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Agency for Toxic Substances and Disease Registry
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Re: Comments on ATSDR “Draft for Public Comment.” Toxicological Profile for Copper

Dear Sirs:

You base your MRLs for copper of 0.02 mg/kg/d for acute and intermediate exposures on a study by Pizarro et al. (1999a) who found that gastrointestinal symptoms occurred when adults were exposed to a concentration of copper ≥ 3 mg/L but that there were no increases in symptom incidence at 1 mg/L. This study, however, as were other studies by this group, was designed to address issues of safe water concentrations of copper. Other endpoints may be more valid for determining MRLs for copper, particularly those which are protective of liver disease, a particularly sensitive toxic endpoint. Unless noted within the text of these comments, I determined dose using default values published in the Exposure Factors Handbook (USEPA, 1997) when information on weight or ingested formula (in infants) was missing.

Introduction

Copper is a necessary micronutrient with deficiencies associated with perinatal anemia, diarrhea and growth retardation, impaired reproductive performance with low birth weight infants, and disorders of white blood cell phagocytosis with associated increased incidence of infections, bone disorders with osteoporosis (Uauy, et al. 1998). Menkes syndrome, where there is a prenatal tissue deficit of copper, severe mental retardation ensues. Infants require 0.1-0.135 mg Cu/kg/d to prevent anemia. Children ages 3-6 years require 1-1.6 mg Cu daily to maintain health. The normal daily copper balance is maintained at 1.3 mg in children ages 6-10 years and 2-5.8 mg in adults. (Underwood, 1971). The FDA Reference Daily Intake for copper is 2 mg (FDA, 1999).

Copper and gastrointestinal symptoms

ATSDR's proposed acute and medium MRLs are based on the threshold for nausea, vomiting, and/or abdominal associated with copper ingestion (ATSDR, 2002). These MRLs are, in turn based on a no adverse effect level (NOaEL) where women were dosed with 0.0272 mg/kg/d copper solutions (1 mg Cu/L) for two week periods (Pizarro et al. 1999a) with symptoms occurring when

using water containing 3 mg Cu/L. They had an additional 0.0266 mg/kg/d copper intake from other sources. Although ATSDR uses dose as their parameter, the appropriate concern may be copper concentration in water. The World Health Organization has set a provisional guideline for the concentration of copper in water of 2 mg/L (Pizzaro, et al. 1999b). Olivares, et al. (2001) in a follow up experiment found that men and women developed acute gastrointestinal symptoms at a copper concentration in drink of 4 mg/L with no effect at 2 mg/L, findings similar to the Pizzaro, et al. (1999a) study. This study, however, used daily doses of 200 mL of Cu-containing water as apposed to an average of 1640 mL in the Pizzaro, et al., study. Araya, et al. (2001) in a similar study found that nausea occurred at concentrations of >4 mg/L in 200 mL drinks. Masking the metallic taste with a flavor increased the NOaEL concentration, further suggesting that this endpoint is concentration dependent and not dose dependent (Olivares, et al. 2001). Buchanan. Et al. (1999) in a study of 145 households and 442 occupants, also found that ingestion of copper-contaminated water, containing up to a median concentration of 2.8 mg Cu/L, was not associated with acute gastrointestinal symptoms. They performed a nested case-control study as well. In this latter study daily doses of copper up to 3 mg/kg/d were not associated with acute gastrointestinal symptoms.

Diarrhea can occur at higher levels of copper exposure. Chuttani et al (1965) found no effects with ingestion of 6 mg Cu/kg as copper sulfate. Diarrhea occurred with ingestion of >6 to 40 mg/kg. The authors felt that the diarrhea was likely secondary to the irritant action of copper sulfate since blood was found in the stool of all symptomatic cases. Araya, et al. (2001) found diarrhea associated with a copper concentration in water of 8 mg/L with an NOaEL of 6 mg/L. Other studies have not found diarrhea with copper concentrations below 6 mg/L. (Pizzaro, et al. 1999a,b; Olivares, et al. 2001).

Acute oral toxicity of copper

ATSDR (2002) discounts reports of acute lethalties in man because of potential reporting bias. A recent study by Liu, et al. (2001) overcomes this shortcoming. Copper sulfate is used as an emetic in China for treating suicidal overdose cases. In these cases there is precise documentation of the amount of copper sulfate that is used. The authors describe the case of a Chinese woman who attempted suicide by ingesting 50 mg of diazepam. She was given 2.5 g of copper sulfate in 1750 mL of water as an emetic, equivalent to 14 mg Cu/kg, assuming a 53 kg body weight (Popkin et al, 1995). This treatment was followed by hemolysis and acute tubular necrosis. The authors report on an additional 11 cases of acute copper sulfate poisoning either when ingested with suicidal intent or when used as an emetic. Nine died of acute renal failure after copper sulfate doses ranging from 9–400 g. Two individuals ingesting 6 and 120 g of copper sulfate recovered. Hayes (1982) reports two children who became ill on eating grapes treated with copper sulfate. One died and one recovered. Measured absorbed copper levels were 12.5 and 16.5 mg/kg.

Similar acute toxic effects have been demonstrated in experimental animals. Vogel (Am J Path 1961; 36: 699-711) gave mice intraperitoneal injections of 22 mg Cu/kg in the form of an ionized copper-albumin complex. Within 6 hours there was evidence of proximal renal tubular accumulation of copper and acute tubular necrosis.

Copper and liver toxicity

The National Research Council (2000) recommends that childhood liver cirrhosis in susceptible populations, i.e., those with idiopathic copper toxicosis or related conditions, should be considered when regulating copper in drinking water. Studies that examine liver effects as an endpoint may be useful for determining medium and chronic MRLs as well. Excessive copper ingestion has been

associated with the development of liver inflammation and chronic liver disease. In India excess copper in milk from boiling formula in copper and brass vessels has resulted in early childhood cirrhosis (Scheinberg & Sternlieb, 1994). In a study by these authors, 5 of 7 children with early childhood cirrhosis had maximum copper levels in their home water that ranged from 3.4-13 mg/L. These 7 infants had excess liver copper levels ranging from 425-3380 ppm (normal 295 ppm or less). Dieter et al (1999) noted that 5 of 8 infants with histologically-confirmed childhood cirrhosis of unknown etiology had been previously fed formulas with high copper content (9-26.4 mg/L). Both acute and subacute human dosing studies with copper salts have looked at serum enzyme changes as indicators of early of liver toxicity. Pizzaro, et al. (1999a) investigated such changes in 60 women who were dosed with copper sulfate intermittently over a period of 12 weeks. No liver effects were seen at the highest dose (0.13 mg/kg/d for 2 weeks) nor during the entire 12 week study where dosing averaged 0.042 mg/kg/d. Pizzaro, et al. (2001) dosed 45 women with copper salts for 9 weeks and found no liver enzyme changes. Using the water intake data of Pizzaro, et al. (1999a), the average copper dose during this interval was 0.073 mg/kg/d. Olivares, et al.(1998) dosed infants with 2 mg Cu/L for 9 months starting at age 3 mos. This is equivalent to 0.32 mg/kg/d at 14 weeks of age and 0.083 mg/kg/d at 12 mos of age. No liver enzyme changes were noted at these doses. In 3 Massachusetts towns where there were 64124 child-years of exposure to high levels of copper in the water (8.5-8.8 mg/L; equivalent to 1.4 mg/kg/d in 3 month old infants and 0.20 mg/kg/d in 5 year old children) between 1969 and 1991, no deaths occurred in children from cirrhosis or any form of liver disease (Scheinberg and Sternlieb, Lancet 1994; 344: 1002-4).

Both liver and kidney disease occur in experimental animals that chronically ingest excessive amounts of copper. Rats given drinking water containing 1250 ppm of copper acetate for one month had no evidence of liver effects by histology or enzyme changes (equivalent to 50 mg Cu/kg/d; Gaeta et al, 1980). Gummow (1996) dosed cattle with 0.6-12 mg Cu/kg/d for 745 d as copper sulfate. No effects were seen at the 0.6 mg/kg/d level. Subclinical effects (enzyme changes) of liver disease were seen at dose levels of ≥ 12 mg Cu/kg/d.

Development of MRLs for acute, medium and chronic exposure to copper based on systemic toxic endpoints

In determining risks levels associated with ingestion of copper, Olivares, et al. (1999) gives the following guidelines: levels should not be below those associated with deficiency state, should not be above those found in a “healthy” population exposed to copper and should take into account populations particularly susceptible to copper deficiency and/or toxicity. The note that infants have unusual needs for copper, up to 2.5 times that of adults per unit body weight, and are 80% as tolerant to excess copper levels as adults per unit body weight. Based on data from Underwood (1971; see above), the minimum daily needs per unit body weight would be expected to be as follows:

Group	Cu Need (mg/kg/d)
Infants	0.1-0.135
Children age 3-6 years	0.05-0.08
Children ages 6-10 years	0.05
Adults	0.03-0.08

Klevay, et al. (1980) in a copper balance study in adult men found the mean daily copper requirement was 0.02 mg/kg/d.

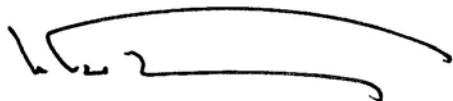
Using Oliveres, et al. (1999) approach, instead of setting the lower bound at the background ingestion rate (which may be at a deficiency level), one could set the background level at the required dose (RD) to prevent a deficiency state. The MRL could then be calculated as

$$\text{MRL} = \text{RD} + [\text{NoaEL dose}]/[\text{uncertainty factor}]$$

This approach differs from that used by ATSDR where the background ingested amount of copper was added to the NoaEL dose prior to applying an uncertainty factor.

NoaELs vary depending on the age of the population being investigated. This age difference is apparent in the attached summary Table 1 for human copper-related effects. It would appear to be reasonable to develop a range of MRLs based on population age. If one applies an uncertainty factor of 3 for human ingestion or population NOaEL endpoints and an additional safety factor 10 to convert from LOEL to NoaEL endpoints, a range of MRLs may be such as that shown in Table 2, attached.

Respectfully submitted,

A handwritten signature in black ink, consisting of a series of loops and a long horizontal stroke that curves upwards at the end.

Woodhall Stopford, MD, MSPH

Table 1: Copper Dose Effects in Humans

Effect	Population	Dose	Reference
Acute ingestion		(mg/kg/d)	
Death/renal failure	Adults	14 (LOEL)	Liu et al '01
	Child	12.5 (absorbed dose; LOEL)	Hayes '82
Liver	Adults	0.13 (NOEL)	Pizzaro et al '99a
Intermediate ingestion			
Liver	Infants	1.5-4.3 (LOEL)	Dieter et al '99
	Infants (3mos)	0.315 (NOEL)	Olivares '98
	Infants (12 mos)	0.083 (NOEL)	Olivares '98
	Infants (population)	1.4 (NOEL)	Scheinberg & Sternlieb '94
	Adults	0.042 (NOEL)	Pizzaro et al '99a
	Adults	0.073 (NOEL)	Pizzaro et al '01
Chronic Ingestion			
Liver	Children (population)	0.20 (NOEL)	Scheinberg & Sternlieb '94

Table 2: Range of MRLs for Copper Exposure

MRL Type	Population	MRL	Reference
Acute		(mg/kg/d)	
	Adults	0.07	Pizzaro et al '99a
Intermediate			
	Infants	0.15	Dieter et al '99
	Infants (3mos)	0.20	Olivares '98
	Infants (12 mos)	0.08	Olivares '98
	Adults	0.034	Pizzaro et al '99a
	Infants	0.57	Scheinberg & Sternlieb '94
	Adults	0.044	Pizzaro et al '01
Chronic			
	Children	0.12	Scheinberg & Sternlieb '94

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