Limits of metabolic tolerance to copper and biological basis for present recommendations and regulations[1,2]

Manuel Olivares and Ricardo Uauy

ABSTRACT Acute copper toxicity is infrequent in humans. The evidence for chronic toxicity is derived principally from patients with Wilson disease and cases of infantile cirrhosis that were related to excessive copper intakes. The evaluation of the safety of a nutrient requires toxicologic studies to determine the limits of safe exposure. The acceptable daily intake (ADI) is calculated by determining the highest no-observed-adverse-effect level (NOAEL). When it is not possible to identify the NOAEL, the lowest observed-adverse-effect level (LOAEL) may be used. For the calculation of the human ADI, the NOAEL or LOAEL obtained is divided by an arbitrary safety factor to provide an adequate margin of security. Drinking water standards have been adopted by the United States, the European Community, the World Health Organization, and other countries. The upper limits of copper concentration in water are based on organoleptic considerations and on debatable toxicity information. Given the importance of copper as an essential mineral for human health, it is conceivable that this and other essential minerals with health significance should be approached differently from nonessential minerals. *Am J Clin Nutr* 1996;63:846S–52S.

KEY WORDS Copper, requirements, toxicity, safety limit, drinking water

REQUIREMENTS

The requirement of a nutrient, as defined by the World Health Organization (WHO), is the smallest amount of the nutrient (absorbed or consumed) needed to maintain optimal function and health (1). Copper requirements can be estimated through use of metabolic balance studies (2, 3). However, it is particularly difficult to estimate surface losses by skin, sweat, and other integumentary sources. For infants, children, and adolescents, an additional amount needed for growth must be estimated. These factorial calculations can be biased because individuals can adapt to nutrient intakes. If copper intake is low, copper losses by gut and other sources decrease. The percentage of copper absorbed is regulated by dietary copper intake (4). Absorption increases in response to low copper intake, and conversely it decreases if copper intake is high (5).

Copper requirements can also be studied through use of experimental diets with different mineral intakes, thus determining the minimal copper intake that prevents the development of biochemical abnormalities or functions (2). However, these experimental diets may also have modifications in other nutrients that could affect copper absorption or influence the biochemical or physiologic indexes used in the assessment of copper status. In addition, some biochemical indexes currently used to measure copper nutriture are not sufficiently sensitive for detecting marginal copper status (6).

Another method is to calculate requirements on the basis of epidemiologic studies of nutrient status carried out in healthy populations with different nutrient intakes (2). Commonly, requirements of infants 0–6 mo of age are derived from the nutrient intake of fully breast-fed infants (2). However, there is increasing concern about the validity of intakes provided in breast milk as an indication of requirements of infants during the first 6 mo of age (7).

These methods will facilitate calculation of the average basal requirement for each age and sex segment of the population. However, the basal requirement does not account for the need to maintain a body copper reserve and the amount sufficient to ensure that copper absorption and retention are not operating at maximum capacity. Therefore, the amount needed to fulfill the basal requirement plus these additional needs constitutes the average normative requirement (1).

Knowledge of the variability of essential nutrient requirements among individuals, within each sex and age category, would answer the amount by which the average normative requirement must be increased to meet the requirements of all healthy subjects (1, 2). For most nutrients this information is limited. Also, it is necessary to provide an additional amount to compensate for the effect of other dietary components on the bioavailability of the nutrient. The final value obtained corresponds to the safe level of intake in the WHO nomenclature (1) or the recommended dietary allowance (RDA) according to the US National Academy of Sciences (2). The US National Academy of Sciences also created the category of estimated safe and adequate daily dietary intake (ESADDI) for essential nutrients for which there is insufficient information to determine the RDA, but for which potentially toxic upper concentrations are known (2). The ESADDI for copper varies from 0.4–0.6 mg/d in infants 0–0.5 y of age to 1.5–3 mg/d in adults (2).

SAFETY LIMITS FOR NUTRIENT INTAKES

Many nutrients consumed in excess can be toxic. Exposure to copper derives from the consumption of food and water and

[1] From the Institute of Nutrition and Food Technology, University of Chile, Santiago.

[2] Address reprint requests to M Olivares, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Casilla 138–11, Santiago, Chile.

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air and skin contact with copper-containing substances. Copper toxicity has been observed in animal species as well as in humans (8–14). Tolerance to high intakes of copper varies greatly from one species to another (8). Sheep are most sensitive to copper poisoning, whereas rats have a higher tolerance to copper excess (8, 15, 16). The susceptibility to copper excess is also influenced by the chemical form of copper and dietary interactions with other components (eg, zinc, iron, and molybdenum) (10). In rats, cupric chloride and cupric carbonate are more toxic than cupric nitrate, cupric acetate, and cuprous oxide (10).

Acute copper toxicity is infrequent in humans and is usually a consequence of the contamination of foodstuffs or beverages (including drinking water) by this mineral. Acute copper toxicity can also occur from accidental or deliberate ingestion of copper salts. However, oral ingestion of copper salts is rare because of their unpleasant taste and emetic properties. Acute symptoms include salivation, epigastric pain, nausea, vomiting, and diarrhea. Ingestion of ≥ 100 g $CuSO_4$ could produce intravascular hemolytic anemia, acute hepatic failure, acute tubular renal failure, shock, coma, or death (10, 12–14).

Toxicity to chronic doses has been studied less. Studies performed in animals (rats, dogs, pigs, and rabbits) showed that chronic copper excess produces liver failure associated with a massive hepatic accumulation of copper (10, 12, 14). Infrequently, chronic copper poisoning causes hemolytic anemia. In humans, the evidence is derived principally from patients with Wilson disease, a genetic disorder of copper metabolism. In Wilson disease, the accumulation of copper in the liver and brain is associated with altered structures and functions (11, 17). The occurrence of infantile cirrhosis in areas of India and isolated clusters of cases in Bavaria have been related to excessive copper intakes (18–26). The sources of copper in these cases have been linked to the use of copper utensils for cooking or to a high copper content of drinking water (21–28). The published data of O'Neill and Tanner (29) suggest that the toxic intakes of infants who received milk boiled in brass vessels are $\approx 14.2\ \mu mol \cdot kg^{-1} \cdot d^{-1}$ ($900\ \mu g \cdot kg^{-1} \cdot d^{-1}$) (29), which is more than 10 times the 1.3 μmol/kg (80 μg/kg) recommended by WHO. These rare situations and the special cases of Wilson disease that offer a genetic model for defective copper excretion are the only conditions in humans in which copper toxicity can be evaluated.

Studies of copper toxicity in animals cast doubt on a single cause-effect relation between copper ingestion and cirrhosis and suggest that other toxic factors may be implicated. For example, in rats and sheep a synergistic effect between copper and pyrrolizidine alkaloids was found (30, 31). On the other hand, with other copper hepatic toxins such as galactosamine, a high copper concentration prevented liver damage (32). The frequency of familial occurrences and of consanguinity in the parents of patients with Indian childhood cirrhosis or idiopathic copper toxicosis strongly suggests an inherited disorder (33–39). Some cases of Indian childhood cirrhosis or idiopathic copper toxicosis have occurred despite the virtual absence of copper in the drinking water or even in exclusively breast-fed infants, suggesting that drinking water cannot be the sole cause of these illnesses (34, 38, 40, 41). Furthermore, cultured fibroblasts from a child with Indian childhood cirrhosis had reduced basal and metal-induced metallothionein synthesis (42). The induction of metallothionein is a protective mechanism for liver copper toxicity. This finding is a demonstration that there is a genetic predisposition in at least some cases of early copper cirrhosis. In addition, an epidemiologic study performed in Massachusetts in three towns with copper concentrations in the drinking water ranging from 8.5 to 8.8 mg/L reported no deaths due to liver disease (43). Recently, Thomas et al (44) showed that some mutations that completely disrupt the Wilson disease gene can produce liver disease in early childhood. It is conceivable that some cases of Indian childhood cirrhosis or idiopathic copper toxicosis could correspond to an early-onset form of Wilson disease. In summary, copper-related childhood cirrhosis is a heterogenous condition that can be the consequence of environmental factors or genetic disorders of copper metabolism, or both.

The evaluation of the safety of a nutrient requires toxicologic studies that find the limits of safe exposure. These studies include evaluation of the function and morphology of different tissues and assessment of embryotoxic, teratogenic, reproductive, mutagenic, and carcinogenic effects (12, 45). The Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives has defined the acceptable daily intake (ADI) as the amount of a nutrient that can be ingested daily over a lifetime without demonstrable toxicity risks (45). The ADI is calculated by determining, in animals or preferably in humans, the highest no-observed-adverse-effect level (NOAEL) (12, 45). When it is not possible to identify the NOAEL, the lowest observed-adverse-effect level (LOAEL) may be used (12, 45). For the calculation of human ADI, the NOAEL or LOAEL obtained is divided by a safety factor used to provide an adequate margin of security (12, 45, 46). The safety factor considers mainly *1*) variability among human subjects, *2*) uncertainty of extrapolation to humans the results obtained in animals, *3*) use of subchronic studies as representative of chronic exposure, *4*) if the LOAEL is used, the incertitude of the margin between the LOAEL and the NOAEL, *5*) the significance of the adverse effect, and *6*) the existence of counterbalancing beneficial effects (12, 45–47). Usually, the Joint FAO/WHO Expert Committee uses a composite safety factor of 100 in setting the ADI on the basis of long-term animal studies (47, 48). This safety factor is calculated by assuming that humans are 10 times more sensitive than the animals tested and that the variability of the sensitivity within the human population is in a 10-fold range. However, a safety factor of 10 is usually used when the ADI is derived from data obtained in humans (12, 45–47).

The range of nutrient intake of a normal population may be divided into three zones (**Figure 1**). Low and high intakes are unsafe due to the risk of nutrient deficiency or toxicity, respectively. The intermediate zone represents the safe amount of nutrient intake, across which the risks of either deficiency or toxicity are very low (1, 46, 49). However, populations with nutrient metabolism abnormalities can experience toxicity within the range of nutrient intakes considered low to safe for a normal population. For instance, individuals with some genetic disorders (Wilson disease and glucose-6-phosphate dehydrogenase deficiency), probable genetic abnormalities (idiopathic copper toxicosis and Indian childhood cirrhosis), or liver damage are likely to be more susceptible to the toxic effects of copper (12).

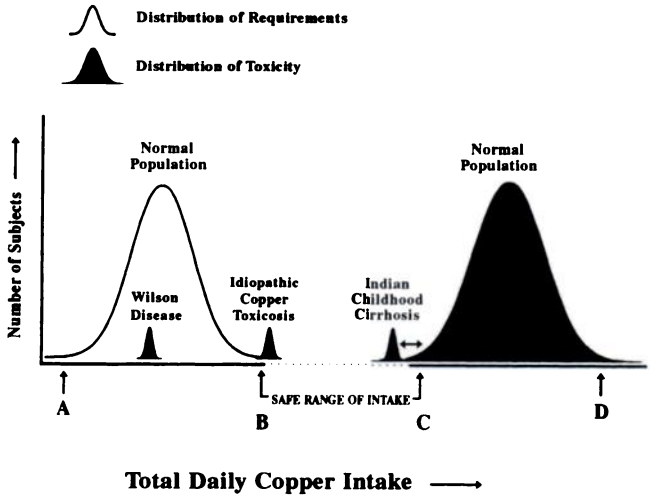

FIGURE 1. Model to illustrate distribution of individual copper requirements and toxicity in a normal population and populations with genetic abnormalities in copper metabolism. Point A represents the mean requirement minus 2 SDs, indicating the limit below which 97.5% of the normal population will suffer from copper deficiency. Point B represents the mean requirement plus 2 SDs, indicating the limit over which 97.5% of the normal population will meet their requirements. Points C represents the mean intake minus 2 SDs, indicating the limit below which 97.5% of the normal population will not suffer from toxicity. Point D represents the mean intake plus 2 SDs, indicating the limit over which 97.5% of the normal population will suffer from toxicity.

RECOMMENDED DIETARY COPPER INTAKE

In 1973 WHO (50) suggested a recommended daily intake of copper of 1.3 μmol/kg (80 μg/kg) for infants and young children and 0.6 μmol/kg (40 μg/kg) and 0.5 μmol/kg (30 μg/kg) for older children and adult males, respectively. The Scientific Committee on Human Nutrition of the Commission of the European Communities established daily population reference intakes (PRIs) for many nutrients including copper (7). They defined the PRI as a mean requirement plus 2 SDs. As mentioned above, the National Academy of Sciences recommended the ESADDI for copper (2). The PRI and ESADDI are shown in **Table 1**. At all ages, the PRI is consistently lower than the range of the ESADDI.

The Joint FAO/WHO Expert Committee on Food Additives (51), in their 14th report, proposed a maximum tolerable daily copper intake of 0.5 mg/kg. To establish this value, the Joint Committee considered the essentiality of copper. They applied a safety factor of 10 to the NOAEL of 5 mg/kg, which was calculated from data of animal experiments (50). In 1982 the Joint Committee (10) established a provisional mean tolerable daily intake (PMTDI) of 0.5 mg Cu $\cdot kg^{-1} \cdot d^{-1}$. This value was based primarily on a study conducted in 1972 in beagles (52). Dogs consumed copper gluconate in concentrations equivalent to 3, 15, and 60 mg $\cdot kg^{-1} \cdot d^{-1}$. After 6 mo of the study, two animals of each sex per group were killed. Copper accumulated in liver, kidneys, and spleen at the highest dose. After 1 y of observation, minimal liver function abnormalities were observed in 1 of 12 dogs receiving the 60-mg/kg dose. This change disappeared after a 12-wk withdrawal period. This study performed in beagles was not published and it has not been subjected to the peer-review process. On the other hand, this study did not take in account the differences in copper metabolism between dogs and humans. The occurrence of interspecies differences in response to foreign compounds complicates the extrapolation of animal toxicity data to humans (45).

TABLE 1
Population reference intake (PRI) and estimated safe and adequate daily dietary intake (ESADDI) for copper

Group and age	PRI	ESADDI
	mg/d	
Infants		
0–5 y	—	0.4–0.6
0.5–1 y	0.3	0.6–0.7
Children		
1–3 y	0.4	0.7–1.0
4–6 y	0.6	1.0–1.5
7–10 y	0.7	1.0–2.0
Adolescents		
11–14 y	0.8	1.5–2.5
15–17 y	1.0	1.5–2.5
≥18 y	1.1	1.5–3.0
Women		
Pregnant	1.1	—
Lactating	1.4	—

To extrapolate reliably from animals to humans, the goal should be to select a species that most closely resembles humans in terms of copper metabolism (45). There is evidence that the use of dogs as a surrogate for human subjects is inappropriate because dogs are more susceptible to copper overload and have differences in the metabolism of copper (53, 54). For example, albumin does not bind copper in dogs whereas it does in humans (54). Furthermore, the NOAEL established by the Joint FAO/WHO Expert Committee on Food Additives is for copper gluconate, a substance not likely to be found in foods or water. The chemical form in which copper occurs in the intestinal lumen markedly affects its absorption (55). Thus, in assessing the NOAEL, LOAEL, or a safe intake for copper it is important to distinguish ionic copper ingested in water or as supplements from dietary copper in foods, which is mainly present as organic compounds.

Fitzgerald (56) recently revised the NOAEL for copper derived from the study performed in beagles. He discovered a mistake (transcription error?) in the NOAEL of 5 mg $\cdot kg^{-1} \cdot d^{-1}$ published by the Joint FAO/WHO Committee. He assumes that the correct value is 15 mg copper gluconate $\cdot kg^{-1} \cdot d^{-1}$. In addition, the reported value is for copper gluconate. Thus, the NOAEL should be 2.1 mg elemental copper $\cdot kg^{-1} \cdot d^{-1}$. Hence, the corresponding values for the PMTDI should be 1.5 mg copper gluconate $\cdot kg^{-1} \cdot d^{-1}$ and 0.21 mg elemental copper $\cdot kg^{-1} \cdot d^{-1}$.

INTERNATIONAL REGULATIONS FOR COPPER IN DRINKING WATER

Drinking water standards have been adopted by the United States, the European Community, WHO, and other countries. In the United States the copper content of drinking water is regulated under the Safe Drinking Water Act (amended in 1986), which was promulgated by the US Environmental Pro-

tection Agency as the "lead and copper rule" (57). The rule establishes a maximum contaminant concentration of 1.3 mg Cu/L in water from the tap after 6 h of stasis in the plumbing system. However, under a secondary, nonenforceable (nonmandatory) regulation, the rule recommends a maximum of 1 mg/L. The limit of 1.3 mg Cu/L is based mainly on organoleptic considerations and to a lesser degree on toxicity information. The drinking water copper limit of 1.3 mg/L was calculated assuming ingestion of 2 L drinking water daily for an adult and a safety factor of 2 ([(5.3 mg/d)/(2 L/d)]/2 = 1.3 mg/L). Wyllie (58) reported acute gastrointestinal symptoms in a group of nurses after the consumption of a cocktail prepared in a copper cocktail shaker. The amounts of copper ingested were not measured. However, in a reconstruction, the amount of copper ingested varied from 5.3 to 32 mg. The dose of 5.3 mg/d was considered an LOAEL. The European Community, in the directive 80/778/EEC concerning water for human consumption, listed copper as an item that was considered undesirable in excessive amounts (59). The directive recommended a guide level (nonenforceable) of 0.1 mg Cu/L at the treatment plant and 3 mg/L after 12 h in piping. No maximum admissible concentration was stated.

WHO, in new provisional guidelines for drinking water quality published in 1993, included copper in the group of chemicals of health significance in drinking water (60). Previously, copper was included in the comfort table, in which the limits for copper contained in drinking water were based on taste consideration and staining characteristics. In this provisional guideline, copper is included jointly with lead, heavy metals, and arsenic among the inorganic constituents with health significance. All in this category except copper have no benefits and have recognized toxicity to humans; the importance of copper to human health is ignored. This recommended change in the status of copper is based mainly on a cluster of cases of infant cirrhosis in Bavaria (Germany), research in beagles, and taste considerations. Because the new provisional limit of 2 mg Cu/L drinking water was considered to be below the taste threshold, copper was included in the list of chemicals with health significance. The argument for keeping iron and zinc in the comfort table given by WHO, despite their known toxicity, is that these elements have a taste threshold below the safe limit in water. As mentioned previously, the scientific information concerning the possible involvement of copper in causing early childhood cirrhosis is by no means conclusive. The evidence for iron overload in causing hemochromatosis is clearly stronger; furthermore, the rather frequent occurrence of genetic susceptibility to this disease is widely recognized (61), yet iron was not included in the list of chemicals with health significance.

The copper concentration of drinking water varies greatly according to the natural mineral content, the pH of the water, and the plumbing system (12, 62). Depending on individual taste acuity, different copper concentrations may produce unpleasant sensations described as metallic, astringent, or bitter (63, 64). Béguin-Bruhin et al (64) showed that only the stable, dissolved Cu^{2+} is of importance in determining taste threshold concentration. The few studies conducted to study the taste threshold concentration of copper in water, performed in a small number of subjects, showed that the threshold is greatly influenced by individual variability and by water quality (63, 64). The threshold is higher in drinking water than distilled water and mineral water with carbon dioxide (63, 64). Probably, the higher values obtained in drinking water are a consequence of a greater proportion of insoluble copper (64). Depending on water characteristics, the taste threshold varied from 1 to 5 mg/L (63, 64). It is important that additional studies be conducted in larger groups of subjects to evaluate the copper taste threshold in drinking water with different pH and hardness, as well with different copper ionic species.

WHO also recommended a new provisional guideline value for copper of 2 mg/L drinking water (60). This limit was derived from the PMTDI of 0.5 mg/kg suggested by the Joint FAO/WHO Expert Committee on Food Additives (based on the erroneous NOAEL derived from the old study in beagles) and from an estimation that no more than 10% of copper intake must come from drinking water. Thus, for a 60-kg man, the maximum intake should be 30 mg daily and water should provide no more than 3 mg/d. Because a typical adult ingests 2 L water/d, 1.5 mg Cu/L was derived as a limit. In practice this led to a recommendation that drinking water copper content should not exceed 2 mg/L (rounded figure). However, according to the analysis performed by Fitzgerald (56), the PMTDI should be 1.5 mg copper gluconate $\cdot kg^{-1} \cdot d^{-1}$. Consequently, the limit of copper in water should be 4.5 mg copper gluconate/L (0.63 mg elemental copper/L). In view of the remaining uncertainties of copper toxicity in humans, WHO considered the guideline value as provisional.

The proposed limit for copper in drinking water does not consider copper bioavailability. Copper is present in drinking water as free Cu^{2+} or in complexes with organic and inorganic ligands (12, 65). Some of these complexes are insoluble; therefore, they are not likely bioavailable (11, 65). Because copper absorption can vary from 25% to 60%, the limit for water copper content should be adjusted considering that only a fraction of the copper ingested will be absorbed (66). On the other hand, assuming a maximum tolerable daily total copper exposure of 30 mg for an adult of 60 kg (based on the Joint FAO/WHO Expert Committee on Food Additives value of 0.5 mg/kg), the margin that could be provided safely by drinking water depends on daily copper intake from food. Thus, considering a hypothetical daily copper intake from food of 3 mg, water could supply 27 mg, whereas if copper in food was 27 mg, water could supply only 3 mg. On the basis of a daily water ingestion of 2 L, the maximum safe limit for copper in drinking water should be 13.5 mg/L in the former hypothetical situation and 1.5 mg/L in the latter. Studies of dietary intake have shown that the copper content of most Western-style diets is below the 1–3 mg of the ESSADI recommended by the US National Academy of Sciences (67–74). Therefore, the assumption that no more than 1.5 mg/L or 10% of copper must come from water is not reasonable considering that the contribution of drinking water to total copper exposure is minimal.

Recently, the European Commission decided to revise its existing directive concerning drinking water for human consumption (75). The commission responsible for the environment has proposed a new standard of 2 mg Cu/L drinking water. Additionally, the commission proposed moving this mineral from the list of substances undesirable in excessive amounts to a list of chemicals with health significance (76). This list also includes well-known toxic, nonessential elements (eg, arsenic, lead, and mercury). They considered that "the available scientific evidence relating the toxicity to the liver

TABLE 2
Copper limits for total intake or drinking water content in regulations of the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the US National Academy of Sciences (NAS), the European Community (EC), and the US Environmental Protection Agency (EPA)[1]

Agency and reference	Upper limit	Observations
Total intake		
FAO/WHO (10)	0.5 mg/kg	Based on an NOAEL of 5 mg/kg obtained in dogs (52)
US NAS (2)	10 mg[2]	—
EC (7)	10 mg[2]	Over this dose, there are higher risks of nausea and vomiting
Water content		
WHO (60)	2.0 mg/L	Based on the FAO/WHO maximum tolerable daily intake of 0.5 mg/kg, and estimating that no more than 10% of copper must come from drinking water
EC (59)	NS[3]	Recommended guide level: 0.1 mg/L at treatment plant; 3 mg/L after the water has been standing for 12 h in piping
US EPA (57)	1.3 mg/L	Based in an LOAEL of 5.3 mg in humans (57) and assuming ingestion of 2 L water/d, a healthy advisory value of 1.3 mg/L was calculated by using a safety factor of 2
US EPA (57)	1.0 mg/L	Alternative, nonenforceable concentration of 1 mg/L was based on organoleptic and aesthetic considerations

[1] NOAEL, no-observed-adverse-effect level; LOAEL, lowest observed-adverse-effect level.
[2] In adults.
[3] No standard.

indicates that a parametric value of 2 copper mg/L is appropriate for the protection of human health. This is near or below the threshold for taste, and so it is necessary to include copper in the list of chemical parameters. However, it is understood that the evidence relating to the toxicity of copper will be subject to further review, and this may lead to a need to modify the classification and parametric value" (76). The changes proposed are mainly based in the WHO provisional guideline for copper in drinking water.

A summary of regulations for total copper intake and copper content of drinking water is shown in **Table 2**. Given the importance of copper as an essential mineral for human health and the importance of water as a source, we propose that copper and other essential minerals with health significance be listed separately from chemicals and elements known to be toxic but of no benefit to humans. Listings in the separate category should indicate the benefits of providing these elements with water and the potential problems of deficit and excess within the context of total daily exposure to foods, drinking water, and air and possible genetic susceptibility.

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