EVALUATION OF THE HEALTH ASPECTS OF ACONITIC ACID
AS A FOOD INGREDIENT

October, 1974

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 72-85
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
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NOTICE

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) food substances being made by the Federation of American Societies for Experimental Biology (FASEB) under contract with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

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Life Sciences Research Office
FASEB
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I. INTRODUCTION

Under terms of FDA Contract 72-85, FASEB's Life Sciences Research Office was requested to evaluate the health aspects of using aconitic acid as a food ingredient. The evaluation has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1970.* To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally recognized as available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of September 23, 1974 (39 FR 34218) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using aconitic acid as a food ingredient. The Select Committee received no requests for such a hearing on aconitic acid.

Aconitic acid is a food substance that has been generally recognized as safe (GRAS) under the provisions of the Code of Federal Regulations (21 CFR 121.101, revised April 1, 1974). As indicated in the Food Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarking clearance that is required for food additives. It is stated in 21 CFR 121.1 that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. This section of the Code also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA recognizes further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconduted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on aconitic acid and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.

II. BACKGROUND INFORMATION

Aconitic acid, 1,2,3-propenetricarboxylic acid, occurs in the leaves and tubers of Aconitum napellus L. and other Ranunculaceae. It is also found in yarrow (Achillea sp.), and horsetails (Equisetum sp.) as well as in other plants such as beets and sugar cane. Depending on the natural source, aconitic acid is also called achilleic acid, citridic acid, and equisetic acid (1).

Aconitic acid of the \textit{trans} configuration can be isolated during the processing of sugar cane, by precipitating it as the calcium salt from the cane syrup or molasses. The concentration in molasses ranges from 1.8 to 2.5 percent (2). Aconitic acid may be synthesized from citric acid by dehydration with sulfuric acid, or by catalytic dehydration. The \textit{cis} configuration is somewhat unstable and is readily rearranged to the \textit{trans} form by heating. \textit{Trans}-aconitic acid is decarboxylated to itaconic acid by heating to 180. The \textit{cis} form occurs in plant and animal tissues as a metabolic intermediate in the Krebs cycle during the isomerization of citric acid to isocitric acid by the action of the enzyme aconitase (1).

Aconitic acid is used as a flavoring and flavor adjuvant in the foods shown in Table I.
Table I

Content of Aconitic Acid in Foods (mg per kg)

<table>
<thead>
<tr>
<th>Food Category</th>
<th>NAS/NRC Report (3)</th>
<th>FEMA Report (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Usual</td>
<td>Maximum</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>15.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Baked goods, baking mixes</td>
<td>8.9</td>
<td>25.2</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>5.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Nonalcoholic beverages</td>
<td>1.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Soft candy</td>
<td>22.4</td>
<td>31.2</td>
</tr>
</tbody>
</table>

*All candy

These data were derived from a NRC subcommittee report (3) providing 1970 data, and a report from the Flavoring Extract Manufacturers' Association (4) containing data collected from 1958 to 1964. These figures suggest that use of aconitic acid in non-alcoholic beverages, baked goods and frozen dairy products may have increased from 1964 to 1970. However, the Select Committee has no information to indicate whether or not actual usage of aconitic acid in the food categories listed in Table I has changed significantly in recent years.

III. CONSUMER EXPOSURE DATA

The National Research Council subcommittee survey (3) has supplied information on the possible daily human intakes of aconitic acid added to the total diet for individuals in various age groups (Table II). The Select Committee has converted these figures to possible intakes per kilogram of body weight.
Table II

Possible Daily Intake of Aconitic Acid

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total intake, mg</th>
<th></th>
<th>Intake, mg per kg body weight*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Maximum</td>
<td>Average</td>
<td>Maximum</td>
</tr>
<tr>
<td>0-5 mos.</td>
<td>0.04</td>
<td>0.11</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>0.37</td>
<td>0.89</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>12-23 mos.</td>
<td>0.73</td>
<td>1.79</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>2-65+ yrs.</td>
<td>2.16</td>
<td>4.86</td>
<td>0.04</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Calculations based on an average weight of 60 kg for an adult (5) and the following estimated weights of infants by age groups: 0-5 mos., 5 kg; 6-11 mos., 8 kg; and 12-23 mos., 11 kg (6).

These figures should also be considered in respect to total quantity of aconitic acid used in foods. The NRC subcommittee has pointed out that its calculations of intakes in most cases are overstated, often by considerable margins.** That this is probably true in the case of aconitic acid is supported by the following calculation: The subcommittee has provided data indicating that the use of aconitic acid for food purposes in the United States was 62.3 kg in 1970 (3). This figure is reported to comprise between 60 and 70 percent of the total poundage used in food. On the basis of 60 percent adjusted to 100 percent (103.8 kg), and a U.S. population of 210 million, the per capita per day average intake would be 0.00135 mg of aconitic acid rather than the adult average daily intake of 2.16 mg given in Table II.

**An explanation for such overstatements is detailed in Section XI "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (3). The Select Committee finds this explanation reasonable, and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey on the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
On the basis of the above considerations, therefore, the Select Committee believes that the NRC subcommittee consumption levels are not likely to be achieved by any of the age groups.

IV. BIOLOGICAL STUDIES

The form of aconitic acid used in food is trans-aconitic acid (7); its isomer, cis-aconitic acid, is an intermediate in the tricarboxylic acid cycle of cellular metabolism. The conversion of the trans to the cis form is an endothermic reaction and does not occur spontaneously. While an isomerase for the interconversion has been detected in Pseudomonas sp. (8) and sugar cane (9), the enzyme has not been identified in mammalian tissues.

There are few definitive studies on possible toxicity of trans-aconitic acid. For many years, aconitic acid has been implicated in the ruminant syndrome, grass tetany, because the acid is present in Equisetum arvense, the common horsetail, as well as several grasses and other plants. Grass tetany results in toxic symptoms in cattle, sheep, and horses. However, Camp et al. (10) were not able to produce the syndrome in six pregnant ewes by oral administration of either the free acid or potassium aconitate as a 25 percent solution in water. The dose of potassium salt was 1.0 g per kg body weight per day for 7 days and subsequently increased in increments of 0.5 g per kg body weight for each week until a level of 4.5 g per kg was reached (56 days). In sheep receiving potassium aconitate, there was a decrease in serum magnesium level and an increase in the serum phosphate level. There was a significant decrease in the average serum potassium level of sheep similarly treated with trans-aconitic acid, but no significant changes in serum levels of magnesium or phosphate occurred. Animals killed by lethal doses of either the free acid or salt (4 g per kg) showed non-specific changes in kidneys, liver, heart, and lungs.

In another study, Kennedy (11) fed sheep diets containing 0.1 and 0.2 mole per day (about 387 to 774 mg per kg of body weight) of partially neutralized trans-aconitic acid for five days. Experimental animals appeared healthy and exhibited normal levels of blood citrate, ketones, and aconitate but increased urinary citrate. Kennedy also injected 1.0 millimole per kg doses of neutralized trans-aconitic, cis-aconitic, and cis-citric acid into the jugular vein of sheep. The intravenous injection of 1.0 millimole (192 mg per kg) of cis-citric acid was lethal to one of the five experimental
animals. No adverse reaction followed the intravenous injection of 1.0 millimole (174 mg per kg) of trans-aconitic acid. It rapidly disappeared from the serum; 40 percent of the injected material was recovered in the urine in 24 hours. Subsequent experiments indicated that injection of 57.6 mg per kg of cis-citric acid (0.3 millimole) over a 20 minute period was "close to the toxic dose", and resulted in excessive urinary calcium and citrate excretion without marked change in urinary magnesium excretion. Metabolic changes or toxic reactions to cis-aconitic acid were not mentioned. In another experiment, Kennedy noted that trans-aconitate was not affected by incubation with rumen fluid in vitro, but it disappeared from the rumen following intra-ruminal administration in vivo.

Metabolic studies with other animals are less extensive. Lomba et al. (12) perfused rabbits intravenously with a number of organic acids. They observed that trans-aconitic acid had approximately one-tenth the toxicity of citric acid and was less active in disturbing electrolyte balance of the blood. Wright and Wolff (13) reported that single oral doses of sodium trans-aconitate slightly reduced the blood serum magnesium levels of guinea pigs dosed at 666 mg per kg, but did not reduce serum magnesium of sheep given 0.29 moles of the salt by stomach tube (equivalent to about 2.1 g per kg). No other effects were observed. In other experiments, guinea pigs fed a diet containing 6.8 percent trans-aconitate did not show any ill effects after 13 days of feeding. Weight gains were comparable to controls. Labeled citrate injected into guinea pigs previously dosed with trans-aconitate (560 mg per kg) did not effect release of radioactive CO₂.

Trans-aconitic acid competitively inhibits the enzyme aconitase in vitro and thus blocks the conversion of citric acid to isocitric acid by way of the intermediary formation of cis-aconitic acid. Wright and Wolff (13) indicate that this effect has not been observed in vivo although increases in citric acid excretion have been observed occasionally.

Sodium trans-aconitate, in vitro, markedly increased the clotting time of blood although the increase was less than that produced by citrate (14).

No reports are available to the Select Committee that contain information on possible carcinogenic, mutagenic, reproductive or teratogenic effects from feeding aconitic acid to animals or man.
V. OPINION

The limited data on trans-aconitic acid indicate it to be less toxic than citric acid. Trans-aconitate salts appear to be excreted readily by the kidneys. There is no direct evidence that trans-aconitic acid is utilized as is the cis-aconitic acid isomer in mammalian metabolism although non-specific oxidation probably occurs.

The Select Committee has weighed all of the foregoing and concludes that:

There is no evidence in the available information on aconitic acid that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in future.
VI. REFERENCES CITED


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Report submitted by:

November 4, 1974

Date

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Select Committee on GRAS Substances