The Acute Oral Toxicity in Rats of Several Diet–Arsenic Trioxide Mixtures

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The effect that diet may have upon the acute oral toxicity of arsenic trioxide, when this compound is incorporated dry into diets of protein, fat, or carbohydrate, has been evaluated. When fed dry, as a mixture with protein or carbohydrate, the LD_{50} of arsenic trioxide is approximately 150 mg. per kg. of body weight. However, when it is fed in a fat diet, the toxicity is greatly reduced, LD_{50} approximately 230 mg. per kg.

Arsenic trioxide or "white arsenic" as it is commonly called has been employed as an insecticide and rodenticide for more than 200 years. In recent times baits containing arsenic have been used by farmers in the United States in an attempt to control and eventually eliminate rodent infestations from agricultural areas. Because of the extensive use of arsenic trioxide as a rodenticide and insecticide, and because other commercial arsenical agents used for these purposes are expressed in equivalents of white arsenic, it is essential to have complete knowledge of the toxicity of arsenic trioxide by itself as well as in combination with various diet mixtures. Insect and rodent baits are often composed of food material in addition to the toxic ingredient and such information may be of value in formulating more efficient arsenic-containing bait products.

The literature is replete with acute toxicity data (LD_{50}) for arsenic trioxide or white arsenic with values which range from 8 to 500 mg. per kg. (*1*, *2*). These variations have been explained by many investigators on the basis of species or strain difference of the test animal (*2*, *3*), solubility of the arsenical (*4*), particle size of the sample (*3*), and trace impurities present in the compound (*2*).

The physical and chemical composition as well as the acute oral toxicity of commercial arsenic trioxide (97.8%) and pure arsenic trioxide (99.999%) in several strains of mice and in rats have been compared (*2*). A comparison was also made of the acute oral toxicity of an aqueous solution of pure arsenic trioxide and of dry arsenic trioxide in a standard rat diet mixture: protein 21%, carbohydrate 50 to 60%, and fat 8%.

The striking variation in toxicity between the aqueous solution of pure arsenic trioxide and the dry diet mixture of pure arsenic trioxide led to the investigation of the acute oral toxicity of various diet–arsenic trioxide mixtures.

The present report is concerned with results of a determination of the acute oral toxicity, in rats, of mixtures of pure arsenic trioxide (99.999%) with diets composed almost entirely of either protein, carbohydrate, or fat.

Methods

Male, Sprague Dawley albino rats weighing between 125 and 175 grams were employed throughout the study. Food was removed from the animals 24 hours prior to each test and water was supplied *ad libitum*. The arsenic trioxide, incorporated in 3 grams of the individual diet, was then placed in the feeding cup and offered to the fasted animals. The test animals consumed the "experimental diet" containing the arsenic within 1 hour. For the remainder of the day (24 hours), the animals received a diet of either carbohydrate, protein, or fat depending upon the experimental diet they had received with the test dose. Observations were made over a 2-week period and autopsies were performed on animals that died.

Constituents, particle size, and distribution of pure arsenic trioxide are presented in Table I.

Experimental diets were prepared by carefully triturating individual doses of the pure arsenic trioxide with 3 grams of each of the following: sucrose-starch mixture (equal parts), powdered egg albumin, and strained bacon fat.

Acute oral toxicity data are expressed

Table I. Constituents, Particle Size, and Distribution of Pure Arsenic Trioxide

Chemical Constituents		Particle Size and Distribution	
As_2O_3	Constituents	μ	%
Sb	0.0001%	589	0
Cu	0.00001%	246–589	10.1
		177–246	11.6
		149–177	7.3
		74–149	17.4
		44–74	17.4
		44	36.2

Table II. Acute Oral Toxicity in Rats of Pure Arsenic Trioxide–Sucrose–Starch Mixture

Dose, Mg. As/Kg.	Number Deaths / Number Animals: 24 hours	48 hours	96 hours	14 days
25	0/5	0/5	0/5	0/5
50	0/5	0/5	0/5	0/5
100	1/10	2/10	2/10	2/10
150	3/10	5/10	5/10	5/10
200	4/10	7/10	7/10	7/10
250	9/10	9/10	9/10	9/10

LD_{50}, 48 hours = 154 mg. ± 19 mg. As/kg.

Table III. Acute Oral Toxicity in Rats of Pure Arsenic Trioxide

Dose, Mg. As/Kg.	Number Deaths / Number Animals: 24 hours	48 hours	96 hours	14 days
Egg Albumin Mixture[a]				
25	0/5	0/5	0/5	0/5
50	0/10	1/10	1/10	1/10
100	3/10	3/10	3/10	3/10
150	5/10	6/10	6/10	6/10
200	8/10	8/10	8/10	8/10
250	5/5	5/5	5/5	5/5
Bacon Fat Mixture[b]				
25	0/5	0/5	0/5	0/5
50	0/5	0/5	0/5	0/5
100	0/10	0/10	0/10	0/10
150	2/10	2/10	2/10	2/10
200	2/10	4/10	4/10	4/10
250	5/10	7/10	7/10	7/10
300	9/10	9/10	9/10	9/10

[a] LD_{50}, 48 hours = 133 mg. ± 16 mg. As/kg.
[b] LD_{50}, 48 hours = 225 mg. ± 12 mg. As/kg.

Table IV. Acute Oral Toxicity in Rats of Several Diet–Pure Arsenic Trioxide Mixtures

As_2O_3 Dry Diet Mixture	LD_{50}, Mg. As/Kg.
Standard rat diet	
Protein 21%	
Fat 8%	145
Carbohydrate 50–60%	
Protein	
Dried egg albumin	133
Carbohydrate	
Sucrose-starch, equal parts	154
Fat	
Strained bacon fat	225
As_2O_3 solution	
(distilled water)	15

[a] 48 hours.

as milligrams of elemental arsenic per kilogram of body weight.

Results

The acute oral toxicity, expressed as LD_{50}, of pure arsenic trioxide incorporated in a diet of carbohydrate or protein, was calculated to be 135 to 150 mg. of elemental arsenic per kilogram of body weight (Tables II and III). When arsenic trioxide was administered in a diet of bacon fat, the acute oral toxicity was reduced to 225 mg. of elemental arsenic per kg. of body weight (Table III). Calculations were based on the deaths observed up to 48 hours.

Upon autopsy there was no evidence of gastrointestinal hemorrhage, although some slight mucosal irritation was noted in all animals. This confirms previous observations (*3*).

Comparative acute oral toxicity data summarizing the effect of diet–arsenic trioxide mixtures are presented in Table IV.

Discussion

Incorporation of arsenic trioxide in diets of protein or carbohydrate during this investigation has not significantly altered the acute oral toxicity from the previously observed value. However, when pure arsenic trixoide was fed to rats in a bacon-fat mixture, the acute oral toxicity was markedly reduced from the 145 mg. of arsenic per kg. observed in the standard diet mixture, to 225 mg. of elemental arsenic per kg. of body weight.

One may assume that the feeding of an arsenic-fat mixture reduces the toxicity by coating the intestinal mucosa, thus decreasing absorption of the arsenic or by physically coating or chemically binding the arsenic compound, decreasing its availability for absorption. Therefore, the coadminstration of fat with arsenic trioxide decreases the acute oral toxicity of arsenic.

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PHOSPHATE SOLUBILITY

Evaluation of Water-Insoluble Phosphorus in Fertilizers by Extraction with Alkaline Ammonium Citrate Solutions

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Water-insoluble phosphorus in fertilizers is present mainly as dicalcium phosphate and hydroxyapatite. Agronomic tests show that dicalcium phosphate is an effective phosphorus fertilizer, but hydroxyapatite is much less effective. Official methods of analysis do not distinguish between these two compounds. An alkaline ammonium citrate extraction method was tested, whereby amounts of phosphorus present as dicalcium phosphate and hydroxyapatite can be estimated. Heavily ammoniated superphosphates contained a high proportion of hydroxyapatite; nitric phosphates contained predominantly dicalcium phosphate. This method should be useful in assessing the value of water-insoluble phosphorus in fertilizers.

In agronomic tests to evaluate the effect of water solubility of phosphorus in fertilizers, the quality of the water-insoluble phosphorus is often an unknown and uncontrolled variable. This circumstance may give rise to anomalies in agronomic data and may cause different investigators to reach divergent conclusions. The aim of the present work was to provide a convenient method for roughly quantitative characterization of water-insoluble phosphorus in fertilizers.

About 80% of the fertilizer phosphorus used in the United States is in the form of mixed fertilizer. The great majority of the mixed fertilizers are manufactured by processes that involve ammoniation of superphosphate. Ammoniation decreases the water solubility of phosphorus by converting the monocalcium phosphate into ammonium phosphates, dicalcium phosphate, and more basic calcium phosphates. Also, ground limestone or dolomite often is incorporated in mixed fertilizer or added to superphosphates; these materials also decrease the water solubility of the phosphorus.

As a result of these practices, less than half of the available phosphorus in superphosphates and mixed fertilizers was water soluble in 1955–56 (*4*). The water solubility of phosphorus in individual samples ranged from 1 to 98%.

The principal water-insoluble phosphorus compounds that are formed by reaction of superphosphate with ammonia, limestone, or dolomite are di-