse effects were observed. Post-mortem examination did not reveal any abnormalities. Subsequent to these observations, Gage [26] recommended that the epichlorohydrin concentration in the occupational environment where employees are working continually without respiratory protection should not exceed 5 ppm. However, it is noted that Gage did not attempt to investigate any long-term effects of epichlorohydrin inhalation since the longest exposure lasted only 19 days. The recommendation of 5 ppm was therefore based only on effects resulting from short-term exposure.

In 1961, Kremneva and Tolgskaya [27] reported the effects of epichlorohydrin on mice, rats, and rabbits. Routes of administration were stomach intubation, subcutaneous injection, inhalation, and skin and eye application. Three groups of 10 mice and 5 rats each were administered aqueous solutions of epichlorohydrin by stomach tube in doses of 500, 325, and 250 mg/kg. All animals given doses of 500 or 325 mg/kg died within the first 2 days. In both rats and mice, the same type of intoxication pattern was evident: low mobility, slow and labored breathing, hyperemia of the skin, subcutaneous bleeding, ataxia, periodic body tremor, and distention of the abdomen. Microscopic examination of tissues of the dead animals revealed plethora of the internal organs, hemorrhage and edema of the lungs and pulmonary tissue, vacuolization of the liver cells with what was described as fatty degeneration, and degenerative processes in the epithelium of the convoluted tubules accompanied by some necrosis in the kidneys. Foci of necrosis in the stomach and intestinal mucosa also were observed. At the 250-mg/kg dose, no visible signs of intoxication were evident during the 14-day observation period.

A total of 50 mice were injected with epichlorohydrin at doses of 500, 375, 250, and 125 mg/kg. [29] The results of the injections were not described except that, at doses of 500 and 375 mg/kg, all of the mice died, and, at 250 mg/kg, 7 of 10 mice died. Epichlorohydrin at a dose of 125 mg/kg was tolerated without visible alteration in the behavior of the mice.

The skin-penetrating ability of epichlorohydrin was tested when the tails of mice (2 groups, 10 mice each) were lowered into test tubes containing pure epichlorohydrin. Six of ten mice died within 3 days from a single 1-hour exposure. All of the 10 mice in the second group subjected

to repeated 20-30 minute immersions died after 2 or 3 exposures. Morphologic examination of dead animals revealed congestion of the internal organs, edema, brain hemorrhage, and severe degenerative alterations or necrosis of the epithelium of the convoluted tubules of the kidneys. The authors [29] noted that the signs of intoxication in these mice were similar to those observed in animals injected subcutaneously with epichlorohydrin. Thus, the evidence indicates that epichlorohydrin can effectively penetrate the skin and induce severe systemic poisoning.

For inhalation exposures, 70 mice and 63 rats in groups of 10-18 each were placed in a 100-liter static chamber. [27] Epichlorohydrin was placed in the chamber and allowed to evaporate freely. Air was sampled from the chamber after 15-30 minutes and again 90 minutes after the start of the 2-hour exposure. The actual concentrations were determined by a method based on the reduction of chlorohydrins to chloride ions. Because of the static chamber, the measured concentrations at these times are not equal to either the average or the total exposure. Exposure to epichlorohydrin at concentrations ranging from 7.5 to 9.0 mg/liter (1,950-2,340 ppm) caused the death of all 10 animals of both species during the 1st day. There were no deaths in rats and mice exposed to epichlorohydrin at 0.9-1.2 mg/liter (approximately 234-312 ppm). Once again, it should be noted that the concentrations of epichlorohydrin reported give only an approximate index of exposure.

A group of 10 rats was exposed for 3 hours daily to epichlorohydrin vapor at 0.02-0.06 mg/liter (5.2-15.6 ppm) for up to 6.5 months. [27] No deaths or signs of intoxication were observed. The gain in body weight lagged behind that of the controls. The threshold of excitability of the

nervous system increased in the exposed animals during months 2-5, but returned to the preexposure value by the 6th month. The blood pressure of the intoxicated animals fluctuated around a normal value of 95-100 mmHg, in contrast to the normal 90-95 mmHg. Oxygen consumption by the exposed animals increased during the first 2 months but decreased after 5-6 months. No significant variations were observed in the composition of the peripheral blood. Pathomorphologic study revealed an occasional, slight thickening of the alveolar septa and catarrhal bronchitis. Degenerative alterations in the liver and kidneys were insignificant. The authors [29] concluded that, in rats, concentrations of 0.02-0.06 mg/liter (approximately 5.2-15.6 ppm) approximated the threshold. Although the authors did not define threshold, it is interpreted to mean the concentration at which no measurable adverse effect would occur in rats.

Kremneva and Tolgskaya [27] also studied the effects of epichlorohydrin on the mucous membranes of the eyes. A drop of epichlorohydrin placed into the conjunctival sac of a rabbit's eye caused blepharospasm, hyperemia of the mucous membrane, lacrimation, pupillary constriction, reduction of the eye slit, clouding of the cornea, and edema of the lids. Recovery occurred within 10 days. The persistent skin damage following dermal contact with epichlorohydrin seen in humans [35] is confirmed by these animal studies. The suggestion of Kremneva and Tolgskaya [27] that measures be taken to provide skin and eye protection to avoid damage is supported by others. [11,19,25,35,37]

Based on these extensive studies, Kremneva and Tolgskaya [27] suggested 0.001 mg/liter (approximately 0.26 ppm) as a tentative maximum permissible concentration of epichlorohydrin in the occupational

environment. Bartlett [59] reported the pulmonary ventilation rate as a function of the degree of the metabolic activity which ranged from sleep to maximum work. A ventilation rate of 25 liters/minute or 1.5 cu m/hour is intermediate between light and medium work. Consequently, this ventilation rate is an estimation of a spectrum of work activities in the manufacture, use, and handling of epichlorohydrin. For an individual working 10 hours/day with a ventilation rate of 25 liters/minute or 1.5 cu m/hour, the approximate amount inhaled during an 8-hour workday would be 0.21 mg/kg for a 70-kg man.

In 1967, Pallade et al [29] found the subcutaneous LD50 of epichlorohydrin in rats to be 150 mg/kg. An 8% epichlorohydrin solution in propylene glycol was used and the animals were observed for 2 weeks. Application of 0.5 ml of epichlorohydrin to the skin of an unspecified number of rabbits for 24 hours caused a lesion with a central zone of coagulation necrosis surrounded by one of a hard edema involving the superficial layer of the dermis. A zone of erythema with punctiform hemorrhages extending beyond the area of contact with epichlorohydrin appeared at the periphery. In all cases, the areas of necrosis and erythema became covered with a bloody fibrinous scab after 2-3 days that persisted for up to 30 days. Application of 0.1-0.2 ml of epichlorohydrin for 24 hours caused similar, but less severe and smaller, lesions. The authors reported that all rabbits on both application schedules recovered.

Effects of epichlorohydrin [29] by the cutaneous route were determined by immersing the tails of 10 mice for 15-20 minutes in test tubes containing undiluted epichlorohydrin. [29] Seven of the mice died, and the three survivors showed local lesions leading to the loss of the

distal portion of the tail.

Effects on the kidneys were studied in 57 rats divided into four groups. Epichlorohydrin was administered by an unspecified route in single doses of 180, 150, 125, and 100 mg/kg. [29] The animals were placed in metabolism cages for a period of 2 weeks. Daily urine outputs were examined for albumin concentration and sediment. Methods of analysis were not reported. At 100 mg/kg, 22 of 27 rats (81.5%) had oliguria, and one (3.7%) had anuria. Anuria was observed at 125 mg/kg in 7 of 12 rats, (58.3%) and, at 150 mg/kg, in 10 of 12 rats (83.3%). Following the administration of epichlorohydrin at 125 or 150 mg/kg, the rest of the animals had oliguria. At a dose of 180 mg/kg all six (100%) animals had anuria. Mortality rate was 66.7% for animals receiving 180 or 150 mg/kg epichlorohydrin. Mortality rates were 50 and 7.4%, respectively, in the 125 mg/kg and the 100 mg/kg groups.

Epichlorohydrin at a single dose of 150 mg/kg was injected by an unspecified route into 80 rats. [29] Blood catalase (Enzyme Commission Number, E.C. 1.11.1.6) and carbonic anhydrase (E.C. 4.2.1.1) activities were determined in 55 and 25 animals, respectively, on days 1, 3, 5, 7, 9, and 16 after injection. Blood catalase and carbonic anhydrase activities were measured in 26 and 12 untreated control rats, respectively. The catalase activity of the treated animals at the end of the 16-day period showed a statistically significant decrease of 20% (P value equal to or less than 0.05) in contrast to the 2.5% decrease observed in control rats. Decreases in carbonic anhydrase activity of about 10% also were observed in the treated animals. The long duration of these effects on enzyme activities is noteworthy, and, as the authors pointed out, this persistence

suggests irreversible, and perhaps cumulative, changes.

For microscopic studies, [29] 23 rats were treated with epichlorohydrin at 150 mg/kg, and 14 rats were treated at 180 mg/kg. A similar, or probably the same, study was reported in greater detail in Rumania by Rotaru and Pallade, [30] who reported administering 150 or 180 mg/kg of epichlorohydrin by single subcutaneous injections to 23 and 14 albino rats, respectively. Tissues from animals killed at 24 or 48 hours, or 5 or 10 days after the injection and from those which expired during the experiment were examined microscopically. Immediately after death, samples from the myocardium, lungs, liver, kidneys, spleen, stomach, intestine, adrenals, and brain were taken for study. The induced effects were similar at both doses, but were more severe at the higher dose. The kidneys were the most damaged organs. Animals examined at 24 hours showed ischemia of the cortex and congestion of the deep cortical layers near the medulla. Various degrees of congestion and interstitial edema with hemorrhagic foci also were present in the medulla. In all cases, prevailing lesions of degenerative or necrotic nature were evident in all nephrons but were especially severe in the proximal parts of the nephrons. Diffuse, cloudy intumescences, granular alterations, granular vacuoles, and homogeneous eosinophilic coagulation also were observed. The authors attributed these observations to a marked cellular metabolic imbalance. The epithelium was severely affected and showed nuclear damage, as evidenced by karyolysis, karyorrhexis, and karyopyknosis. This damage was attributed by the authors to the irreversible nature of the degenerative lesions which became necrotic. Examination of the tubular basal membranes revealed that, in some cases, they were discontinuous for various lengths, and that

disruptive cortical lesions were especially prevalent. Minimal perinecrotic inflammatory infiltrates appeared 48 hours after treatment at both doses. On the 5th day, epithelia with hypertrophic nuclei in the necrotic zones and occasional binuclear regeneration were present. On the 10th day, most of the tubules consisted of regenerated epithelium, and the ischemic necrotic zones were no longer recognizable. In general, the gross and microscopic tissue damage detected during the first 48 hours was no longer present, despite the persistence of changes in the activity of blood enzymes. The signs observed in the animals as a result of epichlorohydrin exposure appear to be those caused by a general systemic poison. The authors concluded that epichlorohydrin had a marked primary nephrotoxic action complicated by its action on the vascular system. They further concluded that regeneration could reestablish the structural integrity of nephrons in the surviving animals. However, the evidence is insufficient to conclude that the functional activity of the nephrons was fully restored.

Examination of other organs revealed generalized damage. [30] Alvèolar septal congestion, desquamative bronchial catarrh, and sporadic edema of the peribronchovascular connective tissue were detected in the lungs. The spleen exhibited frequent areas of sludged blood and small hemorrhagic foci. The stomach and intestine showed occasional slight congestion and edema in the mucosa with eventual desquamative catarrh. The liver and heart exhibited no appreciable microscopic alterations, and only a slight degree of congestion was present in the brain and adrenal glands.

In 1967, Soloimskaya [42] reported the effects of single subcutaneous injections of 125, 250, or 500 mg/kg of epichlorohydrin in 112 rats. For

controls, 93 untreated animals were used. Two days later, the blood of the rats injected with epichlorohydrin was examined for pyruvic acid, oxalacetic acid, and total citric and isocitric acids. Free aromatic amines were determined for animals treated at all three doses. At 250 mg/kg, increases in pyruvic acid, total citric and isocitric acids were observed, and at all three doses, increases in the free aromatic amine concentrations occurred in the blood. The concentration of oxalacetic acid in the blood decreased in animals given 250 mg/kg. Elevated concentrations of pyruvic acid and total citric and isocitric acids in the blood indicate altered rates of metabolism. After grinding the liver and incubating the mixture for 3 hours, its histaminase activity (E.C. 1.4.3.6) was determined. The average histaminase activity of the liver in 10 control animals was 130 ± 1.84 μg/g. Animals injected with a dose of 500 mg/kg of epichlorohydrin had a sharp decrease in histaminase activity to 42 ± 0.54 μg/g and those injected with epichlorohydrin at a dose of 250 mg/kg had a decrease to 83 ± 2.43 μg/g. In animals injected at a dose of 125 mg/kg, there was a decrease to 98 ± 1.56 μg/g. Thus, compared with the control animals, the rats at all three doses had sharply decreased histaminase activity in the liver. Although there is no evidence of decrease in histaminase activity in humans exposed to epichlorohydrin, the decrease in histaminase activity could be of clinical significance, particularly in people susceptible to allergies and sensitization. Other reports on the effects of epichlorohydrin on histaminase activity have not been found.

In 1972, Lawrence et al [31] reported on a series of experiments intended to determine acute and subacute effects of epichlorohydrin. The oral and ip LD50's for epichlorohydrin were determined by using unspecified

numbers of mice, rats, guinea pigs, and rabbits. The animals were observed for 7 days after epichlorohydrin administration. The ip LD50 ranged from 0.10 to 0.14 ml/kg for mice, rats, guinea pigs, and rabbits, while the oral LD50 was 0.20 ml/kg for mice and 0.22 ml/kg for rats. The dermal LD50 was 0.64 ml/kg for rabbits. Inhalation of epichlorohydrin vapor by mice resulted in an LT50 of 9.13 minutes at 71.89 mg/liter (18,690 ppm). The concentrations were not verified by analytical techniques. However, it should be noted that the animals were probably exposed to epichlorohydrin at an increasing concentration up to 71.89 mg/liter (18,690 ppm).

Tissue culture experiments were performed by the agar-overlay method with mouse fibroblasts. [31] Epichlorohydrin in cottonseed oil was applied to paper disks which were placed on the surface of the agar and incubated at 37 C for 24 hours. A cytotoxic response had occurred when a clear zone of lysed cells surrounded the disk. Epichlorohydrin at 0.00122% v/v (0.00016 M) was cytotoxic to the cells, but, at 0.000486% v/v (0.000062 M) or less, no effects were observed. The hemolytic activity of epichlorohydrin was evaluated by estimating the amount of hemoglobin released by addition of 0.2 ml of oxalated whole rabbit blood to 10 ml of various saline solutions of epichlorohydrin. When the epichlorohydrin concentration was 0.01 M, hemolysis was first detected; when it was 0.0375 M, 50% hemolysis occurred. In the absence of further experimental details, it is not possible to draw conclusions about the dose-response relationship for epichlorohydrin-induced hemolysis.

To assess the irritant effects on eyes, 0.1 ml of epichlorohydrin solution in cottonseed oil was instilled into the right eyes of the rabbits; the left eyes served as the untreated controls. [31] The eyes

were examined every 30 minutes for 3 hours. Corneal damage was present in 80% of the animals and a lesser degree of irritation occurred in the rest of the animals. Epichlorohydrin was found to be a strong ophthalmic irritant.

There was no evidence of sensitization to epichlorohydrin, as determined by the maximization test in 5 Hartley-strain guinea pigs. [31] Groups of 12 male Sprague-Dawley rats were injected ip daily for 30 consecutive days with 0.00955 or 0.01910 ml/kg of epichlorohydrin or 0.01910 ml/kg of cottonseed oil. At the end of 30 days, hemoglobin values were increased significantly at the low dose but decreased significantly at the higher dose (P values less than or equal to 0.05). The concentration of neutrophilic metamyelocytes increased significantly (P value less than or equal to 0.05) in the high-dose group but remained equal to that of controls for the rats administered epichlorohydrin at the lower dose. Lymphocytes showed an insignificant dose-related decrease in frequency. A slight, insignificant dose-related increase in clotting time was also observed. Hepatic function, as measured by the BSP test, was not impaired. The heart-to-body weight ratio increased in a dose-related way but the increases were not statistically significant. The ratio of the weight of the kidney to body weight increased significantly with both doses (P value less than or equal to 0.05). The ratio of brain-to-body weight of the rats was higher in the epichlorohydrin-treated rats than in the controls. Microscopic examination did not reveal changes in any organs except in the lungs. Lesions in the lungs were evident in all groups, but the incidence and severity were "somewhat greater" in the treated animals than in the controls. It is noteworthy that the authors did not detect any kidney

damage in the microscopic examination.

Lawrence et al [31] also investigated the effect of administering epichlorohydrin ip to rats on Mondays, Wednesdays, and Fridays for 12 weeks. Four groups of male Sprague-Dawley rats received either 0.04774 ml/kg of cottonseed oil (control) or 0.0095, 0.0190, or 0.04774 ml/kg (1/10, 1/5, and 1/2 the ip LD50) of epichlorohydrin in cottonseed oil. The number of animals in each group was not specified. A significant reduction (P value less than or equal to 0.01) in the body weight gain accompanied by reduced food intake was seen in the group receiving epichlorohydrin at the highest dose. A dose-related (the higher the dose, the greater the reduction) and statistically significant (P less than or equal to 0.05) decrease in hemoglobin was observed. All animals treated with epichlorohydrin had lower hematocrit and erythrocyte counts than the control rats, but this was significant (P value less than or equal to 0.05) only for the hematocrit value of the middle-dose group. An increase in the concentration of platelets in the blood also was observed in the epichlorohydrin-treated animals. Total leukocyte counts were lower in the low-dose group and higher in the highest-dose group than in the control group. These differences were not significant (P value less than or equal to 0.05). Leukocyte counts for the middle-dose and control groups were the same. A dose-related increase in the average percentage of segmented neutrophils was observed but was significant (P value less than or equal to 0.01) only for the high-dose group. The percentage of eosinophils increased in all experimental groups, the increases in the low- and the high-dose groups being significant (P value less than or equal to 0.05). Significant reductions in the percentage of lymphocyte in the total

leukocyte count were observed in the animals treated with the two highest doses (P values less than or equal to 0.05 and 0.01, respectively). The ratio of the weight of the brain to that of the body was significantly lower (P value less than or equal to 0.01) in the animals treated with epichlorohydrin at the highest dose than in the control animals; this is in contrast to the effect obtained in animals receiving 30 daily doses of epichlorohydrin. The change in the brain-to-body weight ratio suggests abnormal changes in the CNS. The organ weight-to-body weight ratios for heart, kidneys, and liver were significantly (P values less than or equal to 0.01, 0.01, and 0.05) higher for the animals treated with the highest dose than for the controls. The ratio of spleen-to-body weight was not significantly different from that found in the controls. The results indicate that repeated doses induce a cumulative effect on basic cellular growth with a subsequent perturbation of the normal rates of growth of these internal organs.

The effect of epichlorohydrin on pentobarbital-induced sleeping time was studied in mice. [31] Male ICR-strain mice in groups of 10 were administered 1/10, 1/5, or 1/2 of the acute ip LD50 dose (0.14 ml/kg) of epichlorohydrin. Control mice were administered ip saline injections. Similar groups of mice were exposed to epichlorohydrin vapor at 1/10, 1/5, or 1/2 of the LT50 dose (71.89 mg/liter, 9.13 minutes). Control mice were placed in an inhalation chamber and were exposed to uncontaminated air. Twenty-four hours after exposure to epichlorohydrin by either route, 50 mg/kg of sodium pentobarbital was administered ip. A dose-related increase in sleeping time was observed in all the epichlorohydrin-treated animals; however, the only significant increases (P value less than or equal to

0.01) were in the groups receiving the highest ip dose or the highest inhalation exposure. Based on these extensive studies, it can be concluded that epichlorohydrin is severely irritating to the skin and the eyes, and perhaps to the lungs. Further, it also affects some metabolic processes of the liver. In most cases, the severity of its effects appears to be dose dependent.

In 1972, Lawrence and Autian [44] further examined the effect of epichlorohydrin on pentobarbital-induced sleeping time. Groups of 10 male ICR mice were exposed to epichlorohydrin vapor at 98.20 mg/liter (approximately 25,500 ppm) for 0.92, 1.83, or 4.58 minutes daily for 3 days (0.1, 0.2, or 0.5 times the inhalation LT50). Based on the assumptions discussed at the beginning of this section, the approximate amounts inhaled were 333, 663, and 1,600 mg/kg, respectively. Pentobarbital-induced sleeping time was measured 24 hours after the last exposure. Compared with that of the control animals, an increased sleeping time was apparent in the test rats, suggesting an effect on microsomal processing enzyme systems of the liver.

Fomin [28] exposed three groups of 15 male albino rats to epichlorohydrin vapor for periods of 24 hours/day for 98 days at 20 ± 0.026, 2 ± 0.007, or 0.2 ± 0.001 mg/cu m. These concentrations approximate 5.2 ± 0.01, 0.5 ± 0.002, or 0.05 ± 0.0003 ppm and the total amounts inhaled are estimated to be 1,722, 172, or 17 mg/kg, respectively. Epichlorohydrin concentrations were determined colorimetrically. A fourth group of 15 animals served as a control. Peripheral blood was sampled for analysis of fluorescence of leukocytes from five rats in every group, at first once a week and then once every 2 weeks. Compared with the controls, animals

receiving epichlorohydrin at the highest dose had a sevenfold increase in the number of leukocytes with altered fluorescence. The nucleic acid concentration in the blood of the same animals decreased to 90.73 mg% in contrast to 127.07 mg% in the control group. An increase in the amount of urinary coproporphyrin, 2.68 μg compared with 1.07 μg in the control group, occurred in the animals receiving epichlorohydrin at the highest dose. Reduced weight gains and prolonged latent periods of the motor defense reaction were also observed in this group of animals. Gross and microscopic examinations disclosed emphysema, bronchopneumonia, edematous areas, and loosening and swelling of the adventitia of blood vessels in the lungs. Cloudy swelling of the epithelium of the convoluted tubules in the kidneys and foci of interstitial hemorrhage and venous congestion in the heart were evident. Severe lesions of the neurons in the medulla oblongata, Ammon's horn, and cerebellum were also present.

Animals inhaling epichlorohydrin at 0.5 ppm showed an increase in leukocytes with altered fluorescence, a decrease in the nucleic acid content of the blood, but no significant effects on the amount of urinary coproporphyrin. [28] The concentration of leukocytes with altered fluorescence increased significantly (95% probability of not being a variation by chance), but the effect was less marked than on the animals treated at the highest dose. Animals in the third group, inhaling epichlorohydrin at 0.05 ppm, did not show similar effects. There were no morphologic differences between the control animals and those in the last two groups.

Based on the results of this continuous inhalation study, Fomin [28] recommended that the mean diurnal maximum permissible concentration of

epichlorohydrin in the atmosphere not exceed 0.2 mg/cu m (approximately 0.05 ppm). The exposure in this study was continuous and the effect of intermittent recovery periods was not evaluated.

In 1969, Golubev [45] recorded changes in the diameters of the pupils of the eyes of rabbits. Groups of six rabbits were used to ascertain the irritating effects of eight different chemicals, including epichlorohydrin. The rabbits' eyes were illuminated with a uniform reflected light, and the diameters of the pupils were measured with an instrument referred to as a tangential pupilometer. A 0.25 M solution of epichlorohydrin was instilled into the conjunctival sacs and the pupil diameters were measured 1, 3, 5, 10, 15, 20, 25, and 30 minutes later. In the control rabbits, the pupil diameters were measured both before instillation and at similar time intervals after instillation of 0.05 ml of saline solution into the conjunctival sacs. Epichlorohydrin caused constriction ranging from 1 to 16% during the first 20 minutes, the initial diameter being considered 100%. The author noted that this effect was elicited at an epichlorohydrin concentration that caused no visible changes in the conjunctivae or cornea. Thus, epichlorohydrin at 0.25 M (2.3%) has a measurable effect on the eyes.

In 1968, Pallade et al [46] subcutaneously injected 67 white rats with a single dose of epichlorohydrin at 125 mg/kg to investigate epichlorohydrin-induced kidney damage. Prior to injection, the urine of each animal was examined for protein, potassium, and sodium; thus, each animal served as its own control. The animals were kept in metabolism cages; their urines were examined 1, 2, 3, and 8 days after epichlorohydrin administration. Oliguria or anuria was exhibited by 53 (79%) animals and polyuria by 4 (6%). The remaining 10 (15%) produced urine at normal rates.

Of the 53 rats that produced little or no urine during the period immediately after the administration of epichlorohydrin, 7 (13.2%) entered a stage of polyuria within 2-3 days after the administration of the dose. The mortality rate was 13.4%, death occurring only among the oliguric and anuric animals. Urinary protein was determined by spectrophotometry and urinary potassium and sodium were measured by flame photometry. Although it was evident in all animals, proteinuria was more marked in the oliguric animals. The excretion of protein in the urine returned to normal after 8 days. Sodium concentration in the urine was low and remained so even after 8 days; the potassium concentration was elevated initially but returned to normal within 8 days.

Serum was examined 48 hours after epichlorohydrin was administered to 60 rats. [46] Protein concentrations were determined by a modified Weichselbaum method, sodium and potassium by flame photometry, and serum lipids by microphotometry. Reductions in serum protein and sodium concentrations and an elevation in the serum potassium concentration were observed. The concentrations of total serum lipids and the lipase activity of serum were not different from those in 40 untreated rats serving as controls.

Pallade et al [46] noted that proteinuria and the parallel oliguric and anuric conditions indicated the occurrence of renal damage. If tissue repair and regeneration had occurred, the animals survived. They concluded that the signs of renal disorders confirmed that epichlorohydrin is nephrotoxic to rats. Therefore, medical examinations oriented to the detection of renal disorders were recommended for workers exposed to epichlorohydrin.

In 1966, Shumskaya and Karamzina [47] used epichlorohydrin as a known nephrotoxin to evaluate various measures of kidney function in rats. Since the intent of these investigations was not to identify epichlorohydrin toxicity, details such as time of exposure and number of animals used were generally not specified. Polyuria, secretion of urine of low specific gravity, proteinuria, reduced urinary chloride concentrations, and increased concentrations of nitrogen-containing substances in the urine were found.

In 1971, Shumskaya et al [32] conducted another study similar to the one discussed in the previous paragraph. [47] Experiments with single 4-hour inhalations of epichlorohydrin at 0.35 mg/liter (91 ppm), at 0.02 mg/liter (5.2 ppm), and at 0.007 mg/liter (1.8 ppm) were conducted on three groups of 60 male white rats each; the control group comprised 60 rats. Information on how epichlorohydrin vapor was generated and information on the size of the chamber were not provided. Neither was it indicated whether the chamber was operated in the static or the dynamic mode. The total amounts of epichlorohydrin inhaled during each of the 4-hour exposure periods are estimated to have been approximately 51, 2.9, and 1.0 mg/kg, respectively. The same effects seen in the previous experiment [47] were observed. In addition, the weights of the liver and kidneys were increased, whereas those of the lungs and the spleen were decreased. Removal of BSP from blood was decreased on the day of exposure, but not reliably on the day after the exposure. The production of urine was increased by all three levels of exposure, with concomitant decreases in the specific gravity of the urine. The daily output of chlorides in the urine on the day after exposure was usually increased by a larger factor

than the production of urine. The daily excretion of protein in the urine was also increased, but less than that of chlorides. No additional studies of pulmonary and splenic function were reported. Reductions in both oxygen consumption and body temperature also were observed. A significant (P value not given) stimulating influence on the mobility of spermatozoa also occurred in animals exposed to epichlorohydrin at the two lower concentrations. Even though the intent of these experiments was to evaluate various tests as indices of intoxication, important information on the toxicity of epichlorohydrin was generated. The results are summarized in Table XIII-2. They demonstrate that even short-term exposure to epichlorohydrin at approximately 1.8 ppm induces perturbations in basic physiologic processes such as consumption of oxygen and regulation of body temperature in mammals. These experiments [32,47] suggest that epichlorohydrin affects the function of the liver, as indicated by the BSP tests, and the kidney, as indicated by the production of urine and the reabsorption and filtration of chloride and protein. Lungs and spleen were also affected, but no assessment of the impacts on the functions of these organs was made. The authors concluded that epichlorohydrin affects the kidneys, liver, lungs, and nervous system.

Since a number of enzymes are involved in reabsorption and tubular secretion in the kidneys, Pallade et al [48] investigated the effects of epichlorohydrin on the activity of several enzymes. White rats of both sexes were administered epichlorohydrin subcutaneously at single doses of 125 mg/kg dissolved in propylene glycol. Animals were killed 2.5 or 24 hours after the administration of the dose and examined for various enzyme activities in the urine, renal tissue, and serum. Cytochrome oxidase (E.C.

1.9.3.1), succinic dehydrogenase (E.C. 1.3.99.1), and carbonic anhydrase (E.C. 4.2.1.1) activities were determined by manometric methods. Alkaline phosphatase (E.C. 3.1.3.1) activity was determined by the Gomori method, the transaminases activity by a colorimetric method, and catalase (E.C. 1.11.1.6) activity by an iodometric method.

Renal cytochrome oxidase activity was determined in 14 rats and in 10 controls 24 hours after they had been injected with epichlorohydrin. [48] Statistically significant (P value less than or equal to 0.01) inhibitions were observed in treated animals. Renal succinic dehydrogenase activity, determined 24 hours after epichlorohydrin administration, was similar for 10 experimental and 6 control animals. Examination of 40 treated and 30 control rats showed reductions in renal carbonic anhydrase activity of 6-11% 24 hours after the administration of epichlorohydrin. This reduction was not statistically significant. Glutamic-pyruvic transaminase (GPT) (E.C. 2.6.1.2) activity was measured 2.5 and 24 hours after administration in both renal tissue and serum. There were 30 animals in the group examined at 2.5 hours after epichlorohydrin administration and 40 in the group examined 24 hours postdose. At 2.5 hours, no change was observed in the kidneys, but a statistically significant (P value less than or equal to 0.01) increase in SGPT activity was observed at both the 2.5-hour and the 24-hour intervals. A significant reduction (P value less than or equal to 0.01) was observed at 24 hours; there were 0.230 units of activity in the controls in contrast to 0.100 units in the experimental group. The average SGPT activity in the experimental group ranged from 0.051 ± 0.022 units at 2.5 hours to 0.058 ± 0.018 units at 24 hours, in contrast to the control group average of 0.034 ± 0.010 units. Glutamic-oxaloacetic transaminase

(GOT; E.C. 2.6.1.1) activities in the kidney were similar for the controls and the intoxicated animals at 2.5 hours, but significantly (P values less than or equal to 0.01) different at 24 hours in the experimental rats (0.261 ± 0.046 units) versus 0.302 ± 0.040 units in the controls. SGOT activity was significantly increased in the experimental rats, 0.045 ± 0.009 units at 2.5 hours (P value less than or equal to 0.02) and 0.046 ± 0.010 units at 24 hours, (P value less than or equal to 0.01) in contrast to 0.040 ± 0.008 units observed in the controls. Alkaline phosphatase activity was assayed in 30 experimental animals and in 30 controls. The animals given epichlorohydrin showed a significant reduction (P value less than or equal to 0.01) in the mean kidney phosphatase activities (0.029 ± 0.008 units) at 2.5 hours but had a mean alkaline phosphatase activity comparable with that in the controls (0.033 ± 0.014 units) at 24 hours. However, serum alkaline phosphatase activity decreased progressively at both 2.5 and 24 hours. Catalase (E.C. 1.11.1.6) activity was measured in the kidneys and in the urine of 20 treated and 20 control animals. Renal catalase activity was only 3.93 ± 1.86 units in the experimental animals, whereas it was 7.91 ± 2.67 units in the controls. The average of urinary catalase activity of the epichlorohydrin-treated animals was about nine times that of the controls.

Pallade et al [48] stated that the observed changes in serum, urine, and kidney enzyme activities resulted from renal lesions or were consequences of general toxic effects in other tissues. In view of the reports on the nephrotoxicity of epichlorohydrin, [32,46,47] it seems likely that the altered serum enzyme activities may be due to renal lesions. As the authors [48] noted, however, damage to such other tissues

as liver and heart also can cause elevated blood enzyme activities. This study does not rule out the possibility that such effects are involved in the genesis of the observed changes in the enzymes of serum. In addition, direct chemical inhibition of enzyme-active centers or alteration of the rates of production or degradation of the enzyme could have caused the effects noted, but this was not discussed or evaluated by the authors. In particular, catalase, an enzyme participating in cellular oxidation-reduction reactions, is distributed widely in renal and hepatic cells and in erythrocytes. The reduced renal catalase activity observed may be attributed to renal damage, but, as the authors pointed out, the observed increase in urinary catalase activity could have been due to the presence of erythrocytes, leukocytes, or bacterial contamination as well as to leakage from damaged renal epithelial cells. Increases in serum transaminase activities (SGOT and SGPT), accompanied by reductions in these activities in the kidneys of experimental animals, were attributed by the authors to cellular lesions or to changes in mitochondrial permeability which precede cell destruction. The results of this study clearly indicate that exposure to epichlorohydrin induces major changes in several basic biochemical functions of the kidneys in laboratory mammals. The observed increase in urinary catalase activity suggests that examination of this activity may be a useful test to monitor for acute overexposure to epichlorohydrin in humans.

Grigorowa et al [60] determined the LC50 of epichlorohydrin in albino rats weighing 230-270 g and in albino mice weighing 18-26 g. Groups of 20 animals of each species were exposed to epichlorohydrin at concentrations of 0.190, 0.390, 0.855, 0.915, or 1.680 mg/liter (49.4, 101.4, 222.3,

237.9, or 436.8 ppm, respectively) for 4 hours in the case of the rats and for 2 hours in the case of the mice. One-half the animals in each group were subsequently exposed for 45 minutes to an environmental temperature of 35 C and a relative humidity of 35-50%. Survivors were counted 72 hours after exposure. The exposure to heat had no appreciable effect on the LC50. The values with and without heat were 2.2 and 2.4 mg/liter (582 and 635 ppm) for rats and 4.0 and 3.0 mg/liter (1,060 and 793 ppm) for mice.

Grigorowa et al [60] further exposed 2 groups of 60 male albino rats weighing 230-270 g to epichlorohydrin at concentrations of 0.6 or 0.06 mg/liter for 4 hours/day for 8 days. An additional group of 60 rats served as controls. One-half the animals in each group had a 45-minute exposure to an environmental temperature of 35 C and a relative humidity of 35-50% after each epichlorohydrin exposure; the other half received no temperature stress. Ten rats in each subgroup were killed on days 2, 4, or 11 of the experiment. Exposure at 35 C for 45 minutes on each of 8 days within an 11-day period had no effect on body weight. Inhalation exposure at a concentration of 0.6 mg/liter of epichlorohydrin for 4 hours/day for eight times during 11 days decreased body weight by about 9%. Combination of these two exposures, that to heat following immediately after that to epichlorohydrin, had the same effect on body weight as exposure to the chemical alone. This finding agrees with that relating to the LC50. In conclusion, the amount of heat stress had no effect on these two responses of rodents to epichlorohydrin.

Despite these two negative findings, there were a few instances in which addition of heat stress seemed to modify toxic actions by the chemical stressor. Thus, rats exposed to both heat and epichlorohydrin

were reported to have had somewhat less marked alterations of the structure of the liver and the thyroid follicles, but more pronounced ones of the adrenal medulla, than those exposed to epichlorohydrin alone. Five rats showed moderate accumulations, resembling abscesses in some instances, of leukocytes around blood vessels in the interstitial tissue of the thyroid. The animals tended to have increased relative weights of lungs, liver, kidneys, and adrenals (though not for each organ at all times of slaughter) and increased whole blood catalase (E.C. 1.11.1.6) and serum lactate dehydrogenase (E.C. 1.1.1.27) activities. Urinary volume and protein concentration decreased; changes in serum sodium, potassium, and leucine aminopeptidase (E.C. 3.4.1.1) were inconsistent. At the 0.06 mg/liter epichlorohydrin exposure, fewer effects from the addition of heat stress were observed and consisted principally of increases in the whole-blood catalase and serum leucine aminopeptidase activities. Therefore, exposure to an elevated environmental temperature sometimes altered some effects of epichlorohydrin exposure, most notably increasing the blood-catalase activity.

Effects on Reproduction

Epichlorohydrin-induced sterility has been reported in animals. [39-41] Cooper et al [39] administered epichlorohydrin suspended in arachis oil orally to adult male Wistar rats. No controls were used. Five daily doses of 20 mg/kg (a total of 100 mg/kg), or of 50 mg/kg (a total of 250 mg/kg), or a single dose of 100 mg/kg were given to five animals at each level. Animals receiving 5 daily doses of 20 mg/kg lost fertility during the first 2 weeks, but completely regained it by the third week. The

fertility of these rats was tested for 10 weeks. The sperm cycle in rats is 8 weeks. Rats receiving the five 50 mg/kg of epichlorohydrin doses were rendered completely sterile throughout a 10-week period. Microscopic examinations of reproductive tissues were done weekly for 20 consecutive weeks after the 100 mg/kg dose. Sterility occurred within the 1st week after treatment with epichlorohydrin at a dose of 100 mg/kg. Fertility returned during the 2d week, but the average litter size was reduced by about one-third. By 12 weeks, probable permanent sterility had occurred. Apart from small spermatoceles in the efferent ductules of the testes, histologic examination revealed no abnormalities up to 8 weeks after the administration of epichlorohydrin at a dose of 100 mg/kg. However, by 12 weeks, large retention cysts were present in the efferent ductules and the proximal caput in four of five sterile animals. Therefore, when the 100 mg/kg dose is administered in five equal daily doses, a reversible functional sterility occurs, while at a single dose of 100 mg/kg, spermatoceles form and permanent sterility may result.

Jones et al [40] investigated the antifertility effects of epichlorohydrin on Wistar rats. A single oral or ip administration of epichlorohydrin at a dose of 50 mg/kg in an aqueous solution produced "antifertility effects resembling those due to alpha-chlorhydrin." Details about measurement of antifertility, duration of the sterility, the time between dosage and onset of sterility, and the number of animals used were not reported.

In 1970, Hahn [41] gave daily oral doses of 15 mg/kg of epichlorohydrin for 12 days (a total of 180 mg/kg) to adult male rats of demonstrated fertility. Control animals were also observed. Two preestrus

female rats were placed in the cage of each male at different times during the study. After 7 days, the fertility of each male was evaluated on the basis of the number of uterine implantations in the females. Within 1 week, the males became infertile, but fertility was restored within the next week. On the 12th day, histologic examination of the testes, epididymis, prostate, and seminal vesicles showed no differences between the experimental and the control animals. The number of experimental animals was not reported. The kinetics of the effect cannot be evaluated since sterility observed at week 1 could have occurred on the 1st or the 7th day. Thus, the inference may be made that the sterility induced in male rats by epichlorohydrin is dose and time dependent. [39,41]

In 1972, Epstein et al [61] reported the effects of epichlorohydrin on the rate of pregnancy in ICR/Ha Swiss mice in a dominant lethal assay. A dose of 150 mg/kg of epichlorohydrin was administered to 10 male mice by ip injection. Each male was then caged with three undosed virgin female mice for 1 week. The females were replaced each week for 8 weeks and then killed and examined for pregnancy. The numbers of total live implants, early fetal deaths, and late fetal deaths were recorded. Since, in general, late fetal deaths were exceedingly rare, total implants and early fetal deaths were the only features of pregnancy analyzed. The authors did not report the observation of any effects of epichlorohydrin on early fetal deaths or other reproductive characteristics, including male fertility. Therefore, it can be concluded that epichlorohydrin at 150 mg/kg given to male mice in a single ip injection does not increase the ratio of early fetal deaths to the total number of implantations occurring in the uterus of the female mice to which they are mated.

A summary of the results of the animal studies, other than for carcinogenicity and mutagenicity, is presented in Table III-1.

Carcinogenicity

In 1963, Kotin and Falk [62] reported at a conference, and Falk confirmed in a written communication of September 1975, that skin papillomas and cancer of the lymphatic system and of the lungs were observed in C3H strain mice injected with epichlorohydrin. A dose of 5 μM in 0.1 ml of ethyl laurate or tricaprylin was administered in a single subcutaneous injection to each of 30 mice, which were then observed for about 2 years. Control animals were injected with either solvent. Four experimental animals developed malignant lymphomas at 3.25, 3.25, 5.5, and 6 months after the beginning of the experiment. The incidence of lymphomas in the control group was about half that observed in the experimental animals. In the mice injected with epichlorohydrin, there was a skin papilloma after 11.5 months, a hepatoma in another mouse after 13 months, and 2 lung adenomas in 1 mouse after 24 months. The control animals had occasional hepatomas and lung adenomas, but no skin papillomas. The survival of the epichlorohydrin-treated animals was poor. During the 1st year, 12 mice died; after the 2nd year, only a few survived. Falk noted that, except for the single skin papilloma, the tumors were generally of the same type and frequency of occurrence as in the control groups. Thus, the experiment was inconclusive.

In 1963, Weil et al [63] reported the results of a skin carcinogenic assay with 60 epoxy compounds, including epichlorohydrin, on 90-day-old C3H-strain mice. Hair was removed from the backs of about 40 mice and

undiluted liquid epichlorohydrin was painted onto the midline 3 times/week for 25 months. The amount of epichlorohydrin applied was not measured. At the end of 12 months, 37 mice survived; at the end of 17 months, 30 were alive; and, at the end of 24 months, only 1 survived. Examinations showed no tumors and no signs of toxicity. Based on the mortality rate, the authors stated that epichlorohydrin was the most toxic of the epoxides tested. The only control substance was methylcholanthrene, a positive control, which induced tumors. Thus, it can be concluded that, under the conditions of this experiment, dermally applied epichlorohydrin does not induce significant numbers of skin tumors in C3H-strain mice.

In 1974, Van Duuren et al [64] reported the final results of dermal application, subcutaneous injection, and ip injection of epichlorohydrin on female ICR/Ha Swiss mice. Previously, Van Duuren had published two papers reporting preliminary [65] and intermediate results. [66] Since the complete report is now available, [64] the earlier papers are not discussed in this document. In the skin-application experiments, 50 mice were shaved initially and whenever necessary during the experiment. A dose of 2.0 mg of epichlorohydrin in 0.1 ml of acetone was applied three times/week to the interscapular region. The test lasted for 580 days (83 weeks). Skin lesions were diagnosed as papillomas when they reached 1 cu mm in size and persisted for 30 days or more. No papillomas or carcinomas occurred in the animals to which epichlorohydrin, acetone, or nothing was applied.

A group of female ICR/Ha mice had 2.0 mg of epichlorohydrin in 0.1 ml of acetone applied to the skin followed 2 weeks later by three applications each week, and throughout the study (total duration, 385 days), of 2.5 μg

of phorbol myristate acetate (PMA), a promoter, in 0.1 ml of acetone. [65] In the group of 50 mice to which epichlorohydrin was applied, nine mice developed papillomas and one a carcinoma. Control animals to which solvent (30) alone or no chemical (100) was applied produced no tumors. Of the mice to which PMA alone (30) was applied, three developed papillomas.

For an assay by subcutaneous injection, 50 mice were injected weekly in the left flank with 1.0 mg of epichlorohydrin in 0.05 ml of tricaprylin. [64] This test also lasted for 580 days. Of the animals given epichlorohydrin, six developed sarcomas and one an adenocarcinoma (P value less than or equal to 0.05). In comparison, 1 mouse of 50 injected with tricaprylin alone developed sarcoma, and none of the control animals developed any tumors. The significance of subcutaneous sarcomas which occur at the injection site has been discussed by Grasso and Golberg. [49] In an ip assay, 30 mice received weekly injections into the lower abdomen of 1.0 mg of epichlorohydrin in 0.05 ml of tricaprylin. The experiment was terminated after 450 days. None of the mice developed local sarcomas, but 11 had papillary lung tumors. Of mice given tricaprylin alone, 10 had papillary tumors of the lungs and 1 developed a local sarcoma.

The results of these studies raise concern about an additional risk of carcinogenesis for the segment of human population continuously exposed to epichlorohydrin throughout the working lifetime. Available experimental evidence [67] indicates that the risk of induction of cancer in animals and in humans can be reduced by reducing the maximum allowed cumulative dose to which they are exposed. Based on these reports, further tests involving long-term inhalation exposures of animals to epichlorohydrin must be done to determine the degree of occupational risk following chronic inhalation

of epichlorohydrin. A tabular summary of results of the carcinogenic studies is presented in Table III-2.

Mutagenicity

Definitions of various terms used in this section are given in the Glossary, Appendix IV. [68,69]

One of the earliest mutagenicity studies was undertaken by Rapoport [70] who, in 1948, reported that epichlorohydrin induced 0.7% mutations (4 mutations in a total of 526 chromosomes) in Drosophila melanogaster. A control series showed no mutations (0 mutations in 887 chromosomes). Although lack of details about the experimental design prevents a thorough evaluation of this report, it provides evidence that epichlorohydrin induces mutations in this eukaryotic organism.

In 1951, Loveless [71] defined radiomimetic activity as a highly specific effect upon the resting cell producing an alteration in the genetic material. He postulated that this alteration can be shown by chromosome breakage and rearrangement in subsequent divisions. Root tip meristems of Vicia faba (broad bean) were treated with epichlorohydrin solutions of unspecified concentrations. Exposure was limited to 1 hour, and epichlorohydrin at a wide series of concentrations was tested. At 4-6 hours after treatment, samples were examined for immediate effects on those cells already in division at the time of treatment and again at 18, 24, 36, or 48 hours to determine the effect upon cells in the resting stage at the time of treatment. The author [71] rated epichlorohydrin as having a "low activity" but did not state what was meant by this term; he indicated that the results of this study would be discussed fully in a later paper.

However, such a report has not been found.

The induction of mutations in microorganisms has been used as an ancillary method to study genetic responses to environmental agents. [72] Microbiologic assays offer a number of unique experimental advantages over mammalian assays. They can use large populations so that they have the capacity to detect events which occur infrequently. Also, microbial test systems are available which can distinguish between mechanistically different classes of microscopic mutations (base-pair substitution and frameshift mutations) and macroscopic alterations (chromosomal translocations and deletions). All these genetic alterations have been observed in human populations. [7,72] However, routine mammalian mutagenicity test systems which are capable of detecting base-pair substitutions and frameshift mutations in the offspring of mammals are not simple or very practical. From a practical viewpoint, microbial assays are rapid and relatively inexpensive. With the exception of certain viruses, the genetic material of all organisms consists of deoxyribonucleic acid, often complexed with ribonucleic acid and protein. [72] It is, therefore, clear that evidence of mutation induced by a chemical in microbial systems is a cause for concern for human populations but does not provide a basis for establishing the degree of risk to human populations.

In 1955, Kolmark and Giles [73] studied the induction of mutations by epichlorohydrin and five other monoepoxides. A purple mutant of Neurospora crassa lacking adenine, strain 38701, was used for the induction of reverse mutation. The spontaneous mutation rate at the loci governing adenine independence for Neurospora crassa is 0.0008-0.029 mutations/100,000 gametes. [69] Epichlorohydrin at a concentration of 0.15

M was added directly to a suspension of macroconidia in sterile distilled water. The suspension was kept at 25 C and gently agitated for 15-60 minutes, centrifuged, and then washed. At various intervals, portions were then plated onto a minimal agar medium. Controls were treated similarly except that no epichlorohydrin was added to the suspension. The number of viable, surviving conidia was determined by plating diluted samples on minimal medium supplemented with adenine. Out of a million surviving conidia, 8.5 reverse mutations were induced when treated with epichlorohydrin for 15 minutes. When the treatment was for 60 minutes, 411 mutations/million surviving conidia were induced. Thus, under the optimal conditions of 0.15 M epichlorohydrin for a 45-minute exposure, 7.36 x 10*7 (* means "to the power of") conidia were treated and yielded 4,140 revertants. The background was 39 revertants; the survival was 41.5%. This yields an average value of 135.2 induced premutational lesions/million surviving conidia, or 20 premutational lesions/mole of epichlorohydrin/minute. The biochemical nature of the original mutations in the conidia that were reversed by epichlorohydrin was not discussed. This test gives an underestimate of the true back-mutation frequency at this locus, since macroconidia were tested, and, if each such conidium contained three nuclei, a reversion in more than one nucleus would still be scored as a single revertant.

In 1960, Strauss and Okubo [74] reported the mutagenic actions of ultraviolet radiation and alkylating agents. One of the alkylating agents selected was epichlorohydrin. A tryptophan-requiring mutant of Escherichia coli, strain B/r, was used. The measure of mutation was the change from tryptophan requirement to independence, a reverse mutation. The number of

revertants present in a particular sample was determined by plating a 0.1-ml portion of the bacterial suspension on plates of minimal medium on a tryptophan-free nutrient agar. The total number of viable cells was determined by plating after dilution on nutrient agar containing tryptophan. The cells from overnight cultures were collected, washed twice with 0.1 M phosphate buffer of pH 7.2, and suspended in the same buffer at approximately 7 x 10*8 cells/ml. Epichlorohydrin (2 ml) was suspended in 3 ml of ethanol, but it did not go completely into solution. One milliliter of this mixture was added to 100 ml of the bacterial suspension and 1-ml portions were removed and plated without being washed. Cells were plated on either minimal agar or minimal agar supplemented with a small amount of nutrient broth to allow several divisions of the cells to enhance the expression of the revertant phenotype. Cells were also harvested, washed to remove excess epichlorohydrin, and resuspended in the buffer before plating. In the control, there were 2.2 x 10*8 viable cells plated. A total of 6.4 revertants/10*8 cells plated on the minimal medium and 11.4 revertants/10*8 cells plated on the minimal medium containing the nutrient broth were observed. When cells were harvested immediately after the addition of epichlorohydrin, there were 1.7 x 10*8 viable cells plated. Cells plated on the minimal medium showed 0.3 revertants/10*8 cells. Cells plated on the medium containing the broth showed 28.0/10*8 revertants. When cells were incubated with epichlorohydrin for 15 minutes before harvesting, there were 1.5 x 10*8 viable cells plated. There were no revertants in the cells plated on the medium alone and 38.3/10*8 revertants in cells plated on the medium containing the broth. Thus, the revertants are calculated to have increased at the rate of 30 premutational

lesions/mole of epichlorohydrin/minute during the 60-minute treatment. Survival was approximately 80% at the end of the treatment.

In 1973, Mukai and Hawryluk [75] reported testing epichlorohydrin in Escherichia coli and in Salmonella typhimurium. Since the details of the tests were not given, Mukai (written communication, October 1975) reported that 11.8 mg of epichlorohydrin in 0.1 ml of dimethyl sulfoxide was applied to sterile filter paper disks and placed in the center of duplicate test plates. Base-pair substitutions in Escherichia coli and both base-pair substitutions and frameshift mutations in Salmonella typhimurium were induced by epichlorohydrin. He further reported that greater than twentyfold increases in revertants over control plates were observed in both organisms.

In 1973, Voogd [76] reported in an abstract testing several epoxy compounds, including epichlorohydrin, for their abilities to induce mutations in a Klebsiella pneumoniae auxotroph requiring uracil and proline for growth. Mutations to streptomycin resistance were scored. The author stated that nearly all the tested substances displayed a certain degree of mutagenicity and that the mutagenic activity of the epoxy group was often slightly increased by electrophilic groups on carbon atoms next to the epoxy group. Voogd provided additional information in a written communication of October 1975. Increases in mutations in both Klebsiella pneumoniae and Salmonella typhimurium were induced by epichlorohydrin. The experiment was a Luria-Delbruck fluctuation test, [77] so that a true mutation rate, and not a mutation frequency, was measured. In the Klebsiella pneumoniae assay, the mutation frequency was based on a forward mutation to streptomycin-resistance or streptomycin-dependence. In

general, such mutations alter a ribosomal protein, and chemicals which cause frameshift mutations or lead to deletions are not detected as mutagens in such an assay. The cells were suspended in broth in a concentration of 100 cells/ml, and 105 samples of 2.5 ml were treated for 20 hours with 0.00637 M epichlorohydrin. After treatment with epichlorohydrin, the maximum reported frequency of mutations was 7.617/10*9 or 45.4 times the background of 0.1676/10*9. In the Salmonella assay, his G-46, TA1530, TA1535, TA100, and TA98 strains were also tested for mutagenesis by a procedure similar to the Klebsiella assay. The first four strains contain the same base-pair substitution mutation. The reverse mutation to histidine-independence was increased in all four strains by 0.001 M epichlorohydrin. Strain TA98 contains a frameshift mutation and did not revert when treated with epichlorohydrin.

Recently, workers exposed to epichlorohydrin were monitored for the presence of mutagens in their urine (DJ Kilian, written communication, April 1976). The study group included six people exposed to epichlorohydrin at concentrations from 0.8 to 4.0 ppm, measured as 8-hour TWA concentrations, and two people exposed by a large epichlorohydrin spill that generated a concentration in air in excess of 25 ppm. Five subjects who had not been exposed to epichlorohydrin served as controls. Mutagenic activity was evaluated using Salmonella typhimurium strains 1535, 1537, 1538, 100, and 98. Results are summarized in Table XIII-3. All samples except the two from individuals with high exposures were comparable with those from the control subjects; preparations derived from the urine of these two individuals indicated the presence of materials which influenced the genetic mechanism of Salmonella typhimurium. With the 1535 strain, an

increase in mutants in excess of twofold over the control was evident. In strain 1537, a statistically significant decrease in the number of mutants, relative to the controls, was observed. Although there were only six people in the data base, this report does indicate that exposure to high concentrations of epichlorohydrin may give positive results. While it is not possible to draw any dose-effect relationship from this small group of subjects, the results of the study tend to indicate that a genetically active material is present within the human body after a sufficiently large exposure to epichlorohydrin.

In summary, the induction of reverse mutations by epichlorohydrin in N crassa, [73] E coli, [74] and S typhimurium, [75] and forward mutations in K pneumoniae [76] have been observed. Most of the microbial mutations could be accounted for by base-pair substitutions. Following exposure to epichlorohydrin, mutations have occurred in the fruit fly (Drosophila melanogaster). [70] Thus, apparently epichlorohydrin can induce point mutations in some organisms, both prokaryotic and eukaryotic. Other than the dominant lethal assay, [61] reports of experiments designed to detect epichlorohydrin-induced inheritable genetic alterations in mammals have not been found, so that a reasonable basis for estimating the risk to the human population has not been established. However, it has been shown that a substance that can induce mutations is present in the urine of people who are overexposed to epichlorohydrin (DJ Kilian, written communication, April 1976). The results of the mutagenicity experiments discussed in this section are summarized in Table III-3.

Correlation of Exposure and Effect

Occupational exposure to epichlorohydrin occurs chiefly by inhalation and skin contact and, to a limited extent, by ingestion. Cases of delayed skin burns resulting from contact with epichlorohydrin in the occupational environment have been reported. [35] Burning, itching, blisters, skin erosion, redness, and residual erythema have been the usual signs. Usually, there has been a latent period of a few minutes to several hours between the dermal contact and the resulting effects. The severity of the burns has been dependent upon the duration of the contact. One worker who failed to remove his epichlorohydrin-contaminated shoes for 6 hours developed severe skin damage with painful, enlarged lymph nodes in the groin; he was hospitalized for 22 days. Another worker who washed his hands with soap and water after wiping up an epichlorohydrin-methanol mixture from the floor developed itching, burning, redness, and swelling, and was hospitalized for 11 days. Edema, ulceration, hyperemia, and necrosis of rabbit skin resulting from dermal application of epichlorohydrin also have been observed. [27,29] Kremneva and Tolgskaya [27] reported also that, when the tails of 10 mice were immersed for an hour in test tubes containing epichlorohydrin, 6 animals died within 3 days.

Thus, experience in both humans [35] and animals [27,29] indicates that epichlorohydrin may induce, in addition to severe damage to the skin, systemic poisoning following dermal absorption. The intensity of these effects appears to increase with the dose and the duration of exposure.

There are very few reports of the effects on humans of epichlorohydrin inhalation. [11,33,37,38] One report [33] involved a

worker who was acutely exposed to epichlorohydrin at an unspecified but probably very high concentration. Eye and throat irritation, nausea, vomiting, headache, and dyspnea were the immediate effects. Bronchitis, liver damage, and hypertension were detected during the 2 years following the accident. An industrial report [37] stated that exposure to epichlorohydrin at 100 ppm, even for a short period, would be intolerable to humans. This was based on the observation that exposure to epichlorohydrin at 40 ppm for an hour caused eye and throat irritation lasting 48 hours and that exposure at 20 ppm for one hour caused temporary burning of the eye and nasal passages. Wexler [38] reported that exposure to epichlorohydrin at concentrations greater than 100 ppm induced lung edema and kidney lesions in humans. Pet'ko et al [11] observed changes in the reticulocyte count, bilirubin, cholesterol, and protein concentration of the blood in people occupationally exposed to epichlorohydrin. These changes were not statistically significant. An epidemiologic study (DJ Kilian, written communication, April 1976) of workers exposed to epichlorohydrin revealed no abnormalities. Because of the lack of a control group, lack of information on the exposure concentrations, and because the longest exposure period was only 16 years, it is difficult to make any final conclusions regarding chronic effects, especially those with long latent periods.

Fomin [28] reported that inhalation of epichlorohydrin at 0.08 ppm induced statistically significant (P value not given) changes in the EEG recordings in all of five volunteers; no effects were observed at a concentration of 0.05 ppm. Since the exposure period was not given, it is not possible to estimate the dose of epichlorohydrin absorbed. If the

concentrations stated in this paper are correct, it can be concluded from this study that inhalation of epichlorohydrin at 0.08 ppm induces measurable effects on brain function in humans.

Epichlorohydrin-induced sterility in animals has been reported. [39,41] Cooper et al [39] found that 12 repeated doses of epichlorohydrin at 100 mg/kg caused an apparently permanent sterility of male rats as indicated during a 12-week period, while Hahn [41] found that the dose of 15 mg/kg/day of epichlorohydrin produced a reversible sterility. It can be concluded that, in rats, epichlorohydrin causes sterility which is both dose and time dependent. The induction of sterility following repeated doses of 15 mg/kg is another example of the time-dependent cumulative nature of effects induced by epichlorohydrin. The results from the experiments in mice and rats are insufficient to permit any inference of the rate at which epichlorohydrin must be absorbed to induce inheritable genetic alterations in the offspring of mammals, although the evidence suggests that epichlorohydrin may influence the spontaneous mutation rate in mammals.

Animal studies in which inhalation was the principal route of entry have been extensively reported. [26-28] Gage [26] found that labored breathing, profuse nasal discharge, loss of weight, leukocytosis, increased urinary protein concentration, and peripheral atrophy of the cortical tubules in the kidney occurred in rats exposed to epichlorohydrin at 120 ppm. Respiratory distress, nasal discharge, and weight loss were evident in rats inhaling epichlorohydrin at 56 ppm; these effects were still present but less severe at 27 ppm. No effects were observed on rats exposed to epichlorohydrin at 17 ppm; however, at 9 ppm, pulmonary

infection occurred in two rats. Fomin [28] reported increases in leukocytes with altered fluorescence and urinary coproporphyrin, accompanied by decreases in the nucleic acid content of the blood, in rats exposed to epichlorohydrin at 5 ppm; in addition, lung and kidney damage were present. Some of these effects were also present in rats exposed to epichlorohdyrin at 0.5 ppm, while none were observed at 0.05 ppm. Kidney damage and disrupted liver function were observed in animals exposed at 5.2 and 1.8 ppm. [32] From these studies, it can be concluded that epichlorohydrin causes liver and kidney damage in animals. There are two conditions of exposure at which epichlorohydrin induced no effects: 27 ppm, 6 hours/day, for 18 days [26] and 0.05 ppm, 24 hours/day, for 98 days. [28]

Carcinogenicity, Mutagenicity, and Teratogenicity

Van Duuren et al [64] have reported that epichlorohydrin induces tumors in mice at subcutaneous injection sites when administered alone. Weil et al [63] and Van Duuren et al [64] found that epichlorohydrin induced no tumors in mice by skin painting assay. The data of the skin applications followed by a promotor suggest that epichlorohydrin may initiate the carcinogenic process in mice. [64] Weekly ip injections of 1.0 mg epichlorohydrin resulted in 11 of 30 mice developing papillary lung tumors, which was similar to the observations in the control group.

The induction of mutations following exposure to epichlorohydrin in microbial species has been reported. [71,73,75] The experiments indicate that epichlorohydrin can induce base-pair substitution mutations. It is evident that epichlorohydrin induces a high frequency of mutations in

fungi, [71] microbial organisms, [73-76] and in the fruit fly. [70] The most plausible molecular basis of the epichlorohydrin-induced mutations in all of these organisms is the covalent bonding of epichlorohydrin to the cellular genetic material, DNA. In view of the virtual irreversibility of these reactions, the degree of genetic damage may be expected to increase with exposure time in these lower organisms as well as in higher forms. [78]

Reports of experimental attempts to induce the formation of terata by exposing pregnant female animals to epichlorohydrin have not been found.

TABLE III-1

EFFECTS OF EPICHLOROHYDRIN IN ANIMALS

Routes of Exposure	Animals	No.	Exposure Concentration and Duration	Effects	Reference
Inhalation	Mice	30	16,600 ppm 30 min	Nose and eye irritation, mortality 100%	25
"	"	20	8,300 ppm 30 min	Mortality 100%	25
"	"	10	2,370 ppm 1 hr/d x 16 d	"	25
"	"	30	2,370 ppm 1 hr	No deaths	25
"	Rats	6	250 ppm 4 hr	Death of 2-4 rats	58
"	"	8	120 ppm 6 hr/d 5 d/wk x 11 d	Labored breathing, profuse nasal discharge, weight loss, leukocytosis, increased urinary protein, peripheral atrophy of cortical tubules	26
"	"	60	91.0 ppm 4 hr	Kidney damage, liver function disrupted	32
"	"	60	5.2 ppm 4 hr	"	32
"	"	60	1.8 ppm 4 hr	Kidney damage and disrupted liver function less severe than with 91.0 and 5.2 ppm	32
"	"	8	56 ppm 6 hr/d 5 d/wk x 18 d	Respiratory distress, nasal discharge, weight loss	26

TABLE III-1 (CONTINUED)

EFFECTS OF EPICHLOROHYDRIN IN ANIMALS

Routes of Exposure	Animals	No.	Exposure Concentration and Duration	Effects	Reference
Inhalation	Rats	8	27 ppm 6 hr/d 5 d/wk x 18 d	Mild nasal irritation	26
"	"	8	17 ppm 6 hr/d 5 d/wk x 19 d	No effects	26
"	"	8	9 ppm 6 hr/d 5 d/wk x 18 d	Pulmonary infection	26
"	"	15	5.2 ppm 24 hr/d x 98 d	More leukocytes with altered fluorescence, increased urine coproporphyrin, kidney and lung damage, decrease in blood nucleic acid	28
"	"	15	0.5 ppm 24 hr/d x 98 d	Increased modified leukocytes, reduced blood nucleic acid	28
"	"	15	0.05 ppm 24 hr/d x 98 d	No effect	28
Subcutaneous	"	120	500 mg/kg 250 mg/kg 125 mg/kg	Reduced blood histamine activity	42
"	"	14	180 mg/kg	Necrotic lesions in nephrons; nonspecific lung, brain, and adrenal gland damage	30
"	"	23	150 mg/kg	"	30

TABLE III-1 (CONTINUED)

EFFECTS OF EPICHLOROHYDRIN IN ANIMALS

Routes of Exposure	Animals	No.	Exposure Concentration and Duration	Effects	Reference
Subcutaneous	Rats	-	150 mg/kg	LD50	29
"	"	67	125 mg/kg	Oliguria, anuria, polyuria, kidney damage	46
"	Mice	10	0.23 ml/kg/d x 4 d	Mortality 100%	25
"	"	10	0.08 ml/kg/d x 21 d	"	25
Oral	"	15	0.5 ml/kg	"	25
"	"	15	0.23 ml/kg/d x 4 d	"	25
"	"	15	0.23 ml/kg	No deaths	25
"	"	-	0.20 ml/kg	LD50	31
"	"	15	0.08 ml/kg/d x 21 d	Mortality 100%	25
"	Rats	-	0.22 ml/kg	LD50	31
Dermal	Rabbits	-	0.64 ml/kg	LD50	31
"	Rats	20	2 ml/kg x 1 hr	Mortality 80%, local irritation	25
"	"	10	1 ml/kg x 1 hr x 3	Mortality 40%, local irritation	25
"	"	10	1 ml/kg x 1 hr	Mortality 20%, local irritation	25

TABLE III-1 (CONTINUED)

EFFECTS OF EPICHLOROHYDRIN IN ANIMALS

Routes of Exposure	Animals	No.	Exposure Concentration and Duration	Effects	Reference
Dermal	Rats	10	0.5 ml/kg x 1 hr x 4	Mortality 100%, local irritation	25
"	"	10	0.5 ml/kg x 1 hr	Local irritation, no deaths	25
iv	Cats	-	0.08 ml/kg	Minimum lethal dose	25
"	"	3	0.008 ml/kg	Transitory fall in blood pressure	25
"	Dogs	-	0.08 ml/kg	Minimum lethal dose	25
"	"	2	0.008 ml/kg	Transitory fall in blood pressure	25
ip	Mice, rats, guinea pigs, and rabbits	-	0.10 to 0.14 ml/kg	LD50	31

TABLE III-2

CARCINOGENIC EFFECTS OF EPICHLOROHYDRIN IN MICE

Routes of Exposure	Strain	No.	Exposure Concentration and Duration	Effects	Reference
Subcutaneous	C3H	30	5 μM	4 malignant lymphomas, (about double the control value)	Falk*
"	ICR/Ha Swiss	50 F	1 mg** 1/wk x 83 wk	1 adenocarcinoma, 6 sarcomas	64
Controls	"	50 F	vehicle only	1 sarcoma	
ip	"	30 F	1 mg** 1/wk x 64 wk	11 papillary lung tumors	64
Controls	"	30	vehicle only	10 papillomas 1 sarcoma	
Painting on clipped backs	C3H	27 - 30	Undiluted 3/wk x 25 mon	0 tumors	63
Application to shaved backs	ICR/Ha Swiss	50 F	2.0 mg in 0.1 ml acetone 3/wk x 83 wk	"	64
"	"	30 F	2.0 mg in 0.1 ml acetone***	9 papillomas, 1 carcinoma	64
"	"	30 F	controls****	3 papillomas	

*(H Falk, written communication, September 1975)
**Dissolved in 0.05 ml tricaprylin
***Beginning 2 weeks after the one epichlorohydrin application, 2.5 μg of PMA was applied three times/week for 53 weeks.
****2.5 μg of PMA only was applied three times/week for 53 weeks.

TABLE III-3

MUTAGENICITY STUDIES

Species	Dose	Results	Reference
Drosophila melanogaster	Unspecified	Epichlorohydrin-induced mutations, 0.7%; none in control series	70
Neurospora crassa	0.15 M	Epichlorohydrin-induced premutational lesions, 20/mole/minute, over the background	73
Escherichia coli	0.017 M	Epichlorohydrin-induced (reverse) premutational lesions, 30/mole/minute, over the background	74
Escherichia coli	1.27 M in dimethyl-sulfoxide	A 20-fold increase in revertants over control	75
Salmonella typhimurium	"	"	75
Klebsiella pneumoniae	0.00637 M	A 45.4-fold increase in mutations over background	76
Salmonella typhimurium	0.001 M	An increased number of induced mutations over control	76