EVALUATION OF THE HEALTH ASPECTS OF CHOLINE CHLORIDE
AND CHOLINE BITARTRATE AS FOOD INGREDIENTS

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
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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 of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) or prior sanctioned food substances 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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Life Sciences Research Office
FASEB
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I. INTRODUCTION

This report concerns the health aspects of using choline chloride and choline bitartrate 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; 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 22, 1976 (41 FR 3332 to 3334) 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 choline chloride and choline bitartrate as food ingredients. The Select Committee received no requests for such a hearing on choline chloride and choline bitartrate.

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, 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

*The document (PB-223 845/9) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
risk to the public health. While the Select Committee realizes that a conclu-
sion 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 Com-
mittee, 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 choline chloride and choline bitartrate
and submits its interpretation and assessment in this report, which is
intended for the use of FDA in determining the future status of these sub-

II. BACKGROUND INFORMATION

Choline, 2-hydroxyethyl trimethylammonium hydroxide, is a natural
constituent of foods as a component of lecithin, sphingomyelin, and other
compounds. This nutritional factor is widely distributed in plants and animals
and present in highest concentrations in egg yolk, meat, fish, milk, cereals,
and legumes. In animals, choline occurs as a component of the phosphatides,
which are major constituents of the fatty matter of the brain, kidney, and
liver, and it is present in some form in virtually all tissues. The edible seeds,
leafy material, and roots of most plants contain significant amounts of
choline (1, 2).

Commercially, choline salts are generally synthesized from trimethyl-
amine and ethylene chlorhydrin or ethylene oxide (3). Choline bitartrate
(2-hydroxyethyl trimethylammonium bitartrate) and choline chloride (2-hydroxy-
ethyl trimethylammonium chloride) are listed in the Code of Federal Regula-
tions as nutrients and/or dietary supplements that are generally recognized
as safe (GRAS) (4).

The Food Chemicals Codex specifications for food grade choline chloride
and choline bitartrate provide that neither should assay less than 98 percent
of the anhydrous salt or contain more than 3 ppm arsenic, 10 ppm lead, and
20 ppm heavy metals (as lead) (5). Choline chloride is a colorless or white,
hygroscopic, crystalline powder. Choline bitartrate is a white crystalline
powder having an acidic taste. Both salts usually have a faint odor of tri-
methylamine and are very soluble in water.

Choline chloride and choline bitartrate were used in food in the
United States at least by 1960 (6). Between 1960 and 1970 there was a
slightly greater than twofold increase in the amount of choline chloride used each year in foods; the amount of choline bitartrate used was essentially unchanged during the same period (Table I).

TABLE I

Consumption of Choline Salts Based on Total Quantity Used Annually in Foods (6)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative amounts used&lt;sup&gt;a&lt;/sup&gt; 1970/1960</th>
<th>Total used&lt;sup&gt;b&lt;/sup&gt; 1970 kg</th>
<th>Total used, calculated as choline&lt;sup&gt;c&lt;/sup&gt; kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline bitartrate</td>
<td>1.1</td>
<td>28,000</td>
<td>12,000</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.3</td>
<td>6,900</td>
<td>5,200</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based only on the reports from those respondents to the National Research Council (NRC) survey who submitted information for both 1960 and 1970 (6).

<sup>b</sup> Total usage is based on the sum of kilograms used in foods as supplied by NRC and the Flavoring Extract Manufacturers' Association (FEMA) recalculated to 100 percent from survey data that the NRC estimated to represent about 60 percent of the actual usage.

<sup>c</sup> Choline bitartrate = 41 percent choline; choline chloride = 75 percent choline.

According to the comprehensive survey conducted by the National Research Council subcommittee (6) choline chloride and choline bitartrate are currently used in infant formulas and milk products in the concentrations (weighted means of usual levels of use) shown in Table II. Choline salts are added to these foods to assure the presence of choline in an amount approximating that present in milk. Cow's milk is reported to contain 15 mg per 100 ml or about 0.015 percent as choline chloride (3); another estimate is slightly lower, 0.012 percent as choline chloride (7). Human milk is reported to have an average content of 10 mg of choline chloride per 100 ml (7) or 5 to 14 mg total choline per 100 ml (8).
TABLE II

Level of Addition of Choline Salts to Foods by Food Category (6)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Choline bitartrate</th>
<th>Choline chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weighted mean *</td>
<td>Weighted mean *</td>
</tr>
<tr>
<td></td>
<td>percent</td>
<td>percent</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>0.04</td>
<td>None added</td>
</tr>
<tr>
<td>Infant formulas</td>
<td>0.11 b</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Level of addition of choline salts is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean see Section X and Exhibit 50 of reference 6.

bAll analyses of choline bitartrate content of infant formulas available to the Select Committee are considerably below 1,100 mg per liter. It is probable that 0.11 percent refers to concentration in dry formula powder rather than to concentration of the choline salt in the diluted formulas, as consumed.

III. CONSUMER EXPOSURE DATA

The National Research Council subcommittee has estimated the average daily intake of the two choline salts as dietary supplements, as shown in Table III (6). The Select Committee has converted these figures to intakes per kilogram of body weight, and included them in this report for comparative purposes. However, because some of the figures in Table III are derived from uncertain data in Table II (see footnotes in that table), alternative means for estimating intakes of the choline salts have been explored.
### TABLE III

Possible Average Daily Intake of Added Choline Salts by Age Group (6)\(^a\)

<table>
<thead>
<tr>
<th>Substance</th>
<th>0-5 Months</th>
<th>6-11 Months</th>
<th>12-23 Months</th>
<th>2-65+ Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>mg/kg</td>
<td>mg</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>382</td>
<td>76</td>
<td>104</td>
<td>13</td>
</tr>
<tr>
<td>Choline chloride(^b)</td>
<td>32</td>
<td>6</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Possible average daily intake of choline salts by age group was estimated from the data in Table II and other factors as explained in the exhibits cited in the footnote to Table II. Calculated intake, mg/kg body weight, was based on an average weight of 60 kg for an adult (9) and 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).

\(^b\) Choline chloride is only used in infant formulas and special dietary formulas such as protein hydrolysate and tube feeding formulations.
Essentially all of the choline chloride used by the food industry is added to milk-free infant formulas at a concentration of about 120 mg per liter, and choline bitartrate is consumed by infants only in one milk-based formula at a concentration of about 220 mg per liter.* Accordingly it is possible to estimate the consumption of choline salts by babies less than 1 year old by another method. Of the 3.1 million babies born each year (11), approximately 10 percent receive milk-free formulas during the first 4 months, 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 18 mg of choline chloride per 100 kcal of milk-free formula, it may be calculated that approximately 4,500 kg of choline chloride are consumed by infants in milk-free formulas. This quantity accounts for the major portion of the 6,900 kg of choline chloride added to foods in 1970 (Table I). On this basis, the infants (up to 6 months old) fed milk-free formulas receive about 70 to 80 mg of added choline chloride per day (about 15 mg per kg per day); older infants receive progressively decreasing amounts from this source. These intakes, which are more than double those indicated in Table III, are regarded as reasonable since they compare favorably with intakes of 90 and 60 mg of naturally occurring choline chloride consumed per day by infants of the same age receiving milk-based formulas and breast milk, respectively.

Choline bitartrate, because of its low hygroscopicity, is used in certain powdered and special diets for children and adults. Its major use is in a milk-based infant formula marketed as a concentrated or ready-to-feed liquid. Calculations similar to those made with milk-free formulas, based on a concentration of 33 mg of choline bitartrate per 100 kcal of formula, indicate that about 14,900 kg of choline bitartrate can be accounted for by addition to this formula. This is more than half of the total amount estimated to have been added to foods in 1970 (Table I). On this basis, the infants (up to 6 months of age) fed this milk-based formula, receive about 125 to 160 mg of added choline bitartrate per day (about 30 mg per kg per day); older infants receive progressively decreasing amounts from the same source. These intakes, which are half those given in Table III, are regarded as the more realistic in the light of available information.

The remaining half of the choline bitartrate used in foods in 1970, is less readily accounted for. The Select Committee does not have information on the amounts employed in special diets and dietary supplements for older children and adults.

Although the human requirement for choline is not known, it has been estimated that the daily adult intake of choline in an average mixed diet is of the order of 150 mg or more (2). The effects of choline deficiency are discussed by Sebrell and Harris (3). It is believed unlikely that a choline deficiency can occur, except possibly in infants or young children with

* Based on data supplied by manufacturers of infant formulas.
substantially protein-deficient diets rich in highly refined products and lacking in other lipotropic agents (13). The Joint FAO/WHO Expert Committee on Food Additives has not specified an acceptable daily intake (ADI) for choline salts (14).

IV. BIOLOGICAL STUDIES

Metabolism

Choline has several important physiological functions. It is a lipotropic agent and appears to be the most active compound in preventing or reversing experimentally-induced fatty infiltration of the liver in laboratory animals. As a constituent of lecithin, it is essential for the normal transport of fat. Choline is also a methyl group donor in intermediary metabolism, supplying methyl groups for the biosynthesis of methionine, and for certain one-carbon metabolites via folic acid and cyanocobalamin. Choline is also a precursor of acetylcholine through its acetylation by the enzyme, cholineacetylase (3).

Choline is present in animal tissues in much higher concentrations than the substances usually considered to be vitamins. It can be synthesized in vivo from serine provided that there is an adequate supply of the methyl donor methionine. Thus, in order to produce a choline deficiency in experimental animals, it is necessary to restrict the dietary intake of choline and of methyl donors such as methionine (3).

Although young rats, young pigs, calves, and other animals develop fatty livers and hemorrhagic lesions of the kidney on choline restricted diets, the relevance of these findings to the question of need for dietary choline in human nutrition is not clear. It has been reported that choline decreases the concentration of fat in the livers of children with protein-calorie malnutrition (15). As pointed out in Olson's review (16) of W. H. Griffith's work on choline in children "It is becoming increasingly clear that the amino acid lack may greatly limit the transport of lipids not only of triglycerides but trace lipids such as the fat-soluble vitamins." Olson also states that "It appears that choline lack blocks the assembly of low density lipoproteins and this, in turn, results in the accumulation of cytoplasmic triglyceride in the liver cell."

Choline chloride is well absorbed from the intestinal tract of rats; its absorption is not appreciably changed by ingestion of antibiotics (17). Other workers report that a portion of orally administered choline in rats
and human patients is converted to trimethylamine and its oxide by intestinal bacteria (18). In man, the free choline concentration in blood and tissues is very low. Ingested choline appears to be quickly metabolized or converted to choline-containing phospholipids (19).

The Joint FAO/WHO Expert Committee on Food Additives has noted that choline, while pharmacologically active when administered parenterally, is without appreciable adverse action when given orally. The Committee concludes that the use of choline as a food additive presents no toxicological hazards (20).

**Short-term studies**

Estimates of the oral LD₅₀ of choline chloride for rats vary from 3,400 to 6,700 mg per kg (2, 21, 22). The intraperitoneal LD₅₀ dose is about a tenth of these values (23). Intravenous choline chloride is much more toxic; 1.1 mg per kg is lethal in rabbits and 35 mg per kg is lethal in cats (21).

Feeding experiments with dietary supplements of choline added to the basal diet have been carried out in various species. Chicks given a supplement of choline chloride of 0.15 percent (providing a total dietary choline of 0.29 percent) grew well; growth was depressed without any choline supplement, and growth increased proportionally with added choline up to 0.15 percent (24). Over three times as much choline was required for maximum growth of chicks when they were fed a diet containing 64 percent protein as when they were fed 13 percent protein. A high protein diet markedly increased the incidence of perosis in chicks fed a choline-deficient diet. When diets containing 13 percent protein were supplemented with 0.84 percent methionine, the choline required for prevention of perosis and support of maximum growth rate was 800 mg per kg of diet, as compared with 300 mg per kg when the basal diet was fed without the high level of methionine. These studies show that the choline requirement of chicks is elevated by high dietary levels of methionine and protein (25).

Young mice weighing about 10 g, given 8 to 12 mg per day of choline chloride (equivalent to about 1 g per kg of body weight) as a supplement to the basal diet (choline content not stated) for 30 days, showed no greater growth rate than controls (26).

The effects of various levels of added choline chloride in the diet of rats was studied (27) for 3 to 4 months; growth rate was depressed by 20 percent with 2.7 percent choline chloride, by 45 percent with 5 percent choline chloride, and by 100 percent with 10 percent choline chloride (equivalent to nearly 5 g of choline chloride per kg of body weight). As much as one percent choline in the diet had no effect on growth rate.
Food consumption was reduced in the rats receiving 5 percent or 10 percent choline, but no consistent histological changes were found that could be attributed to choline.

Adult rats tolerated a choline chloride supplement of 1.35 percent (equivalent to about 700 mg per kg) to the basal diet for 20 days (28). Higgins et al. (29) gave a total dose of 160 mg per day of choline chloride by stomach tube and in the drinking water to young rats (equivalent to about 1 g per kg) and observed that the rats developed a reddish-brown hair pigmentation in 3 weeks. A diet enriched with yeast extract or liver extract prevented the pigmentation, as did increasing the dietary content of dextrin.

Rabbits given 0.12 percent choline chloride (equivalent to about 36 mg per kg) with a choline-deficient diet were protected from the effects of choline deficiency which include fatty, cirrhotic livers, edema, and early death (30).

A macrocytic, hyperchromic anemia was produced in dogs on a diet of dog chow and rolled oats given a daily dose of 10 mg per kg choline chloride by stomach tube for 25 days; the onset of the anemia was earlier if 10 mg per kg doses were given two or three times a day (31).

Special studies

Choline, through the lipoproteins, plays an important role in the maintenance of normal fat transport in the body. It effects the removal of, or decreases the deposition of, liver fat in the rat (16). Hemorrhagic kidney degeneration in the rat is produced in choline deficiency (2, 32). The action of choline in reducing the lipid content of experimentally-induced fatty livers in several species amply demonstrates its lipotropic properties; the species include rats (33), mice (34), rabbits (35), chickens (36), and monkeys (2).

Dietary choline decreases cholesterol levels of the blood and liver of rats and rabbits fed high cholesterol diets (35, 37) but this effect is only partial. It has been suggested that choline increases the conversion of cholesterol to bile acids. These effects and the lipotropic action of choline have led to the suggestion that choline might be of therapeutic value in patients with fatty livers or hypercholesterolemia. However, there is no convincing evidence that dietary supplementation of choline salts is of value under these conditions if the diet is adequate in protein and other essential nutrients (2).

No long-term toxicity studies of choline, or studies of its carcinogenic, mutagenic, or teratogenic potential, have come to the attention of the Select Committee.
V. OPINION

Despite the significant endogenous synthesis of choline, it is an important dietary constituent for normal growth and well-being in all of the species that have been studied. Its metabolic role in fat transport is established. In addition, it is a contributor of methyl groups in essential transmethylation reactions in the animal body. However, the precise nutritional significance of dietary choline for man is not clear.

In evaluating the possible effects of choline salts as added food ingredients, particularly on the infant which is the largest consumer of these supplements, the Select Committee recognizes the approximate nature of current estimates of total daily intake and the scarcity of chronic toxicity data. The latter information might be supplied by studies on preweanling animals. However, the available evidence raises no suspicion that choline chloride and choline bitartrate have harmful effects at dosage levels that are several orders of magnitude greater than the most generous current estimates of human intake.

The Select Committee has weighed the foregoing and concludes that:

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


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