EVALUATION OF THE HEALTH ASPECTS OF INOSITOL
AS A FOOD INGREDIENT

1975

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 INOSITOL

AS A FOOD INGREDIENT

1975

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.

C. Jelleff Carr, Ph.D., Director
Life Sciences Research Office
FASEB

- ii -
CONTENTS

<table>
<thead>
<tr>
<th></th>
<th>Introduction</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>Background information</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>Consumer exposure data</td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>Biological studies</td>
<td>4</td>
</tr>
<tr>
<td>V</td>
<td>Opinion</td>
<td>8</td>
</tr>
<tr>
<td>VI</td>
<td>References cited</td>
<td>9</td>
</tr>
<tr>
<td>VII</td>
<td>Scientists contributing to this report</td>
<td>13</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using inositol as a food ingredient. 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; 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 February 13, 1976 (41 FR 6787 and 6788) 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 inositol as a food ingredient. The Select Committee received no requests for such a hearing on inositol.

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 the Code of Federal Regulations 21 CFR 121.1, revised April 1, 1975 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 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

---

*The document (PB-223 361/6) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
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 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 inositol 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

Inositol (hexahydroxycyclohexane) is a widely distributed natural constituent of plant and animal tissues. The animal tissues richest in inositol are brain, heart, stomach, kidney, spleen, and liver, where it occurs free or as a component of phospholipids. Among plants, cereals are rich sources of inositol, particularly in the form of polyphosphoric acid esters, called phytic acids. Although there are several possible optically active and inactive isomers, considerations of inositol as a food additive refer specifically to optically inactive cis-1,2,3,5-trans-4,6-cyclohexanehexol, which is preferably designated myo-inositol (2).

Pure inositol is a stable, white, sweet, crystalline compound. The Food Chemicals Codex specifies that it assay not less than 97.0 percent, melt between 224 and 227°, and contain not more than 3 ppm arsenic, 10 ppm lead, 20 ppm heavy metals (as Pb), 60 ppm sulfate, and 50 ppm chloride (3).

Inositol was thought for a time to be a vitamin because experimental animals on a synthetic diet developed clinical signs that were corrected by inositol supplementation (4). However, no cofactor or catalytic function for inositol has been found; it can be synthesized and occurs in relatively high concentration in animal tissues. These factors argue against its classification as a vitamin. A dietary requirement in man has not been established (5).

Inositol is included among the Generally Recognized as Safe food substances as a nutrient and/or dietary supplement (6). It is added to infant formulas and has also been added to certain special dietary foods. The latter use is not included in FDA's recently proposed regulations for special dietary uses, but their effective date has been stayed pending final revision of these regulations(7).
A National Research Council (NRC) subcommittee has provided data to show that about 18 times as much inositol was added to foods in 1970 as in 1960. The subcommittee has also provided data from which it can be calculated that 5,400 kg of inositol were added to foods in 1970 (8).

III. CONSUMER EXPOSURE DATA

The report of the NRC subcommittee (8) contains information on the levels of added inositol in infant formulas. Similar information is not available for levels of inositol added to special dietary foods. The NRC subcommittee surveyed food manufacturers by questionnaire and, based on the information supplied, calculated a weighted mean of 0.0035 percent for the usual percentage addition of inositol to infant formulas. It is to be noted that the weighted mean does not express the highest percentage of inositol added by any manufacturer. Information obtained in direct communications with several manufacturers of infant formulas indicates that the usual level of addition of inositol to these products is 0.01 percent (9).

The NRC subcommittee (8) has provided data on the possible average daily human intake of inositol by various age groups (Table I); the report provides no data for inositol intake of adults. The Select Committee has converted these figures into possible intakes per kilogram of body weight.

TABLE I

<table>
<thead>
<tr>
<th>Age group</th>
<th>Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
</tr>
<tr>
<td>0-5 mo</td>
<td>12</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>2</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>1</td>
</tr>
</tbody>
</table>

Calculated intake, mg/kg body weight, was based on the following estimated weights of infants by age groups: 0-5 mo, 5 kg; 6-11 mo, 8 kg; 12-23 mo, 11 kg (10).

Since food consumption data were not requested in the subcommittee survey of the food industry, intake estimates were derived by utilizing Market Research Corporation of America data on mean frequency of eating foods by category, U.S. Department of Agriculture data on mean portion size, and by assuming that all infant formulas contained inositol at the weighted mean level of 0.0035 percent. However, since all babies in the age groups
indicated in Table I do not consume formulas containing added inositol, the Select Committee has explored alternative means of estimating intakes of added inositol.

Inositol is added only to milk-free infant formulas, and it is current commercial practice to add 100 mg of inositol per liter of these formulas (9). Of the 3,100,000 infants born per year (11), approximately 10 percent receive milk-free formulas during the first four months of life and decreasing percentages thereafter (12). Utilizing the estimates of Fomon (12) concerning 50th percentile calorie intakes and percentage of calories from milk or formula at various ages and a concentration of 15 mg of inositol per 100 kcal of milk-free formula, it may be calculated that approximately 3,750 kg of inositol are consumed by infants in milk-free formulas. This quantity therefore accounts for the major proportion of the 5,400 kg of inositol estimated to be added to foods in 1970 (8). For infants less than six months of age (i.e., the major consumers of milk-free formulas), it may be calculated on the basis of estimates previously cited (12) that intakes of added inositol average 65 mg per infant per day. The Select Committee regards this value as a closer approximation than the figures given in Table I of the usual daily intake of that fraction of the infant population under six months old that consumes formulas to which inositol has been added.

It is of interest to compare the inositol content of milk-free formulas to which inositol is added to a level of 100 mg per liter, with the inositol content of milk. Cow's milk contains an average concentration of 80 mg per liter (range, 60 to 120 mg); human milk averages 450 mg per liter (range, 390 to 560 mg)(13). Thus, considering the content of endogenous inositol in milk and other infant foods, the amount of added inositol consumed by the small percentage of infants fed formulas containing added inositol would be within the range of usual intake by babies fed other formulas.

In adults, inositol consumption varies depending on food choices but a good mixed diet (2,500 calories) provides about 1 g or more of inositol per day (14), or about 16 mg per kg of body weight. On a weight basis, the usual intake of inositol by infants and children consuming milk, milk-based, and milk-free formulas would be near this value except for very young infants receiving human milk, whose inositol intake could be highest.

IV. BIOLOGICAL STUDIES

Absorption, metabolism, excretion

Much of the biological information on inositol has resulted from studies of its possible role in nutrition as a lipotropic agent and its metabolic relationship to other carbohydrates. The particular role of inositol in
human nutrition is poorly understood; neither is it clear how important dietary inositol is in the synthesis of the inositol-containing constituents of the tissues, versus inositol synthesized de novo from glucose or other carbohydrate sources.

Although inositol has demonstrable lipotropic activity, it is considerably less effective than choline (15-17). Under certain experimental conditions, inositol has a synergistic effect with choline (18,19), but inositol does not prevent hemorrhagic kidneys in young rats receiving a diet low in choline and methionine; indeed, it seems to increase the severity of the renal pathology in these animals (17,20). It also appears, at least in rats, that the lipotropic activity of inositol depends upon the dietary intake of other nutrients, particularly biotin and thiamin (15,16). Signs of inositol deficiency have been demonstrated in several species of animals, but these observations have been complicated by interactions with such nutrients as biotin, pyridoxine, choline, and p-aminobenzoic acid (2). Moreover, possible therapeutic uses of inositol in the management of adult patients with diseases associated with disturbances in fat transport and fat metabolism have failed to provide any convincing evidence of beneficial effects in man (21).

Biosynthesis of inositol from glucose via glucose-6-phosphate involves enzymes which catalyze the cyclization of glucose-6-phosphate (cyclase) and removal of the phosphate (phosphatase)(2). These two enzymes are present in fetal rat liver but decrease in activity promptly after birth (22). Studies by Nixon (23) on sheep fetuses suggest that fetal blood inositol is of fetal rather than maternal or placental origin. However, it was also observed that blood inositol concentration declines after birth which is suggestive of placental origin. While a possible explanation of these findings was offered, the question regarding the site of inositol synthesis was not resolved. Naccarato and Wells (24) have recently isolated a new disaccharide containing galactose and inositol (6-0-β-D-galactopyranosyl myo-inositol) from the mammary gland of the lactating rat. While the significance of this inositol derivative is not known, its discovery emphasizes the importance of further investigations on inositol metabolism in early development.

In animals, ingested inositol appears to be absorbed slowly. Fasted rats given 250 mg of inositol orally (about 1.25 g per kg of body weight) required 24 to 48 hours for nearly complete absorption and only about one percent was excreted in the urine (25). Anderson (26) found inositol (2 g per kg of body weight) to be absorbed very slowly in the dog and largely eliminated in the feces. The daily oral intake of 1.5 g of inositol in man for three weeks produced only a moderate increase in plasma inositol concentration (27). If given at a dose level of 0.5 g per kg body weight per day, inositol produced diarrhea in man, which subsided after a few days even with continued inositol administration; about 9 percent was excreted in the urine and the remainder of the ingested dose was not accounted for (28). Others have found
that adults given 1 to 2 g of inositol per day for several weeks experienced no apparent harmful effects (29).

Stetten and Stetten (30) demonstrated with deuterium-labeled inositol that a significant fraction of an intraperitoneal dose of 2 g, divided in four portions and given at 150 minute intervals, was converted to glucose in a phlorhizinized rat. Coots (31) showed that intraperitoneal $^{14}$C inositol is incorporated intact in complex tissue components, such as phospholipids, and is vigorously metabolized to CO$_2$ by the male rat, 20 to 40 percent of the dose being excreted in the respiratory CO$_2$ within 8 hours. Moscatelli and Larner (32) found significant amounts of parenteral $^{14}$C inositol to be incorporated into lipids of the brain, liver, kidney, and heart of the rat. Howard and Anderson (33) found that the kidney may be the major site of inositol metabolism since oxidation of labeled inositol to CO$_2$ did not occur in nephrectomized rats.

Acute and short-term studies

The maximum tolerated intraperitoneal dose of inositol in the mouse is 500 mg per kg of body weight (34). Human subjects have been given up to 4 g of inositol intravenously, dissolved in isotonic saline (up to about 67 mg per kg of body weight), without reported adverse reactions, although there was a reduction in basal metabolic rate (35).

Young Wistar rats weighing about 50 g were fed diets providing doses of inositol of 0.5, 5, 10 and 50 mg daily for 45 days (36). At a dose of 0.5 mg per day, weight gain was greater than in control animals, but at 50 mg per day (about 1 g per kg of body weight) there was retardation of growth, staining of the hair, and general hyposthenia. When the experiment was repeated in 3-month-old rats no growth inhibition occurred with daily oral inositol supplements up to 5 g per kg body weight for a month. Further studies in young rats showed that the growth inhibition by an inositol intake of 50 mg per day was largely prevented by increasing either the protein content or by adding choline to the basal diet (37). Interestingly, in earlier work (38), inositol fed to female rats for 60 days at a level of 1 g per kg body weight had no effect on growth rate. In these experiments the diet contained 30 percent casein and was supplemented with choline.

Herrmann (39) fed 36 hens 0.5 g inositol daily for 30 to 68 days. Blood cholesterol decreased by about 20 percent, indicating that inositol acts as a decholesterizing agent in hens.

No long-term toxicity studies on inositol in animals have been reported.
Carcinogenicity, mutagenicity, teratogenicity studies

Lazlo and Leuchtenberger (40) and subsequently Hesselbach and Burke (41) found that intravenously injected inositol inhibited the rate of growth of transplanted Sarcoma 180 in female mice. Inhibition was dose-related. Oral and subcutaneous administration were ineffective.

Inositol fed at 1 percent of the diet (about 2 g per kg body weight) for 60 days to female rats prior to mating had no effect on growth rate. In continuing feeding after mating, no effects were observed in reproductive performance, litter size, or weight of offspring, and no gross structural abnormalities were found in the offspring (38).

A study designed to evaluate mutagenic potential by several in vitro assay procedures revealed that inositol did not exhibit mutagenic activity. The assay procedures included estimation of reversion or conversion frequencies of three strains of Salmonella typhimurium and two strains of Saccharomyces cerevisiae, with and without activation by tissue homogenates derived from mouse, rat, or monkey liver, lung or testis. Concentrations up to 5 percent inositol were used (42).

Inositol displayed no teratogenicity at levels up to 200 mg per kg when aqueous solutions were injected into the air cell or yolk of unincubated eggs or eggs after 96 hours of incubation. An \( \text{LD}_{50} \) could not be determined for any of the four test conditions. Scattered minor abnormalities were observed but in no instance were they significantly higher in incidence or different in nature from those observed in the solvent-treated or untreated control eggs (43).

Other studies

Inositol, fed at a dose of 280 mg to patients with cancer of the gastrointestinal tract and elevated fat in their livers with some expectation that it might reduce the fat content, decreased liver lipid to about the same extent as an 8 g dose of lipocaic, a crude mixture containing 2.6 percent choline and 3.5 percent inositol. Neither inositol nor lipocaic caused as great a reduction in hepatic lipid as did a 3 g dose of choline chloride (44). It should be noted that in rats, inositol had a lipotropic effect only when animals consumed a fat-free diet, and the effect was greater when choline also was given (19).

Hypercholesterolemic patients given 2 g inositol daily for 6 to 10 weeks showed slightly reduced blood levels of cholesterol and cholesterol esters, but the investigator did not consider the reduction to be significant (45). Attempts to treat high-tone deafness and alopecia with inositol were unsuccessful when 1 to 3 g daily were given orally in divided doses for as long as 12
months (29, 46). These and other therapeutic trials (2) indicate that appreciable amounts of inositol can be tolerated in the diet of adults without adverse effects.

V. OPINION

Inositol is a naturally-occurring substance that is widely distributed in plant and animal tissues and synthesized in animals and man. Orally administered inositol is absorbed slowly and is metabolized. The available information from toxicological studies in animals suggests no adverse effects associated with consumption of inositol at levels considerably in excess of those now consumed by humans.

Despite the demonstration of signs of inositol deficiency in several animal species, no requirement for dietary inositol in man has been established. The high inositol concentration of human milk, and the relatively low concentration in cow's milk, together with the inadequate understanding of inositol metabolism and utilization during neonatal development in animals and man, suggest the possibility that basic infant formulas, particularly milk-free preparations, may contain less inositol than is necessary for optimal growth and development. The rationale for adding inositol to certain infant formulas is based on an assumption that the greater intake insures against a possible deficiency of inositol during early growth and development, when the need for dietary sources of inositol might be maximal. However, if future investigations should clearly demonstrate a need for additional inositol in infant formulas, and if that need should be greater than the amount now added, it is unlikely that the recommended level of intake would exceed that supplied by human milk.

The Select Committee has considered the foregoing and concludes that:

There is no evidence in the available information on inositol 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 the future.
VI. REFERENCES CITED


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 of Chemistry and Special Assistant to the Vice Chancellor for Research and Development, 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), Research Associate Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

C. Jelleff Carr, Ph.D., Director
Kenneth D. Fisher, Ph.D., Associate Director
Richard G. Allison, Ph.D., Research Associate
Samuel B. Detwiler, Jr., Research Associate
Andrew F. Freeman, Research Associate
Frederic R. Senti, Ph.D., Research Associate
John M. Talbot, M.D., Research Associate

Report submitted by:

June 3, 1976
Date

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