# Toxicity of Triethylene Glycol and the Effect of Para-Amino-Benzene-Sulfonamide upon the Toxicity of This Glycol

*By W. M. Lauter and V. L. Vrla**

The recent investigations of Kesten, Mulinos and Pomerantz (1) on the toxicity of diethylene glycol, of Holck (2) on glycerine, ethylene glycol and the work done by Geiling and others (3) while investigating the toxicity of solutions of *p*-amino-benzene-sulfonamide in diethylene glycol solutions made it seem that "the ether linkage of the di-glycols may be the portion of the molecule responsible for the degeneration of epithelial cells of parenchymatous organs, especially of the kidney." (Kesten and others.) It was therefore interesting to determine the toxicity of triethylene glycol ($HO.C_2H_4OC_2H_4OC_2H_4OH$) which has two ether linkages in the molecule. It might be expected that its toxicity would be greater than that of the diethylene glycol with only one ether linkage.

Haag and Ambrose (5) stated that concentrations of 3 and 10 per cent, respectively, of diethylene glycol in the drinking water of rats proved rapidly fatal. Holck (2) states that when rats drank from a 5 per cent solution, the average duration of life was only eight days. This author also states that even in a concentration of 0.25% some indication of impaired growth was detectable. He also states that pregnancy did not occur when males and females were mixed and both received 0.5% of pure diethylene glycol in the drinking water.

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## EXPERIMENTAL

The triethylene glycol used (commercial grade obtained from the Carbide and Carbon Chemicals Corp.) is a colorless liquid, has a specific gravity of 1.125 at 20° C. and boils at 288° C. It is hygroscopic. It is a good solvent for *p*-amino-benzene-sulfonamide. Ten per cent solutions remained clear at low temperatures.

CHART I.—TOXICITY OF TRIETHYLENE GLYCOL ADMINISTERED BY STOMACH TUBE

| Expt. No. | No. of Rats | Cc. Triethylene Glycol per Kg. Body Weight, Daily Dosage | Method of Administration | Time of Treatment, Days | Time of Post-Observation, Days | Weight Increases During Treatment | Weight Increases During Post-Treatment Period | Remarks |
|---|---|---|---|---|---|---|---|---|
| *A* | 5 | 0.1 | As 5% aqueous solution | 30 | 15 | Normal | Normal | Animals looked healthy. All females had normal litters. No deaths |
| *B* | 5 | 3.0 | As 30% aqueous solution | 30 | 15 | Normal | Normal | Animals looked healthy. Females (2) had litters, one of which was small. No deaths |
| *C* | 5 | 10.0 | Undiluted | 30 | 15 | Slow | Slow during first week, but increased in 2nd week | Animals showed definite toxic symptoms, such as loss of hair and diarrhea. One female had a small litter. No deaths |
| *D* | 5 | 20.0 | Undiluted | 30 | 15 | 3 animals died within 24 hours, 2 within 48 hours | | All animals died. Toxic symptoms were severe. Food was refused, the animal lay on its side, respirations increased in rapidity and depth. The fur became ruffled |

Albino rats (100–210 Gm. weight) were used. A standard laboratory feed preparation (Purina Mills) was employed. The triethylene glycol was given by the stomach tube daily for 30 days in succession and the post-treatment observation period lasted 15 days.

Chart I shows that rats will not be killed by a dosage of 10 cc. per Kg. body weight, although the toxic symptoms are pronounced. In contrast to diethylene glycol, rats receiving 3 cc. per Kg. body weight had litters, and even one female of the 5 cc. per Kg. body weight had a litter, although small.

CHART II.—TOXICITY OF TRIETHYLENE GLYCOL IN AQUEOUS SOLUTION

| Expt. No. | No. of Rats | Weight of Rats, Gm. | Concentration in Water | Time of Treatment, Days | Time of Post-Observation, Days | Weight Changes | Remarks |
|---|---|---|---|---|---|---|---|
| *E* | 5 | 140–198 | 5% by vol. = 5.6% by weight | 30 | 15 | No increase in weight in all animals. Those that died refused food and lost weight. The survivors showed increase beginning the 39th day | 1 animal died on the 8th day<br>1 animal died on the 21st day<br>1 animal died on the 28th day<br>2 recovered—all showed severe toxic symptoms |
| *F* | 5 | 130–196 | 10% by vol. = 11.3% by weight | 30 | 15 | Weight decrease noticeable on the 4th day | 2 animals died on the 6th day<br>1 animal died on the 8th day<br>1 animal died on the 9th day<br>1 animal died on the 12th day |
| *G* | 5 | 30– 34 | 3% by vol. = 3.4% by weight | 30 | 15 | The animals showed no signs of toxicity symptoms. They weighed from 115–154 Gm. (average 129 Gm.) on the 31st day | The young rats drank correspondingly far larger amounts of fluids than the adult animals. All animals alive |
| *H* | 5 | 28– 32 | 5% by vol. = 5.6% by weight | 30 | 15 | The rats gained weight very slowly during the first two weeks. Thereafter improvement in behavior and weight. Weight on the 31st day 78–111 Gm. (average 92 Gm.) | Severe toxic symptoms during the first two weeks. Improvement thereafter. 1 animal died on the 15th day |
| *I* | 5 | 29– 34 | Water only. Control | 30 | 15 | Weight on the 31st day, 96–124 Gm. (average 115 Gm.) | All animals alive |

Albino rats were used. Two series were mature rats (130–200 Gm.) and 3 series were 3-week-old rats (28–34 Gm.). A standard laboratory feed preparation (Purina Mills) was employed. The triethylene glycol was given in aqueous solution. The rats were kept on these solutions for 30 days, and for 15 days on water for post-treatment observation.

The rats were kept on a diet of a standard laboratory feed preparation (Purina Mills).

The experiments were divided into four groups:

*A*. Triethylene glycol given by stomach tube daily for 30 days. (Chart I.)

*B*. Triethylene glycol given as aqueous solution in the drinking water for 30 days. (Chart II.)

*C*. Triethylene glycol given as aqueous solution in the drinking water for 30 days to young rats. (Charts II and III.)

*D*. Triethylene glycol solutions of *p*-aminobenzene-sulfonamide in high, but not lethal dosages given daily by stomach tube to adult rats for 30 days. (Chart IV.)

*Group B*.—The animals receiving triethylene glycol in the drinking water (adults drinking approximately 30–40 cc. per day) demonstrated that it is apparently immaterial whether this glycol is given undiluted by stomach tube, or whether it is taken with the daily drinking water.

*Group C*.—The results obtained with this group of animals induced us to see whether the toxicity for aqueous triethylene glycol solution for adult rats which appears to be around 4% would be lower in young animals.

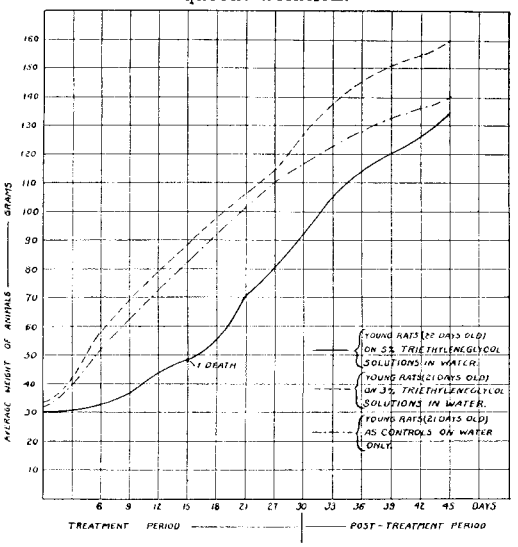
We used three groups of young rats, 21 and 22 days old. It was shown (Charts II and III) that young rats will barely tolerate a 5% concentration, despite the fact that 5 animals drank daily from 120 cc. in the beginning to about 210 cc. after the 22nd day. That would mean from 24–42 cc. daily. The fact that the 3% series progressed splendidly demonstrates again the lower toxicity of triethylene glycol compared with diethylene glycol.

On the basis of previous reports (see above) it seems that triethylene glycol is decidedly less toxic when taken orally than diethylene glycol.

According to Holck, 2.92% solutions of commercial ethylene glycol killed rats usually within 4 days, while propylene glycol in 3.58% solutions was tolerated, but toxic symptoms were observed.

The findings of Geiling and others have shown that diethylene glycol solutions of *p*-amino-benzene-sulfonamide seemed to have no particular effect.

Chart III.—Toxicity of Triethylene Glycol in Aqueous Solution.




We also found this to be the case when this sulfanilamide was dissolved in triethylene glycol.

Chart IV shows that rats tolerated 10% solutions of sulfanilamide in this solvent when they received such large dosages as 1% of their weight daily for 30 days of this solution by stomach tube.

The toxicity of other glycols when given as intramuscular single injections has been described by several authors. We found (see Chart V) that these injections caused considerable local irritation. The lethal dose appears to be approximately 7.5 cc. per Kg. body weight (about 8.4 Gm./Kg. body weight). This places triethylene glycol between propylene glycol and ethylene glycol. Hanzlick (4) shows that the M. L. D. for ethylene glycol is 4.4 Gm./Kg. body weight when given intramuscularly to white rats, while Haag and Ambrose (5) showed that the M. L. D. for diethylene glycol is 7 cc. (7.8 Gm.) per Kg. body weight when injected into the muscles of albino rats. Propylene glycol, however, has a toxicity of 14.7 Gm. per Kg. body weight for intramuscular injections.

## CONCLUSIONS

Triethylene glycol is less toxic than diethylene glycol when fed to rats. It is, therefore, doubtful that the ether linkage in the higher glycols is responsible for the increase in toxicity reported previously.

The lethal dosage when given by stomach tube undiluted is between 10 and 15 cc. triethylene glycol per Kg. body weight. Ten cubic centimeters per Kg. body weight show definite toxic symptoms and retarded weight increase.

Water solutions do not seem to be more toxic than when the glycol is given undiluted. Adult rats are killed by 5% solutions while young rats barely survive this concentration. Young rats thrive well on 3% solutions and the increase in weight is absolutely normal. If one considers that as low as 0.25% of diethylene glycol showed impairment of growth in rats, it seems that further investigations of triethylene glycol and glycols of higher molecular weight might be worth while.

The M. L. D. for intramuscular injections for white rats is approximately 8.4 Gm. per Kg. body weight.

CHART IV.—EFFECT OF *p*-AMINO-BENZENE-SULFONAMIDE ON THE TOXICITY OF TRIETHYLENE GLYCOL

| Expt. No. | No. of Rats | Weight before Treatment, Gm. | Average Weight before Treatment, Gm. | Average Weight after Treatment, Gm. | Time of Treatment, Days | Time of Post-Treatment Obs., Days | Gm. Sulfanilamide per Kg. Body Weight | Cc. Triethylene Glycol per Kg. Body Weight | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| *K* | 5 | 138–144 | 161 | 189 (30 days)<br>230 (45 days) | 30 | 15 | 1 | 10 | No animal died |
| *L* | 5 | 124–134 | 120 | 122 (30 days)<br>158 (45 days) | 30 | 15 | 0.75 | 7.5 | No animal died |

A solution of *p*-amino-benzene-sulfonamide in triethylene glycol was given to adult rats by means of a stomach tube One cubic centimeter of this solution contained 0.1 Gm. of the sulfonamide.
Both series of course showed decided sulfanilamide toxicity symptoms, but appeared normal on the 45th day.

CHART V.—TOXICITY OF TRIETHYLENE GLYCOL ADMINISTERED INTRAMUSCULARLY

| Expt. No. | No. of Rats | Dosage in Cc. per Kg. Body Weight | Animals Died | Remarks |
|---|---|---|---|---|
| *M* | 5 | 5.0 cc. = 5.6 Gm. | None | Toxicity symptoms are slight. Animals appeared normal after 48 hours |
| *N* | 5 | 7.5 cc. = 8.4 Gm. | 3 died | Animals died within 36 hours. 2 survivors recovered in 3 days |
| *O* | 5 | 10.0 cc. = 11.3 Gm. | 5 died | Death occurred within 16–36 hours |

Triethylene glycol was given intramuscularly into the leg muscles of albino rats (120–145 Gm. weight).

REFERENCES

(1) Kesten, Mulinos and Pomerantz, *J. A. M. A.*, 109, 1509 (1937).

(2) H. G. O. Holck, *Ibid.*, 109, 1517 (1937).

(3) Geiling, Coon and Schoeffel, *Ibid.*, 109, 1532 (1937).

(4) Hanzlick, Seidenfeld and Johnson, *J. Pharmacol. and Exper. Therap.*, 41, 387 (1931).

(5) Haag and Ambrose, *Ibid.*, 59, 93 (1937).

# A Preliminary Study of the Anthelmintic Activity *in Vitro* of Fresh Pineapple Juice*

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## INTRODUCTION

Recently Berger and the author (1) published a short note pointing to the anthelmintic activity exhibited *in vitro* by fresh pineapple juice.

The purpose of the present paper is to report some further experiments, as well as to summarize the literature related to bromelin, the proteolytic enzyme probably responsible for the digestive activity of the juice.

The existence of a proteolytic enzyme in pineapple juice was discovered by the Venezuelan chemist Marcano (2) in 1891. The name bromelin, derived from *Bromeliaceæ*, the family to which pineapple belongs, was given to this enzyme. Soon afterward a concern in Detroit, Michigan,[1] patented the use of pineapple juice for the preparation of pre-digested foods (3), (4), (5), (6).

Later in that same year (1891) Chittenden (7) learned of Marcano's discovery and undertook a series of experiments with pineapple juice. He showed that the active enzyme is concentrated in the protein precipitate that can be separated by saturation of the juice with ammonium sulfate, sodium chloride or magnesium sulfate. In a second paper (8) published in 1893, particular attention was paid to the nature of the isolated enzyme as well as to its action on different proteins. He reports that inactivation of the enzyme takes place at 60–70° C.

Vines (9) observed that peptolysis as well as fibrin digestion by pineapple juice occurred both in acid and alkaline media.

Caldwell (10) studied the effect of metallic salts upon the action of bromelin, finding that silver salts were most poisonous. He also suggested the presence of two proteolytic enzymes in bromelin.

Willstätter, Grassmann and Ambros (11) showed that bromelin is activated by HCN as well as by $H_2S$. They report that the optimum cleavage of gelatin takes place at $p_H$ 4.5–5. In the case of albumin peptone the $p_H$ is 5. They describe in detail a method for obtaining very active bromelin preparations.

Ambros and Harteneck (12) claim that the juice from the green fruit is inert toward peptone. According to them the ripe fruit alone yields the activated enzyme. It is their theory that during the process of maturation the natural activator present in the cell juice from the interior of the fruit increases considerably. The inactive enzyme is present in the outer layers of the fruit; the combination of these two principles on crushing the tissue yields then the active bromelin. On the other hand Tanaka (13) found that the largest yields of crude bromelin are obtained from unripe fruits. According to this investigator the proteolytic activity of the juice decreases with increased maturity.

Maschmann (14) has recently studied the effect of different activators on plant proteases including bromelin.

Bergmann and co-workers (15) after experimenting with different synthetic substrates concluded that bromelin contains two enzymes which they have designated as Bromelin I and Bromelin II. These same investigators have effected synthesis using bromelin as promoter (16).

There are records in the literature of the use of pineapple juice as an anthelmintic by the native population of Brazil (17) and India (18). However, up to the present

* Presented before the Scientific Section, A. PH. A., Atlanta meeting, 1939.

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[1] Mosquera-Julia Food Co.