(11) A. Suzuki, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, **59**, 644(1970).
(12) *Ibid.*, **59**, 651(1970).
(13) D. R. Reese, G. M. Irwin, L. W. Dittert, C. W. Chong, and J. V. Swintosky, *J. Pharm. Sci.*, **53**, 591(1964).
(14) "Documenta Geigy," Geigy Pharmaceuticals, Basle, Switzerand, 1962, p. 314.

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Toxicity of Ethylene Chlorohydrin II: Subacute Toxicity and Special Tests

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Abstract □ Ethylene chlorohydrin may be found as a reaction product when plastic items are sterilized with ethylene oxide. A previous publication presented results of an acute toxicity study of ethylene chlorohydrin in animals and tissue culture. This paper presents additional toxicity information derived from a study of rats receiving the compound intraperitoneally three times a week for 3 months, and from a similar study in which the compound was administered daily for 1 month. No adverse effects were observed in the 1-month study from 6.4 mg./kg. daily of ethylene chlorohydrin (one-tenth of the acute LD_{50}), or from 12.8 mg./kg. administered three times a week for 3 months. Additionally, special tests were included to determine the effect of the compound upon: cardiovascular, respiratory, and neuromuscular functions in rabbits; sensitizing potential in guinea pigs; and pentobarbital sleeping time in mice.

Keyphrases □ Ethylene chlorohydrin—subacute toxicity, cardiovascular, respiratory, neuromuscular, sensitizing potential, sleeping time effects □ Ethylene oxide sterilization—ethylene chlorohydrin toxicity evaluation □ Toxicity, subacute—ethylene chlorohydrin evaluation, rats

During the late sixties, a growing awareness of a lack of pertinent information relating to the toxicity of ethylene chlorohydrin (2-chloroethanol) prompted a number of studies. For many years, industrial workers have experienced local and systemic adverse reactions from improper handling of this compound; more recently, the treatment of foods and the sterilization of polymeric substances with ethylene oxide have resulted in the formation of ethylene chlorohydrin when chlorides were present even in minute amounts in the exposed material. The acquisition of additional toxicological data is essential to alert medical care personnel to the potential hazards associated with ethylene chlorohydrin when hospitalized patients come in contact with ethylene oxide-sterilized devices, to warn the general public of the dangers of consuming ethylene oxide-treated foods, and, finally, to inform those who come in contact with large quantities of the chemical in industry.

In a previous paper (1), results of an acute toxicity study of ethylene chlorohydrin gave LD_{50} values within a rather narrow range (64–98 mg./kg.) when the compound was administered to mice, rats, rabbits, and guinea pigs, which were independent of the route of administration. The compound was easily absorbed through the intact skin; thus, the LD_{50} produced by topical administration was comparable to that from intraperitoneal or oral administration. Its vapors, when mixed with air, killed 50% of the test animals after 13.3 min. of exposure. The compound was found to be highly irritating intradermally or from ophthalmic application. Little irritation was noted, however, when the compound was applied to the skin of rabbits.

Because it is possible for people to be repeatedly exposed to ethylene chlorohydrin in very small concentrations by the parenteral, dermal, or oral[1] routes, it was deemed appropriate to extend the previous studies into an investigation of subacute toxicity and to conduct certain other special studies.

EXPERIMENTAL

Materials—Ethylene chlorohydrin[2] was used.

Subacute Toxicity Studies—*Phase One: 12-Week Study*—In these experiments, male Sprague-Dawley rats were used, having initial weights of 57–64 g. In the first series of experiments, two dose levels were administered intraperitoneally to groups of 12 rats. The dose levels were based upon one-fifth and one-half the acute LD_{50} dose or 12.8 and 32.0 mg./kg., respectively. When possible, the compound was administered as such or, when necessary, it was diluted in distilled water and administered using microliter syringes. The respective dose levels were administered three times a week (Monday, Wednesday, and Friday) over a 12-week period. A control group of rats received normal saline in a volume equivalent to the test samples and at the same time intervals. The animals were housed two in a cage and given food and water *ad libitum*.

All animals were observed daily to assess general health and mortalities; they were weighed three times per week, just prior to injection of the chlorohydrin, and the weights were recorded. Food consumption was measured during the 1st, 2nd, 5th, 9th, and 12th weeks. Hematologic tests were performed on six rats selected at random from each test group and saline controls at the end of the 12th week. Several drops of blood were obtained from each animal by clipping the end of the tail for determination of the following:

[1] In addition to the possibility that it may be contained in certain foods, some hospitals sterilize plastic drinking tumblers and pitchers with ethylene oxide and then fill these with drinking water, which, of course, contains chlorides and possibly fluorides.

[2] Matheson, Coleman, and Bell, East Rutherford, N. J. The liquid was established as being more than 99% ethylene chlorohydrin.

Table I—Mortalities in Subacute Toxicity Studies of Ethylene Chlorohydrin in Rats

Dose[a]	Ratio (Deaths/Total)
Phase One	
0.20 LD_{50}	4/12[b]
0.50 LD_{50}	6/12
Normal saline (control)	0/12
Phase Two	
0.10 LD_{50}	0/12
0.20 LD_{50}	0/12
Normal saline (control)	0/12

[a] LD_{50} in rats intraperitoneally is 64.0 mg./kg. [b] These rats died in the first 1–2 weeks and were most likely due to errors in dosage. The experiment was repeated in Phase Two with no deaths.

hemoglobin, hematocrit, erythrocytes, total leukocytes, platelets, and differential leukocyte counts.

At the end of the study, six animals in each group were sacrificed and autopsied for gross pathologic lesions. Organ-to-body weights were determined for the adrenals, brain, gonads, heart, kidneys, liver, lungs, and spleen; these organs were preserved in 10% buffered formalin, sectioned, and stained with hematoxylin-eosin for histopathologic evaluation.

Phase Two: 12-Week Study—During the early course of the subacute toxicity study reported in Phase One, four rats in the 0.20 LD_{50} group died but it was believed that these deaths may have been caused by an overdose of chlorohydrin; thus the experiment was repeated following the procedures already described. A second 12-week subacute toxicity study was also conducted in a manner similar to the previous studies except that a lower dose of chlorohydrin was employed: 0.10 LD_{50} (6.4 mg./kg.). In each of these studies, an additional test was utilized to ascertain if possible liver damage may have occurred by selecting six animals from each group and evaluating the rate of disappearance of sulfobromophthalein[3] from serum. The method of Gaebler (2) was used for sulfobromophthalein determination.

Phase Three: 30-Day Cumulative Study—Another study was undertaken to determine if cumulative toxic effects might be seen when animals were administered daily doses of the test compound for 30 days. Two dose levels were administered to male rats of the same strain. Doses of 0.10 LD_{50} (6.4 mg./kg.) and 0.20 LD_{50} (12.8 mg./kg.) were employed, and a control group of 12 rats received a similar volume of normal saline. All animals were observed daily for mortalities and general health, and weights of the animals were recorded daily. At the end of the 30-day period, representative animals from each group were sacrificed and autopsied for gross pathological lesions. The adrenals, brain, gonads, heart, liver, kidneys, lungs, and spleen were removed from each animal and weighed to permit calculation of the organ-to-body weight ratio. Sections of each organ were prepared for histopathologic examination.

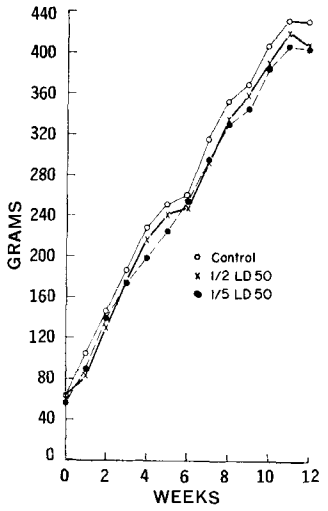

Figure 1—*Effect of ethylene chlorohydrin on growth rate of rats (injected three times a week).*

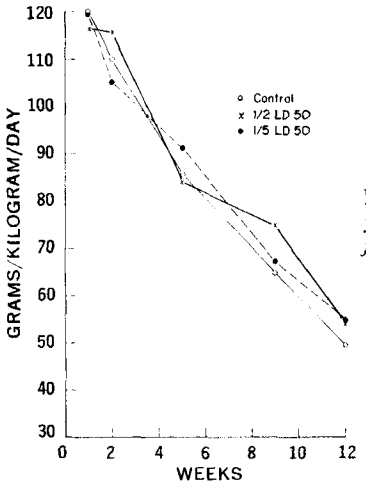

Figure 2—*Effect of ethylene chlorohydrin on food consumption in rats.*

Special Tests and Studies—*Sensitization Test*—Sensitization tests were performed on Hartley strain guinea pigs, using the "guinea pig maximization test" of Magnusson and Kligman (3) with 2,4-dinitrochlorobenzene as the positive control and cottonseed oil and petrolatum as the negative control. The test, as conducted here, involved an intradermal injection of 0.1 ml. of 10% ethylene chlorohydrin in one group of guinea pigs along with Freund's Complete Adjuvant[4], as described by Magnusson and Kligman (3), and 0.1 ml. of 5% ethylene chlorohydrin in a second group. This was followed in 7 days with a topical application of the same quantity of test compound. Two weeks later, the hair was shaved from another site and the test material was applied topically with an occluding bandage for 24 hr. The bandage then was removed, and the area was cleansed with alcohol. After an additional 24 hr., the test sites were evaluated for response to the challenging test agent. The results were scored as follows: 0 = no reaction; 1 = scattered, mild redness; 2 = moderate and diffuse redness; and 3 = intense redness and swelling. The challenging dose of the positive control (25% 2,4-dinitrochlorobenzene in petrolatum) produced a 3 reaction. Five animals per group were used in this test.

Cardiovascular, Respiratory, and Neuromuscular Effects—Rabbits, of approximately 2 kg., were anesthetized by intravenous injection of 30–40 mg./kg. of pentobarbital sodium approximately 15 min. after an intraperitoneal injection of 500 mg./kg. of urethan (ethyl carbamate). All recordings were made with a Grass model 7 polygraph in conjunction with appropriate preamplifiers[5]. Standard surgical procedures were employed in cannulation or isolation of various anatomical structures. Systemic blood pressure was determined by cannulating a branch of the left femoral artery and connecting it to a Statham pressure transducer. Electrical activity of the anterior tibialis and/or gastrocnemius muscle was monitored on the oscilloscope of a dual-beam Neurophysiograph[6], while the mechanical response was recorded using a Grass force-displacement transducer. Shielded electrodes were placed on the sciatic nerve, and the stimulus (voltage, frequency, duration, and delay) was supplied and controlled by a Grass S-4 stimulator through a matching stimulus isolation unit. Respiration was recorded using an impedance pneumograph, and the ECG was obtained from appropriately placed subdermal electrodes.

In these experiments, each rabbit was administered a specific dose of chlorohydrin solution intravenously. The doses administered were as follows: 38.3, 78.9, 157.7, 606.5, and 1577 mg./kg. Cardiovascular, respiratory, and neuromuscular effects were recorded before and after injections.

Sleeping Time—Male, ICR albino mice, weighing 25–30 g., received intraperitoneal injections of ethylene chlorohydrin at three dose levels (0.10, 0.20, and 0.50 LD_{50}) 24 and 48 hr. prior to sleeping time determinations. Pentobarbital sodium, 50 mg./kg., was injected intraperitoneally into each mouse; the mice were observed

[3] Bromsulphalein, brand of sulfobromophthalein sodium injection, by Hynson, Westcott & Dunning, Inc., Baltimore, Md.

[4] Difco Laboratories, Detroit, Mich.
[5] Grass Instruments Co., Quincy, Mass.
[6] E. & M. Instrument Co., Inc., Houston, Tex.

Table II—Subacute Toxicity of Ethylene Chlorohydrin in Rats[a], Hematologic Values[b] (Mean ± *SE*)

Item	Saline Control	0.20 LD_{50} (12.8 mg./kg.)	0.50 LD_{50} (32.0 mg./kg.)
Hemoglobin, g./100 ml.	17.73 ± 0.72	15.67 ± 0.18	16.76 ± 0.65
Hematocrit, %	54.83 ± 1.22	52.17 ± 1.42	55.25 ± 1.59
Erythrocytes, $\times 10^6$/mm.^{3}	9.965 ± 0.616	9.465 ± 0.259	9.625 ± 0.669
Total leucocytes, $\times 10^3$/mm.^{3}	15.81 ± 2.26	17.74 ± 3.18	13.33 ± 1.54
Platelets[c]	185.00 ± 30.72	233.00 ± 43.56	189.00 ± 25.95
Differential leucocyte count, as %			
Segs	9.25 ± 1.48	8.42 ± 0.98	12.50 ± 1.79
Lymphs	83.42 ± 1.35	87.75 ± 1.20	80.33 ± 1.67
Monocytes	1.83 ± 0.60	0.833 ± 0.146	1.33 ± 0.16
Eosinophils	1.417 ± 0.25	1.50 ± 0.36	1.42 ± 0.33
Meta	0.250 ± 0.145	0.083 ± 0.076	0.083 ± 0.076
Juvenile	1.00 ± 0.26	0.033 ± 0.172	0.58 ± 0.13
Bands	2.33 ± 0.73	0.917 ± 0.249	3.92 ± 0.69

[a] Drug injected intraperitoneally three times per week for 12 weeks. [b] Based on six rats per group. $SE = \sqrt{\frac{(X - \bar{X})^2}{n - 1}} \cdot \frac{1}{\sqrt{n}}$. [c] Number observed per 10 oil immersion fields.

closely for loss and then return of the righting reflex. Sleeping time was considered as that time period during which the righting reflex was absent. Ten mice were used for each dose level.

RESULTS

Subacute Toxicity Studies—*Phase One and Two Studies*—Table I summarizes the mortalities that occurred in the Phase One and Two studies. Four animals out of 12 died in Phase One at a 0.20 LD_{50} dose. As was already mentioned, these deaths occurred in the early portion of this study and some suspicion was cast upon the dose actually administered. When this experiment was repeated (Phase Two), no deaths occurred. As will be seen from Table I, when the dose was increased to 0.50 LD_{50}, six animals died during the 12-week period. No deaths were seen for animals administered the 0.10 LD_{50} dose nor for the saline-treated controls. The remaining animals in all groups remained in good health throughout the study.

Figure 1 graphically represents the weekly weight gain (mean value) throughout the 12-week study for those rats receiving 12.8 and 32 mg./kg. of chlorohydrin as well as for the saline controls. Animals receiving 6.4 mg./kg. of chlorohydrin (not included in Fig. 1) showed weight gains similar to the control animals. A careful examination of the weight gain–time curve reveals a very slight decrease in the mean weight gains for the experimental animals *versus* the controls. No significant difference (by *t*-test) was apparent in the average weight gain over the 12-week period for the experimental animals when compared to the controls.

There was no appreciable difference in food consumption between the saline-treated controls and those receiving ethylene chlorohydrin.

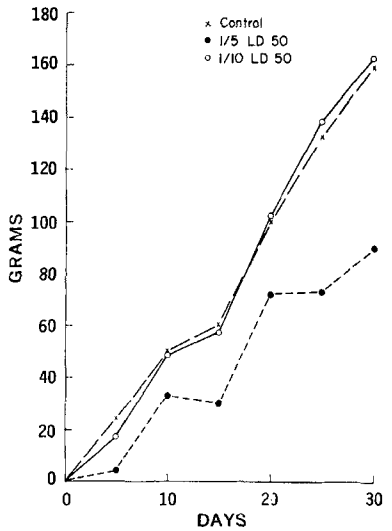

Figure 3—*Effect of daily administration of ethylene chlorohydrin on weight gain in rats.*

This may be seen in Fig. 2, where the amount of food consumed, as grams/kilogram/day, is plotted against weeks of the study. This figure shows data for the two higher dose groups (12.8 and 32 mg./kg.) and the controls. Data for the group receiving 6.4 mg./kg. were not included; however, this group was not significantly different from the saline-treated controls.

Results of hematologic tests conducted on chlorohydrin-treated (12.8 and 32 mg./kg.) rats and controls are shown in Table II. There appears to be no remarkable dose-related effects among the hematologic parameters tested. In the instances of differences between the test animals and the controls, the variations did not follow a dose–response pattern. Results of hematology tests for the animals treated with 6.4 mg./kg. of chlorohydrin did not differ significantly from the control animals.

Table III includes organ-to-body weight ratios from rats administered chlorohydrin over the 12-week period. The data include the average values for animals receiving 0.20 and 0.50 LD_{50} and for the control group (saline treated). Since the ratios for the animals treated with 0.10 LD_{50} were similar to the control group (saline treated), these values were not included in the table.

Histopathologic evaluation of the various organs studied in both the experimental animals (treated with the various dose levels of chlorohydrin) and the control animals did not reveal any significant pathologic features which could be attributed to the administration of the compound.

Phase Three (30-Day Cumulative Study)—During the 30-day period, no deaths occurred in the saline-treated controls or in the group receiving 0.10 of the LD_{50} (6.4 mg./kg.) dose of chlorohydrin (Table IV). At the higher daily dose (0.20 LD_{50}), seven of the 12 animals died (58.3%). Figure 3 presents the weight gain of both groups of experimental animals and the control animals. The curves reveal that the treated group on the highest dose (0.20 LD_{50}) exhibited a marked reduction in the rate of growth. These differences were statistically significant at 15 days ($p = < 0.20 > 0.10$) and at 30 days ($p = 0.01$).

Table III—Organ-to-Body Weight Ratio of Rats Administered Ethylene Chlorohydrin over 12-Week Period[a] (Expressed as Percent ± *SE*)

Organ	Control (Normal Saline)	Ethylene Chlorohydrin 0.20 LD_{50} (12.8 mg./kg.)	Ethylene Chlorohydrin 0.50 LD_{50} (32.0 mg./kg.)
Adrenals	0.019 ± 0.005	0.013 ± 0.001	0.010 ± 0.002
Brain	0.340 ± 0.018	0.350 ± 0.014	0.410 ± 0.015
Gonads	0.780 ± 0.023	0.800 ± 0.029	0.780 ± 0.021
Heart	0.290 ± 0.012	0.290 ± 0.009	0.310 ± 0.008
Kidneys	0.650 ± 0.008	0.660 ± 0.022	0.680 ± 0.037
Liver	3.550 ± 0.146	3.580 ± 0.215	3.700 ± 0.131
Spleen	0.180 ± 0.033	0.210 ± 0.018	0.200 ± 0.016

[a] Animals in each group were administered, intraperitoneally, control solution or test solutions three times a week up to and including the 12th week. Values are based upon results from six rats per group.

Table IV—Mortalities in 30-Day Cumulative Studies of Ethylene Chlorohydrin in Rats

Dose	Ratio (Deaths/Total)
0.10 LD_{50} (6.4 mg./kg.)	0/12
0.20 LD_{50} (12.8 mg./kg.)	7/12
Normal saline (control)	0/12

Histopathologic examinations of the adrenals, brain, gonads, heart, kidneys, liver, lungs, and spleen did not reveal any unusual pathologic lesions which could be attributed to the administration of the compound. Organ-to-body weights, expressed as percentages, are shown in Table V. Several of the organs (adrenals, brain, gonads, kidneys, and lungs) tended to show a relative increase in weight with an increase in the dose of chlorohydrin.

Special Studies—*Sensitization Test*—Chlorohydrin did not produce a sensitizing response in guinea pigs under the experimental conditions employed.

Cardiovascular, Respiratory, and Neuromuscular Effects—The acute effects of chlorohydrin upon cardiovascular function of the anesthetized rabbit may be divided into two categories, depending upon the quantity administered. With the lower doses (38.8, 78.9, and 157.7 mg./kg.), intravenous administration was followed by a fall in diastolic pressure, presumably from vasodilation since heart rate, systolic pressure, and the ECG pattern remained essentially unchanged. About 2–2.5 hr. following the administration of the agent, systolic pressure in some, but not all, of the animals declined. Higher doses (606.5 mg./kg. or more) produced a dose-related reduction in both systolic and diastolic pressures, culminating in death of the animal usually between 1 and 2 hr. after the agent was administered. With a dose of 1577 mg./kg., the largest dose used, marked dyspnea was produced and a precipitous fall in both systolic and diastolic pressure. Death usually occurred in about 5 min., which was preceded by respiratory failure.

It is difficult to pinpoint the exact cause of death in these experiments, but it is believed to be due to failure of the respiratory center of the medulla; however, one should not rule out a direct cardiotoxic or neuromuscular effect. The effects on blood pressure are shown diagrammatically in Fig. 4.

The respiratory system of the rabbit is susceptible to the action of chlorohydrin. Low doses, as used, had litle or no effect upon the rate or depth of respiration, while moderate doses tended to diminish it and larger doses to arrest it.

The response of skeletal muscle (anterior tibialis and gastrocnemius) of the rabbit to electrical stimulation of the sciatic nerve was impaired and eventually blocked by the administration of ethylene chlorohydrin. Electrical stimuli were supplied to the nerve at a frequency of one per second and a duration of 0.3 msec., with a voltage sufficient to produce nearly a maximum twitch response. After injection of the compound, the latency period was prolonged, followed by an increase in threshold and finally failure to respond to 5–10 times the initial voltage. Observation of the electrical activity of the muscle, displayed on an oscilloscope, showed the development of

Table V—Organ-to-Body Weight Ratio of Rats Administered Ethylene Chlorohydrin over 30-Day Period[a] (Expressed as Percent ± *SE*)

Organ	Control (Normal Saline)	Ethylene Chlorohydrin— 0.10 LD_{50} (6.4 mg./kg.)	0.20 LD_{50} (12.8 mg./kg.)
Adrenals	0.010 ± 0.001	0.012 ± 0.001	0.022 ± 0.004
Brain	0.387 ± 0.018	0.496 ± 0.013	0.715 ± 0.011
Gonads	0.906 ± 0.059	0.990 ± 0.038	1.130 ± 0.069
Heart	0.263 ± 0.009	0.291 ± 0.016	0.291 ± 0.008
Kidneys	0.634 ± 0.022	0.719 ± 0.061	0.782 ± 0.030
Liver	3.850 ± 0.132	4.218 ± 0.278	3.855 ± 0.211
Lungs	0.303 ± 0.013	0.368 ± 0.041	0.448 ± 0.067
Spleen	0.244 ± 0.014	0.263 ± 0.028	0.249 ± 0.012

[a] Animals in each group were administered, intraperitoneally, saline or test solutions each day up to and including the 30th day. Values are based upon six rats per group, except in the 0.20 LD_{50} group in which only five rats survived to the end of the study.

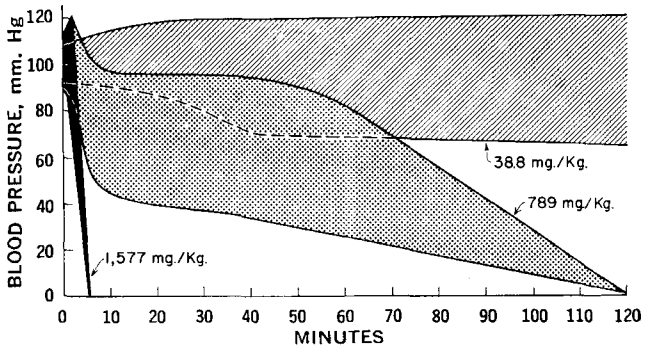

Figure 4—*Representative effects of intravenous ethylene chlorohydrin upon blood pressure of rabbits at three dosage levels.*

fasciculations within the muscle which persisted until it completely lost its contractile response to nerve stimulations. This syndrome was elicited in many of the animals, even at the low range of the doses used. The effect produced was not one of total skeletal muscle paralysis, as might be produced by succinylcholine or tubocurarine, since stimulation of a quiescent muscle group resulted in a prompt response.

Intravenous injection of the middle and high doses (*i.e.*, 606.5 mg./kg. and above) evoked a pain response (a cry and movement) in the rabbit even though anesthesia was satisfactory for surgical procedures.

Sleeping Time—As may be noted from Fig. 5, there was a dose-related increase in mean sleeping time of the treated animals. The mean sleeping time of the mice receiving 0.20 LD_{50} of chlorohydrin was significantly different from the saline control group, according to Student's t test, at the 90% level ($p = 0.10$), while that for the group receiving 0.50 LD_{50} was significant at the 99% level ($p = 0.01$).

DISCUSSION

Subacute Toxicity—Ambrose (4), in a 7-month feeding study in rats, noted that there were no apparent ill effects when the concentration of ethylene chlorohydrin in the diet was 0.08% or less. When the concentration was increased to 0.12 or 0.16%, the weight gain of the treated animals was less than the controls, but no animals died during the experimental period. However, when the quantity was increased to 0.24% or more, mortalities ranged from 40 to 100% of the test groups. Histopathology of organs from all surviving animals was essentially negative. From the data presented by Ambrose (4), it is impossible to determine accurately the quantity of ethylene chlorohydrin each rat consumed in these experiments; however, this may be approximated based upon daily food consumption (per rat per day) and concentration of the toxicant.

The highest concentration of ethylene chlorohydrin that produced no inhibition of growth was 0.08% in food, or approximately 34.6 mg./kg. of rat/day; the highest concentration at which there were no fatalities was about 73.4 mg./kg. of rat/day (0.16% in food). In previous studies (1) in this laboratory, the acute, oral LD_{50} of ethylene chlorohydrin was 71.3 mg./kg. for rats, while Johnson (5) reported the acute, oral LD_{50} to be 77 mg./kg. The "no effect" daily dose in the feeding studies of Ambrose (4) was found to be approximately one-half the acute LD_{50} of ethylene chlorohydrin. A reduced rate of weight gain, but no mortalities, was noted by Ambrose (4) in the group of rats consuming a daily quantity of ethylene chlorohydrin essentially equal to the acute LD_{50}.

More recently, Carson and Oser (6) studied the oral toxicity of ethylene chlorohydrin in the food of rats, dogs, and monkeys for 90 days. They indicated that a "no adverse effect" could be estimated to be 45 mg./kg./day, which, for the rat, represented about 60% of the acute LD_{50}.

The results of the study reported here showed a "no adverse effect" in a group of rats who received 10% of the acute LD_{50} dose (6.4 mg./kg. i.p.) daily for 30 days, which is, in essence, in agreement with that of Johnson (7) who found that a life-long oral intake of 12% of the acute, oral LD_{50} (about 9 mg./kg.) produced no adverse effects.

A review of the oral toxicity data of Ambrose (4), Carson and Oser (6), and Johnson (7), as well as some parenteral data reported

here, shows several common features. First, the initial sign of toxicity (other than death) manifests itself by a reduced rate of growth which is dose dependent. Second, relatively large doses given daily over extended periods of time tend to produce no histological evidence of toxicity. Even in groups of rats receiving quantities of the compound that were lethal to some of the group, the surviving animals did not show unusual histopathology of organs or of tissues.

Apparently, ethylene chlorohydrin is detoxified rapidly in animals, thus preventing a build-up to toxic levels over these periods of time. Johnson (7), based upon considerable biochemical studies, explained that the rather innocuous effects of daily amounts of ethylene chlorohydrin are due to the compound being absorbed through the portal system and being detoxified rapidly in the liver by enzymatic conversion to *S*-carboxymethylglutathione. More reduced glutathione is then synthesized to replace the depleted glutathione, thus tending to protect more sensitive (or critical) organs from the very toxic metabolite, chloroacetaldehyde. At these intracellular sites, the formed chloroacetaldehyde is conjugated with glutathione and removed to prevent a toxic biologic response. If the concentration of chloroacetaldehyde is increased, however, it will conjugate more or all of the glutathione, thereby destroying the body's ability to protect itself from the toxic action of chloroacetaldehyde.

In the subacute toxicity study reported here, ethylene chlorohydrin was administered intraperitoneally three times a week over a 3-month period; the results showed no lethality or adverse effects at one-tenth and one-fifth the acute LD_{50} dose in rats. Increasing the dose to one-half the acute LD_{50}, however, produced death in six out of the 12 animals in the test group. When the compound was administered daily for 30 days, no apparent adverse effects were noted for the group receiving the 0.10 LD_{50} dose. Very toxic effects (deaths and impaired weight gain), however, resulted when the daily dose was increased to one-fifth the LD_{50}. Comparisons of these results with those of other investigators who used the oral route indicate that the parenteral subacute toxicity of ethylene chlorohydrin is considerably greater than that seen from incorporation of the compound into the animal's food (4, 6). Since acute toxicity experiments have shown the compound to have essentially the same toxicity orally as it does intraperitoneally in rats, factors other than inherent toxicity, must be responsible for this apparent discrepancy. First, when a dose is injected, the animal receives its "daily dose" all at once; when it is contained in food, the animal receives the compound gradually over a period of several hours, thus allowing more time for metabolic transformation and glutathione replenishment before the cells become unprotected from glutathione depletion. Second, when the compound is absorbed from the gastrointestinal tract, it enters the portal circulation to permit the liver to remove much of it, thereby helping to protect cells of other organs. Third, the daily consumption of the compound by the animal is less certain and more difficult to control when the material is contained in the diet. Finally, due to the volatility of the compound, special feeding techniques may be needed to ensure that the compound is not lost from the diet.

When, in spite of the body's attempts to detoxify it, sufficient quantities of ethylene chlorohydrin reach the intracellular sites, the compound is converted to chloroacetaldehyde, as postulated by Johnson (7). If the quantity of chloracetaldehyde accumulation is sufficient to deplete the pool of glutathione (by conjugation) within the cell, toxic manifestations result.

Hayes (8) introduced the term "chronicity factor" and equated this factor to the ratio of a one-dose LD_{50} to a repeated-dose LD_{50} given over a specific time period. Even though Hayes suggested that the denominator be the LD_{50} dose for a 90-day period (daily doses), he indicated that other time periods can be used. If the data from this study are used, and it can be assumed that in the 30-day study the LD_{50} is 12.8 mg./kg. (actually, as will be seen from Table I, this dose killed 58% of the animals), an approximate chronicity factor of 5 (64.0/12.8) would result. Hayes stated that a chronicity factor of 2 or greater indicates a cumulative effect of the compound and, as this number increases, there is an increase in the cumulative effect. With a slight modification, the chronicity factor for the 3-month study (with 36 doses administered three times a week) can be estimated to be approximately 2 (64.0 mg./32.0 mg.), thus indicating much less of a cumulative toxic effect than produced by the 30 daily doses of ethylene chlorohydrin. This would indicate that its toxic biologic half-life (*i.e.*, cumulative toxic effect) is rather short, a matter of hours rather than days or weeks.

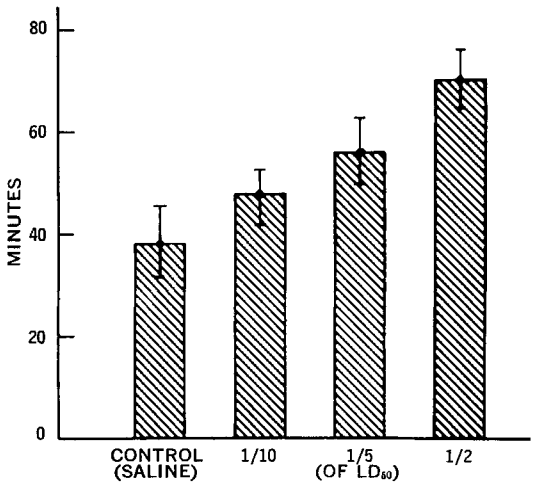

Figure 5—*Effect of ethylene chlorohydrin pretreatment upon pentobarbital sleeping time in mice (mean ± SE).*

Special Tests and Studies—Ethylene chlorohydrin was found not to be a sensitizing agent, according to the test method of Magnusson and Kligman (3). This method of testing was found to be a more sensitive test (3) than the method of Draize (9), which admittedly may not detect weak sensitizing agents.

Results from acute cardiovascular, respiratory, and neuromuscular studies did show that relatively high doses (606.5 mg./kg.) produced a dose-related reduction in both systolic and diastolic pressure, leading to death. Lesser doses produced a slight decline in diastolic pressure and, later, systolic pressure. Low doses (<40 mg./kg.) appeared to have little effect upon the rate or depth of respiration; but as the dose was increased, rate and depth were diminished and, eventually, at higher doses, completely arrested. Death of animals was generally attributed to failure of the respiratory center of the medulla, even though there may be a direct cardiotoxic or neuromuscular action. Ethylene chlorohydrin showed an effect upon the *in vivo* nerve–muscle complex, impairing and eventually blocking nerve–muscle function. Goldblatt (10) demonstrated that ethylene chlorohydrin in cats led to a fall in blood pressure and inhibition of respiration. He found that a 1% solution of the compound applied to an isolated nerve of a frog or cat could completely block the response to electrical stimulation of the nerve. The block, however, was reversible.

The sleeping time experiment in mice demonstrated that ethylene chlorohydrin can increase the mean sleeping time, suggesting that the parent compound or a metabolic product inhibits or antagonizes one or more enzymes or enzyme systems concerned with the metabolic detoxification of pentobarbital or, alternatively, the compound or metabolite produces more generalized liver damage which, in turn, would impede metabolic detoxification of pentobarbital. If, however, generalized hepatic damage is produced, it has not been apparent from histopathologic examination of hematoxylin-eosin-stained sections of liver from ethylene chlorohydrin-treated rats.

Ethylene Chlorohydrin and Devices—It has been demonstrated that repeated parenteral administration of ethylene chlorohydrin to rats over periods of time is more toxic than similar quantities given by the oral route (feeding experiments). Since it is possible that plastic and rubber items that have been ethylene oxide sterilized could contain ethylene chlorohydrin, the question may be asked as to what might constitute a "safe concentration" of ethylene chlorohydrin per day parenterally in man. If a dose of 6.4 mg./kg./day is taken as that dose which has been found to produce a "no adverse effect" in rats (for 30 days), then no medical device should be capable of releasing to a 70-kg. man an amount equal to 448 mg. of the agent.

Since it is dangerous to extrapolate rodent data to man, every attempt should be made to reduce the ethylene chlorohydrin concentration in plastic and rubber items to a minimum and, if possible, to a zero level. One must also consider that a plastic item may contain ethylene oxide and ethylene glycol as well as other reaction products, including ethylene chlorohydrin; thus, the subacute and chronic toxicity profile may be quite different from such combinations than when ethylene chlorohydrin is considered alone. It should

also be apparent that plastic or rubber devices most likely should not be repeatedly sterilized with ethylene oxide if there is any possibility that chlorides are present. Many hospitals apparently reuse disposable devices a number of times and feel that general washing of the item and sterilization with ethylene oxide ensure the safety of the item. This practice should be discouraged.

REFERENCES

(1) W. H. Lawrence, J. E. Turner, and J. Autian, *J. Pharm. Sci.*, **60,** 568(1971).

(2) O. H. Gaebler, *Amer. J. Clin. Pathol.*, **15,** 452(1945).

(3) B. Magnusson and A. M. Kligman, *J. Invest. Dermatol.*, **52,** 268(1969).

(4) A. M. Ambrose, *Arch. Ind. Hyg. Occup. Med.*, **21,** 591(1950).

(5) M. K. Johnson, *Biochem. Pharmacol.*, **14,** 1383(1965).

(6) S. Carson and B. L. Oser, "Oral Toxicity of Ethylene Chlorohydrin, A Potential Reaction Product of Ethylene Oxide Fumigation," Abstract No. 51, Society of Toxicology, 8th Annual Meeting, Williamsburg, Va., March 1969.

(7) M. K. Johnson, *Biochem. Pharmacol.*, **16,** 185(1967).

(8) W. J. Hayes, Jr., *Toxicol. Appl. Pharmacol.*, **11,** 327(1967).

(9) J. H. Draize, in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics," Association of Food and Drug Officials of the United States, Austin, Tex., 1959, pp. 50–52.

(10) M. W. Goldblatt, *Brit. J. Ind. Med.*, **1,** 213(1944).

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Relative Substituent Effects on Alkaline Solvolysis of β-Lactams (2-Azetidinones) and Amides

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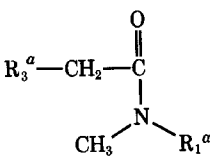
Abstract □ The effect of aliphatic and aromatic substituents, in the 1- and 3-positions of β-lactams, on the rate of solvolysis were examined and compared to analogous substituent effects in the model linear amides. It was found that substituent effects in the β-lactams could be quantitated using the Taft equation, and that a greater sensitivity to polar effects exists in the β-lactams as compared to the model linear amides. In addition, for single substituents in the 1- and 3-positions of the lactams, there is less steric influence on the rate of solvolysis as compared to linear amides. The mechanism of alkaline hydrolysis of these β-lactams appears to be the rate-limiting attack of hydroxide ion at the carbonyl carbon of the amide group and not the breakdown of an ionized tetrahedral intermediate as has been proposed for γ-lactams. Hydrolytic studies on β-lactams at 80° yield a different pathway of degradation, because deamination of the amino acid appears to be rate limiting; thus, elevated temperature studies on β-lactams should be carried out with caution. Finally, it is concluded that β-lactams are not unusually stable in their reactivity toward nucleophiles, such as hydroxide and methoxide ions, and their lability closely follows the parent linear amides.

Keyphrases □ 2-Azetidinones and linear amides—solvolysis mechanism, substituent effects □ β-Lactams, unfused, and linear amides—solvolysis mechanism, substituent effects □ Solvolysis (alkaline) mechanism, substituent effects—unfused β-lactams and linear amides

An enormous effort has been expended investigating various physicochemical and biological aspects of penicillins and cephalosporins (1–4), but comparatively little work has been done on the physicochemical properties of β-lactams. This is somewhat surprising, considering that present thinking (5–8) concerning the mode of action of the penicillins and cephalosporins centers around the acylating potential of the β-lactam moiety in these compounds. Recent work (9) demonstrated that fusion of the β-lactam ring to other rings can introduce considerable strain into the β-lactam ring which, when coupled with polar inductive effects arising from the C-3 side chain and the prevention of amide ground-state resonance, helps explain the unusual lability of these antibiotics to nucleophilic attack. However, the question still remains whether β-lactams are unusual in their stability toward nucleophiles, such as hydroxide and methoxide ions, as compared to corresponding linear amides. In addition, a mechanism of solvolysis of these agents has not been reported.

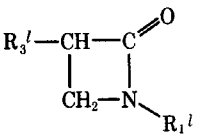
A summary of the effect of substituents on reactivity for a variety of β-lactams was reported (10), and these effects are all qualitative in nature. The only quantitative results of substituent effects on reactivity appears to be the work by Holley and Holley (11–13). These investigators examined the solvolysis of some substituted β-lactams, amides, and penicillins in 85% ethanolic solutions. They discussed, in qualitative terms, the effect of structural variation on reactivity. Recent work concerning the effect of ring size in fused ring β-lactams on the rate of hydrolysis was published by Earle *et al.* (9) and Moll (14).

The purposes of the present study were: (*a*) to investigate substituent effects on the rates of alkaline hydrolysis and solvolysis of unfused β-lactams and (*b*) to compare these effects to analogous substituent effects exhibited in normal amide solvolysis to determine if β-lactams are



amide structure

I



β-lactam structure

II