EVALUATION OF THE HEALTH ASPECTS OF GLYCEROPHOSPHATES AS FOOD INGREDIENTS

1976

Prepared for

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

Contract No. FDA 223-75-2004
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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 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.

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I. INTRODUCTION

This report concerns the health aspects of using glycerophosphates as food ingredients. It 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 1973.* 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; recent literature searches by the Toxicology Information Response Center, Oakridge, Tennessee; 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 January 7, 1977 (42 FR 1519 to 1521) 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 glycerophosphates as food ingredients. The Select Committee received no requests for such a hearing on glycerophosphates.

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 Code of Federal Regulations 21 CFR 121.1, revised April 1, 1976, 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 (PB-228-543/5) 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. While the Select 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 Select Committee, there are insufficient data upon which to base a conclusion. The Select 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 reconducted, 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 glycerophosphates 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

Calcium, manganese and potassium glycerophosphates are GRAS substances listed in the Code of Federal Regulations (2) under nutrients and/or dietary supplements [21 CFR 121.101(d)(5)]. The calcium and magnesium salts are regulated for use as stabilizers in the manufacture of food packaging materials [21 CFR 121.2005(g)](2). Calcium, magnesium and potassium glycerophosphates are described and limits of impurities are set in Food Chemicals Codex (3).

Potassium glycerophosphate is used in tonics, and calcium glycerophosphate is used in dentifrices, baking powder and in foods as a food stabilizer (4). In 1965 it was reported that calcium glycerophosphate was used in infant dietary formulas (5). The Select Committee has found no evidence of its current use in infant formulas.

L-α-Glycerophosphate (α-glycerol 3-phosphate) occurs in plants and animals and is required for the synthesis of triacylglycerols (6). Two enzymes, one a mitochondrial membrane-associated flavoprotein, and the second a NAD-dependent cytoplasmic enzyme, catalyze reactions with glycerol 3-phosphate as a normal part of energy metabolism.
The glycerophosphates may be salts of either β- or α-glycerophosphoric acid. Three isomers are described for calcium glycerophosphate: β-glycerophosphate and the D(+) and L(-) forms of α-glycerophosphate (4). Commercial preparations of the glycerophosphates would be expected to be a mixture of isomers.

III. CONSUMER EXPOSURE DATA

A subcommittee of the National Research Council (NRC) surveyed manufacturers concerning the addition of GRAS substances to foods in 1970 and estimated the possible average daily intake of these substances by various age groups (7). Calcium, manganese and potassium glycerophosphates were included in the survey, but there were no reports from the manufacturers surveyed that these glycerophosphates were added to any foods. The Select Committee is aware of the use of calcium glycerophosphate in some special low residue diets intended for use under medical supervision and its use in some pudding mixes at a level equivalent to 0.12 to 0.85 g of calcium glycerophosphate per serving (8). The NRC survey specifically excluded addition to animal feeds, packaging materials, and vitamin and mineral supplements incorporated in tablets or other dosage forms or other non-food items.

From 1965 to 1973, imports of glycerophosphoric acid and its compounds (including calcium, magnesium and manganese glycerophosphates) ranged from 14,000 to 27,000 kg per year (9); no trend was apparent. If all of these imports were added directly to foods the per capita daily consumption would be less than 0.4 mg (about 0.006 mg per kg body weight).

The Select Committee, in the absence of contrary evidence, believes that glycerophosphates as direct food ingredients do not contribute significantly to the intake of calcium, manganese or potassium and that only trace amounts of calcium and magnesium glycerophosphate could migrate to food from packaging material. For this reason the biological effects of these cations are not reviewed in this report.

IV. BIOLOGICAL STUDIES

While several feeding studies have reported the use of glycerophosphates as antirachitic agents, no reports of acute oral toxicity, few feeding studies, and no reports of teratogenicity, mutagenicity, and carcinogenicity testing specifically designed to demonstrate the safety of using glycerophosphates in food have been found by the Select Committee in its search of scientific reference material.
Absorption, metabolism, excretion

The in vitro hydrolysis of glycerophosphate by cat and dog intestinal enzymes suggested that orally administered glycerophosphates would be largely hydrolyzed to glycerol and phosphate before absorption (10, 11). Other reports of the Select Committee have considered the health aspects of glycerol and certain phosphates as food ingredients (12, 13). Oral doses of 0.3 to 1.2 g of calcium glycerophosphate were reported as a medical use to provide calcium and phosphorus dietary supplements (4).

Anghileri (14) reported on the fate in the rat of the following intravenously administered β-glycerophosphates: $^{51}$Cr, $^{64}$Cu, $^{56}$Mn, $^{140}$La, $^{65}$Zn, and $^{85}$Sr. Most of the radioactivity was accumulated in the liver, kidney, and spleen; the main excretory route was via the urine. All compounds tested had similar patterns of cation distribution and excretion.

The efficacy of calcium glycerophosphate as a dietary calcium supplement to rachitogenic diets was greater than calcium carbonate, dicalcium phosphate, tricalcium phosphate and calcium lactate when fed to weanling female mice (15). The α- and β-glycerophosphates of sodium, calcium and magnesium were comparable to monocalcium phosphate as antirachitic agents in rats (16-18). The subcutaneous injection of 0.1 to 0.5 g of potassium glycerophosphate into guinea pigs (estimated dose of 0.1 to 0.5 g per kg body weight) resulted in an increase in blood glycerophosphatase activity within 30 minutes (19).

The addition of calcium glycerophosphate (50 mg per kg) to a high cholesterol diet (0.5 g per kg per day) resulted in an earlier onset of hypercholesterolemia and higher aortic cholesterol levels in experimental than in control rabbits receiving cholesterol to produce atherosclerosis (20). The addition of glycerin to the high cholesterol diet produced similar results. In another study involving rabbits fed a high cholesterol (0.5 g per kg body weight per day) diet, the addition of calcium glycerophosphate (0.1 g per kg body weight per day) resulted in a higher serum level of esterified cholesterol but no effect on free cholesterol in blood (21).

In a five-month feeding study of a mixture of α- and β-isomers of calcium glycerophosphate (200 mg per kg), treated rabbits had a slightly increased blood cholesterol level, a decreased blood protein-bound cholesterol level, and an increase in the deposition of total lipids and cholesterol in liver, lung and kidney tissues (22). No atherosclerotic changes were noted in blood vessels, although changes in other organs suggested experimental atherosclerosis to the author.
Calcium glycerophosphate (50 mg per kg per day) was reported to decrease the respiration rates in rabbit brain, liver and heart muscle after a five-month feeding period with or without the addition of cholesterol to the diet (23, 24). Cera and Bellini (25) reported that the absorption of neutral fats in rats is enhanced by the addition of sodium glycerophosphate to the fat.

**Special studies**

The only reported study concerned with the effects of glycerophosphate on reproduction and/or teratology is that of Landauer and Sopher (26). Using fertilized chicken eggs, they reported that sodium DL-α-glycerophosphate inhibited the teratogenic effects of 3-acetylpyridine and 6-aminonicotinamide, but potentiated the teratogenic effects of acetazolamide and insulin.

Bowen (27) reported that the feeding of 1 percent calcium β-glycerophosphate (a 75:25 mixture of the β isomer and α isomers) in a cariogenic diet to monkeys for 30 months resulted in a marked decrease in the incidence of caries. The effectiveness of calcium and sodium α- or β-glycerophosphate as an anticaries agent in rats fed a cariogenic diet was reported by Regolati and Hotz (28). In this study calcium glycerophosphate at a level of 0.6 percent phosphorus added to the diet caused reduced feed and water intake and retarded growth in weanling rats. The daily intake of calcium glycerophosphate tetrahydrate was about 3 g per kg body weight. Federov (29) reported that calcium glycerophosphate was an active anticaries toothpaste component when tested in rats fed a cariogenic diet.

**Pharmacological effects**

The intravenous administration of sodium β-glycerophosphate to rabbits with fractured right radii resulted in accelerated healing and larger callouses as compared to control animals (30). These authors reported that administration of the following doses did not elicit any adverse effect: the intravenous administration of 100 mg (50 mg per kg body weight) into a rabbit daily for 55 days, and 10 daily intravenous injections of 10 g to a 17 kg dog. However, the intraperitoneal administration of 1.5 to 3 g per kg for two to five days to rabbits was fatal.

In a review of some of the general pharmacological effects of sodium glycerophosphate, Velazquez (31) noted its uterine spasmolytic and respiratory stimulatory properties. Aragon (32) also demonstrated the spasmolytic action of sodium-β-glycerophosphate on uterine smooth muscle. A hypoglycemic effect of subcutaneously injected sodium-β-glycerophosphate in rabbits was reported by Fernandez and Aguilar (33). The mechanism the authors proposed was that of insulin reinforcement attributable to the phosphoric acid moiety as an activator of glycolysis.
The simultaneous administration of sodium glycerophosphate with aureomycin to rats and guinea pigs resulted in a two to threefold increase in serum levels of the drug when compared to controls (34). Citric acid, trisodium citrate, malic acid, tartaric acid, tricarballylic acid, monosodium phosphate, malonic acid, pyruvic acid, and lactic acid produced larger increases, but calcium glycerophosphate did not produce an increase. The "Lamson glucose effect" (intraperitoneal injection of certain solutions into an animal just awakening from barbiturate-induced sleep causes the animal to return to sleep) also was induced with β-glycerophosphate in guinea pigs (35). Sodium β-glycerophosphate, intravenously administered, was reported to enhance the hypertensive effects of epinephrine; similar doses of sodium α-glycerophosphate did not (36).

V. OPINION

The glycerophosphate salts that are considered to be GRAS could provide absorbable sources of glycerol, phosphate, and their respective cations. However, glycerophosphates are not now widely used in foods. The Select Committee believes that the level of consumer exposure is very low and that use under limitations as a nutrient or dietary supplement will not present a hazard to the public. In previous evaluations of glycerol and certain phosphates no evidence was found of a hazard to the public from the hydrolysis products of the glycerophosphates.

In light of the information reviewed and reported herein, the Select Committee concludes that:

There is no evidence in the available information on calcium glycerophosphate, potassium glycerophosphate, and manganese glycerophosphate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used as nutrient supplements or as they might reasonably be expected to be so used in the future.

There is no evidence in the available information on calcium glycerophosphate and magnesium glycerophosphate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used in food packaging materials as now practiced or as they might be expected to be used for such purposes in the future.
VI. REFERENCES CITED


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Report submitted by:

March 15, 1977
Date

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