EVALUATION OF THE HEALTH ASPECTS OF CITRIC ACID, SODIUM CITRATE, POTASSIUM CITRATE, CALCIUM CITRATE, AMMONIUM CITRATE, TRIETHYL CITRATE, ISOPROPYL CITRATE, AND STEARYL CITRATE AS FOOD INGREDIENTS

1977

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

Contract No. FDA 223-75-2004
EVALUATION OF THE HEALTH ASPECTS OF CITRIC ACID, SODIUM CITRATE, POTASSIUM CITRATE, CALCIUM CITRATE, AMMONIUM CITRATE, TRIETHYL CITRATE, ISOPROPYL CITRATE, AND STEARYL CITRATE AS FOOD INGREDIENTS

1977

Prepared for

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

Contract No. FDA 223-75-2004

Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 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 to the public 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.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Introduction</td>
</tr>
<tr>
<td>II.</td>
<td>Background information</td>
</tr>
<tr>
<td>III.</td>
<td>Consumer exposure data</td>
</tr>
<tr>
<td>IV.</td>
<td>Biological studies</td>
</tr>
<tr>
<td>V.</td>
<td>Opinion</td>
</tr>
<tr>
<td>VI.</td>
<td>References cited</td>
</tr>
<tr>
<td>VII.</td>
<td>Scientists contributing to this report</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. It has been based partly on the information contained in two scientific literature reviews (monographs) furnished by FDA (1, 2), which summarize the world's scientific literature from 1920 through 1973/4.* 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 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, an announcement was made in the Federal Register of September 2, 1977 (42 FR 44284-44285) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation, or in lieu of an oral presentation, submit a written statement of data, information and views on the health aspects of using citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. The Select Committee received no requests for a public hearing but received one statement on ammonium citrate (dibasic) from Pfizer, Incorporated, New York, New York.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (3) [21 CFR 170.3 and 170.30] 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. These sections of the Code also indicate 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 (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The documents (PB-223 850/9 and PB-241 967/9) are 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 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. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee, is aware that its 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 citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

Citric acid, 2-hydroxy-1, 2, 3, -propanetricarboxylic acid, and its salts are natural constituents and common metabolites of plants and animals. Citric acid is an intermediary compound in the Krebs cycle linking oxidative metabolism of carbohydrate, protein and fat. The concentration of naturally occurring citrate is relatively higher in fruits, particularly citrus fruits and juices, than in vegetables and animal tissues (4-6). Typical concentrations, fresh weight, are about 1 percent in orange juice and up to 8 percent in underripe lemon juice as compared to less than 0.1 percent in peas, corn, and cabbage and about 0.1 percent in human milk.

Citric acid [21 CFR 182.6033 and 182.1033], calcium citrate [21 CFR 182.6195 and 182.1195], potassium citrate [21 CFR 182.6625 and 182.1625], and sodium citrate [21 CFR 182.6751 and 182.1751] are GRAS substances listed in the Code of Federal Regulations (3) as sequestrants and as multiple purpose GRAS food substances; calcium citrate [21 CFR 182.5195] is listed also as a nutrient and/or dietary supplement. Identity standards provide for the addition of citric acid, calcium citrate, potassium citrate and sodium citrate as optional ingredients to certain cheeses [21 CFR 133], ice cream [21 CFR 135], jellies and preserves [21 CFR 150], canned vegetables [21 CFR 155.156], nonalcoholic beverages [21 CFR 165], and dressings [21 CFR 169].

Under provisions of the Code of Federal Regulations (7), citric acid is used to increase the effectiveness of antioxidants in lard or shortening at 0.01 percent; in dry sausage at 0.001 percent; and in fresh pork sausage and dried meats at 0.01 percent. It may be used to protect the flavor of oleomargarine and to flavor chili con carne at levels sufficient for that purpose. Citric acid or sodium citrate may be used as a curing accelerator in combination with a curing agent and as an anticoagulant at 0.2 percent with beef blood. Sodium citrate, potassium citrate and ammonium citrate are prior sanctioned food ingredients that may be used as stabilizers in manufacturing food packaging material [21 CFR 181.29] (3). Such uses do not contribute significantly to total citrate intake from all sources.

Food grade specifications limit citric acid, potassium citrate and sodium citrate to 3 ppm arsenic and 10 ppm heavy metals (as Pb); calcium citrate may contain 3 ppm arsenic and 20 ppm heavy metals (as Pb) but is limited to 10 ppm lead (8, 9). These citrates are water soluble, white or colorless powders or crystalline solids that are most frequently used in foods for pH control and as flavoring agents or flavoring enhancers (10). The metal ion complexing properties of citrates make them useful as sequestrants, antioxidants, and preservatives.
Food grade specifications are not given for all forms of the citrate salts. The form of potassium citrate described in the Food Chemicals Codex (8) is C₆H₅K₃O₇·H₂O; specifications are not given for monopotassium citrate. In the case of sodium citrate, specifications are given for trisodium citrate, C₃H₅Na₃O₇·2H₂O, and not for disodium citrate. For diammonium citrate, C₆H₁₄N₂O₇, no food grade specifications are listed.

The isopropyl, stearyl, and ethyl esters of citric acid are evaluated separately in this report, although these esters share some of the properties of the citrate salts and yield citrate by hydrolysis. The Code of Federal Regulations (3) lists as GRAS: monoisopropyl citrate [21 CFR 182.6511], stearyl citrate [21 CFR 182.651] with a tolerance of 0.15 percent, and isopropyl citrate [21 CFR 182.6386] with a tolerance of 0.02 percent as sequestrants. The Code lists triethyl citrate [21 CFR 182.1911] in dried egg whites with a tolerance of 0.25 percent as a multiple purpose GRAS food substance. Monoisopropyl citrate, mono-, di-, and triestearyl citrate, and triethyl citrate [21 CFR 181.27] may be used as plasticizers in the manufacture of food packaging materials. The standard of identity for margarine and oleomargarine [21 CFR 166.110] permits the use of up to 0.15 percent stearyl citrate, or up to 0.02 percent isopropyl citrate mixture.

Triethyl citrate is odorless, nearly colorless, oily liquid and the food grade material may contain no more than 3 ppm arsenic and 10 ppm heavy metals as lead (8). Extremely low concentrations of triethyl citrate occur naturally in sour cherries and red currants (11). Expressed as a weighted mean, its usual level of addition to certain foods by food category in 1970 was: baked goods, 36 ppm; frozen dairy products, 6 ppm; soft candy, 32 ppm; gelatin and puddings, 7 ppm; nonalcoholic beverages, 12 ppm; alcoholic beverages, 15 ppm; hard candy, 9 ppm; and chewing gum, 502 ppm (10). Maximum average levels of addition are known to be higher in most instances, for example, maximum average level of addition to baked goods was 125 ppm (12).

The composition of commercially available isopropyl citrate is 65 to 80 percent monoisopropyl, 15 to 30 percent diisopropyl, and 5 to 10 percent triisopropyl citrate (13). In 1970, it was added to one category of foods, the category of fats and oils; the weighted mean level of addition of isopropyl citrate ranged from a usual level of 33 ppm to a maximum of 100 ppm, which is half of the permitted level of 200 ppm (10).

Stearyl citrate is also a mixture; it contains 10 to 15, 70 to 80, and 10 to 15 percent, respectively, of the monostearyl, diestearyl, and triestearyl derivatives (13). Its use in food was not reported in a 1970 survey of the food industry (10).

Food grade specifications for isopropyl citrate and stearyl citrate are not given in the Food Chemicals Codex (8).
III. CONSUMER EXPOSURE DATA

A National Research Council (NRC) subcommittee surveyed the 1970 industrial food use of GRAS substances and calculated possible average daily intakes for each GRAS substance resulting from its addition to processed foods (10). This calculation was based on Market Research Corporation of America data on the mean frequency of eating foods by food category, U.S. Department of Agriculture data on mean portion size of foods in these categories and the assumption that all food products within a given category contained the GRAS substance when it was added to any product in that category. The NRC subcommittee suggested these calculated possible intakes often represent considerable overestimates of the actual average daily intakes, and the Select Committee believes this is true for the case of the citrates evaluated in this report.

For the age group 2 to 65+ years, the calculated possible average intakes for citric acid, sodium citrate, potassium citrate, triethyl citrate, calcium citrate, and isopropyl citrate were 3100, 1600, 280, 7.4, 6, and 0.6 mg per day, respectively. The actual average daily intakes are probably nearer to the quantities used by the food industry expressed on a per capita basis as shown in Table I. These per capita estimates are about tenfold smaller and are derived from NRC survey data which represents the poundage used in 1970 by the survey respondents. If the per capita figures are expressed as citrate ion, about 480 mg of citrate was added per capita to foods by industrial processing. Thus, the addition of these compounds to foods represents only a fraction of the daily citrate intake for most individuals, e.g. one 8-ounce glass of orange juice provides about 2 g of citric acid.

For adults, citric acid and sodium citrate are the major sources of added citrate. They were added to at least one food product in nearly all of the food categories used in the NRC survey. The level of addition was usually below 0.5 percent, expressed as a weighted mean (10). Potassium citrate was added at similar levels but was used in fewer food categories. Calcium citrate was added to foods in only two categories; it was used as a firming agent in gelatins, puddings, and fillings, and as a nutrient supplement in baby formulas.

Ammonium citrate was not included in the list of GRAS substances utilized by the National Research Council in their 1970 survey of industry. However, one or more of the respondents to the survey indicated that the substance was added to foods (10). The Select Committee has been informed that ammonium citrate is used in the media for cheese cultures where it functions as a buffer and fermentation aid resulting in a usual level in cheese and whey of 0.00295 percent with a corresponding maximum level of
# TABLE I

## Quantity of Citrates Added Annually to Foods and Per Capita Daily Intake Calculated Therefrom (10)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative quantities added(^a)</th>
<th>Total quantity added (^b)</th>
<th>Per capita daily &quot;intake&quot; (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1970/1960</td>
<td>1970 (^b)</td>
<td>kg</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1</td>
<td>27,000,000</td>
<td>360</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>6</td>
<td>12,000,000</td>
<td>160</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>5</td>
<td>440,000</td>
<td>5.9</td>
</tr>
<tr>
<td>Isopropyl citrate</td>
<td>1</td>
<td>30,000</td>
<td>0.4</td>
</tr>
<tr>
<td>Calcium citrate</td>
<td>4</td>
<td>14,000</td>
<td>0.2</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>1</td>
<td>8,000</td>
<td>0.1</td>
</tr>
</tbody>
</table>

\(^a\) Based only on the reports of those respondents to the National Research Council (NRC) survey submitting information for both 1960 and 1970.

\(^b\) Recalculated to 100 percent from data estimated to represent 60 percent of actual usage.

\(^c\) Based on a U.S. population of 205 million.

0.00516 percent (14). These levels of use appear consistent with levels reported for ammonium salts previously reviewed by the Select Committee (15).

The NRC subcommittee's calculated possible average daily intakes of added citrates for the 0 to 5 month age group are for citric acid, 610 mg; potassium citrate, 560 mg; and calcium citrate, 330 mg. The relative contribution of these compounds to the intake of added citrates differs from that of the 2 to 65+ year age group in part from the formulation of certain baby formulas with potassium and calcium citrate. Citric acid is used in many categories of baby foods at a weighted mean level of addition below 0.5 percent. There was no reported addition of the isopropyl, stearyl and ethyl esters of citric acid to baby foods.

Consumer exposure to the citrate esters appears to be small, based on 1970 usage data. Stearyl citrate was included in the NRC survey and no addition of this compound to foods was reported. The calculated possible average daily intake of isopropyl citrate for persons over age 2 years was about 0.6 mg and the per capita industrial usage was about 0.4 mg per day. Fewer than four industrial firms reported adding isopropyl citrate to any product in the category of fats and oils. Triethyl citrate was used in a greater variety of foods than isopropyl citrate but the per capita industrial usage was less, 0.1 mg daily.
IV. BIOLOGICAL STUDIES

This report emphasizes biological studies in which citric acid, citrates, and citrate esters were given orally. Results obtained by parenteral administration of these compounds such as the use of citrate as an anticoagulant are not relevant to an evaluation of the safety of citrates and citric acid as food ingredients. The major physiological effects from large amounts of citric acid taken orally are related to its strong carboxylic acid nature and to its ion chelating properties, particularly in binding calcium ions.

A. Citric acid and its sodium, potassium, calcium and ammonium salts

Absorption and metabolism

The biochemical reactions involved in the biosynthesis and metabolism of citric acid are well established because of its involvement in the Krebs cycle (6). The human body contains about 80 g of citrate, most of this as a component of bone. Whole blood citrate concentration is about 2 mg per dl, and 0.2 to 1.0 g of citrate is excreted daily in urine (16,17). Orally administered citric acid is well absorbed and largely metabolized. Exogenous as well as endogenous citric acid can be completely metabolized and serve as a source of energy, furnishing 2.47 kcal per g. Infants demonstrate efficient metabolism of citric acid and the kidney tubules reabsorb most (about 90 percent) of the filtered load of citric acid (18,19).

Acute toxicity

The acute oral LD$_{50}$ of citric acid (produced by Candida sp fermentation of normal paraffin) in mice was about 5 g per kg body weight and 12 g per kg body weight in rats (20). The oral LD$_{50}$ of sodium citrate in mice was 7.1 g per kg, all mice tested at a dose of 4.8 g per kg survived (21). The signs of acute toxicity from orally administered citric acid in mice and rats are those of organic acidosis and of calcium deficiency. Animals given citric acid orally in lethal doses demonstrated hemorrhage of the gastric mucosa at necropsy.

Acute oral toxicity studies of citric acid in man have not been reported. On the basis of tolerated chronic oral doses of citric acid in dogs and rabbits, Nazario (22) estimated that an adult 70 kg man should be able to tolerate 53 g of citric acid daily without damage to health. However, he reviewed one report of a young woman who vomited and almost died after ingesting 25 g of citric acid as a single dose.
Short-term studies

Six young rats weighing about 75 g were fed a diet supplemented with 2.5 percent citric acid (about 2 g per kg body weight) for 9 days (23). The experimental group showed weight loss or no appreciable gain in weight during the first few days and then recovered their expected rate of growth.

Rogers et al. (24) found no benefit to the growth of male, weanling rats (50 to 60 g) during a 2-week period from the addition of 2.47 percent diammonium citrate (2.5 g per kg per day) to a basal diet containing 16 percent amino acids when the diet contained the necessary level of indispensable amino acids and the total dispensable amino acid nitrogen was not low. However, growth was reduced if two or more of the glutamic-proline-arginine group of amino acids were omitted.

Yokotani et al. (20) fed groups of 10 SD-JCL male rats (98 to 112 g body weight) citric acid (a refined product of yeast fermentation) for 6 weeks at 1.2, 2.4, and 4.8 percent of the diet; measured mean intakes of citric acid were 1.15, 2.26, and 4.67 g per kg body weight per day, respectively. Food intake was depressed as compared to a control group by 0.7, 2.6, and 4 percent, respectively. Growth rate was slightly reduced at all levels of intake. Total plasma protein concentration was significantly less than that of controls only at the 2.4 percent dietary level; slight decreases in blood cell counts and hemoglobin were not statistically significant. At the highest dietary level, plasma cholesterol concentration decreased, serum glutamic oxalacetic transaminase activity increased, the thymus weights were lower, and slight atrophy of the thymus and splenic follicles was found at necropsy.

Daily oral citric acid administration of 600 mg per kg (1.2 percent in the diet) to rats for more than 90 days produced no abnormalities in body weight gain, blood, histopathology of the viscera or reproduction (25). Also, daily oral administration of citric acid to dogs, 1.38 g per kg, for 112 to 120 days was shown to produce no behavioral, biochemical or histopathological abnormalities.

Wehrbein et al. (26) included diammonium citrate for the partial replacement of nonessential amino acid nitrogen in experimental diets fed to replicate groups of three male and three female young Yorkshire-Hampshire pigs. The basal diet fed the control group contained 16 percent crude protein (Nx6.25) at the start of the experiment, and when the pigs reached a weight of about 50.5 kg the protein content was reduced to 14 percent. The experimental diets provided diammonium citrate at an average rate of 230, 465, and 930 mg per kg by replacing 5, 10, and 20 percent of the nitrogen of the basal diet with an equimolar mixture of diammonium citrate and diammonium phosphate. The experiment lasted 81 days. Average daily weight gain and feed intake decreased with increasing levels of added salts. In comparison
to controls, the growth depression was only significant at the 20 percent replacement level. The depressed feed intake and growth were partly reversed by addition of lysine, methionine and tryptophan to the experimental diets. The total blood nitrogen was not affected by feeding the ammonium salts; however, there was a significant depression in blood urea nitrogen levels with increasing levels of the salts.

Rats maintained on a "high protein diet" and given a single oral dose of \[^{15}N\] ammonium citrate (about 70 mg per kg) excreted the ammonia nitrogen almost quantitatively within 48 hours, while rats on a "low protein diet" incorporated a significant fraction of the ammonia nitrogen into proteins (27). Similar results occurred after administration of diammonium citrate (about 5 mg per kg) to a human subject on a normal diet and to a patient suffering from Addison's disease on a low protein diet. The authors concluded that ammonia is extensively utilized for protein synthesis only when there is a deficiency of dietary amino acids.

Premature infants fed a formula with added citric acid, 680 mg per kg per day, developed metabolic acidosis without clinical signs when a high protein diet (3.19 g protein per 100 ml formula) but not when a lower protein diet (1.62 g protein per 100 ml) was given (28).

Swendseid et al. (29) found that a combination of diammonium citrate and glycine was as effective as a mixture of nonessential amino acids in maintaining nitrogen equilibrium of four young adult subjects fed minimal amounts of essential nitrogen in the form of egg protein in a basal diet. Diammonium citrate (430 to 580 mg per kg) included in the experimental diets as an isonitrogenous mixture with glycine for periods of 6 or 7 days was better utilized as a source of nonessential nitrogen than glycine alone under the experimental conditions.

Scrimshaw et al. (30) replaced isonitrogenously up to 30 percent of the nitrogen contributed by the essential amino acid of whole egg protein with a mixture of glycine and diammonium citrate (each providing equal amounts of nitrogen) without affecting the nutritive value of egg protein in the experimental diets fed to groups of three and eight 17- to 22-year-old male subjects. The eleven subjects received sufficient calorie intake to maintain body weight. In one experiment, three subjects received protein at 0.42 g per kg per day for 13 days, then the protein intake was adjusted by 0.06 g per kg per day for 5-day periods to establish minimum requirements as determined by urinary nitrogen excretion. At this point, the protein of the diet was replaced isonitrogenously by the glycine-diammonium citrate mixture at the rate of 0.06 g per kg for 5-day periods. One 71 kg subject eating a basal diet providing 0.36 g protein per kg was adjudged by the investigators as probably receiving too much of a dilution when 33 percent of the protein nitrogen had been replaced; on this diet the subject was receiving 77 mg diammonium
citrate per kg per day. In a subsequent experiment, the nitrogen contributed by egg protein was increased from 78.6 percent, used in the first experiment, to 90 percent and four of the eight subjects showed no significant difference in urinary nitrogen excretion at a dilution of 40 percent for 8 to 12 days and one 70 kg subject did not increase urinary nitrogen at 50 percent for 4 additional days (124 mg diammonium citrate per kg per day).

Kies et al. (31) measured the nitrogen retention of 10 male subjects fed a basal diet and an isonitrogenous mixture of glycine and diammonium nitrate as a nonspecific nitrogen source. The basal diet provided suboptimal amounts of protein and a daily nitrogen intake of 4.5 g, of which 4 g nitrogen was from dry skim milk solids, white degerminated corn meal, enriched white flour or unenriched polished rice. When added, the salt mixture provided 4 or 8 g of nitrogen daily (215 or 430 mg diammonium citrate per kg body weight), and it was taken in three divided doses in water solution with meals. Nitrogen balance was measured during 5-day periods when the subjects had daily nitrogen intakes of 4.5 g (basal diet alone), 8.5 g or 12.5 g. A negative balance occurred on all diets except one, the basal diet containing milk solids supplemented with 8 g of the glycine and diammonium citrate mixture. However, the degree of nitrogen loss was reduced at each level of total dietary nitrogen increase, and the authors concluded that the nonspecific nitrogen source had a sparing effect on protein requirements.

Long-term studies

Long-term toxicity studies on citric acid have been carried out in rats. Three successive generations of albino Wistar rats were fed citric acid in the diet at 0.15, 0.45, and 1.20 percent, providing an average intake of 100, 300, and 800 mg per kg per day, respectively (32, 33). No effects were noted on growth, reproduction, mortality or blood components after the feeding period of up to 12 months. The teeth were not harmed by the acid diets. Metabolic studies utilized female rats; nitrogen balance, mineral balance, acid-base balance and the gross and microscopic appearance of the tissues were normal. Decrease in ash content of the tibia with an increase in calcium was found, and a slight increase in calcium was observed in muscle. Small changes in tissue composition were not considered evidence of adverse effects; the liver sodium content was decreased and the muscle sodium content was increased; the total muscle phosphorus content was also decreased.

In 1957, Horn et al. (34) reported feeding citric acid for 2 years at 3 and 5 percent of the diet to a group of 20 young male albino Carworth rats (average daily intake was 1.2 and 2.0 g per kg, respectively). Both experimental groups grew more slowly than controls, but survival rates were not decreased. At the time of sacrifice (2 years) there were no differences in organ weights of controls and experimental groups. Results of microscopic
examination of thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small and large intestines, pancreas, bone marrow, and testes were within normal limits.

**Special studies**

Fatal experimental tuberculosis progressed more rapidly in mice given a diet containing 8 to 10 percent sodium citrate (about 5.5 g per kg body weight) (35). Adding 2 percent sodium citrate to the drinking water (or 1 percent sodium glutarate) also caused accelerated rate of mortality in the animals. The effect on bacterial resistance was not explained but the investigator noted that citrate addition to the diets of control mice markedly reduced the rate of weight gain.

Citric acid and sodium citrate were found to interfere with calcium absorption in vitamin D-deficient rats on a low phosphorus diet (36). Citrates have an antirachitic effect in rats receiving an adequate phosphorus intake (37).

Citric acid was found to decrease the teratogenic effects of insulin and of trypan blue in chicken embryos (38, 39). Incubation of transplantable tumors (Walker carcinoma and Pliss lymphosarcoma) in sodium citrate at concentrations above 30 mg per kg inhibited their growth (40).

A 37-year-old man was reported to be allergic to several organic acids and developed canker sores, headache, general lassitude and irritability from eating foods containing citric acid (41). Direct application of citric acid crystals to the oral mucosa repeatedly produced canker sores but potassium citrate was without effect.

Teratological evaluation of citric acid in pregnant mice (≤241 mg per kg administered on days 6 through 15 of gestation), rats (≤295 mg per kg administered on days 6 through 15 of gestation), hamsters (≤272 mg per kg administered on days 6 through 10 of gestation) and rabbits (≤425 mg per kg administered on days 6 through 18 of gestation), gave no indications of adverse effects on nidation, maternal or fetal survival, and the number of abnormalities seen in either soft or skeletal tissues of the groups did not differ from the number occurring spontaneously in the sham-treated controls (42).

Citric acid was not mutagenic in Salmonella typhimurium strains TA-1530 and G-46 in the host-mediated assay (43). Although it appeared to induce mitotic recombination in Saccharomyces cerevisiae strain D3 in both in vitro and host-mediated assay tests, these tests were repeated at a higher dose level (3.5 g per kg) and all results were negative. Citric acid produced no detectable significant aberration of the bone marrow metaphase chromosomes of rats when given orally up to 3 g per kg per day for 5 days. There
were also no significant chromosomal (anaphase) aberrations in human embryonic lung culture cells (WI-38) when tested up to 600 µg per ml. Citric acid was considered to be nonmutagenic in rats in the dominant lethal assay when tested at levels up to 3 g per kg per day for 5 days.

Tests of citric acid in developing chick embryos showed that at dose levels of 10 mg per kg of egg or above there was higher mortality after air cell treatment at 96 hours and after yolk treatment at 0 and 96 hours. A dose level of 5 mg per kg increased mortality after yolk treatment at 96 hours of embryonic development. No abnormalities were observed in the hatched chicks for the test conditions employed (44).

Neither potassium nor sodium citrate was considered mutagenic when evaluated in microbial assays with and without the addition of mammalian metabolic activation preparations (45, 46). The indicator microorganisms were \textit{S. cerevisiae} D4 and \textit{S. typhimurium}, strains TA-1535, 1537, and 1538. Potassium citrate and sodium citrate showed no teratogenicity in the developing chicken embryo (47, 48).

B. Ethyl, isopropyl and stearyl esters of citric acid

\underline{Absorption and metabolism}

Isopropyl citrate (predominantly the monoisoisopropyl ester) in a mono- and diglyceride vehicle at levels up to 10 percent of the diet was nearly completely absorbed and did not lower the digestibility of margarine in rats (49). Stearyl citrate, predominantly distearyl citrate, fed at 2.5 to 10 percent of the ration was poorly absorbed by the rat and incomplete digestion of stearyl citrate owing to inefficient hydrolysis of the ester in the gastrointestinal tract was described. The dog was able to digest stearyl citrate more effectively than the rat.

\underline{Acute toxicity}

The oral LD$_{50}$ for 20 percent stearyl citrate in cottonseed oil in rats was greater than 5.4 g stearyl citrate per kg of body weight (50). Isopropyl citrate (38 percent isopropyl citrate esters in a mono- and diglyceride vehicle) given orally to rats gave an LD$_{50}$ value of greater than 20.7 g per kg for male rats and greater than 18.8 g per kg for female rats. The LD$_{50}$ was 2.8 to 3.7 g per kg body weight when the isopropyl citrate esters were dissolved in 10 percent ethanol; this LD$_{50}$ approximated that expected for the hydrolysis products of this ester. Single doses of 12 g per kg of isopropyl citrate plus vehicle (2.25 g per kg of isopropyl citrate) and 5 g per kg of stearyl citrate were not fatal to dogs.
Finkelstein and Gold (51) included triethyl citrate in a study of the toxicity of several citrate esters administered orally to rats and cats. The single oral \( \text{LD}_{50} \) for triethyl citrate was about 8 g per kg in rats and 4 g per kg in cats. Lethal doses in cats produced nausea, vomiting, ataxia, weakness, muscle twitching, tremors, reflex hyperexcitability, lowering of body temperature, gasping and shallow respiration, prostration, convulsions, respiratory failure, and death. One cat surviving a dose of 6.2 g per kg body weight was examined at 2-week intervals for 2 months and no toxic effects were shown as judged by weight, blood counts, hemoglobin levels or blood nitrogen and urine analysis.

**Short-term studies**

Adult rats fed stearyl citrate at various levels up to 10 percent of the diet (over 5 g per kg per day) and rabbits given 2 and 10 percent of the diet (over 4 g per kg per day at the high level) as stearyl citrate for 6 weeks showed no adverse reactions as measured by growth, mortality, or tissue pathology (50). Similarly, rats fed up to 5.3 percent of the diet (over 2 g isopropyl citrate per kg per day) as isopropyl citrate, in a mono- and diglyceride vehicle, and rabbits fed the compound up to 8.5 percent (over 3 g per kg per day) of the diet for 6 weeks showed no signs of toxicity. A group of four dogs was fed a diet containing 0.06 percent isopropyl citrate plus the glyceride vehicle and a similar group was fed a diet with 3 percent stearyl citrate added; no evidence of toxicity was reported.

Young rats were fed triethyl citrate in their diet at an initial rate of 1, 2, and 4 g per kg body weight for 8 weeks (51). Periodic urine examinations and blood counts and growth revealed no toxic effects. At necropsy no gross abnormalities were seen in the thoracic and abdominal organs; histological sections of the heart, lungs, gastrointestinal tract, liver, pancreas, spleen and kidneys were comparable to those from controls. Cats receiving daily doses of triethyl citrate approximating 7 percent of the \( \text{LD}_{50} \) for an 8-week period did not differ from controls with respect to weight, blood count, hemoglobin, blood sugar and blood nitrogen. However, weakness, ataxia and depression appeared after the fourth or fifth dose and progressed to an advanced degree; the animals appeared normal within 1 to 4 days after treatment was discontinued.

Two groups of four young adult, male and female, beagle dogs were given daily doses of triethyl citrate of 0.05 and 0.25 ml per kg for 6 months (52). The parameters of body and organ weights, blood and urine analyses, and the results of histological examination of tissues revealed no adverse effects. Increasing the daily dose to 2.5 to 3.5 ml per kg for 7 to 12 weeks resulted in a characteristic liver pathology in three treated dogs. A fourth dog that had reacted adversely to a dose of 2 ml per kg showed none of the histological changes after receiving 1.5 ml per kg daily for an additional month.
Long-term studies

Stearyl citrate and isopropyl citrate have been evaluated in a 2-year feeding study and in a multigeneration feeding study in rats (50). The rats were fed stearyl citrate at levels up to 10 percent of the diet in the 2-year study (about 5 g per kg for an adult rat) and either 1.9 or 9.5 percent stearyl citrate in a four-generation study with no adverse effects on growth, mortality, fertility, gestation or lactation, and histopathology.

Isopropyl citrate was fed to weanling rats at a level up to 1.06 percent of the diet (about 1 g per kg) in a 2-year study and in a 5-generation study with no adverse effects (50). The liver, kidney, heart, brain, lung, spleen, stomach, small intestine, large intestine, pancreas, adrenal, and testicle or ovary were examined for histopathological changes at necropsy. Metastatic calcification and tumor formation were noted in the tissues of both test and control rats and were not attributable to the ingestion of isopropyl citrate.

Three groups of 15 male and 15 female weanling Sprague-Dawley rats were fed diets containing 0.33, 1.0, and 3.0 percent triethyl citrate in a lifetime feeding study (2 yr) (53). The dose of triethyl citrate initially ranged from about 0.2 to 2 g per kg body weight. Weight gain and food intake were reduced below that of the control groups when the level of the ester in the diet was increased. Blood and urine studies, survival, and gross and histopathology examinations showed no adverse effects attributable to triethyl citrate ingestion.

Special studies

The intraperitoneal administration of doses in excess of 400 mg per kg of triethyl citrate produced a loss of righting reflex in Swiss albino mice, an effect reversible within 15 minutes (54). Signs of stimulation and a more rapidly reversible loss of righting reflex were observed in Wistar rats dosed at 400 mg per kg. Intravenous administration of a 100 mg per kg dose of the compound to rabbits produced marked increases in motor activity and respiration. A group of 20 mice given intraperitoneal doses of 350 mg of triethyl citrate per kg daily for 14 days had a slightly lower growth rate than controls but no differences were seen in red and white blood cell count, clotting time and hemoglobin levels. Examination of liver, lung, and kidney tissues of two animals at necropsy revealed no pathological cellular changes. Triethyl citrate had a local anesthetic effect and blocked neural transmission when placed in direct contact with a nerve trunk.

Triethyl citrate displayed no teratogenicity to the developing chick embryo when tested in ethanol (as solvent) via the air cell and yolk at pre-incubation (up to 10 mg per egg) and at 96 hours (up to 0.4 mg per egg) of
incubation (55). After yolk administration, the percent mortality was significantly different from solvent control ($p \leq 0.05$); however, the mortality was not dose dependent from 0.5 to 10 mg per egg preincubation or from 0.02 to 0.40 mg per egg at 96 hours. Verrett concluded that triethyl citrate showed very little toxicity under the four test conditions.

It was not mutagenic in plate and suspension tests using *Salmonella typhimurium* TA 1535, TA 1537, and TA 1538 and *Saccharomyces cerevisiae* D4 with and without tissue homogenates (56).
V. OPINION

The citrate ion is widely distributed in plants and animals and is a naturally occurring component of the diet. It is a common metabolite in oxidative metabolism and an important component of bone. Exogenous citrate administered to infants and adults as a component of commonly consumed diets is considered completely metabolizable. The addition of citric acid to foods is considered equivalent to adding citrate salts except in foods of very high acidity. The amount of citrate added to foods by food processors is about 500 mg per person per day. This amount occurs naturally in 2 ounces of orange juice and does not constitute a significant addition to the total body load. Although data on acute and chronic effects of orally administered sodium citrate, calcium citrate and potassium citrate are limited, no biological effects of the citrate-containing substances evaluated in this report cause concern about the safety of these GRAS substances used in reasonable amounts and in accordance with prescribed tolerances and limitations.

In light of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, isopropyl citrate, stearyl citrate, and triethyl citrate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when used at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


44. Anonymous. Investigation of the toxic and teratogenic effects of GRAS substances to the developing chicken embryo: citric acid. [Report supplied by Mississippi State University to Food and Drug Administration, Washington, D.C., under contract no. FDA 72-342. 7 pp.]


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

   Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

   Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Ind.

   Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

   Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

   John R. McCoy, V. M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N.J.

   Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

   Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

   Michael B. Shimkin, M.D., Professor of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, Calif.

   Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

   John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

   George W. Irving, Jr., Ph.D., (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

Kenneth D. Fisher, Ph. D., Director
Frederic R. Senti, Ph. D., Associate Director
C. Jelleff Carr, Ph. D., Director Emeritus
Richard G. Allison, Ph. D., Staff Scientist
Herman I. Chinn, Ph. D., Senior Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M. D., Senior Medical Consultant
Michael J. Wade, Ph. D., Staff Scientist

The Select Committee expresses its appreciation to the following organizations who contributed information and data:

Fleischmann Laboratories, Standard Brands, Inc., Betts Avenue, Stamford, Conn.

Pfizer Central Research, Pfizer, Inc., 235 East 42 Street, New York, N. Y.

Report submitted by:

April 4, 1978
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances