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TOXICITY OF PROPYLENE GLYCOL.*

BY J. H. WEATHERBY,[1] PH.D., AND H. B. HAAG,[1] M.D.

INTRODUCTION.

During the past several years suggestions have been made that propylene glycol is a suitable solvent for a variety of purposes, pharmaceutical and otherwise. As a result of this interest several papers (1, 2) have appeared in which attention was directed particularly to toxicological studies on this substance. In connection with another investigation involving the use of propylene glycol it was considered advisable to extend some of these studies for the purpose of completing our own data on this subject. Some of the results to be described confirm those of other authors; and some are believed to be new observations not previously described in the literature. The propylene glycol used in these studies was the alpha form (1, 2 propane diol) obtained from the Carbide and Carbon Chemicals Corporation.

Seidenfeld and Hanzlik (1) reported acute fatal doses for propylene glycol when given intramuscularly and intravenously to white rats and rabbits. They also investigated the effects in growing rats of the continued drinking of water containing various amounts of propylene glycol. The intramuscular fatal doses were found to be about 14 Gm. per Kg. body weight for rats, and about 7 Gm. per Kg. for rabbits. The intravenous fatal doses were found to be about 16 Gm. per Kg. for rats, and about 5 Gm. per Kg. for rabbits. Practically no effects were observed in rats from drinking water containing less than 10% propylene glycol; but with higher concentrations the fluid intake was greatly restricted, and the animals lived only a few days. No definite microscopic changes were observed in organs of the animals sacrificed at the end of the experimental period of 140 days. It is interesting to note that these authors found the intravenous fatal dose for rats to be greater than the intramuscular fatal dose, a relationship rarely observed. This is particularly significant since the intravenous fatal dose for another rodent (rabbit) was found to be considerably less than the intramuscular fatal dose, which is the usual relationship.

Braun and Cartland (2) investigated the acute toxicity of propylene glycol for rats by subcutaneous, intramuscular and intravenous injections, and obtained results which were in satisfactory agreement with those reported by Seidenfeld and

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[1] Medical College of Viriginia, Dept. of Pharmacology.

Hanzlik. In addition Braun and Cartland investigated the acute toxicities on oral administration to rabbits.

EXPERIMENTAL METHODS AND RESULTS.

Acute Toxicity.—In the acute experiments propylene glycol was administered intravenously to rabbits; and orally, subcutaneously, intramuscularly and intravenously to white rats. All animals were adults, but otherwise unselected as to age, source, weight or sex, and were maintained on standard diets (Purina). The animals given propylene glycol orally received no food for twenty-four hours prior to the experiment.

The results of the acute toxicity experiments are shown in Table I. The Average Fatal Dose is taken as the quantity required to kill 50% of the animals. This figure was arrived at by plotting dose against per cent mortality, and then deriving the fatal dose for 50% of the animals from the curve so constructed. In Table II is shown a summary of the data published by various authors to whom references are made. These figures also represent doses fatal to 50% of the animals, and were derived from data published by the original authors. In many instances the number of animals used for each dose was so small as to make the accuracy of such a procedure questionable. One author used only four mice in the entire series. However, since there is no consis-

TABLE I.—TOXICITY OF PROPYLENE GLYCOL.

Dose (per Kg. Body Wt.). Cc.	Gm.	Number Animals.	Died.	Lived.	% Mortality.
		* Oral Administration: Rats.			
25	26.0	5	0	5	00
30	31.2	5	2	3	40
35	36.4	5	3	2	60
		Subcutaneous Administration: Rats.			
20	20.8	5	1	4	20
25	26.0	5	4	1	80
30	31.2	5	5	0	100
		Intramuscular Administration: Rats.			
12	12.48	5	0	5	00
14	14.56	5	3	2	60
16	16.64	5	4	1	80
18	18.72	5	5	0	100
		Intravenous Administration: Rats.			
6	6.24	10	1	9	10
7	7.28	5	4	1	80
8	8.32	10	8	2	80
9	9.36	5	5	0	100
10	10.40	5	4	1	80
12	12.48	5	5	0	100
		Intravenous Administration: Rabbits.			
5	5.2	3	0	3	00
6	6.24	5	1	4	20
7	7.28	5	4	1	80
8	8.32	5	5	0	100

* Larger doses were impractical due to limited capacity of rat's stomach.

TABLE II.

Animal.	Method of Administration.	Propylene, Gm./Kg.	Diethylene, Gm./Kg.	Ethylene, Gm./Kg.
Mouse	Subcutaneous	...	...	5.6 (3)
Rat	Oral	33.5 (6)	16.8 (4)	...
Rat	Subcutaneous	22 (2)	15.7 (4)	...
		22.5 (6)	...	...
Rat	Intramuscular	14 (1)	7.87 (4)	4.2 (5)
		15.5 (2)	...	...
		14 (6)	...	...
Rat	Intravenous	16 (1)	4.75 (4)	...
		18.9 (2)	...	...
		6.8 (6)	...	...
Rabbit	Oral	19.3 (2)	...	...
Rabbit	Intramuscular	7 (1)	3.9 (4)	6 (5)
Rabbit	Intravenous	5 (1)	2.25 (4)	4.7 (5)
		6.5 (6)	...	...

Numbers in parentheses refer to papers from which these data were obtained. The data represent Average Lethal Doses (50% mortality) calculated from data published by the various authors.

tency in the per cent mortality taken by various authors as indicating Average Fatal Dose, it is believed that this treatment of the data gives results which are more nearly comparable. The intravenous fatal dose for rabbits, and the intramuscular fatal dose for rats agree satisfactorily with those reported by Seidenfeld and Hanzlik, but the intravenous fatal dose for rats was found to be far less than that reported by these authors. Also, results obtained from subcutaneous and intramuscular injections in rats were in excellent agreement with those reported by Braun and Cartland, but again there was a marked discrepancy in the intravenous fatal doses for rats. When this discrepancy first became apparent it was thought that perhaps diet, age or some other uncontrolled factor may have been responsible, but the experiment has since been repeated on two different occasions on animals from other sources of supply. Essentially the same results were obtained on all three groups of rats, those from two groups being included in Table I.

During the early attempts at injections into veins of the tails of rats it was observed several times that the needle passed completely through the vein and into loose tissue surrounding the vein. Then, upon aspiration with the syringe, a droplet of blood was drawn into the syringe, giving a false indication of the position of the needle. This droplet of blood was the result of leakage from the punctured vein, and was not obtained directly from the lumen of the vein. Upon injecting the contents of the syringe the vein was obliterated by external pressure of the injected material on the vein. This material passed toward the body through tissues surrounding the vein, and ultimately most of it accumulated in muscular and subcutaneous tissues at the base of the tail. Such injections gave many of the appearances of intravenous injections without actually having been intravenous. It was found that such accidents could be obviated almost entirely by first placing the rat's tail in water at a temperature of about forty degrees Centigrade for one or two minutes before attempting the injection. This caused dilatation of the veins so that the needle could be introduced quite easily, and with little danger of failure.

Neither Seidenfeld and Hanzlik nor Braun and Cartland mentioned the veins selected by them for injections. Concerning these injections Braun and Cartland state: "The intravenous injection of undiluted propylene glycol produced rapid obliteration of the veins making administration very difficult. At doses of 16 to 19 cc. per Kg. intravenously, all rats showed marked muscular tremors and later became comatose." The authors of this communication did not find the administration of propylene glycol difficult after previous dilatation of the vein as mentioned above, nor were the veins rapidly obliterated. With sub-lethal doses, the veins were seen to refill with blood just about as promptly after withdrawal of the needle as if saline or glucose solutions had been injected. Following fatal doses death occurred within a short time in most instances, although with barely fatal doses the animals sometimes lived for ten or fifteen minutes; exceptionally one lived as long as several hours.

The data presented by Seidenfeld and Hanzlik in their Table I suggests that some such accident as previously described may have happened in many of their injections. An increase in dose from 9 cc. to 12 cc. per Kg. was accompanied by a drop in fatalities from 30% to 20%, and a further increase in dose to 15 cc. per Kg. was accompanied by an increase in fatalities to only 40%. To increase the number of fatalities from 30% to 70% it was found necessary to increase the dose from 9 cc. to 18 cc. per Kg. One would predict just such a relationship between dose and number of fatalities if approximately 30% to 40% of the injections actually were made into veins. Then, upon approaching the intramuscular fatal dose (variously reported between 14 and 15.5 Gm. per Kg.) the mortality rate would naturally approach that for the intramuscular method of administration. The fact that the intravenous fatal dose was found by Seidenfeld and Hanzlik actually to exceed the intramuscular fatal dose may have been due to retention of part of the injected material in the tissues of the tail, from which absorption was exceedingly slow because of obliteration of the vein.

Symptoms following the administration of fatal or near fatal doses of propylene glycol were found to be essentially as described by Seidenfeld and Hanzlik and Braun and Cartland. The fatal period varied from a minimum of two or three minutes following intravenous injections to a

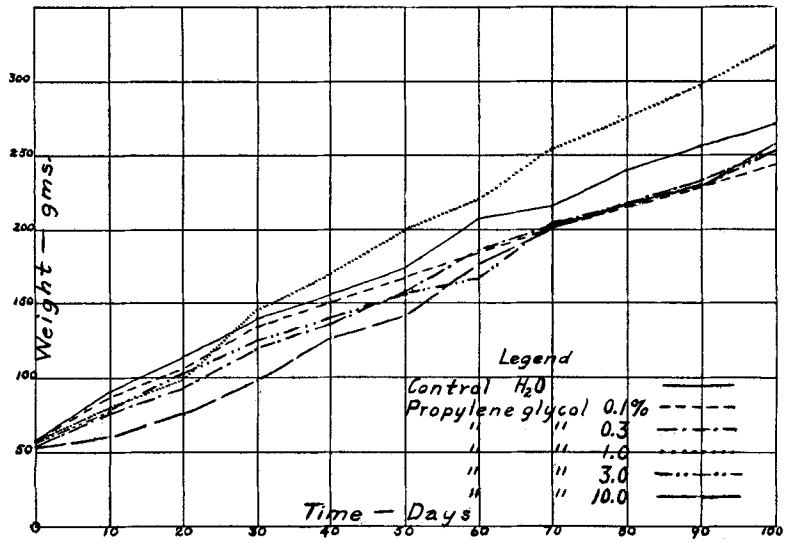

Fig. 1.—The effect on the growth of white rats of adding various concentrations of propylene glycol to the drinking water.

maximum of about two days following oral administrations. Muscular tremors and mild clonic convulsions occurred frequently. The animals became comatose more or less rapidly depending on the method of administration. Hematuria was observed frequently, especially after intravenous injections.

Chronic Toxicity.—The chronic toxicity of propylene glycol was investigated by administering the substance to growing rats by way of their drinking water. Male rats at weaning age (about 55 Gm. average weight) were used in groups of five animals each. Propylene glycol was added to the drinking water in concentrations of 0.1%, 0.3%, 1.0%, 3.0% and 10.0% by weight. A control group received distilled water instead of propylene glycol solution. All animals received the diet recommended by Osborn and Mendel. Food and drink were allowed ad libitum. Weights of the animals and consumption of food and drink were recorded at five-day intervals for the duration of the experiment—100 days. At the end of this period all surviving animals were sacrificed, and all internal organs were prepared for microscopic examination. During the first ten days of the experimental period those animals which received 10.0% propylene glycol increased in weight less rapidly than the remainder of the animals, and appeared to be in poor physical condition. However, very soon thereafter, those animals recovered from their poor start, and began to increase in weight at a rate about equal to that of the control group. Two animals which received

10.0% propylene glycol and one which received 1.0% died before the termination of the experiment. Growth curves for the six groups of animals are shown in Fig. 1. No significant differences were noted in consumption of food and drink by the various groups. It is worthy of note that those animals which received 10.0% propylene glycol solution consumed from 9.6 to 15.8 Gm. per Kg. per day, whereas, the acute oral fatal dose was found to be 33.5 Gm. per Kg.

Microscopic examination of organs from these animals was made by Doctor G. Z. Williams, Associate Professor of Pathology of this institution. In no way were the organs from animals which had received propylene glycol found to differ from those of the control group, which had received distilled water. These results substantiate those reported by Seidenfeld and Hanzlik from a similar experiment.

Hemolysis.—The frequent occurrence of hematuria following administration of sub-lethal doses of propylene glycol to rats, and the previous mention of this action by Von Oettingen and Jirouch (3), prompted a study of this phenomenon *in vitro*. Isotonic solutions of diethylene glycol and propylene glycol were prepared (0.278 molar). These solutions were mixed with varying quantities of 0.9% NaCl solution so that a series of mixtures resulted which contained isotonic glycol and isotonic saline in the proportions of 9 to 1, 8 to 2, 7 to 3, 6 to 4, and so on. A separate series of solutions was prepared by diluting isotonic saline with distilled water in the same manner. These last solutions were used for ascertaining the fragility of the red corpuscles, and whether or not the blood used was normal in this respect. One drop of human blood was added to each tube, which contained 5 cc. of one of the solutions prepared as described above. The results are shown in Table III. Three such experiments were performed, each with blood from a different individual.

TABLE III.—HEMOLYSIS FROM DIETHYLENE GLYCOL AND PROPYLENE GLYCOL.

Tube No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Dilution	0	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10
Molar conc.	0.278	0.250	0.222	0.195	0.167	0.139	0.111	0.083	0.056	0.028
Diethylene	xxxx	xxxx	xxxx	xxxx	xxxx	x	0	0	0	0
Propylene	xxxx	xxxx	xxxx	xxxx	xxxx	x	0	0	0	0
Saline	0	0	0	0	0	x	0	0	0	0
Conc. saline	0.9%	0.81%	0.72%	0.63%	0.54%	0.45%	0.36%	0.27%	0.18%	0.09%

xxxx indicates complete hemolysis. 0 indicates no hemolysis.

Fragility of corpuscles was normal in each instance, and there was no significant variation in the effects of the glycols. The line of demarcation between hemolysis and non-hemolysis was very definite. Methemoglobin could not be detected in any of the tubes by means of a small hand-spectroscope.

NOTE: Since the above experiments were completed, Lehman and Newman (7) reported results from somewhat similar experiments. These authors found that if propylene glycol solutions were prepared with a final concentration of 0.9% NaCl, then no hemolysis occurred with glycol concentrations of 30% or less; but that if distilled water were used as a diluent, hemolysis occurred in all dilutions. Thus, 0.9% NaCl seems to have afforded marked protection against hemolysis. The explanation for this apparent protection probably lies in the fact that certain substances penetrate the red cell membrane so rapidly that they exert no appreciable osmotic effect on the cell. Therefore, aqueous solutions of propylene glycol behave essentially as distilled water, and produce rapid and complete hemolysis; whereas, the presence of 0.9% NaCl provides an approximately isotonic medium in the presence of less than 30% glycol. This explanation was offered for the same type of phenomenon observed by Johnson, Carlson and Johnson (8) in connection with the hemolytic action of glycerine. These latter authors also stated that a similar situation exists with respect to aqueous solutions of urea. On the basis of data presented in Table III, it appears that dilution of 0.9% NaCl solution with 0.278 molar solutions of either propylene or diethylene glycol was equivalent to dilution with distilled water in so far as production of hemolysis was concerned. These results are therefore in accord with the observations of Lehman and Newman and Johnson, Carlson and Johnson.

SUMMARY.

1. In rats the acute Average Fatal Dose of propylene glycol was found to be 33.5 Gm. per Kg. orally, 22.5 Gm. subcutaneously, 14 Gm. intramuscularly and 6.8 Gm. intravenously. These figures are in agreement with those reported by other authors, with the exception of the intravenous fatal dose, which is somewhat less than half that reported by other authors. Reasons are given for believing the lower figure to be correct for the intravenous fatal dose.

2. In rabbits the acute Average Fatal Dose on intravenous administration was found to be 6.5 Gm. per Kg.

3. The chronic toxicity of propylene glycol was studied by the administration of the material to growing rats by way of their drinking water. Concentrations of 3% or less caused no appreciable change in rate of growth. A concentration of 10% caused a temporary slowing in rate of growth which lasted for about ten days. Rate of growth after this initial slowing became essentially normal. No significant changes were found on microscopic examination of organs of these animals sacrificed at the end of the experimental period of 100 days.

4. Hematuria was observed following the intravenous administration of sublethal doses of propylene glycol to rats. Hemolysis was produced *in vitro* by both diethylene glycol and propylene glycol in concentrations greater than 0.14 molar. This *in-vitro* action seems to be due to the dilution of isosmotic NaCl solution with the osmotically inactive glycol solution, rather than to a specific hemolytic action of these glycols in the dilutions used.

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LIQUOR ANTISEPTICUS.*

BY H. S. HOWELL,[1] LOUIS GOLDBERG,[1] R. K. SNYDER[1] AND E. N. GATHERCOAL.[1]

BORIC ACID IN LIQUOR ANTISEPTICUS.

The following statement in the N. F. VI monograph on Liquor Antisepticus has been criticized on the basis that the limits on these residues were not always attainable:

"Evaporate 10 cc. of the solution at 100° C.: not more than 0.184 Gm. of a white crystalline residue remains. Add 10 cc. of alcohol to this residue and ignite: the flame is enveloped with a green mantle and not less than 0.042 Gm. of residue remains."

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* National Formulary Research Project F7 at the University of Illinois College of Pharmacy. This is but an abstract of the original paper which is given in full in the National Formulary Bulletin, pages 2682-2689.

[1] National Formulary Laboratory.