# Toxicity Profile of Epichlorohydrin

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**Abstract** □ A series of tests was conducted to obtain a profile of the toxicity of epichlorohydrin since this compound finds a number of industrial applications, including synthesis of some epoxy resins which may be formulated into certain biomedical materials. These included acute toxicity by the oral, intraperitoneal, dermal, and inhalation routes; toxicity to mouse fibroblast cells in culture; and hemolytic activity against rabbit blood. Irritancy activity was determined in rabbits by the intradermal, topical (dermal), ophthalmic, and muscle implant methods. Cardiovascular activity of the compound was investigated in rabbits. Alteration of pentobarbital sleeping time in mice was determined following administration by inhalation and intraperitoneal injection. Cumulative and subacute toxicity studies in rats were also conducted. In general, the compound proved to be quite toxic and very irritating in the systems employed.

**Keyphrases** □ Epichlorohydrin—toxicity profile □ Toxicity profile—epichlorohydrin □ Packaging materials—toxicity profile of epichlorohydrin

Epichlorohydrin, or 1-chloro-2,3-epoxypropane, is a colorless liquid at room temperature. It has limited aqueous solubility but is infinitely miscible with alcohol or ether. It has a molecular weight of 92.53, a density of 1.1801 g./ml. (at 20°), a melting point of −48°, and a boiling point of 116.5° (1). Epichlorohydrin finds many industrial applications, including its use as a starting material for a number of epoxy resins which may be used as medical and dental biomaterials.

Hine and Rowe (2) reviewed some of the toxicological properties of this compound. They indicated that its mode of toxic action is that of CNS depression and irritation to the respiratory tract. Cause of death in acute experiments is generally attributed to depression of the respiratory center, whereas chronic toxicity may be the result of a nephrotoxic effect of epichlorohydrin. The threshold limit value for epichlorohydrin was listed as 5 p.p.m. (19 mg./m.$^3$) in 1970 (3); however, Formin (4) recommended only 0.2 mg./m.$^3$ as the average daily permissible concentration in atmospheric air. Most of the recent toxicological data relating to industrial hygiene of epichlorohydrin is being published by Russian investigators.

Chronic inhalation of 20 mg./m.$^3$ of epichlorohydrin produced a loss of weight in white mice, an increase in the latent time of the motor-defense reaction, an increase in the content of nucleic acids in blood, an increase in the quantity of coproporphyrin in the urine, and morphological changes in the lungs, heart, kidneys, and the CNS (4). Concentrations of 2 mg./m.$^3$ under the same conditions affected certain physiological reactions and increased the number of leukocytes (4). Gusev and Minaev (5) presented confirmatory data that 20 mg./m.$^3$ in the atmosphere tends to inhibit reflex reactions in laboratory animals. Pallade *et al.* (6) found evidence of renal insufficiency in about 80% of rats within 24–48 hr. after administering 125 mg./kg. of epichlorohydrin. Hahn (7) produced reversible infertility in male rats by the oral administration of 15 mg./kg. of epichlorohydrin. The males became infertile within 1 week of treatment, and the effect was reversible about a week after discontinuance of treatment.

Bulycheva *et al.* (8) pointed out that epichlorohydrin, along with other potential toxicants, may be evolved in curing various epoxy resins. Thus, they suggested that the concentration of these volatile toxicants (including epichlorohydrin) in the building be monitored and controlled when using polyethylene–polyamide as the curing agent for epoxy resins.

## MATERIALS AND METHODS

The epichlorohydrin used in these experiments was obtained as 1-chloro-2,3-epoxypropane[1]. Due to its limited solubility in water, cottonseed oil was employed as the solvent whenever dilutions were required and was also employed as the negative control. Since procedures employed in this study were described in detail in previous publications (9–11), only a brief outline will be presented here.

**Acute Toxicity**—Oral and intraperitoneal $LD_{50}$ values were determined by administering graded doses of the compound to groups of animals and observing the animals for mortalities during the next 7 days. The $LD_{50}$ was calculated by Cornfield and Mantel's (12) modification of Karber's method. Dermal toxicity was determined by applying measured quantities of the compound to a Webril patch, applying the patch to the shaved rabbit's back with an occlusive bandage, and allowing it to remain in contact for 24 hr., after which the bandage was removed and the animals were observed an additional 6 days for deaths. Inhalation toxicity was determined by placing groups of mice in an 8.75-l., all-glass chamber and passing air, saturated with epichlorohydrin vapors (by bubbling the air through the liquid epichlorohydrin), into the chamber at the rate of 2 l./min. The concentration of vapor was calculated from weight loss during the experiment divided by the quantity of air passed through the epichlorohydrin. Animals were exposed for specific periods of time and were observed 7 days for mortality; an $LT_{50}$ in minutes (lethal time 50%) was calculated from these data using the same methodology as employed for $LD_{50}$ determinations.

**Tissue Culture**—Mouse fibroblast cells (NCTC clone 929, Strain L, Earle) were employed in these tests. The agar-overlay method, as described by Guess *et al.* (13), was used in which 0.2 ml. of the specified concentration of epichlorohydrin in cottonseed oil was applied to a paper disk, the disk was then placed firmly on the agar and incubated at 37° for 24 hr. A cytotoxic response was recorded when a clear zone of lysed cells was observed surrounding the test sample. The protein assay method of Oyama and Eagle (14) was

[1] From Matheson, Coleman and Bell.

utilized in the evaluation of the concentration of epichlorohydrin in the nutrient medium required to inhibit 50% of cell growth ($ID_{50}$) as described in a previous publication by Lawrence *et al.* (9).

**Hemolysis**—The hemolytic activity of epichlorohydrin was evaluated by determining the quantity of hemoglobin released by various concentrations of epichlorohydrin in saline when 0.2 ml. of oxalated, whole rabbit blood was added to 10 ml. of epichlorohydrin–saline solution as described previously by Lawrence *et al.* (11). Normal saline was employed as a negative control (0% hemolysis), and 1% sodium carbonate in saline was the positive control (100% hemolysis). Extracellular hemoglobin was determined spectrophotometrically and expressed as percent hemolysis produced by each concentration of epichlorohydrin. The results were graphed, and the concentration of epichlorohydrin producing 50% hemolysis ($H_{50}$) was determined from the curve.

**Irritant Tests**—Intradermal irritation was determined by injecting 0.2 ml. of the test solution intradermally on the shaved backs of albino, New Zealand rabbits, followed 15 min. later by an intravenous injection of 1 ml./kg. of a 1% trypan blue solution to visualize better the irritant response. All test sites were evaluated at specific time intervals up to 60 min. and evaluated on a 0–3+ scale. The positive control (3+) was 20% ethanol in saline, and the negative control (0) was the diluent, cottonseed oil. Dermal irritation involved the application of 0.2 ml. of the test solution to a small Webril patch [1.27 cm. (0.5 in.) square], which was placed on the shaved backs of rabbits and covered with an occlusive bandage for 24 hr. An 8% (w/v) aqueous solution of sodium lauryl sulfate was used as the positive control, and cottonseed oil was used as the negative control. After removal of the bandage, all sites were evaluated for an irritant response on a 0–3+ scale. For ophthalmic irritation, 0.1 ml. of the test solution was instilled into the superior temporal quadrant of the rabbit's right eye, with the left eye serving as the untreated control. The eyes were examined every 30 min. for 3 hr. and scored for the degree of irritation, using the following scale:

0 = no irritation, comparable to control eye
± = doubtful irritation
1+ = definite conjunctiva and palpebral irritation with significant edema
2+ = iritis and palpebral irritation with significant edema
3+ = corneal damage

The irritant and/or cytotoxic effect of epichlorohydrin to rabbit muscle was determined by placing implant samples of a polyvinyl chloride material, previously determined to be nonreactive by this test, in epichlorohydrin and allowing them to remain for 24 hr. At the time of implantation, each sample was removed, blotted lightly to remove the excess liquid, and implanted into the rabbit muscle (15). Seven days later the rabbit was sacrificed and the implant sites were examined grossly and histologically for tissue reaction. The nonreactive polyvinyl chloride was used as a negative control, while another polyvinyl chloride sample, known to produce a positive reaction by this test, was used as the positive control.

**Sleeping-Time Test**—Groups of 10 male, ICR mice, weighing 20 ± 5 g., were treated with one-tenth, one-fifth, or one-half of the acute intraperitoneal $LD_{50}$ or $LT_{50}$ of epichlorohydrin. Twenty-four hours after the last pretreatment, 50 mg./kg i.p. of sodium pentobarbital was administered to each mouse and the mouse was observed for time of loss and return of righting reflex. Control mice were placed in the inhalation chamber for an equivalent period of time each day but received only air, while another group received intraperitoneal injections of saline as pretreatment.

**Sensitization Test**—The "guinea pig maximization" test of Magnusson and Kligman (16) was employed to evaluate the sensitizing potential of epichlorohydrin. A group of five Hartley strain guinea pigs was employed in this test.

**Cardiovascular Effects**—Healthy New Zealand rabbits, weighing approximately 2 kg., were anesthetized with an injection of 500 mg./kg. i.p. of urethan (ethyl carbamate) followed about 15 min. later with 30–50 mg./kg. i.v. of sodium pentobarbital. The left femoral artery was surgically isolated, cannulated, and connected *via* a polyethylene tube to a pressure transducer[2] for the continuous recording of arterial pressure with a polygraph[3].

**Cumulative Toxicity**—Groups of 12 male Sprague-Dawley rats were employed in which one group received injections of 0.01910 ml./kg. i.p. of cottonseed oil and two groups received epichlorohydrin, 0.00955 and 0.01910 ml./kg., representing 0.1 and 0.2 of the acute $LD_{50}$ dose, respectively. Injections were given daily for 30 consecutive days. At the end of 30 days, clotting time was measured by the capillary tube method; platelet counts were obtained by determining, in duplicate, the ratio of platelets to erythrocytes in the differential slide and by using the erythrocyte count for the particular rat, converting this to number of platelets per cubic millimeter of blood. The other hematologic parameters were obtained by standard clinical procedures. The rate of disappearance of sodium sulfobromophthalein from the plasma was determined using six rats from each group. Plasma concentrations were determined by the method of Gaebler (17) 15, 30, and 45 min. after intravenous injection of 75 mg./kg. of sodium sulfobromophthalein. At autopsy, the rat was weighed, the organs (adrenals, brain, gonads, heart, kidneys, liver, lungs, and spleen) were removed and weighed, and the results were expressed as percent of body weight. Organs were preserved in 10% buffered formalin, sectioned, and stained with hematoxylin and eosin for histologic evaluation.

**Subacute Toxicity**—Four groups of male Sprague-Dawley rats were used in this test. One group received 0.04774 ml./kg. of cottonseed oil (control group), while the others received 0.0095, 0.0190, and 0.04774 ml./kg. of epichlorohydrin, respectively, by intraperitoneal injection 3 days per week (Monday, Wednesday, and Friday) for 12 weeks. The three dose levels of epichlorohydrin utilized represent 0.1, 0.2, and 0.5, respectively, of the acute $LD_{50}$ in each injection. Platelet concentrations were determined by a clinical method of counting the number of platelets contained in 10 oil immersion fields of the differential slide. Evaluation of the various other criteria followed the same methodology as outlined in the preceding section (*Cumulative Toxicity*).

## RESULTS AND DISCUSSION

**Acute Toxicity**—Acute $LD_{50}$ determinations indicate the compound to be rather toxic by all routes of administration in the species of animals tested. When administered by intraperitoneal injection, the $LD_{50}$ ranged from about 0.10 to 0.14 ml./kg. for mice, rats, guinea pigs, and rabbits. Oral (intragastric) administration to mice and rats gave rather consistent values of 0.20 and 0.22 ml./kg. Dermal (topical) application to rabbits produced an $LD_{50}$ of 0.64 ml./kg., but the 95% confidence limits overlapped values for the oral and intraperitoneal routes. Inhalation of epichlorohydrin vapors by mice produced an $LT_{50}$ of 9.13 min., at which time the chamber should have reached about 88% equilibrium with the incoming air–vapor mixture (18). Under the conditions of these experiments (room temperature of 23° and barometric pressure of 30.18), the air–vapor mixture contained 71.89 mg. epichlorohydrin/l. Inhalation toxicity tests conducted on 3 separate days under these conditions gave good reproducibility for the vapor concentration, with the maximum deviation from the above value being 1.26 mg./l. or 1.75%.

Tissue culture tests, using the agar-overlay method and a series of dilutions of epichlorohydrin, indicated that concentrations of 0.001215% (v/v) or greater of epichlorohydrin were cytotoxic to the cells while concentrations of 0.000486% or less were noncytotoxic. Assessment of epichlorohydrin toxicity by the protein assay method of tissue culture revealed that a concentration of $1.6 \times 10^{-5}$ *M* in the medium would inhibit protein synthesis by 50% ($ID_{50}$). Tests for hemolysis of rabbit blood indicated the beginning of hemolysis at about 0.01 *M* epichlorohydrin in saline, with 0.0375 *M* producing 50% hemolysis ($H_{50}$)[4].

A summary of these data is presented in Table I.

**Irritant Tests**—Epichlorohydrin demonstrated considerable irritant activity by all of the tests employed. The undiluted material produced irritation equal to, or greater than, the positive control (3+) when tested intradermally, dermally, or ophthalmically in the rabbit. The irritant range of concentrations (*i.e.*, 0–3+) for epichlorohydrin dissolved in cottonseed oil was determined for each of these tests (Table II). One can note from Table II that the relative

[2] Statham P23Dc.
[3] Grass model 7.

[4] Tissue culture and hemolysis data from Cellular Toxicology Section, courtesy of Dr. E. O. Dillingham.

**Table I**—Acute Toxicity of Epichlorohydrin

| Test Animal | $LD_{50}$[a], ml./kg. | 95% Confidence Limits, ml./kg. |
|---|---|---|
| **Intraperitoneal Administration** | | |
| Mice, male, ICR | 0.1439 | 0.1297–0.1597 |
| Rats, male, Sprague-Dawley | 0.0955 | 0.0802–0.1136 |
| Guinea pigs, male, Hartley, albino | 0.1000 | 0.0250–0.4000 |
| Rabbits, male, New Zealand, albino | 0.1356 | 0.0709–0.2595 |
| **Intragastric Administration** | | |
| Mice, male, ICR | 0.2000 | 0.1615–0.2476 |
| Rats, male, Sprague-Dawley | 0.2203 | 0.1242–0.3908 |
| **Dermal (Topical) Application** | | |
| Rabbits, male, New Zealand, albino | 0.6389 | 0.3254–1.2232 |
| **Inhalation** | | |
| Mice, male, ICR | 9.13[b] | 8.49–9.81 min. |
| **Tissue Culture, L-Cells** | | |
| Agar overlay (cytotoxic/noncytotoxic) | 0.001215%/0.000486% (v/v) | |
| Protein assay, $ID_{50}$ | $1.6 \times 10^{-5}$ *M* | |
| **Hemolysis of Rabbit Blood** | | |
| 50% Hemolysis ($H_{50}$) = 0.0375 *M* | | |

[a] All values are expressed in terms of undiluted epichlorohydrin. [b] $LT_{50}$ (lethal time 50%) in minutes at a vapor concentration of 71.89 mg./l.; air flow = 2 l./min.; chamber volume = 8.75 l.

sensitivity, from most sensitive to least, of these irritant tests, as indicated by the lowest concentration required to produce a specified degree of relative irritation, is in the order of intradermal, dermal, and ophthalmic.

The liquid compound was tested for its compatibility (irritant or cytotoxic effect) with rabbit muscle. As indicated in Table II, the treated samples produced a necrotic response surrounding the implant which, by both gross and histologic examination, was equivalent to, or greater than, the positive control. Untreated implants of the same polyvinyl chloride sample, as previously indicated, did not exhibit this type of response.

**Pentobarbital Sleeping-Time Test**—The effect of epichlorohydrin upon liver function was investigated by determining what, if any, effect sublethal doses of the compound would have upon hepatic function as reflected by altering the duration of sleep of mice to a standard dose of sodium pentobarbital. A dose-related increase in sleeping time was observed with both intraperitoneal injection and inhalation of epichlorohydrin (Fig. 1). From inhalation, these increases were significantly greater than the controls at the 99% level ($p = 0.01$) for pretreatment with one-fifth and one-half the $LT_{50}$ of epichlorohydrin; intraperitoneal administration of epichlorohydrin revealed no significant differences at the 99% level ($p = 0.01$), but the increased sleeping time for the high dose group was significant at the 95% level ($p = 0.05$).

**Table II**—Irritation Tests: Epichlorohydrin

| Test in Rabbits | Response[a] |
|---|---|
| Intradermal (0.2 ml./site) | 3+ = 0.5%[b]<br>2+ = 0.125%<br>1+ = 0.031%<br>± = 0.008%<br>0 = 0.002% |
| Dermal (0.2 ml./site) | 3+ = 5.0%<br>2+ = 2.5%<br>1+ = 1.25%<br>± = 0.625%<br>0 = 0.3125% |
| Ophthalmic (0.1 ml./eye) | 3+ = 80%<br>2+ = 40%<br>1+ = 20%<br>± = 10%<br>0 ± 5% |
| 7-Day muscle implant[c] | Gross = 3+<br>Histopathology = 4 (marked toxicity) |

[a] Gross responses graded on a 0–3+ scale; histopathologic response graded on a 0–4 scale. [b] All in cottonseed oil. [c] A nonreactive polyvinyl chloride material was placed in epichlorohydrin for 24 hr. prior to implantation.

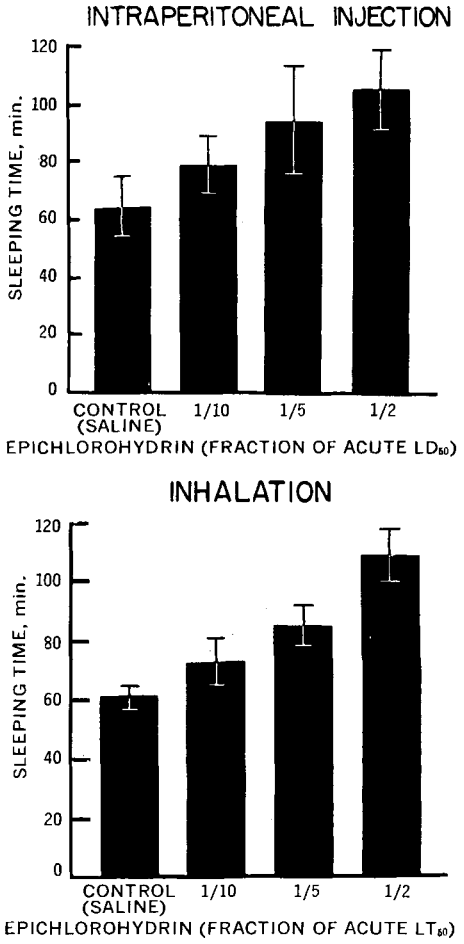


**Figure 1**—*Effect of epichlorohydrin pretreatment upon pentobarbital sleeping time in mice (mean ± SE).*

**Sensitization Test**—Epichlorohydrin was tested for sensitization potential by the guinea pig maximization test. Due to the inherent irritant and lethal action of the compound, it was necessary to employ a rather dilute solution of the material, *i.e.*, 0.01% in cottonseed oil. With this concentration, no evidence of a sensitivity reaction was seen in any of the five guinea pigs tested.

**Cardiovascular Effects**—In acute experiments of a qualitative nature, using anesthetized rabbits, a dose-related deterioration of car-

**Table III**—Cumulative Toxicity of Epichlorohydrin: Body Weight Gain in Grams (Mean ± *SE*)

| Days | Cottonseed Oil Controls | 0.00955 ml./kg. | 0.01910 ml./kg. |
|---|---|---|---|
| 5 | 28.17 ± 1.96 | 36.25 ± 5.73 | 26.00 ± 1.54 |
| 10 | 66.00 ± 2.44 | 71.92 ± 6.15 | 63.67 ± 2.68 |
| 15 | 111.25 ± 3.39 | 105.25 ± 7.24 | 91.33 ± 4.33[a] |
| 20 | 145.67 ± 3.75 | 123.42 ± 4.83[a] | 131.58 ± 2.87[a] |
| 25 | 157.25 ± 4.38 | 145.67 ± 6.44 | 153.92 ± 7.33 |
| 30 | 183.33 ± 5.59 | 167.67 ± 7.27 | 158.50 ± 6.82[b] |

[a] Significantly different from controls at 99% level ($p = 0.01$) by Student's *t* test. [b] Significantly different from controls at 95% level ($p = 0.05$) by Student's *t* test.

**Table IV**—Cumulative Toxicity of Epichlorohydrin: Sodium Sulfobromophthalein Liver Function Test in Rats[a] (Mean ± *SE*)

| After, min. | Cottonseed Oil Controls | Dose Level of Epichlorohydrin —Pretreatment— 0.00955 ml./kg. | 0.01910 ml./kg. |
|---|---|---|---|
| **Sodium Sulfobromophthalein Concentration (mg. %) in Plasma** | | | |
| 15 | 22.14 ± 0.36 | 25.15 ± 1.25 | 19.06 ± 2.12 |
| 30 | 5.89 ± 1.10 | 9.47 ± 1.69 | 5.67 ± 1.52 |
| 45 | 2.76 ± 0.54 | 5.54 ± 3.26 | 2.06 ± 0.50 |
| **Percentage of Sodium Sulfobromophthalein Eliminated** | | | |
| Between, min. | | | |
| 15–30 | 74.30 ± 4.83 | 63.51 ± 4.84 | 72.12 ± 5.28 |
| 30–45 | 48.20 ± 9.40 | 44.81 ± 7.06 | 57.38 ± 8.18 |

[a] Test was performed at the conclusion of the 30-day cumulative toxicity study. All rats received 75 mg./kg. of sodium sulfobromophthalein intravenously. Values are from six rats per group.

diovascular function was observed. Doses of 0.02 ml./kg. produced a slight decrease in systolic and diastolic pressure which tended to stabilize after about 20 min.; doses of 0.2 ml./kg. produced a decline in both systolic and diastolic pressure with a reduction in pulse pressure which terminated in death of the animal in about 30 min. Doses of 1.0 ml./kg. resulted in a pattern of response similar to that for 0.2 ml./kg. but much more abrupt in nature, with death ensuing in about 5 min. With these higher doses, cardiac rate was reduced but blood pressure fell to undetectable levels before cardiac function ceased, thus suggesting that cardiac failure was not the primary cause of cardiovascular collapse.

**Cumulative Toxicity**—No deaths occurred in either the treated animals or the cottonseed oil controls during the 30 consecutive daily injections. Weight gain was generally less in the treated animals than controls, with these differences being significant ($p \leq 0.05$) at 20 days for the low dose group and at 15, 20, and 30 days for the high dose group. Table III shows the mean weight gains and standard errors for each group at 5-day intervals.

A sodium sulfobromophthalein disappearance test was conducted at the conclusion of the 30-day treatment to see if the compound had produced detectable alteration of hepatic function. These data are presented in Table IV. Since none of the values was significantly different from the controls ($p = 0.05$), impairment of hepatic function was not detected by the rate of sodium sulfobromophthalein disappearance from plasma.

Hematologic and organ to body weight ratio data showed few statistically significant differences ($p \leq 0.05$). Hemoglobin showed a significant increase at the low dose but a significant decrease at the high dose. Neutrophilic metamyelocytes (metas) were the same for the low dose and controls but showed a significant increase in the high dose group. Lymphocytes showed a dose-related decrease in frequency, but the values were not statistically significant. A slight dose-related increase was observed in clotting time but was not significant. The heart to body weight ratio presented a dose-related, but nonsignificant, increase. The ratio of kidneys to body weight showed a dose-related increase, in which both epichlorohydrin-treated groups were significantly ($p = 0.05$) greater than controls. Hematologic data are presented in Table V, and data for organ to body weight ratios are presented in Table VI.

**Table VI**—Cumulative Toxicity of Epichlorohydrin: Percent Organ to Body Weight of Rats (Mean ± *SE*)

| Organs | Cottonseed Oil Controls | Epichlorohydrin 0.00955 ml./kg. | 0.01910 ml./kg. |
|---|---|---|---|
| Adrenals | 0.014 ± 0.001 | 0.014 ± 0.001 | 0.013 ± 0.002 |
| Brain | 0.475 ± 0.017 | 0.567 ± 0.041 | 0.543 ± 0.033 |
| Gonads | 0.975 ± 0.037 | 0.995 ± 0.051 | 0.971 ± 0.037 |
| Heart | 0.273 ± 0.006 | 0.309 ± 0.022 | 0.378 ± 0.078 |
| Kidneys | 0.654 ± 0.023 | 0.750 ± 0.025[a] | 0.786 ± 0.035[a] |
| Liver | 3.960 ± 0.140 | 4.150 ± 0.230 | 3.916 ± 0.056 |
| Lungs | 0.381 ± 0.027 | 0.409 ± 0.028 | 0.407 ± 0.015 |
| Spleen | 0.236 ± 0.010 | 0.266 ± 0.017 | 0.253 ± 0.012 |

[a] Significantly different from controls at 95% level ($p = 0.05$) by Student's *t* test.

Histological examination of organs from these animals did not reveal any significant changes except for the lungs. Animals from all groups showed some lesions of the lungs including bronchitis, peribronchitis, interstitial pneumonia, bronchopneumonia, and emphysema. However, the incidence and severity of these changes were somewhat greater in the epichlorohydrin-treated animals than in the controls.

**Subacute Study**—Food consumption, as measured during the 1st, 7th, and 12th weeks, indicated that the two higher dose groups consumed less food per body weight of animal; however, this was significant ($p = 0.05$) only for the high dose group during the 1st week and for the middle dose group during the 12th week (Table VII).

Weight gains for the low and middle dose groups were generally equal to, or greater than, the means of the controls, but few of these differences were statistically significant. Throughout the major portion of the study, the high dose group demonstrated a significantly ($p = 0.01$) reduced weight gain when compared to the controls. At the end of the 12-week study, however, there were no statistically significant differences ($p = 0.05$) in mean weight gains for the groups, although the low dose group had gained the most, followed in order by the middle dose group, cottonseed oil controls, and high dose group (Table VIII).

Hematologic studies conducted at the end of the 12th week showed a dose-related, statistically significant ($p \leq 0.05$) reduction in hemoglobin. All treated groups showed lower hematocrit values and erythrocyte counts, but this finding was significant ($p \leq 0.05$) only for the hematocrit value of the middle dose group. When the mean corpuscular volume is calculated (19) for each animal, variations of 30–40% may be seen between animals within the same group; however, the mean values for the groups are quite comparable. Only the low dose group differed from the

**Table V**—Cumulative Toxicity of Epichlorohydrin: Hematologic Values (Mean ± *SE*)

| Item | Cottonseed Oil Controls | Epichlorohydrin 0.00955 ml./kg. | 0.01910 ml./kg. |
|---|---|---|---|
| Hemoglobin, g./100 ml. | 16.300 ± 0.177 | 17.183 ± 0.254[a] | 14.633 ± 0.530[a] |
| Hematocrit, % | 51.000 ± 0.577 | 48.167 ± 1.493 | 48.667 ± 1.054 |
| Red blood cells/mm.$^3$, × $10^6$ | 7.012 ± 0.511 | 7.545 ± 0.965 | 6.946 ± 0.467 |
| Total white blood cells/mm.$^3$, × $10^3$ | 9.292 ± 1.362 | 14.262 ± 2.652 | 12.517 ± 1.913 |
| Platelets/mm.$^3$, × $10^6$ | 1.263 ± 0.148 | 1.402 ± 0.218 | 1.028 ± 0.090 |
| Clotting time, sec. | 1.215 ± 0.095 | 1.272 ± 0.056 | 1.385 ± 0.057 |
| Differential white cell count, % | | | |
| Segs | 13.833 ± 1.986 | 23.750 ± 5.773 | 17.667 ± 2.522 |
| Lymphs | 82.333 ± 1.453 | 71.083 ± 6.463 | 65.667 ± 12.240 |
| Monocytes | 1.333 ± 0.357 | 1.167 ± 0.307 | 1.833 ± 0.422 |
| Eosinophils | 0.583 ± 0.327 | 1.000 ± 0.516 | 0.167 ± 0.105 |
| Basophils | 0.333 ± 0.333 | 0.833 ± 0.401 | 0.333 ± 0.167 |
| Meta | 0.083 ± 0.083 | 0.083 ± 0.083 | 0.750 ± 0.214[a] |
| Juvenile | 0.167 ± 0.105 | 0.000 ± 0.000 | 0.500 ± 0.224 |
| Bands | 1.667 ± 0.587 | 2.167 ± 1.202 | 1.833 ± 0.667 |

[a] Significantly different from controls at 95% level ($p = 0.05$) by Student's *t* test.

**Table VII** —Subacute Toxicity of Epichlorohydrin: Food Consumption in Rats (Grams of Food Consumed/Kilogram of Rat/24-hr. Day) (Mean ± *SE*)

| Week | Cottonseed Oil Controls | Epichlorohydrin 0.0095 ml./kg. | Epichlorohydrin 0.0190 ml./kg. | Epichlorohydrin 0.04774 ml./kg. |
|---|---|---|---|---|
| 1st | 161.68 ± 2.13 | 160.98 ± 4.97 | 151.33 ± 5.13 | 87.07 ± 10.28[a] |
| 7th | 90.54 ± 1.26 | 87.09 ± 1.50 | 87.81 ± 4.06 | 86.03 ± 3.55 |
| 12th | 58.49 ± 3.08 | 77.49 ± 2.71[b] | 45.17 ± 1.79[a] | 50.99 ± 11.50 |

[a] Significantly different from controls at 99% level ($p = 0.01$) by Student's $t$ test. [b] Significantly different from controls at 95% level ($p = 0.05$) by Students' $t$ test.

**Table VIII**—Subacute Toxicity of Epichlorohydrin: Body Weight Gain in Grams (Mean ± *SE*)

| Week | Cottonseed Oil Control | Epichlorohydrin 0.0095 ml./kg. | Epichlorohydrin 0.0190 ml./kg. | Epichlorohydrin 0.04774 ml./kg. |
|---|---|---|---|---|
| 1 | 46.58 ± 2.88 | 38.58 ± 4.50 | 41.42 ± 2.40 | 24.00 ± 3.14[a] |
| 2 | 76.50 ± 6.15 | 94.42 ± 3.05[b] | 86.50 ± 5.14 | 55.09 ± 10.23 |
| 3 | 105.33 ± 5.86 | 119.83 ± 3.72[b] | 111.83 ± 6.79 | 97.00 ± 9.27 |
| 4 | 186.17 ± 5.74 | 193.42 ± 4.74 | 175.58 ± 11.02 | 128.30 ± 11.54[a] |
| 5 | 214.25 ± 8.14 | 228.08 ± 6.80 | 203.50 ± 10.73 | 172.00 ± 11.37[b] |
| 6 | 254.83 ± 6.52 | 254.33 ± 11.07 | 242.75 ± 11.44 | 195.60 ± 10.29[a] |
| 7 | 279.25 ± 7.16 | 267.08 ± 9.11 | 261.25 ± 12.27 | 218.50 ± 11.14[a] |
| 8 | 296.17 ± 7.10 | 289.92 ± 5.98 | 276.00 ± 11.47 | 246.80 ± 12.16[a] |
| 9 | 333.50 ± 9.72 | 330.75 ± 9.59 | 315.67 ± 21.92 | 266.60 ± 13.19[a] |
| 10 | 345.50 ± 13.49 | 359.83 ± 9.64 | 334.00 ± 13.81 | 293.70 ± 14.19[b] |
| 11 | 356.67 ± 14.75 | 370.42 ± 10.82 | 371.17 ± 12.05 | 286.70 ± 21.91[b] |
| 12 | 358.92 ± 13.27 | 383.42 ± 11.22 | 380.92 ± 13.34 | 328.33 ± 18.19 |

[a] Significantly different from controls at 99% level ($p = 0.01$) by Student's $t$ test. [b] Significantly different from controls at 95% level ($p = 0.05$) by Student's $t$ test.

**Table IX** –Subacute Toxcity of Epichlorohydrin: Hematologic Values in Rats (Mean ± *SE*)

| Item | Cottonseed Oil Controls | Epichlorohydrin 0.0095 ml./kg. | Epichlorohydrin 0.0190 ml./kg. | Epichlorohydrin 0.04774 ml./kg. |
|---|---|---|---|---|
| Hemoglobin, g./100 ml. | 16.533 ± 0.152 | 15.517 ± 0.352[a] | 15.208 ± 0.412[a] | 11.795 ± 0.557[b] |
| Hematocrit, % | 48.500 ± 0.671 | 46.833 ± 0.972 | 45.000 ± 1.155[a] | 43.850 ± 2.241 |
| Red blood cells/mm.$^3$, × $10^6$ | 7.593 ± 0.362 | 6.563 ± 0.338 | 6.878 ± 0.268 | 6.938 ± 0.751 |
| Total white blood cells/mm.$^3$, × $10^3$ | 12.333 ± 1.663 | 9.375 ± 0.554 | 12.600 ± 1.325 | 16.842 ± 1.780 |
| Differential white cell count, % | | | | |
| Segs | 8.583 ± 1.890 | 12.500 ± 2.536 | 19.667 ± 4.672 | 22.333 ± 2.068[b] |
| Lymphs | 86.417 ± 1.786 | 85.583 ± 2.115 | 72.333 ± 5.475[a] | 72.500 ± 2.436[b] |
| Monocytes | 1.000 ± 0.289 | 0.500 ± 0.183 | 1.000 ± 0.258 | 0.750 ± 0.382 |
| Eosinophils | 0.667 ± 0.105 | 1.917 ± 0.436[a] | 1.417 ± 0.597 | 1.750 ± 0.335[a] |
| Basophils | 0.083 ± 0.083 | 0.250 ± 0.250 | 0.333 ± 0.167 | 0.083 ± 0.083 |
| Meta | 0.333 ± 0.105 | 0.417 ± 0.201 | 0.833 ± 0.380 | 0.083 ± 0.083 |
| Juvenile | 0.417 ± 0.239 | 0.000 ± 0.000 | 0.333 ± 0.167 | 0.000 ± 0.000 |
| Bands | 2.833 ± 0.279 | 0.417 ± 0.239[b] | 4.000 ± 0.967 | 2.833 ± 0.715 |

[a] Significantly different from controls at 95% level ($p = 0.05$) by Student's $t$ test. [b] Significantly different from controls at 99% level ($p = 0.01$) by Student's $t$ test.

**Table X**—Subacute Toxicity of Epichlorohydrin: Percent Organ to Body Weight of Rats[a] (Mean ± *SE*)

| Organs | Cottonseed Oil Controls | Epichlorohydrin 0.0095 ml./kg. | Epichlorohydrin 0.0190 ml./kg. | Epichlorohydrin 0.04774 ml./kg. |
|---|---|---|---|---|
| Adrenals | 0.023 ± 0.013 | 0.011 ± 0.001 | 0.010 ± 0.001 | 0.013 ± 0.002 |
| Brain | 0.444 ± 0.018 | 0.426 ± 0.012 | 0.439 ± 0.029 | 0.324 ± 0.027[b] |
| Gonads | 0.785 ± 0.028 | 0.707 ± 0.034 | 0.866 ± 0.054 | —[c] |
| Heart | 0.291 ± 0.011 | 0.302 ± 0.018 | 0.306 ± 0.030 | 0.402 ± 0.029[b] |
| Kidneys | 0.670 ± 0.034 | 0.636 ± 0.032 | 0.728 ± 0.049 | 0.917 ± 0.054[b] |
| Liver | 2.983 ± 0.219 | 3.232 ± 0.157 | 3.276 ± 0.186 | 4.070 ± 0.276[d] |
| Spleen | 0.174 ± 0.012 | 0.194 ± 0.010 | 0.208 ± 0.031 | 0.225 ± 0.021 |

[a] Calculated as: (organ weight, g./body weight, g.) × 100 = percent organ to body weight. [b] Significantly different from controls at 99% level ($p = 0.01$) by Student's $t$ test. [c] Gonadal weights were not determined in this group. [d] Significantly different from controls at 95% level ($p = 0.05$) by Student's $t$ test.

controls, and this was on the order of 12% higher. The mean corpuscular volumes obtained for the groups were 64.40 for the controls and 72.29, 65.72, and 65.57 for the 0.0095-, 0.0190-, and 0.0477-ml./kg. dose levels of epichlorohydrin, respectively. Screening for an effect upon platelets was done by a clinical method of counting the number of platelets contained in 10 oil immersion fields of the differential slide; thus, this test must be considered as qualitative or, at best, semiquantitative. The magnitude in differences suggests an increased number of platelets from epichlorohydrin treatment. There were 288 platelets/10 oil immersion fields for the high dose of epichlorohydrin compared to 185 for the cottonseed oil-treated controls.

Total leukocyte counts showed the low dose group to be lower than the controls, the middle dose group equal to the controls, and

the high dose group greater than the controls, but none of these differences was significant The differential count revealed a dose-related increase in percentage of segmented neutrophils (segs), but this difference was significant only for the high dose group. There was a significant reduction in percentage of lymphocytes for the two highest doses The percentage of eosinophils was increased in all treated groups, with the differences being significant for the low and high dose groups Other differential values did not show a dose-related trend and were not statistically significant (except that the low dose showed a significant reduction in bands but was not consistent with a dose-related response). These data are included in Table IX.

An examination of organ to body weight ratios did not reveal any to be significant ($p \leq 0.05$) except in the high dose group, in which the ratio was less than for the controls for the brain but greater for the heart, kidneys, and liver. Although not all of the values were significant, there was an indication that the mean ratios for the heart, liver, and spleen increased with an increase in dose of epichlorohydrin. While the mean body weight of the high dose group at 12 weeks was 8.5% less than for the controls, the organ to body weight ratios for the heart, kidneys, liver, and spleen in the high dose group ranged from 29.1 to 38.1% more than for the controls. On the other hand, the ratio for the brain of the high dose group was 27% less than for the controls. Thus, simple retardation in growth would not be expected to account for these differences (Table X).

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# Binding of CNS Active Drugs to the Peptide Bond: A Model System and Mode of Action Hypothesis


**P. R. ANDREWS**

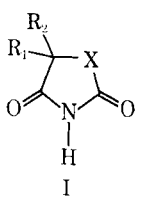


**Abstract** □ The effects of pentobarbital, bemegride, and trimethadione on the NMR spectrum of formanilide show that when possible the *cis*-isomer of the amide forms a doubly hydrogen-bonded complex with the drugs but the *trans*-isomer is favored for the formation of a single hydrogen bond. It is suggested that these CNS active drugs behave as molecular glues, linking together functional groups in protein molecules and thus opposing changes in protein conformation or association state. This hypothesis could account for the seemingly inconsistent structure–activity relationships of drugs with convulsant and anticonvulsant actions.



**Keyphrases** □ CNS drugs—hypothesis and model for binding to peptide bond □ Anticonvulsants—hydrogen binding to peptide bond, mode of action hypothesis □ Convulsants—hydrogen binding to peptide bond, mode of action hypothesis □ Hydrogen bonding—CNS drugs to peptide bond, hypothesis □ Structure–activity relationships—hypothesis for binding of CNS drugs to proteins □ NMR spectroscopy—determination, hydrogen bonding of drugs to peptide bond


Molecules of many structural types display anticonvulsant properties (1), but most drugs used clinically in epilepsy have similar structural features (2), which include two or more groups capable of forming hydrogen bonds. Molecular orbital calculations on 24 drugs of the general structure I indicated that hydrogen-bonding ability is similar for all such drugs (3).

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Cyclic hydrogen-bonded complexes have been observed between several barbiturates and a model com-