EVALUATION OF THE HEALTH ASPECTS OF COPPER GLUCONATE,
COPPER SULFATE, AND CUPROUS IODIDE
AS FOOD INGREDIENTS

1979

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 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
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I. INTRODUCTION

This report concerns the health aspects of using copper (cupric) gluconate, copper (cupric) sulfate, and cuprous iodide 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. In addition, the Select Committee was provided with a review prepared by LSRO (2)* of recent literature on the health aspects of copper salts as food ingredients. 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, Oak Ridge, 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 March 7, 1979 (44 FR 12506-12509) 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 copper gluconate, copper sulfate, and cuprous iodide as food ingredients. The Select Committee received no requests for such a hearing.

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 utilization of animal experimentation data. FDA (3) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*These documents (PB-241 961/2) and (PB-275 749/0) 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. The Committee attempts to reach a judgment even when the information is limited. There are, of course, instances in which the data are so insufficient that no conclusion can be made. 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 copper gluconate, copper sulfate, and cuprous iodide 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

Copper is ubiquitous, being found in the soil, atmosphere, water, plants, and animals (4). It is the prosthetic group for many enzymes and other biologically important proteins, making it an essential nutrient for most plants and animals (5). The most prominent manifestation of copper deficiency in animals is severe anemia, although disturbances of bone and elastic tissue development and of the central nervous system also may result (5). These disturbances include depressed growth, bone disorders, depigmentation of hair, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, cardiovascular defects, and gastrointestinal signs (6). Because of the widespread distribution of this element in the ecosystem, copper deficiency is relatively rare in man, although it has been reported in infants who were severely malnourished or on highly restrictive diets (7,8) as well as in some persons with severe intestinal disturbances (9). A relatively rare genetic disease in the infant, Menkes' kinky hair syndrome, is believed due to copper deficiency produced by a basic defect in its absorption (10). It is characterized by hypothermia, progressive mental deterioration, defective keratinization of hair, low serum and liver copper content, metaphysseal lesions, and degenerative changes in aortic elastin.

Various copper salts are authorized by the Code of Federal Regulations (3) for specific uses in food. Copper (cupric) gluconate at concentrations not exceeding 0.005 percent is classified by the FDA as a nutrient and/or dietary supplement with GRAS status [21 CFR 182.5260]. Cuprous iodide is similarly classified when used as a source of dietary iodine in table salt at levels not to exceed 0.01 percent [21 CFR 182.5265]. Copper (cupric) sulfate is GRAS in paper and paperboard products used in food packaging [21 CFR 182.90]. Unpublished GRAS authorizations for cupric sulfate include its use in special dietary foods, in infant formulas (1 ppm) and, in common with copper (cupric) chloride, copper (cupric) oxide and cupric gluconate, as a nutritional supplement in capsules and tablets not to exceed 2 mg copper per day (11). Cupric chloride and cupric oxide are not evaluated in this report as they are not authorized as GRAS food ingredients.

In addition to these GRAS authorizations, copper compounds are permitted for a variety of regulated uses (3,12,13): as a supplement in animal feeds [21 CFR 582.80]; to clarify and stabilize wine [27 CFR 240.1051]; to control aquatic plants in potential sources of potable water [21 CFR 193.90]; as a stabilizer for polymers contacting food [21 CFR 178.2010]; as a component of adhesives contacting food [21 CFR 175.105]; as a preservative in paper and paperboard materials [21 CFR 176.170]; as a preservative for wood contacting food [21 CFR 178.3800]; as
a slimicide with paper and paperboard products [21 CFR 176.300]; as an accelerator in rubber articles [21 CFR 177.2600]; as pigment or colorant in resins and coatings [21 CFR 175.300 and 21 CFR 177.1460]; and as an accelerator in polyester resins [21 CFR 177.2420].

Of all the copper salts utilized as food ingredients, only cupric gluconate, Cu[CH₂OH(CHOH)₄COO]₂, is described in the Food Chemicals Codex (14). It is very soluble in water but only slightly soluble in ethanol. The food grade compound must contain not less than 98.0 nor more than the equivalent of 102.0 percent of CuC₁₂H₂₂O₁₄. It must contain not more than 3 ppm of arsenic, 10 ppm of lead or 1 percent of reducing substances.

Cupric sulfate is normally hydrated (CuSO₄·5H₂O) and occurs as deep blue crystals. It is very soluble in water and is slightly acidic. It has a nauseous, metallic taste. Specifications for food grade materials have not been established, but the National Formulary (15) requirements specify a loss in drying of 33 to 36.5 percent and a total alkali and alkaline earth content of not more than 0.3 percent.
III. CONSUMER EXPOSURE DATA

The copper intake of man comes almost entirely from food, although under certain circumstances drinking water may contribute significant amounts. A single day's diet may contain 10 mg or more (4) but most estimates of the average adult daily copper intake range from 2 to 4 mg (6). The richest sources of copper are shellfish, liver, nuts, wheat germ, legumes and cocoa with concentrations ranging from 20 to 400 ppm (4,6). Dairy products, nonleafy vegetables, most fresh fruits and refined cereals generally contain less than 2 ppm (6). Drinking water is usually a minor source of copper, although some soft waters, which can corrode copper pipes and containers, may contribute 1 mg or more to the daily intake (16). A 1969 Public Health Service survey of 969 urban water supplies reported 11 cases where levels of 1 ppm (1 mg per liter) were exceeded, with one supply containing 8.35 mg per liter. Small amounts of atmospheric copper can be detected in rural and metropolitan areas, but the intake from this source is negligible, even in the vicinity of copper smelters (4).

The body stores of normal adults are about 100 to 150 mg copper (5). A satisfactory balance can be maintained on an intake of 2 mg per day, or about 30 μg per kg body weight (17). The World Health Organization (WHO) has recommended this amount as the daily intake for an adult, with somewhat larger amounts for infants (80 μg per kg per day) and children (80 μg per kg for young and 40 μg per kg for older) (18).

Level of addition data (Table I) are based on information supplied to a National Research Council (NRC) subcommittee (19) by manufacturers who reported adding either cupric gluconate or cupric sulfate to at least one food in a given category. Only information on the former compound had been requested, but several manufacturers volunteered the indicated data on cupric sulfate addition.

The level of addition shown in Table I does not indicate that any particular product contains that amount of copper salt nor does inclusion of a food category within this table mean that cupric gluconate or sulfate is present in a majority of the foods in that category. Actually, many of the foods within the listed food categories may contain none or much lower levels of the added salt. Thus, the data presented in Table I may represent significant overestimates of the actual average levels.

The NRC subcommittee (19) has used the values shown in Table I together with data on the mean frequency with which foods in each category are consumed (provided by the Market Research Corporation of America) and the mean portion size of these various foods (as determined by the U.S. Department of Agriculture) to calculate the possible average intake of cupric
**TABLE I**

Level of Addition of Cupric Gluconate and Cupric Sulfate to Foods by Food Category (19)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Cupric gluconate</th>
<th>Cupric sulfate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Copper content</td>
</tr>
<tr>
<td>Milk products</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Processed fruits, juices and drinks</td>
<td>4*</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Soft candy</td>
<td>45</td>
<td>7</td>
</tr>
<tr>
<td>Snack foods</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>47</td>
<td>7</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>4</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Level of addition is the weighted mean of the levels reported by manufacturers as their usual addition to one or more food products in a food category. For discussion of weighted mean, see Section X and Exhibit 50 of reference 19. For each of the categories listed, three or fewer firms responded to the survey.

*The original value reported in the NRC Survey was in error (20). The corrected value has been used in calculating the possible average daily intake given on page 7.
gluconate. These calculations suggest that the possible average daily intake of cupric gluconate for individuals over 2 years of age is 1.3 mg or 0.2 mg elemental copper. Data on cupric sulfate addition was too sparse to allow a similar calculation. As pointed out by the NRC subcommittee (19), this procedure is likely to lead to estimates of intakes that are overstated, often by considerable amounts.

Such an overestimation is suggested by reports of the total amount added annually to food by the manufacturers. From data supplied to the NRC (19), it estimated that 2200 kg of cupric gluconate had been added to foods in 1970*. This represents an average per capita consumption of 0.03 mg cupric gluconate or about 0.004 mg added copper per day. Based on reports from respondents to the NRC survey who submitted information for both years, usage of cupric gluconate in 1970 was 5.7 times that of 1960. No information is available on the total amount of cupric sulfate added to food, but it is believed to be less than the added cupric gluconate. The amount of cupric sulfate used in paper and paperboard materials in food packaging is also unknown, but is thought to make only a small contribution to the total copper intake.

Although cuprous iodide is accorded GRAS status in iodized salt, the Select Committee could find no evidence that it is actually used for this purpose. The Food Chemicals Codex (21) states that salt labeled as iodized must contain from 0.006 to 0.01 percent potassium iodide. Officials of the Salt Institute which represents the American salt producers were not aware of any member using cuprous iodide as an iodizing agent (22).

The Joint FAO/WHO Expert Committee on Food Additives (23) has suggested the maximum acceptable daily intake of copper in man should be 0.5 mg per kg body weight, when the dietary levels of constituents known to affect copper metabolism, such as zinc and molybdenum, are within acceptable limits. Supplementing sheep, swine, and poultry feeds with copper salts, especially for animals on low molybdenum diets, may increase their liver copper severalfold (4). To reduce the copper intake of persons consuming foods prepared from such livers, the FDA in 1973 proposed that the permissible level of supplementary copper in feed be lowered from 250 to 15 ppm (24).

*In a 1975 NRC survey (95), four companies reported that 4500 kg were used. This may be compared with 1300 kg reported by seven companies in a 1970 NRC survey (19) which was estimated to have included 60 percent of total usage.
The amount of sulfate, gluconate or iodide consumed in the form of added copper salts is trivial compared with that produced physiologically or found in normal diets (25-27).
IV. BIOLOGICAL STUDIES

Absorption, distribution, metabolism, and excretion

Absorption. The chief sites of copper absorption are the stomach and duodenum (28). Approximately one-third to one-half of the ingested copper is normally absorbed, but the amount is strongly influenced by various factors, including the chemical form of the ingested copper, the presence of other trace inorganic elements and especially the pH of the intestinal contents (9,29-31). Little is known of the chemical forms in which copper exists in foods although it is presumably present both in ionic form and as metallo-organic complexes. Certain small, water-soluble, organic compounds of copper are more readily absorbed than its inorganic salts. Mills (32) has shown that feeding such compounds extracted from pasture herbage resulted in greater copper storage in deficient rats than equivalent amounts of cupric sulfate. Similarly, the sodium salt of a copper-allylthiourea-benzoic acid complex was more effective than inorganic copper in stimulating growth and hematopoiesis in rabbits (33) and ethylenediaminetetraacetic acid (EDTA) increased the utilization of copper in chicks (34). Ascorbic acid, on the other hand, reduced significantly the absorption of copper from isolated segments of the rat gastrointestinal tract (35).

Copper absorption is also reduced when the pH of the intestinal contents is raised or the solubility of the copper compound is reduced. Copper absorption in sheep was markedly reduced by feeding large amounts of calcium carbonate or ferrous sulfide (36). The former compound raised the intestinal pH and the latter formed insoluble cupric sulfide. High dietary levels of molybdenum also reduced absorption of copper, probably by forming an insoluble copper-molybdenum complex (37).

A copper-binding protein, which seems essential for copper absorption, has been detected in the intestinal mucosa of cows (38), chicks (39) and rats (40). This low molecular weight protein binds copper, cadmium and zinc competitively and is similar to, if not identical with, metallothionein (38). Cadmium and zinc (38) and ascorbic acid (41) significantly decreased copper binding by metallothionein and each reduces the absorption of copper. The increased absorption of copper in zinc-deficient rats is consistent with the theory that zinc actively competes for binding positions on metallothionein or a similar protein (42). On usual dietary rations, this intestinal copper transport system is saturated (43) so that much of the ingested copper remains unabsorbed and is excreted in the feces (6).

Distribution. After absorption, copper becomes loosely bound to serum albumin (44) and is rapidly transported to the liver, bone marrow, and other tissues for storage and incorpora-
tion into cuproproteins (4). Human whole blood contains 1 μg copper per ml, about equally distributed between plasma and erythrocytes (5). Approximately 90 percent of the copper in mammalian plasma is in the form of ceruloplasmin, a blue glyco-protein, containing 0.3 percent copper and 8 percent carbohydrate with a molecular weight of about 150,000 (4,5). In contrast, most of the copper in the erythrocytes is associated with another protein, erythrocuprein (5). Ceruloplasmin is synthesized in the liver while erythrocuprein is produced in the bone marrow normoblasts (44,45). In man, the copper content of liver, brain, hair, nails, heart, kidney, retina and corneal epithelium is relatively high (15 to 25 ppm dry weight) while that of skin, muscle and bone is quite low (2 to 5 ppm) (4,46).

Abnormal accumulation of copper in the brain, liver, kidneys and eyes occurs in Wilson's disease (hepatolenticular degeneration) (47). This disease is a well-recognized clinical entity, characterized by a familial tendency, incoordination, ataxia, progressive mental deterioration and hepatic cirrhosis. The plasma concentration of copper and of ceruloplasmin is markedly reduced as is the excretion of copper in the feces. Urinary excretion of copper, on the other hand, is increased. The high concentrations of copper in the brain and liver are of particular importance and are believed responsible for the dysfunctions of these organs. Treatment of the disease is broadly directed toward the removal of the excess body stores of copper by using chelating agents to increase urinary excretion (48).

**Metabolism.** The number of biochemical systems with which copper is associated suggests its involvement in a broad range of biochemical functions. Approximately 20 mammalian copper proteins have been isolated although it is possible that some of these may prove to be identical (4). Most seem involved in oxidative reactions. Cytochrome c oxidase, a copper containing enzyme, is the terminal member of the respiratory chain which transports electrons to oxygen. Ceruloplasmin stimulates the oxidation of ferrous to ferric iron. Erythrocuprein (superoxide dismutase) catalyses the breakdown of superoxide radicals to oxygen and hydrogen peroxide (5). Other copper enzymes catalyze the oxidation of such physiologically important compounds as dopamine, catecholamines, tyrosine, uric acid, galactose and mono- and diamines.

**Excretion.** As indicated above, the intestinal capacity for copper absorption is limited and much of ingested copper remains unabsorbed. Most of the copper actually absorbed is not utilized by the body but is excreted in the feces, by way of the bile. Cartwright and Wintrobe (9) estimated that of the 10 mg or more copper normally consumed daily by man, about 0.6 to 1.6 mg will be absorbed and 0.5 to 1.2 mg of that absorbed will be
excreted in the bile. Only 10 to 60 μg appear in the urine. Similarly, Bowland et al. (49) gave 64Cu as cupric sulfate or sulfide to pigs by mouth and were able to recover less than 0.1 percent of the labeled copper in the urine. Under normal conditions, 90 percent or more of ingested copper is excreted in the feces. Schroeder et al. (50) reported that on a diet containing 3.7 mg copper, man would excrete 3.64 mg in the feces and 60 μg in the urine. Negligible amounts of copper are lost in sweat (51). Concentrations in human milk appear to be approximately 0.6 mg per l during the early weeks of nursing and somewhat less thereafter (6). An average of 0.55 mg copper is lost during each menstrual period (52).

Acute toxicity

Table II summarizes the available oral LD50 data for copper compounds in the rat, the only animal for which such values could be found. No acute toxicity data were available for cuprous iodide or cupric gluconate.

TABLE II

<table>
<thead>
<tr>
<th>Compound</th>
<th>Salt content</th>
<th>Copper content</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupric acetate</td>
<td>710</td>
<td>225</td>
<td>53</td>
</tr>
<tr>
<td>Cupric carbonate</td>
<td>159</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Cupric chloride</td>
<td>140</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>Cupric nitrate</td>
<td>940</td>
<td>247</td>
<td>53</td>
</tr>
<tr>
<td>Cuprous oxide</td>
<td>470</td>
<td>418</td>
<td>53</td>
</tr>
<tr>
<td>Cupric sulfate (anhydrous)</td>
<td>300</td>
<td>119</td>
<td>54</td>
</tr>
<tr>
<td>Cupric sulfate (hydrated)</td>
<td>960</td>
<td>244</td>
<td>53</td>
</tr>
</tbody>
</table>

A number of cases of acute poisoning in man with cupric sulfate have been reported, but the toxic dose is uncertain. Chuttani et al. (55) estimated that the poisoned patients they observed had ingested from "1 gram to 4 ounces" of cupric sulfate. However, much smaller doses of copper can apparently
elicit toxic symptoms. Wylie (56) reported that ten persons at a party exhibited weakness, abdominal cramps, dizziness and headache after consuming from one-half to three glasses of a cocktail prepared in a container with exposed copper lining. On the basis of a subsequent test, it was estimated that the affected persons had ingested 5 to 32 mg copper. Other reports recount poisoning after drinking indeterminate amounts of punch (57), soft drinks (58) or tea (59,60) with copper concentrations of 35 to 260 mg per liter.

The sequelae of serious cupric sulfate intoxication are vomiting, hemolysis, hepatic necrosis, gastrointestinal bleeding oliguria, azotemia, hemoglobinuria, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma or death (4,55). Cupric sulfate has been widely used as an emetic, especially in children, to purge the stomach of ingested poisons (61). This practice has been criticized by Holtzman and Haslam (62) who found significant elevations of serum copper in children after receiving 250 mg cupric sulfate. A fatality was reported in an adult woman given 10 ml of a 10 percent solution of cupric sulfate (about 15 mg per kg) as an emetic in the treatment of alcohol-diazepam intoxication (63). The solution failed to elicit vomiting and despite gastric lavage, symptoms of copper toxicity developed, including hemolytic anemia, hemoglobinuria, hepatic and renal failure and massive gastrointestinal bleeding. The patient died 6 days after the administration of cupric sulfate.

Acute copper intoxication has also been recognized as a complication of hemodialysis (64). Klein et al. (65) reported three patients who developed a characteristic syndrome of gastrointestinal symptoms, leukocytosis, metabolic acidosis, hemolysis, pancreatitis and myoglobinemia after hemodialysis. Each of the patients was markedly hypercupremic. The dialysate mixture was prepared from deionized or tap water which then passed through a copper heating coil. In the two patients whose sera were measured before and after dialysis, the copper concentrations increased from 2.02 to 26.55 mg per l in one and from 0.66 to 13.12 mg per l in the other. Two days after hemodialysis the copper content of blood was still elevated, with the erythrocytes more markedly affected than the serum.

Short-term studies

Numerous studies have been reported on the feeding of copper salts, especially to domestic animals and fowl. There is also an extensive literature on the role of other ions, e.g., zinc, iron, molybdenum and sulfate in modifying the animals' response to copper intake (4). Animals vary widely in their sensitivity to copper. Sheep appear to be particularly sensitive to the toxic effect of copper salts, while the pig and horse are relatively insensitive. Sheep given daily oral doses of 30 mg cupric sulfate (7.6 mg copper) per kg body weight developed
hemolytic crises within 26 to 73 days (66). Severe morpho-
logical changes were seen in liver, kidney and brain. Tissue
levels of both copper and iron were markedly elevated. Approxi-
mately twice this level (about 15 mg copper per kg body weight
per day) fed to yearling ponies for 6 months produced no visible
effects (67). Pigs on even higher relative intakes (about 65 mg
copper per kg body weight daily) actually gained more weight
than their controls during a 9-week feeding study (68). In fact,
it had long been the practice to add copper at levels up to 250
ppm in swine and poultry diets as a means of accelerating growth
rate (69).

However, high dietary levels of copper may cause a signifi-
cant reduction in the weight gain of chicks (70). One-day-old
Hubbard and Leghorn chicks received a basal diet furnishing about
6 mg copper per kg body weight daily or one supplemented with
cupric sulfate to provide approximately 20 times this amount.
After 14 days, the chicks on the higher copper diet showed only
half the weight gain of those on the basal diet.

Kojima and Tanaka (71) administered cupric sulfate in
drinking water for 15 days to groups of 5 male dd mice at con-
centrations of 0.006 to 1.6 percent. Animals receiving the 0.4
percent solution (about 100 mg copper per kg body weight per day)
showed a lower weight gain than the controls. At the highest
level (400 mg copper per kg per day), only one of five mice
survived the test period. Liver copper increased in all groups
of mice ingesting more than about 10 mg copper per kg daily.
At an intake of 200 mg copper per kg daily, the liver contained
178 µg per g wet weight in contrast with 4 µg per g in controls.

Male and female Sprague-Dawley rats were fed diets pro-
viding about 53 or 160 mg copper per kg body weight in the form
of cupric gluconate or cupric sulfate (72). At about the 12th
week, the weight gains of animals on the higher copper intake
began to fall below those of the control rats and those on the
lower copper diet. This effect may have been due, at least in
part, to a decreased food intake, for the gain in weight per g
of food consumed was similar for all groups. Cupric gluconate
exerted a greater adverse effect on growth than cupric sulfate.
Over 80 percent (39 of 47) of the rats on the high cupric
gluconate diet died before the 35th week of treatment in contrast
with 25 percent (12 of 48) deaths among the animals receiving
the same amount of copper as cupric sulfate. A marked increase
in copper deposition in the liver was noted in animals receiving
either copper salt, but the concentration in those on the cupric
gluconate supplement was almost twice that of the cupric sulfate
fed rats.

Copper toxicosis occurred rapidly in rats receiving
even higher dietary supplements in the form of cupric sulfate
(73). All rats receiving 4.0 g copper per kg diet (about 400
mg copper per kg body weight per day) died during the first week on this diet and 12.5 percent of the rats receiving one-half this intake died during the fourth week.

Male and female beagle dogs were fed cupric gluconate at levels of 0.012, 0.06, and 0.24 percent of their diets for 6 or 12 months. These dietary levels approximate dosages of 0.5, 2.4 and 9.6 mg copper per kg body weight daily (74). Food consumption and gains in body weight were similar for control and treated groups. One of 12 dogs receiving the 0.24 percent supplement for 12 months showed minimal liver function change which was reversible when cupric gluconate was removed from the diet. Accumulation of copper in liver, kidney and spleen was detected at the highest dose level. No dog died during the experiment nor was any gross pathology observed that could be attributed to the cupric gluconate.

Long-term studies

Tachibana (75) administered orally 10 ml of 1 percent cupric sulfate to rabbits daily or every other day, for up to 479 days. This represents approximately 12.5 mg copper per kg body weight per administration. Liver damage, somewhat resembling human liver cirrhosis was reported.

Carcinogenicity

No tumors were reported in the experiments described in preceding sections in which cupric sulfate or cupric gluconate were given by mouth to rats, rabbits and dogs for 6 to 18 months.

Schütte (76) reported the development of esophageal tumors of unknown histology in sheep dipped in a mixture of nicotine sulfate and cupric sulfate (about 6 mg copper per kg body weight) for treatment against parasites. Lambs were given this treatment every 3 weeks during their first year of life. About 5 percent developed tumors 3 years later. Very few tumors were stated to occur in sheep not given this treatment.

Parenteral injection of copper salts also has failed to elicit tumors (77,78). The carcinogenic potential of many hepatocarcinogens, including azobenzenes, fluorenes, nitrosamines and ethionine, is reduced in rats fed various copper salts (79). Yamane and Sakai (80) have shown this to be due to a marked elevation of azo reductase activity in rat liver, which accelerates the detoxication of 4-dimethylaminoazobenzene. Both cupric acetate and cupric sulfate were highly effective in increasing this enzymatic activity. However, cupric sulfate given to C57BL/6J or Strain A female mice in drinking water at levels approximating 5 mg copper per kg body weight per day had no effect on the incidence of 7,12-dimethylbenz(α)anthracene-induced adenomas of the lung, lymphomas and breast tumors (81). The cupric sulfate did not prevent the induction of precancerous
lesions in the ovary, but may have delayed the development of granulosa cell tumors.

Mutagenesis

Cupric gluconate was found to be nonmutagenic in various in vitro tests employing Salmonella typhimurium strains TA-1535, -1537, and -1538, and Saccharomyces cerevisiae strain D4 (82).

Cupric sulfate exerted no apparent mutagenic activity on Micrococcus aureus using penicillin and streptomycin resistance as genetic markers (83). Similarly, it had no significant mutagenic activity on a streptomycin-dependent strain of Escherichia coli (84).

Cuprous iodide was nonmutagenic in microbial assays with and without the addition of mammalian activation preparations. The following microorganisms were used in the evaluation: S. cerevisiae, strain D4 and S. typhimurium, strains TA-1535, -1537, -1538, -98, and -100 (85).

Teratogenesis

Cupric gluconate was administered to pregnant albino Swiss-Webster mice by stomach tube on days 6 to 14 of gestation (86). Twenty mice were used at each of the following dosage levels: 0, 0.1, 3.0 and 30 mg per kg per day, equivalent to daily doses per kg of 0, 0.014, 0.42, and 4.2 mg copper. No signs of embryotoxicity or teratogenic potential were demonstrated. The average length and weight of the fetuses, their number per litter and the incidence of skeletal and soft tissue abnormalities did not differ from the control animals. Pregnant albino Wistar rats were given these same doses of cupric gluconate on days 5 to 15 of gestation (87). No significant difference was found between the treated and control rats in any of the parameters analysed.

Two yearling ewes were given 10 mg cupric sulfate per kg per day by mouth (equivalent to about 2.5 mg copper per kg) for the first 45 days of gestation and two others throughout the gestation period (140 to 147 days) (88). One of the two ewes receiving cupric sulfate throughout its pregnancy aborted; the other three gave birth to normal lambs. Verrett (89,90) reported that cupric gluconate is teratogenic to the developing chick embryo when administered via the air cell at preincubation and at 96 h development. Birds with anomalies, especially involving the eyes and beak, were seen at all dose levels above 1.0 mg per kg injected into the air cell at 0 h and above 0.5 mg per kg at 96 h (equivalent to 0.14 and 0.07 mg copper, respectively).
Intravenous injection of copper salts into pregnant hamsters on the 8th day of gestation caused an increase in embryonic resorption as well as developmental malformations in surviving offspring (91). Cupric citrate was more embryotoxic than cupric sulfate. Cupric sulfate was teratogenic in the range of 2 to 10 mg copper per kg body weight and cupric citrate in the range of 0.25 to 4.0 mg per kg.

Reproductive performance

The effect of cupric gluconate on fertility was studied in albino Wistar rats (92). Female rats received 3 or 30 mg cupric gluconate per kg per day (0.42 or 4.2 mg copper equivalent) by stomach tube for 15 days prior to mating with untreated males, during the period of pregnancy and for 21 days postpartum. Male rats received 3 mg cupric gluconate per kg daily (0.42 mg copper) for 60 days prior to mating. One group of males receiving this treatment was mated with untreated females and a second group with females who had also been receiving 3 mg per kg per day for 60 days prior to mating. The administration of cupric gluconate did not impair the fertility potential of the male or female rats. There were no significant differences between control and treated rats with regard to the mean number of fetuses and live pups per litter, implantation sites, resorption sites, duration of gestation, and mean weight per pup. Necropsies of dams and pups of all groups at the end of the 21-day period showed no gross visceral abnormalities.

Other studies

Jecht and Bernstein (93) reported that the motility of twice-washed human sperm cells was inhibited upon exposure to $10^{-4}$M cupric sulfate (6 mg copper per liter) after a latent period of 2 to 4 h. The seminal plasma exerted a protective effect, for sperm in whole semen were generally not affected by tenfold higher concentrations of cupric sulfate.

Elevated serum copper levels occur in many acute and chronic diseases, during pregnancy, with estrogen therapy (48) and in various cancers (94).
V. OPINION

Copper is an essential trace element for most plant and animal species, including man. Its deficiency is characterized by specific biochemical and pathological lesions. The customary adult daily diet provides adequate copper to prevent signs of deficiency. Both copper deficiency and chronic copper intoxication are relatively rare.

The absorption of copper is limited to about one-third to one-half of that ingested under usual circumstances. When large amounts of copper are ingested, the absorptive mechanism becomes saturated and much of the copper remains unabsorbed. Further limitations are imposed by competition for absorption with cadmium and zinc, by organic complexing with ascorbic acid, and by the alkalinity of intestinal contents. Much of the copper that is absorbed is later excreted in the bile so that more than 90 percent of ingested copper is found in feces.

Cupric gluconate, cupric sulfate and cuprous iodide are GRAS in foods for specified purposes: cupric gluconate as a nutrient and/or dietary supplement; cupric sulfate in paper and paperboard products used in food packaging; and cuprous iodide as a source of dietary iodine in table salt.

About 2 mg copper per day is required by the average adult with an acceptable daily intake of 0.5 mg per kg body weight or about 30 mg recommended by international authorities. About 2 to 4 mg copper per day are supplied as natural ingredients in the normal diet. Copper added to food in the form of cupric gluconate is estimated to be about 0.005 mg per capita daily. The amounts added as cupric sulfate or cuprous iodide are unknown but are believed to be less than that from cupric gluconate. Thus, the normal diet supplies several hundred times the amount of copper added to foods. The amount of anions ingested from copper salts added to foods is negligible compared with that produced physiologically or found in normal diets.

Animal toxicity with copper salts was observed only with quantities several orders of magnitude greater than that used as food supplements.

Cupric gluconate, cupric sulfate and cuprous iodide were all nonmutagenic in various microbial tests.

Cupric gluconate and sulfate, as well as other copper salts tested, were noncarcinogenic when given by mouth or parenterally. No reports of carcinogenicity studies on cuprous iodide were available to the Select Committee.
Cupric gluconate produced teratogenic effects in the chick embryo, but not in mice or rats. Cupric sulfate was embryotoxic and teratogenic when injected in large amounts into pregnant hamsters.

In light of these considerations, the Select Committee concludes that:

There is no evidence in the available information on copper (cupric) gluconate or copper (cupric) sulfate 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.

There is no evidence in the available information on cuprous iodide that demonstrates or suggests reasonable grounds to suspect a hazard to the public should it be used at the level and in the manner now authorized.

There is no evidence in the available information on copper (cupric) sulfate that demonstrates or suggests reasonable grounds to suspect a hazard when it is used as an ingredient of paper and paperboard materials in food packaging at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


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