Place the prepared chalk in a mortar and triturate until free from lumps. Add bentonite magma in small portions, triturating thoroughly after each addition until a uniform mixture results. Then add the peppermint water in which the soluble saccharin has been dissolved; transfer this to a graduated vessel and rinse the mortar with enough distilled water to make the product measure 100 cc. Mix thoroughly.

This product is entirely palatable and yields satisfactory preparations when combined in prescription form with elixir of phenobarbital, elixir of Nembutal, compound elixir of pepsin, elixir of pepsin and rennin, Liquid Takadiastase, Caripeptic Liquid, camphorated tincture of opium, milk of magnesia, milk of bismuth, aluminum hydroxide gel, bismuth subcarbonate, bismuth subgallate, bismuth subnitrate, Kaomagma, Kaopectate and tincture of belladonna. Addition of tincture of opium causes excessive thickening.

SUMMARY AND CONCLUSIONS

1. Chalk mixtures containing prepared chalk, soluble saccharin, cinnamon water, distilled water and variable amounts of bentonite were prepared. The bentonite seems superior to the more expensive acacia as a suspending agent for this purpose; concentrations between 0.9% and 2.4% are most suitable. Elimination of fermentable materials (sucrose and acacia) produced a biologically stable preparation, but some samples after long standing developed odors resembling toluene.

2. The toluene-like odor is not produced when cinnamon water is replaced by peppermint water.

3. An improved formula for chalk mixture containing prepared chalk, bentonite (magma), soluble saccharin, peppermint water and distilled water is suggested. This product is palatable and is compatible with many of the drug preparations that are prescribed with chalk mixture.

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The Toxicity of Benzoic Acid for White Rats*

By George P. Hager,† C. W. Chapman and E. B. Starkey

Benzoic acid is widely used in the preservation of food products (1) and pharmaceutical preparations (2), especially those with an acid reaction. Furthermore, because of its antiseptic action, it is used in ointments and dusting powders for the treatment of skin infections, in a 1% solution as a mouth wash (3), in a 0.5% solution for the treatment of chronic suppurating wounds (4) and in preparations for the treatment of tuberculosis, asthma and rheumatism (1).

In view of these many varied uses of benzoic acid, much interest attaches to its toxicity. It has been found that a rather broad margin of safety attends its use in these instances—0.5 Gm. daily produced no demonstrable effects in healthy persons and even 4 Gm. was not injurious (5, 6). Amounts far in excess of the quantities likely to be consumed with food are required to produce ill effects or cause the death of human beings or experimental animals. However, benzoic acid, one of the least harmful of food preservatives, should be avoided by persons with gastro-intestinal or renal diseases (7). Many attempts have been made to prepare compounds related to benzoic acid with even greater margins of safety in the preservation of food. For the purpose of comparing the effect of benzoic acid with that of certain of its substitution derivatives, a preliminary study of the acute toxicity of these compounds for white rats was undertaken.

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EXPERIMENTAL

A portion of the results of this work is the subject of this report. For the comparison of the value of the LD_{50} for white rats found in this investigation with the statements on the toxicity of this substance for human beings and various experimental animals reported in the literature, Table I was drawn up. These other results do not include a measure of the standard deviation and, therefore, the significance of the differences of the reported values cannot be calculated.

Table I.—Summary of the Investigations of the Acute Toxicity of Benzoic Acid

Subject	Dose	Effect
Dogs, cats, rabbits[a]	2 Gm./Kg.	Lethal dose
Rabbits (dry-fed)[a]	1.7 Gm./Kg.	Lethal dose
Rabbits (fasting)[a]	1.52 to 1.83 Gm./Kg.	Lethal dose
Guinea pigs (intraperitoneal)[a]	1.4 Gm./Kg.	Lethal dose
White rats (intravenous)	1.714 ± 0.037 Gm./Kg.	LD_{50}
Human[b]	1 to 1.5 Gm. (capsule)	Gastric pain, nausea and vomiting

[a] See reference (8).
[b] See reference (9).

The method of determining the acute LD_{50} reported here for white rats consisted in injection of an aqueous solution of sodium benzoate of appropriate concentration into the saphenous vein at a rate of 1 cc. per min. Five doses were employed in each test, the first of which was likely to kill none of the animals injected and the last of which was likely to kill all of the animals injected. The doses differed by a constant ratio, the logarithm of which was 0.07; and varied from 1.2 to 2.29 Gm./Kg. Each dose was administered to a group of five rats weighing from 90 to 150 Gm. The solutions employed were of such concentration (12% to 23%) that the dose, based on the weight of the rat, would be contained in about 1 cc. The solutions were prepared by dissolving the highly purified acid in a solution of an equivalent weight of sodium carbonate. Results were recorded hourly for 5 to 6 hrs. to ascertain rate of onset of action, symptoms, etc., and the LD_{50} calculated from the number of animals dead in 24 hrs.

The symptoms of benzoic acid poisoning observed during the course of these experiments were those resulting from a heightening of the reflexes of the central nervous system, and consisted of tremors, clonic and often tetanic convulsions, with death occurring during a remission. These symptoms appeared within a few minutes after the injection and progressed rapidly with deaths often occurring one-half to one hour after injection. Surviving animals generally showed none of the nervous manifestations, but salivation, vomiting and diarrhea were present. A marked diuresis was seen in some cases.

For the calculation of the LD_{50} from these experiments, the use of the maximum likelihood method for the statistical treatment of biological results would be very cumbersome and would likely fail to give values much different from those obtained by a less exacting method. Therefore, the LD_{50}'s were calculated by Kärber's method (10); and the standard deviation of the individual determinations was obtained after first fitting the observed mortalities to a straight line by a method developed by Irwin and Cheeseman (11). The weighted mean of the values thus obtained in three separate determinations was calculated. The standard error of this mean was then obtained in the usual manner. In a different set of experiments carried out in the same way and on the same days, the toxicity of a fluorine-substituted benzoic acid was studied. The results of these experiments are summarized in Table II.

TABLE II

Benzoic Acid	*p*-Fluorobenzoic Acid
LD_{50} ± *S. D.*, Gm./Kg. (P = 0.66)	
1.686 ± 0.105	1.982 ± 0.137
1.855 ± 0.076	1.687 ± 0.109
1.630 ± 0.062	1.434 ± 0.055
Weighted Mean (LD_{50} ± *S. E.*), Gm./Kg.	
1.714 ± 0.062	1.542 ± 0.107
(P = 0.66)	(P = 0.66)
1.714 ± 0.124	1.542 ± 0.214
(P = 0.95)	(P = 0.95)

In experiments of this type in which a small number of animals and a large number of doses are employed, it has been shown that the values obtained by the maximum likelihood method of calculation and by the method outlined above are very close (11).

By plotting the percentage mortalities observed in an individual determination, as ordinates, against the corresponding doses expressed as a logarithm of the ratio of the doses to the LD_{50} calculated from the experiment, the results of the three experiments can be combined in a single curve, the regression line. This can be conveniently done by plotting the percentage mortalities against the ratio on logarithmic probability paper (Fig. 1). Seventy-five animals were employed in the construction of this curve, and its steepness indicates a great uniformity in the response of white rats to benzoic acid.

The significance of the differences between the individual determinations and between the mean values was calculated by the formula

$$\frac{m_1 - m_2}{\sqrt{(S.\,E._1)^2 + (S.\,E._2)^2}}$$

where m is an individual LD_{50} or a weighted mean LD_{50} and $S.\,E.$ is the standard deviation of the corresponding individual LD_{50} or the standard error of the corresponding weighted mean LD_{50}. When the value of this quotient is 2 or greater, the difference between m_1 and m_2 is generally considered significant. That is to say that when the value is 2 or greater, the

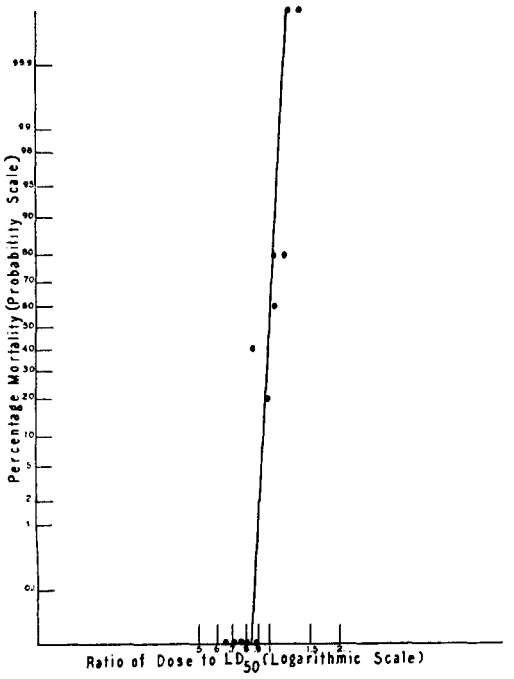

Figure 1.—Regression Line for the Action of Benzoic Acid on White Rats.

observed difference could occur through chance or through an error in sampling only once in twenty times; and the chances are twenty to one that a greater toxicity is to be assigned to the substance whose LD_{50} is m_1 than to that whose LD_{50} is m_2. The application of this formula showed that no significant difference exists between the three values of the LD_{50} determined for benzoic acid. Likewise was the difference between the LD_{50} found for benzoic acid and that found for *p*-fluorobenzoic acid shown to be insignificant. On this basis, one is justified only in saying that benzoic acid and *p*-fluorobenzoic acid were found in these experiments to be of the same toxicity even though the absolute value of the weighted mean LD_{50} of benzoic acid is greater than that of *p*-fluorobenzoic acid. The use of statistical methods is therefore seen to be of great value in the study of the relation of chemical structure and physiological activity from a quantitative standpoint.

SUMMARY

The toxicity of benzoic acid when administered as the sodium salt intravenously to white rats corresponds closely to that reported for other animals. The value of the LD_{50} found in this investigation is 1.714 ± 0.124 Gm./Kg. ($P = 0.95$).

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