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RESEARCH ARTICLES

Toxicity Profile of Chloroacetaldehyde

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Abstract □ Chloroacetaldehyde, a probable metabolite of 2-chloroethanol (ethylene chlorohydrin), was studied in a number of *in vivo* animal systems, in *in vitro* hemolysis tests, and in tissue cultures to obtain a toxicity profile of the compound. Acute toxicity tests were conducted in mice, rats, guinea pigs, and rabbits by one or more routes of administration. Tissue culture tests utilized both the agar-overlay and protein assay methods. Irritant activity was evaluated by intramuscular implantation, intradermal injection, and dermal and ophthalmic applications in the rabbit. Acute cardiovascular effects in rabbits were also determined. Sleeping-time tests in mice were conducted to assess the effect of chloroacetaldehyde pretreatment (inhalation and intraperitoneal) upon drug-metabolizing enzymes. The compound was tested in guinea pigs for its sensitizing potential. Cumulative (30 daily injections) and subacute (three injections per week for 12 weeks) toxicity studies were conducted to evaluate subtle toxic effects (*e.g.*, weight gain, hematology, and histopathology) as well as lethality. Chloroacetaldehyde is a very toxic and irritating compound in acute tests; in tests of longer duration, most of the parameters measured appeared to be normal in animals that survived its lethal activity. The acute toxic effects of chloroacetaldehyde are compared with those of 2-chloroethanol. The former is inherently more toxic and irritating, while the latter exhibits greater ease of quantitative penetration through the GI tract and the intact skin.

Keyphrases □ Chloroacetaldehyde—toxicity, compared to ethylene chlorohydrin □ Toxicity—chloroacetaldehyde, compared to ethylene chlorohydrin

Chloroacetaldehyde ($ClCH_2CHO$) is a liquid at room temperature. As the anhydrous material, it polymerizes on standing (1) and, in aqueous solutions in excess of 50%, forms a half-hydrate which precipitates as white crystals (2). It is intensely irritating to human eyes, skin, mucous membranes, and the respiratory tract (1), and its highly toxic nature is suggested by its "threshold limit value" (TLV) of 1 p.p.m., which should not be allowed to fluctuate above this amount even for short periods of time (3). The uses of chloroacetaldehyde as well as its physical and chemical properties were presented previously (1, 2).

Ethylene oxide sterilization of plastics, spices, and foods, in the presence of chlorides, produces 2-chloroethanol as a reaction product. Johnson (4), in studies conducted on rats, indicated that 2-chloroethanol was

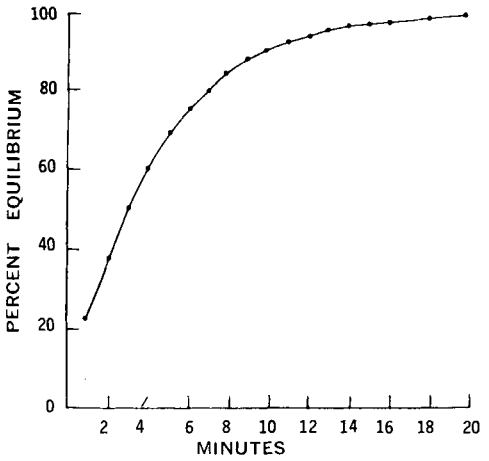

Figure 1—*Percent equilibrium of chamber atmosphere to incoming air–vapor mixture (theoretical curve) (chamber = 8.75 l. and airflow = 2 l./min.).*

oxidized to chloroacetaldehyde *via* a major metabolic pathway. The latter then reacted with reduced glutathione at the cellular level and finally oxidized to *S*-carbomethylglutathione. He thus proposed this mechanism to account for the highly toxic nature of 2-chloroethanol, since simultaneous administration of ethanol (which reduces the rate of chloroacetaldehyde formation by competing with 2-chloroethanol for oxidative enzymes) retards reduced glutathione depletion.

Thus, under appropriate conditions, it might be possible to encounter chloroacetaldehyde toxicity problems as a consequence of the ethylene oxide sterilization process. Therefore, it was of interest to develop a *toxicity profile* for chloroacetaldehyde along the same lines as were previously used for ethylene chlorohydrin (2-chloroethanol).

EXPERIMENTAL

Materials—The chloroacetaldehyde used in these experiments was obtained[1] as an aqueous solution containing either 30 or 45% chloroacetaldehyde. Tests were performed using 30% solutions, aqueous dilutions, or dilutions made isotonic with sodium chloride, as appropriate for the specific test.

LD_{50} Determinations—Experiments were conducted on male ICR mice, Sprague-Dawley and Black Bethesda strains of rats, New Zealand albino rabbits, and guinea pigs of the Hartley strain. Graded doses of the compounds were administered to the animals by the specified route, and the animals were observed 1 week for mortalities. The LD_{50} values and 95% confidence limits were calculated by Cornfield and Mantel's (5) modification of Karber's method or by the method of Weil (6), based upon the 7-day mortalities.

The test material was administered as a single dose by oral intubation, intraperitoneal injection, or topical (dermal) application. Dermal administration to rabbits was accomplished by: (*a*) placing the desired dose on a single Webril patch of sufficient size to absorb the liquid; (*b*) placing the patch in contact with a nonabraded area of the rabbit's skin, previously freed of hair by shaving with a safety razor and commercial shaving cream; and (*c*) securing the patch with a polyethylene overwrap. After 24 hr., the bandage was removed and the rabbit was observed an additional 6 days for signs of toxicity or mortality.

To facilitate comparison of results, all values are calculated in terms of pure chloroacetaldehyde.

Inhalation Toxicity—Groups of male ICR mice, five at a time, were placed in an all-glass inhalation chamber of 8.75-l. capacity. Filtered air was bubbled through a 30% aqueous solution of chloroacetaldehyde at a rate of 2 l./min. and passed into the inhalation chamber. Mice were in the air–vapor environment for a specified period, ranging from 1.44 to 4.40 min., after which the chamber was immediately opened (in a fume hood) and mice were promptly removed. The procedure was repeated for each experimental group to provide 10 mice at each exposure time. All surviving mice were placed in cages and observed 7 days for mortalities. From the 7-day mortality pattern, the time of exposure required to kill 50% of the animals (LT_{50}) and 95% confidence limits were calculated by the method of Weil (6). Under these conditions, the time required for the chamber atmosphere to reach 80% equilibrium with the incoming air–vapor mixture is 7.04 min., as calculated by the method of Silver (7). A plot of the theoretical relationship between time and degree of equilibrium of the chamber atmosphere to the incoming air–vapor mixture is shown in Fig. 1.

Tissue Culture—The cytotoxic activity of chloroacetaldehyde to mouse fibroblast cells (L-cells) in culture was determined by employing graded concentrations in the agar-overlay and protein assay methods. Both of these procedures were described in detail in a previous paper (8).

Dermal, Intradermal, and Ophthalmic Irritations—Albino New Zealand rabbits were employed in the evaluation of the irritancy of chloroacetaldehyde by these routes. Procedures employed and methods for evaluation of results were described in detail in a previous paper (8). However, due to the greater irritant activity of chloroacetaldehyde, more dilute solutions were employed.

Intramuscular Implantation—Small strips (approximately 0.5–1.0 mm. × 1 cm.) of a nonreactive polyvinyl chloride material were placed in a 30% solution of chloroacetaldehyde and allowed to remain for 24 hr. The samples were removed, blotted lightly to remove excess liquid, and implanted into the paravertebral muscle of the rabbit. Similar samples of the same material which had not been soaked in chloroacetaldehyde solution were implanted as a negative control, and samples of another polyvinyl chloride material (known to produce a positive response) were implanted as a positive control. Seven days later, the rabbits were sacrificed and the implanted materials were located and evaluated for tissue response. The responses were graded as follows: 0 = nonreactive, ± = slight or questionable response, 1+ = mild reaction, 2+ = moderate reaction, and 3+ = marked reaction.

Hemolysis Test—To determine the hemolytic threshold and concentrations which produce 50% hemolysis, a series of dilutions of chloroacetaldehyde in normal saline was prepared. Normal saline (without any toxicant) was employed as a negative control, and a 1% solution of sodium carbonate in saline served as the positive

Table I—Acute Toxicity of Chloroacetaldehyde

Test Animal	LD_{50}[a], ml./kg.	95% Confidence Limits	Concentration in Water, %	Number of Animals per Dose
Intraperitoneal Administration				
Mice, male, ICR	0.00598	0.00475–0.00752	30	10
Rats, male, S-D	0.00602	0.00393–0.00906	30	4
Rats, male, BB	0.00827	0.00657–0.01040	30	5
Guinea pigs, male, Hartley, albino	0.00212	0.00068–0.00658	30	3
Rabbits, male, New Zealand, albino	0.00464	0.00357–0.00603	30	4
Intragastric Administration				
Mice, male, ICR	0.06918	0.05063–0.09407	0.5	4
Rats, male, S-D	0.07507	0.04700–0.11989	3.0	4
Rats, female, BB	0.08665	0.07067–0.10151	2.0	5
Dermal (Topical) Application				
Rabbits, male, New Zealand, albino	0.2243	0.1579–0.3186	30	4
Inhalation				
Mice, male, ICR	2.57[b]	2.20–2.99 min.	30	10

[a] All values are expressed in terms of 100% chloroacetaldehyde. [b] LT_{50} (lethal time—50%) = 2.57 min., at which time the chamber had attained approximately 45% of equilibrium with the incoming air–vapor mixture.

[1] K & K Laboratories, Plainview, N. Y.

Table II—Chloroacetaldehyde Irritation Tests

	Concentration, %[a]	Irritant Response[b]
Intradermal, rabbits	TIC-4	0.0195%[c]
Dermal, rabbits	7.5	3+
	3.75	2+
	1.875	1+
	0.9375	±
	0.4688	0
Ophthalmic, rabbits	0.25	3
	0.125	2
	0.0625	2
	0.03125	1
	0.0156	±
	0.0078	0

[a] All solutions, except those used for the dermal test, were made isotonic with sodium chloride. [b] Scored on a 0 to 3+ scale; see *Reference 8* for definitions. [c] TIC-4 = threshold irritation concentration of Luduena and Hoppe (11); equivalent to 1+ response.

control (100% hemolysis). Ten milliliters of saline, sodium carbonate in saline, or chloroacetaldehyde in saline was placed into a 16 × 150-mm. test tube and kept in a water bath at 37° for 30 min. to provide temperature equilibration. Then 0.2 ml. of oxalated, whole rabbit blood was added to each tube and incubated for 60 min. at the same temperature. Each tube was centrifuged for 10 min. at 1000×*g*, and the supernatant was carefully removed with a pipet and placed into spectrophotometric cells; the absorbance of the sample at 540 nm. was then recorded.

The percent hemolysis was calculated as follows:

$$\% \text{ hemolysis} = \frac{\text{absorbance of test sample} - \text{absorbance of negative control}}{\text{absorbance of positive control}} \times 100 \quad \text{(Eq. 1)}$$

The data, as percent hemolysis, were plotted against the molar concentration of chloroacetaldehyde to give the dose–response curve. From this curve, the concentration required to produce 50% hemolysis was determined.

Sleeping-Time Test—Male ICR mice, weighing 25 ± 5 g., were pretreated with chloroacetaldehyde on 3 consecutive days, with the test being performed 24 hr. after the last pretreatment. In one series, mice were pretreated with intraperitoneal injections of chloroacetaldehyde; in the other series, pretreatment was by inhalation. In both instances, 24 hr. after the last exposure, each mouse was administered 50 mg./kg. of sodium pentobarbital intraperitoneally and observed for loss and then return of the righting reflex. The induction time was considered as the time interval between pentobarbital injection and loss of the righting reflex; sleeping time was the duration of the absence of the righting reflex. Ten mice per group were employed in both of these tests.

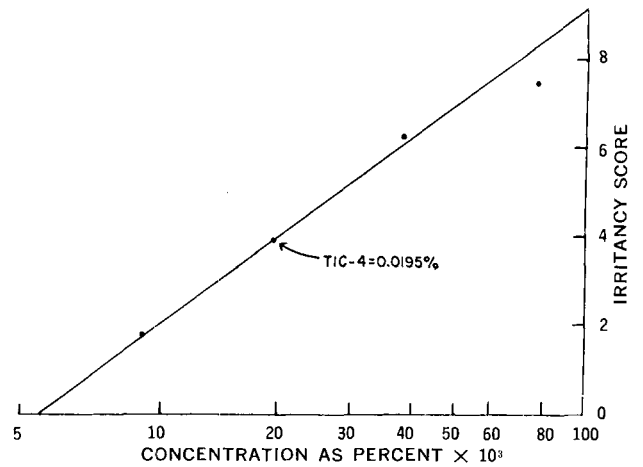

Figure 2—*Intradermal irritation from chloroacetaldehyde (TIC-4 determination).*

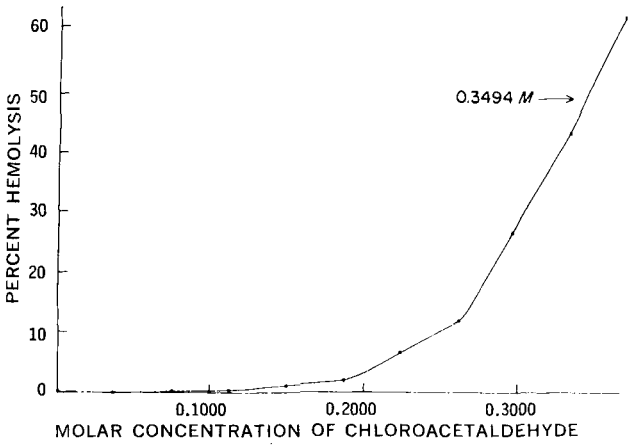

Figure 3—*Hemolysis of rabbit erythrocytes by chloroacetaldehyde; 50% hemolysis = 0.3494* M *(2.75%).*

Sensitization Test—To determine the sensitizing potential of chloroacetaldehyde, the guinea pig maximization test of Magnusson and Kligman (9) was employed using guinea pigs of the Hartley strain as described in a previous paper (10).

Acute Effects upon Cardiovascular, Respiratory, and Neuromuscular Functions—The acute effects of chloroacetaldehyde upon cardiovascular, respiratory, and neuromuscular functions were determined in anesthetized rabbits using the methodology described in a previous publication (10).

Cumulative Toxicity—The cumulative toxic nature of chloroacetaldehyde in male Sprague-Dawley rats was determined by administering daily doses of the compound by intraperitoneal injection for 30 consecutive days. Dose levels employed represent 0.001879 and 0.003758 ml./kg. of pure chloroacetaldehyde. Details of the procedure were described in a previous publication (10).

Subacute (Subchronic) Toxicity—The toxicity of chloroacetaldehyde in male Sprague-Dawley rats was investigated when the compound was administered three times a week for 12 weeks. The dose levels utilized in this study were 0.00032, 0.0008, 0.0016, and 0.0032 ml./kg. calculated as pure chloroacetaldehyde but the doses were injected as 0.5% aqueous solutions. This procedure was described in detail in a previous article (10).

RESULTS AND DISCUSSION

The acute LD_{50} values and their 95% confidence limits when chloroacetaldehyde was administered intraperitoneally, orally, or topically are presented in Table I. These values suggest that guinea pigs are most sensitive to intraperitoneal injection of the compound and the black rats are least sensitive, although overlap of the confi-

Table III—Effect of Chloroacetaldehyde upon Pentobarbital Sleeping Time[a]

Duration of Exposure	Concentration of Compound[b]	Minutes: Induction Time (Mean ± *SE*)	Minutes: Sleeping Time (Mean ± *SE*)
Pretreatment by Inhalation			
Control	Air	4.02 ± 0.25	78.76 ± 9.59
15 sec.	40.0 mg./l.	3.93 ± 0.21	98.25 ± 8.96
31 sec.	41.7 mg./l.	4.15 ± 0.31	127.84 ± 16.84[c]
77 sec.	43.4 mg./l.	3.20 ± 0.14[c]	224.57 ± 21.69[d]
Pretreatment by Intraperitoneal Injection			
Dose of Chloroacetaldehyde Injected			
Controls		3.25 ± 0.28	61.07 ± 5.79
0.00061 ml./kg.		3.13 ± 0.31	100.31 ± 10.63[d]
0.00122 ml./kg.		2.93 ± 0.16	137.54 ± 7.48[d]
0.00305 ml./kg.		3.33 ± 0.23	184.52 ± 12.22[d]

[a] Mice pretreated with chloroacetaldehyde 24, 48, and 72 hr. before intraperitoneal injection of 50 mg./kg. sodium pentobarbital. [b] Calculated as weight loss of 30% chloroacetaldehyde aqueous solution per liter of air. [c] Significant at 95% level ($p = 0.05$) by Student's *t* test. [d] Significant at 99% level ($p = 0.01$) by Student's *t* test.

Table IV—Cumulative Toxicity of Chloroacetaldehyde: Hematologic Values in Rats (Mean ± *SE*)

Item	Saline Control	0.001879 mg./kg.	0.003758 mg./kg.
Hemoglobin, g./100 ml.	15.020 ± 0.489	15.200 ± 0.559	11.700 ± 1.072[a]
Hematocrit	48.080 ± 1.819	47.080 ± 1.819	38.500 ± 2.300[b]
Erythrocytes, mm.^{3} ($\times 10^6$	5.975 ± 0.300	6.673 ± 0.335	4.528 ± 0.355[a]
Total white blood cells, mm.^{3} ($\times 10^3$)	12.050 ± 1.879	17.325 ± 2.043	19.162 ± 4.630
Platelets, mm.^{3} ($\times 10^6$)	0.990 ± 0.109	1.175 ± 0.215	0.690 ± 0.087
Clotting time, sec.	66.500 ± 6.498	79.500 ± 4.817	82.200 ± 9.664
Differential white cell count, %			
Segs	12.833 ± 2.943	31.083 ± 1.680[b]	27.500 ± 5.852
Lymphocytes	82.083 ± 3.145	64.833 ± 1.830[b]	67.875 ± 6.385
Monocytes	0.417 ± 0.154	1.417 ± 0.300[a]	1.00 ± 0.577
Eosinophils	1.083 ± 0.473	0.667 ± 0.248	0.875 ± 0.792
Basophils	0.583 ± 0.417	0.083 ± 0.083	0.125 ± 0.125
Meta	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Juvenile	0.083 ± 0.083	0.250 ± 0.112	0.250 ± 0.250
Bands	1.333 ± 0.401	2.000 ± 0.224	1.250 ± 0.777

[a] Significant at the 95% level ($p = 0.05$) by Student's t test. [b] Significant at the 99% level ($p = 0.01$) by Student's t test.

dence limits does not permit statistical verification of the apparent trend from this data. The magnitude of difference in the LD_{50} produced by changing the route of administration is quite obvious. Absorption of chloroacetaldehyde from the GI tract or through the skin appears to be a significant factor in influencing its toxicity. As discussed later, this is a marked departure from the pattern of response produced by the potential parent compound 2-chloroethanol, which demonstrated equivalent toxicity by each of these three routes of administration.

Chloroacetaldehyde proved to be very lethal by inhalation. Mice were placed into a chamber containing no chloroacetaldehyde, and air containing chloroacetaldehyde vapor was passed into the chamber. The time of exposure required to kill 50% of the animals, LT_{50}, was 2.57 min. In this time, the chamber atmosphere is calculated to have reached approximately 45% of equilibrium with the incoming air-chloroacetaldehyde vapor concentration (Fig. 1). Prior to its entering the inhalation chamber, air was bubbled through a 30% aqueous solution of chloroacetaldehyde and was found to remove 33 mg. of liquid/l. of air.

Tissue culture tests, using both the agar-overlay technique and protein assay, demonstrated the highly toxic properties of chloroacetaldehyde. Application of 0.2 ml. of a solution as dilute as 0.0078% to the agar-overlay produced a cytotoxic effect on the mouse fibroblasts (L-cells), while further dilutions, 0.0039% or less, were noncytotoxic. By using inhibition of protein synthesis in L-cell cultures as the criterion, it was found that a 5.62×10^{-5} M concentration of chloroacetaldehyde in the media was sufficient to reduce protein synthesis by 50% (ID_{50}).

Results of the dermal, intradermal, and ophthalmic irritation tests conducted in rabbits are presented in Table II, which illustrates the highly irritating nature of chloroacetaldehyde. The "threshold irritation concentration" (TIC-4) of Luduena and Hoppe (11) was determined for the intradermal route. The basic 0, ±, 1+, 2+, and 3+ evaluation system was converted to numerical values of 0, 2, 4, 6, and 8, respectively, to be compatible with the system used by Luduena and Hoppe. The odd numbers were used for observed responses that were intermediate between two of the assigned even numbers. Ten observations were made at each of four concentrations for the graphic plot to determine the TIC-4 value (equivalent to 1+ response). Figure 2 shows the straight-line plot of these data and their agreement with a calculated value of TIC-4 = 0.0195%.

Chloroacetaldehyde was extremely irritating to the intact skin of the rabbit. The undiluted material (30% chloroacetaldehyde in water) produced severe and extensive tissue damage, much more intense and over a larger area than was produced by the positive control (8% sodium lauryl sulfate, w/v, in water). Similarly, it was noted in preliminary tests that the more concentrated solutions of chloroacetaldehyde produced extensive damage to the rabbit's eye, while a 0.03125% solution produced definite, but reversible, ophthalmic irritation (score of 1).

Strips of polyvinyl chloride, which had been soaked in 30% chloroacetaldehyde for 24 hr., were implanted into the paravertebral muscle of the rabbit. After 7 days, the rabbit was sacrificed and the implant sites were located in the muscle. The chloroacetaldehyde-treated strips produced an area of necrosis and purulence around the implant equal to or greater than that produced by the positive control (3+). Untreated strips of polyvinyl chloride, identical to those soaked in chloroacetaldehyde, did not produce grossly visible signs of necrosis.

Figure 3 shows the dose-response curve produced by plotting percent hemolysis *versus* concentration of chloroacetaldehyde. Thus, it may be seen that about 10% hemolysis should be produced by 0.2450 M and 50% hemolysis produced by 0.3494 M (or 2.74%) chloroacetaldehyde in saline.

Pretreatment of mice with chloroacetaldehyde produced a dose-related increase in pentobarbital sleeping time. This was true whether the compound was administered by intraperitoneal injection or by inhalation. Dosing of the mice was based upon 0.1, 0.2, and 0.5 of the acute LD_{50} or LT_{50} daily for 3 days prior to pentobarbital administration. The results are presented in Table III. The increase in sleeping time in the groups treated with chloroacetaldehyde intraperitoneally is significant at the 99% level (by t test) for all three dosage levels. For the animals exposed to chloroacetaldehyde vapors

Table V—Cumulative Toxicity of Chloroacetaldehyde as Shown by B.S.P. Liver Function Test in Rats[a]

B.S.P. Concentration (mg. %) in Plasma of Rats (Mean ± *SE*) Dose Level of Chloroacetaldehyde —Pretreatment—			
After	Saline Control	0.001879 mg./kg.	0.003758 mg./kg.
15 min.	25.26 ± 0.81	22.88 ± 1.79	27.38 ± 3.70
30 min.	5.93 ± 0.67	7.13 ± 1.41	10.38 ± 5.71
45 min.	2.74 ± 0.55	3.97 ± 1.45	3.55 ± 1.59
Percentage of B.S.P. Eliminated			
Between			
15–30 min.	76.04 ± 3.21	70.94 ± 8.15	69.91 ± 15.91
30–45 min.	54.72 ± 6.68	42.70 ± 13.37	45.91 ± 11.56

[a] Test was performed at the conclusion of the 30-day cumulative toxicity study. All rats received 75 mg./kg. of B.S.P. intravenously. Values are from six rats per group, except the high dose group in which the test was completed in only three of the four surviving animals.

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

Organ	Saline Control	0.001879 mg./kg.	0.003758 mg./kg.
Adrenals	0.012 ± 0.001	0.018 ± 0.003	0.025 ± 0.006
Brain	0.467 ± 0.015	0.568 ± 0.042[a]	0.758 ± 0.089[a]
Gonads	0.871 ± 0.024	0.982 ± 0.082	1.189 ± 0.083[b]
Heart	0.273 ± 0.008	0.263 ± 0.013	0.321 ± 0.019[a]
Kidneys	0.620 ± 0.014	0.701 ± 0.069	0.769 ± 0.059[a]
Liver	3.505 ± 0.204	3.901 ± 0.384	5.211 ± 0.858
Lungs	0.334 ± 0.011	0.409 ± 0.039[b]	0.565 ± 0.052[b]
Spleen	0.224 ± 0.011	0.321 ± 0.078	0.457 ± 0.085[a]

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

Table VII—Subacute Toxicity of Chloroacetaldehyde: Food Consumption[a] in Rats (Mean ± *SE*)

Week	Saline Control	0.00032 ml./kg.	0.0008 ml./kg.	0.0016 ml./kg.	0.0032 ml./kg.
1st	109.522 ± 8.355	100.934 ± 12.512	92.515 ± 7.246	39.109 ± 5.307[c]	26.828 ± 6.729[c]
2nd	60.008 ± 2.466	70.251 ± 2.832[b]	61.366 ± 4.233	60.750 ± 3.694	74.319 ± 7.036
12th	60.058 ± 2.026	55.141 ± 2.656	56.035 ± 2.195	57.555 ± 3.443	96.376 ± 9.671

[a] Grams of food consumed per kilogram of rat per 24-hr. day. [b] Significant at 95% level by Student's *t* test. [c] Significant at 99% level by Student's *t* test.

Table VIII—Subacute Toxicity of Chloroacetaldehyde: Hematologic Values (Mean ± *SE*)

Item	Saline Control	0.00032 ml./kg.	0.0008 ml./kg.	0.0016 ml./kg.	0.0032 ml./kg.
Hemoglobin, g./100 ml.	17.287 ± 0.234	17.102 ± 0.226	16.885 ± 0.206	16.255 ± 0.507	14.937 ± 0.244[a]
Hematocrit	50.750 ± 0.998	51.500 ± 0.796	51.000 ± 1.335	47.250 ± 1.564	47.500 ± 1.258
Red blood cells, mm.^{3} ($\times 10^6$)	7.752 ± 0.595	6.663 ± 0.539	7.187 ± 0.804	5.237 ± 0.168[a]	5.227 ± 0.424[b]
Total white blood cells, mm.^{3} ($\times 10^3$)	14.608 ± 1.882	14.917 ± 1.792	16.792 ± 2.012	12.133 ± 1.928	16.717 ± 0.985
Platelets, mm.^{3} ($\times 10^6$)	0.637 ± 0.109	0.669 ± 0.079	0.661 ± 0.063	0.769 ± 0.057	0.607 ± 0.119
Clotting time, sec.	80.667 ± 8.123	94.833 ± 4.037	67.000 ± 6.387	119.667 ± 17.021	134.000 ± 6.083[a]
Differential white cell count, %					
Segs	11.667 ± 2.548	11.167 ± 3.206	14.000 ± 2.335	33.167 ± 4.752[a]	31.667 ± 9.493[b]
Lymphs	79.917 ± 2.797	85.583 ± 1.744	77.917 ± 3.177	60.583 ± 5.057[a]	65.333 ± 8.786
Monocytes	2.583 ± 0.597	1.167 ± 0.527	1.500 ± 0.500	2.917 ± 0.455	1.833 ± 0.928
Eosinphils	2.250 ± 0.443	1.083 ± 0.352	1.500 ± 0.465	1.417 ± 0.375	1.000 ± 0.289
Basophils	0.167 ± 0.105	0.000 ± 0.000	0.500 ± 0.258	0.250 ± 0.171	0.000 ● 0.000
Metas	0.000 ± 0.000	0.333 ± 0.167	0.250 ± 0.171	0.000 ± 0.000	0.000 ± 0.000
Juvenile	0.667 ± 0.279	0.333 ± 0.167	0.750 ± 0.359	0.667 ± 0.247	0.167 ± 0.167
Bands	2.917 ± 0.851	2.917 ± 1.179	3.750 ± 1.167	1.083 ± 0.300	0.167 ± 0.167

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

(by inhalation), the increase was not significant for the shortest exposure time (15 sec.), but it was significant at the 95% level for those exposed for 31 sec. and it was significant at the 99% level for those exposed 77 sec./day. Such a response could be explained on the basis of hepatic necrosis or other damage; however, in another study in which chloroacetaldehyde was administered daily for 30 days, hepatic damage was not observed in hematoxylin-eosin-stained specimens from those animals surviving to the end of the experiment.

The sensitizing potential of chloroacetaldehyde was tested in five guinea pigs, using the "guinea pig maximization test" of Magnusson and Kligman (9). Due to the highly toxic and extremely irritating nature of the compound, it was necessary to use a very dilute solution, 0.002%. The test produced negative results, indicating that this concentration of chloroacetaldehyde was not sensitizing by this test.

Experiments were conducted to assess the acute effects of intravenous chloroacetaldehyde on blood pressure, respiration, and neuromuscular function in anesthetized rabbits. The various dose levels employed produced three generalized patterns of response upon blood pressure (Fig. 4). There was no uniform or dramatic effect upon respiration. Doses of 0.02–0.10 ml./kg. of 30% chloroacetaldehyde produced little or no effect upon contraction of the anterior tibialis and gastrocnemius muscles from stimulation of the sciatic nerve; however, 0.2 ml./kg. or more of 30% chloroacetaldehyde inhibited or blocked this response.

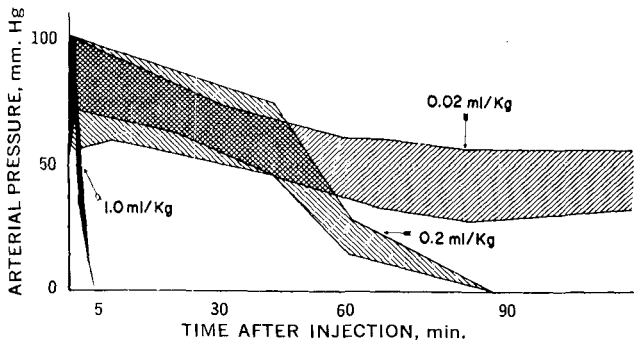

Figure 4—*Acute effects of graded doses of 30% chloroacetaldehyde solution, intravenously, upon blood pressure in the anesthetized rabbit.*

An investigation into the cumulative toxicity of chloroacetaldehyde in which groups of rats received 0.001879 and 0.003758 ml./kg. of chloroacetaldehyde (representing 0.3 and 0.6 of the acute LD_{50} dose, respectively) daily for 30 consecutive days resulted in a mortality of 25 and 66.7%, respectively. During this time, the chloroacetaldehyde-treated rats gained weight more slowly than the controls (Fig. 5). These differences were significant (at 95 or 99% level) for both treated groups prior to the conclusion of the test period. Hematologic tests (Table IV) at the end of 30 days showed that there was a significant (at 95% level or better) decrease in hemoglobin, hematocrit, and erythrocytes in the high dose group; for the low dose group, there was an increase in segs and monocytes with a decrease in lymphocytes. At the end of the experiment, a liver function test, B.S.P. disappearance from the plasma, was conducted on the three groups of animals. The data are presented in Table V; however, there were no significant differences between the controls and the chloroacetaldehyde-treated groups.

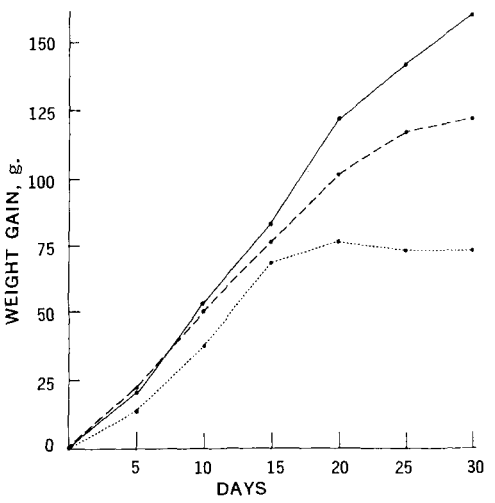

Figure 5—*Cumulative toxicity of chloroacetaldehyde (mean body weight gain of rats). Key: ——, saline control; - - -, 0.001879 ml./kg.; and . . ., 0.003758 ml./kg.*

Table IX—Subacute Toxicity of Chloroacetaldehyde as Shown by B.S.P. Liver Function Test in Rats[a]

After	B.S.P. Concentration (mg. %) in Plasma of Rats (Mean ± SE) — Dose Level of Chloroacetaldehyde Pretreatment — Saline Control	0.00032 ml./kg.	0.0008 ml./kg.	0.0016 ml./kg.	0.0032 ml./kg.
15 min.	29.16 ± 1.80	28.32 ± 1.65	31.19 ± 3.31	30.31 ± 2.68	20.99 ± 4.59
30 min.	15.38 ± 3.19	15.94 ± 2.75	17.97 ± 2.28	10.39 ± 1.87	6.53 ± 2.30
45 min.	8.21 ± 2.03	10.63 ± 3.05	11.38 ± 2.57	2.03 ± 0.27	3.52 ± 1.24
Percentage of B.S.P. Eliminated					
Between					
15–30 min.	49.00 ± 8.52	42.85 ± 10.84	41.34 ± 5.80	65.39 ± 6.32	62.59 ± 19.55
30–45 min.	49.10 ± 5.89	40.41 ± 10.23	39.42 ± 11.07	78.97 ± 2.79[b]	46.16 ± 7.11

[a] Test was performed at the conclusion of the 12-week subacute toxicity study. All rats received 75 mg./kg. of B.S.P. intravenously. Values are from six rats per group, except for 0.0032 ml./kg. in which there were only three surviving animals. [b] Significant at 99% level ($p = 0.01$) by t test.

Table X—Subacute Toxicity of Chloroacetaldehyde: Percent Organ-to-Body Weight of Rats (Mean ± SE)

Organ	Saline Control	0.00032 ml./kg.	0.0008 ml./kg.	0.0016 ml./kg.	0.0032 ml./kg.
Adrenals	0.013 ± 0.001	0.009 ± 0.001[a]	0.014 ± 0.001	0.010 ± 0.000[a]	0.016 ± 0.002
Brain	0.454 ± 0.023	0.363 ± 0.021[a]	0.386 ± 0.012[a]	0.461 ± 0.023	0.804 ± 0.172[a]
Gonads	0.768 ± 0.042	0.728 ± 0.019	0.799 ± 0.034	0.824 ± 0.041	0.808 ± 0.171
Heart	0.283 ± 0.012	0.311 ± 0.022	0.261 ± 0.014	0.245 ± 0.008[a]	0.290 ± 0.023
Kidneys	0.673 ± 0.022	0.637 ± 0.028	0.614 ± 0.017	0.543 ± 0.027[a]	0.663 ± 0.043
Liver	3.086 ± 0.314	3.254 ± 0.194	3.423 ± 0.185	3.355 ± 0.191	4.946 ± 0.198[b]
Lungs	0.387 ± 0.016	0.432 ± 0.112	0.373 ± 0.029	0.340 ± 0.019	0.380 ± 0.056
Spleen	0.212 ± 0.018	0.237 ± 0.009	0.228 ± 0.22	0.244 ± 0.026	0.265 ± 0.022

[a] Significantly different from controls at $p = 0.05$. [b] Significantly different from controls at $p = 0.01$.

After these tests, the animals were sacrificed and certain organs were removed, weighed (for calculation of organ-to-body weight ratio), and fixed in 10% buffered formalin for histopathologic evaluation. The organ-to-body weight ratios, expressed as percentages, are presented in Table VI. The ratios for both the brain and lungs were significantly greater in the low dose group, while the high dose group showed a significant increase in the brain, gonads, heart, kidneys, liver, lungs, and spleen. Hematoxylin–eosin-stained sections of these organs did not reveal any abnormalities which could be attributed to chloroacetaldehyde administration, except for the lungs which showed more severe bronchitis, bronchiolitis, and bronchopneumonia than were seen in the controls.

A series of doses, starting at a lower level, of chloroacetaldehyde was employed in a subacute (subchronic) study. A 0.5% aqueous solution was injected, with the doses being 0.00032, 0.00080, 0.00160, and 0.00320 ml./kg. of pure chloroacetaldehyde administered three times a week (Monday, Wednesday, and Friday) for 12 weeks. Each group consisted of 12 rats, except the 0.00320-ml./kg. group which contained eight rats. With the exception of the high dose group, only two mortalities were produced: one in the low dose group during the 1st week and the other in the 0.00160-ml./kg. group during the 12th week. The 0.00320-ml./kg. dose produced five deaths in the eight animals (62.5%); although an LD_{50} cannot be calculated from these data, it would appear that the subacute LD_{50} would be in the range of 0.0030 ml./kg. or about one-half of the acute LD_{50}.

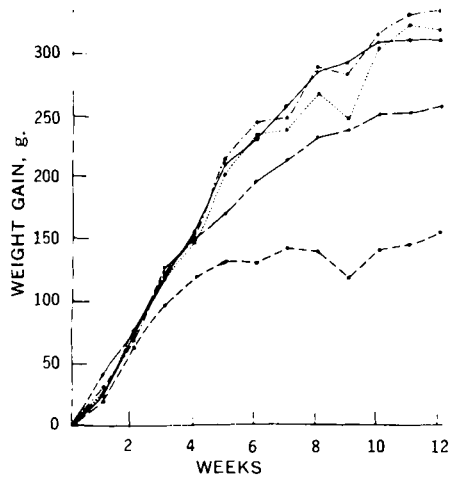

Figure 6 *-Subacute toxicity of chloroacetaldehyde (mean body weight gain of rats). Key: ···, saline controls; ---, 0.00032 ml./kg.; — —, 0.0008 ml./kg., – – –, 0.0016 ml./kg.; and - - -, 0.0032 ml./kg.*

The weight gain of the rats treated with the two lower doses did not differ significantly from the controls; however, there was a significant decrease in weight gain for the two higher dose levels which was dose related (Fig. 6). Food consumption was determined during the 1st, 7th, and 12th weeks. There was a significant decrease in consumption by the two higher dosed groups during the 1st week, but little effect was noted otherwise (Table VII).

Hematologic determinations and B.S.P. liver function tests were performed at the conclusion of the 12th week; these data are presented in Tables VIII and IX, respectively. None of these values was significantly different from the controls (at $p = 0.05$ or less) for the two lowest doses, while 0.0016 ml./kg. showed a decrease in red cell count and lymphocytes, an increase in segmented neutrophils, and an increased rate of B.S.P. disappearance between 30 and 45 min.; the highest dose (0.0032 ml./kg.) showed a significant decrease in red blood cells and hemoglobin with an increase in clotting time and segmented neutrophils.

At autopsy, organ-to-body weight ratios were determined for several organs (Table X). Eight of the 32 values were statistically significant (at $p \leq 0.05$), although there was no apparent dose-related response with the quantities of chloroacetaldehyde employed. However, the ratio for the brain and liver in the high dose group (0.0032 ml./kg.) was considerably higher; this may have been due to a selective effect of the drug or a reflection of smaller body weights of these animals.

Histological examination of hematoxylin eosin-stained sections of these organs revealed significant pathological changes in the lungs of animals receiving the two highest dose levels of chloroacetaldehyde. These changes included focal, chronic bronchopneumonia and certain changes of respiratory epithelium suggestive of a premalignant condition. Similar changes, to a lesser degree, were seen in the two low dose groups and controls, except there was no evidence of atypia of the respiratory epithelium. None of the other organs revealed any changes that could be attributed to administration of chloroacetaldehyde.

In concluding the discussion of chloroacetaldehyde, it might be appropriate to examine it in relation to its biological parent compound 2-chloroethanol. Some comparative data for these two compounds are presented in Table XI.

Table XI—Comparison of Acute Toxicity for Chloroacetaldehyde and 2-Chloroethanol

Test	Chloroacetaldehyde[a]	2-Chloroethanol[a]
Acute toxicity, oral LD_{50}		
Mice, male	0.0692 ml./kg.	0.0671 ml./kg.
Rats, male	0.0751 ml./kg.	0.0588 ml./kg.
Acute toxicity, intraperitoneal LD_{50}		
Mice, male	0.00598 ml./kg.	0.0810ml./kg.
Rats, male	0.00602 ml./kg.	0.0528 ml./kg.
Guinea pigs, male	0.00212 ml./kg.	0.0707 ml./kg.
Rabbits, male	0.00464 ml./kg.	0.0671 ml./kg.
Acute toxicity, dermal LD_{50}		
Rabbits	0.2243 ml./kg.	0.0559 ml./kg.
Acute toxicity, inhalation LT_{50}		
Mice, male	2.57 min.	6.47 min.
Tissue culture, L-cells		
Agar-overlay, cytotoxic/noncytotoxic	0.0078%/0.0039%	10.0%/5.0%
Protein assay, ID_{50}	0.0000562 *M*	0.03193 *M*
Irritation tests		
Rabbits, intradermal	0.0195% = 1+	5.0% = 1+
Rabbits, dermal	7.5% = 3+ 0.4688% = 0	100% = 0
Rabbits, ophthalmic	0.03125% = 1+	1.25% = 1+
7-Day muscle implant[b]	3+	0

[a] All LD_{50} values expressed in terms of pure compound. [b] Strips of a nonreactive polyvinyl chloride were soaked in pure 2-chloroethanol or 30% chloroacetaldehyde for 24 hr. prior to implantation.

Note the very close agreement between the LD_{50} values for chloroacetaldehyde and 2-chloroethanol when they were administered orally to mice and the reasonably good agreement for rats. When the two compounds were administered intraperitoneally, however, chloroacetaldehyde appeared to be about 10–30 times as toxic as 2-chloroethanol. On the other hand, when the compounds were applied topically, 2-chloroethanol was about four times more toxic than chloroacetaldehyde. While the toxic quantity of 2-chloroethanol remained relatively constant between species of animals and routes of administration (approximately 53–81 μl./kg.), a similar comparison for chloroacetaldehyde shows more than a 100-fold variation in its LD_{50} values.

When tested on L-cells in culture, using the agar-overlay technique, chloroacetaldehyde was more than 1200 times as toxic as 2-chloroethanol. ID_{50} values obtained from L-cells indicate chloroacetaldehyde to be 568 times as potent in inhibiting protein synthesis as 2-chloroethanol.

A comparison of the two compounds for their irritant properties also shows chloroacetaldehyde to be more active. In the case of intradermal irritation, it required approximately 250 times as much 2-chloroethanol to produce a 1+ irritant response as was needed for chloroacetaldehyde, while 40 times as much 2-chloroethanol was required to produce a comparable degree of ophthalmic irritation (1+). Dermal irritant tests found solutions of 7.5% or greater of chloroacetaldehyde sufficient to produce a 3+ response, while undiluted (100%) 2-chloroethanol did not produce significant irritation. Seven-day implantation tests in the rabbit muscle, conducted by soaking strips of a nonreactive polyvinyl chloride material in 2-chloroethanol or 30% chloroacetaldehyde for 24 hr. prior to implantation, revealed a marked reaction (3+) to chloroacetaldehyde but no observable response to 2-chloroethanol.

It is apparent that chloroacetaldehyde has a much greater irritant activity and is inherently more toxic than 2-chloroethanol; however, 2-chloroethanol appears to have the greater penetrant capacity. This is particularly noticeable in the dermal toxicity of the two compounds.

In considering the potential danger of accidental exposure to toxic quantities of the two compounds, 2-chloroethanol probably presents the most hazard. Although it is quantitatively less toxic, it produces little or no irritation to the intact skin but penetrates quite readily; thus, contamination and absorption may occur without the individual being aware of them. The pronounced irritation produced by chloroacetaldehyde, coupled with its lesser ability to penetrate, alerts the individual to its presence and encourages him to remove it. Similarly, the more pronounced irritation of chloroacetaldehyde vapors serves as a deterrent to prevent the individual from remaining in an area of exposure to toxic quantities of the vapors, while the less irritating vapors of 2-chloroethanol may not provide such early warning.

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