PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

COPPER

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COPPER
in Drinking Water

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PREFACE

Drinking Water Public Health Goal
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DPH, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR COPPER IN DRINKING WATER

SUMMARY

A revised Public Health Goal (PHG) of 300 parts per billion (ppb) has been developed for copper in drinking water, based on a re-review of the scientific literature since the original PHG, developed in 1997. Copper is an essential nutrient in humans, and has not been shown to be carcinogenic in animals or humans. However, young children, and infants in particular, appear to be especially susceptible to the effects of excess copper. Case reports have attributed adverse effects (diarrhea and weight loss) in infants to rather low levels of copper in drinking water, estimated as 0.22 to 1.0 mg/L or parts per million (ppm) (Stenhammar, 1999) or 1.0 to 6.5 mg Cu/L (Berg *et al*., 1981). High levels of copper in tap water in homes with copper plumbing have been linked to childhood cirrhosis in Germany (Dieter *et al.*, 1999). (Liver is the target organ for systemic copper toxicity). In other studies (Pizarro *et al.,* 1999b; Araya *et al*., 2001, 2004; Olivares *et al*., 2001), consumption by adults of drinking water containing $\geq$ 3 mg/L ionized copper was associated with a significant increase in nausea, abdominal pain, or vomiting.

The PHG is based on infants as a sensitive group, and absence of an adverse effect in the principal study selected (Olivares *et al.*, 1998), which was undertaken to confirm the safety of the World Health Organization (WHO) provisional limit for copper of 2 mg/L during infancy. This is supplemented by data on gastrointestinal effects from other studies as the adverse effect endpoint of concern (Berg *et al*., 1981; Stenhammar, 1999; Pizarro *et al.,* 1999b; Araya *et al*., 2001, 2003a,b, 2004; Olivares *et al*., 2001). Although some differences in biochemical indices of copper nutrition and liver function were observed between exposure groups in the Olivares *et al*. (1998) study, no evidence of adverse or toxic effects were reported in healthy infants (formula- and breast-fed) that consumed water with a copper content of either <0.1 mg/L or 2 mg/L (~30 µmol/L) from 3 to 12 months of age. The no-observed-adverse-effect-level (NOAEL) was 426 µg/kg-day based on the higher drinking water copper concentration administered in the study.

The revised PHG takes into consideration normal copper exposure levels from breast milk or formula. A relative source contribution of 50 percent of total copper intake from powdered formula versus tap water used in its reconstitution was applied, which is consistent with the estimated exposures. A moderate uncertainty factor of 3 is applied to the NOAEL of the Olivares *et al*. (1998) data, based on its application to a sensitive population, infants, who are presumed to be most at risk, using a 95 percent upper confidence level of the expected fluid consumption rate.

The U.S. Environmental Protection Agency (U.S. EPA) Maximum Contaminant Level Goal (MCLG) and Action Level for copper is 1.3 mg/L, as is the California Action Level. The WHO limit for copper in tap water is 2 mg/L (31.48 µmol/L) (WHO, 2004), and according to WHO guidelines, drinking water should not provide more than about one-tenth of the daily requirement for minerals, including copper (WHO, 1993). The revised copper PHG, which is increased from the value of 170 µg/L (170 ppb) in the 1997 PHG

document, is based on new scientific studies and an improved drinking water consumption estimate. We believe the value of 300 ppb is adequate to protect both infants and adults against any adverse acute or chronic effects from copper in drinking water.

INTRODUCTION

The purpose of this document is to reevaluate the PHG for copper in drinking water, originally developed in 1997 (OEHHA, 1997). Copper may be present in source water or may enter tap water in the distribution system of the individual household. Tap water is used for drinking directly and also for the preparation of foods and beverages. Copper is an essential nutrient, but it is toxic at higher intake levels. Children under 10 years of age appear to be particularly susceptible to copper toxicity (Spitalny *et al.*, 1984; Mueller-Hoecher *et al.*, 1988; Klein *et al.*, 1991; IOM, 2001; ATSDR, 2004).

As a required element, copper is incorporated into a number of proteins, such as cytochrome oxidase, lysyl oxidase and superoxide dismutase. Copper is essential for hemoglobin synthesis, carbohydrate metabolism, catecholamine biosynthesis and cross-linking of collagen, elastin, and hair keratin (Solomons, 1985; ATSDR, 2004). The daily nutritional requirement for copper is easily met by food sources; deficiencies are generally associated with disease conditions such as persistent infantile diarrhea or inherited metabolic disorder (Menkes' syndrome) (IOM, 2001; ATSDR, 2004).

Reports of copper intoxication in humans most often arise from accidental poisoning or suicide attempts (Akintowa *et al.*, 1989; ATSDR, 2004). Copper intoxication from the consumption of water containing high copper concentrations is uncommon. Symptoms of mild copper poisoning from ingestion of contaminated water are nausea, abdominal cramps, diarrhea, vomiting, dizziness and headaches. More serious cases involving hepatic and renal necrosis, coma, and death have been reported as "Indian Childhood Cirrhosis" (ICC), a condition affecting primarily children under five years of age, mainly in the Indian subcontinent (Sethi *et al.*, 1993). It is generally believed that milk or water stored in brass or copper containers led to increased dietary copper in these children, possibly combined with variations in genetic susceptibility (McClain and Shedlofsky, 1988; Lee *et al.*, 1989; Sethi *et al.*, 1993; ATSDR, 2004).

In this document we evaluate the available data on the toxicity of copper by the oral route, particularly toxic effects that may result from the ingestion of drinking water with high levels of dissolved copper. To determine a health-protective level for copper in drinking water, sensitive groups are identified and considered, and studies that can be used to identify appropriate levels are reviewed and evaluated.

CHEMICAL PROFILE

Chemical Identity

Copper is a naturally occurring metal with an atomic number of 29 and an average atomic weight of 63.54. The two naturally occurring stable isotopes are ^{63}Cu and ^{65}Cu, occurring in a ratio of approximately 7:3. Two radioactive isotopes of copper, ^{64}Cu and ^{67}Cu, have been useful for clinical and experimental purposes (Marceau *et al.*, 1970; Strickland *et al.*, 1972).

Copper is a metallic element with a bright, lustrous reddish color. It is malleable, ductile, and an excellent conductor of heat and electricity. The melting point of copper is 1,083^{o}C and its boiling point is 2,336^{o}C. The specific gravity of copper is 8.94.

Copper can exist in two valence states: monovalent (cuprous) and divalent (cupric). Copper is found in pure metallic form, or as a component of many minerals, including sulfides, oxides and carbonates. Pure copper can be obtained from these minerals by smelting, leaching or electrolysis.

The copper salt most frequently used in toxicological experiments is cupric sulfate ($CuSO_4$).

Production and Uses

Copper may have been the first metal that human beings smelted and used for manufacturing implements. The manufacture of copper tools and weapons ended the neolithic age (or late stone age) and eventually led to the bronze age when humans learned to alloy copper with tin and other metals. Unalloyed copper is still used to make coins, electrical wiring, casings for ammunition, and water pipes. Copper has excellent electrical and heat conductivity, which makes it useful for electrical wires and for cooking applications. The ductility of copper makes it useful for water pipes that can be bent to fit particular applications.

Bronze (copper alloyed chiefly with tin) is used in a wide variety of applications. Brass (copper alloyed with zinc) is an attractive metal for decorative purposes such as rails and doorknobs, and is used in making musical instruments.

Copper salts are also extensively used as pesticides, with application as antifungals and against moss and other plants. Copper sulfate and copper hydroxide were the tenth and eleventh most-heavily used pesticides (by weight) in California in 2002, the most recent year for which pesticide use data are available (DPR, 2004). Copper hydroxide alone was applied to over one million acres. About seven million pounds of copper salts were used as pesticides in California in 2002.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Copper is a component of many naturally occurring minerals and is extensively used in industry and household products. Therefore, it is very widespread in the human

environment. Copper occurs in virtually all media that humans contact, including air, water and soil (ATSDR, 1990; Nriagu, 1990).

Air

Concentrations of copper in air tend to range between 5 and 200 ng/m^3, although they may be as high as several thousand ng/m^3 in proximity to copper sources such as smelters, mines, and power plants (ATSDR, 2004). Average values around 50 ng/m^3 are common in urban air (Davies and Bennett, 1985). The concentrations of copper detected in air samples from "remote areas" range widely from a high of 110 ng/m^3 to a low of 0.014 ng/m^3 as reported by Wiersma and Davidson (1986). Copper emitted into the air from natural sources amounts to 28 thousand tons annually, whereas anthropogenic sources may contribute another 35 thousand tons (Nriagu and Pacyna, 1988). Natural sources include wind-blown dust, volcanic activity, and spray from ocean waves. The anthropogenic sources are the mining, refining, smelting, and incineration of copper and related metals that are mixed or alloyed with copper in the ores and in the processed forms (ATSDR, 1990).

Soil

Copper is discharged to land from sewage treatment plants, as well as from mining and industry. Based on the 2001 Toxic Release Inventory reports, it was estimated that 92 percent of the 11 million pounds of copper released to the environment by industrial activities is deposited on land (ATSDR, 2004). Large quantities of copper salts used in agriculture are deposited on land over extensive areas (DPR, 2004). The copper in soil can run off to surface water and leach to ground water, thus contaminating drinking water sources (U.S. EPA, 2007).

Water

Copper is found in surface water, groundwater, seawater and drinking water. Surface water concentrations of copper range from 0.5 to 1000 ppb, with a median of 10 ppb (ATSDR, 2004). Most of the copper tends to be bound to sediments. Urban runoff often contains elevated concentrations of copper due to household and industrial uses of water. Sewage is also a major source of copper input to rivers and streams, although some is removed in treatment plants because of its sediment binding properties (ATSDR, 2004). Copper in surface water is a well-known environmental hazard, associated with toxicity to a variety of aquatic organisms (U.S. EPA, 2003, 2007).

The concentration of copper in drinking water can vary widely, depending on variations in acidity/alkalinity (pH), mineral content (hardness), and copper availability in the distribution system. Results from studies in the U.S., Europe and Canada indicate that copper levels in drinking water can range from < 0.005 to > 30 mg/L, with the corrosion of copper pipes serving as the most frequent cause of copper contamination (U.S EPA, 1991; Health Canada, 1992; NRC, 2000).

Well water has a highly variable copper content, dependent on the soil and the underlying water table (Lonnerdal, 1996). Additional copper is added to water due to leaching from the distribution system as drinking water is carried from the water treatment plant to the tap (Lonnerdal, 1996; Sharrett *et al.*, 1982). The use of copper sulfate for water treatment (primarily as an algicide) can also add copper to drinking water.

Copper in drinking water is regulated by the lead and copper rule, a federal and state drinking water standard (Title 22, California Code of Regulations [CCR] section 64672.3) that specifies requirements for copper in drinking water systems, measured at the customers' taps (U.S. EPA, 2001a). The action level for copper refers to a concentration measured at the tap rather in the municipal water supply system because much of the copper in drinking water is derived from household plumbing. The concentration at the tap is affected by water chemistry (pH and various dissolved constituents), which affects the corrosivity of the water. The leaching of copper into drinking water in the home distribution system is greater if the water is slightly acidic or very soft (Lonnerdal, 1996; Sharrett *et al.*, 1982).

The action level of 1,300 ppb for copper is exceeded if the concentration of copper in more than 10 percent of the tap water samples collected during any monitoring period (conducted in accordance with 22 CCR sections 64682 to 64685) is greater than this level. Failure to comply with the applicable requirements for lead and copper is a violation of primary drinking water standards for these substances (22 CCR Chapter 17.5).

The U.S. EPA also has a secondary maximum contaminant level (SMCL) for copper in drinking water of 1.0 mg/L (40 CFR 143). This is an aesthetics guideline based on consideration of taste and the staining of sinks and bathtubs. This is the principal regulatory guideline in many countries. The taste threshold for copper in water is 1 to 5 mg/L (Cohen *et al*., 1960; McKee and Wolf, 1971).

Although the copper content of potable water is generally low, acidic and hard water, particularly if conducted by newly-installed copper pipes, may be highly corrosive. Stagnation is another factor that will increase the copper content of water. First-draw water from household systems that use copper plumbing can contain several mg/L of copper; concentrations are likely to be highest when drawn from the hot water pipes. Copper leaching from pipes tends to decrease over several years, presumably from accumulation of deposits on the inside of the pipes (ATSDR, 2004). Survey data from U.S. municipal water supply systems are not generally available. In the U.S., first-draw copper concentrations (after a minimum 6 hour static period) must be reported to the U.S. EPA if they exceed 1.3 mg/L. The 90^{th} percentile concentration in first-draw water samples taken by 4500 municipal systems (7,307 samples) from 1991 to 1999 was slightly greater than 2 mg/L. Ten percent of the samples with exceedances had copper concentrations in excess of 5 mg/L, and one percent had concentrations greater than 10 mg/L (NRC, 2000). A study of water samples from households in Ohio found about 30 percent exceeded 1 mg/L (Strain *et al.*, 1984). Similarly, a study of households in Seattle, Washington found the median concentration of copper in standing water samples to be 993 µg/L. In those homes with copper pipe (as opposed to galvanized), the 50^{th} percentile for copper concentrations in standing and running water were 760 µg/L and 353 µg/L, respectively; the 75^{th} percentile concentrations were 1,303 µg Cu/L and 758 µg

Cu/L, respectively. For Seattle city employees (males only) chosen as the study subjects, the mean daily copper consumption was 2.2 mg Cu from standing and 1.3 mg Cu from running water. Running water appeared to provide an intake equivalent to more than half the daily copper requirement for their wives and children as well (Sharrett *et al.*, 1982). The WHO (2004) document states that, "Consumption of standing or partially flushed water from a distribution system that includes copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap water."

Drinking water concentrations of copper vary widely, but tap water could typically contribute about 0.08 to 0.3 mg of copper, equivalent to 9 to 30 percent of the adult daily nutritional requirement for copper, which is now considered to be 0.9 mg/day (IOM, 2001). The nutritional requirement of children for copper is lower, ranging from a Recommended Dietary Allowance (RDA) of 0.34 mg/day for infants of one to three years of age to 0.89 mg/day for ages 14 to 18 (IOM, 2001). Assuming that infants drink ~1 liter/day of water, the copper exposure would represent 24 to 88 percent of their nutritional needs. However, food provides an adequate amount of copper except in special cases (IOM, 2001). According to WHO guidelines, drinking water should not provide more than about one-tenth of the daily requirement for minerals, including copper (WHO, 1993).

There exist few published reports on direct measurements of copper intake by oral pathways in community settings. To improve public health protection, more data are needed. In addition, most copper intake studies are designed to assess aggregate daily intake of copper from ingestion of drinking water, beverages and solid food as a whole.

Food

Food is a principal source of copper exposure for humans. As part of a total diet study (Pennington *et al.*, 1986), the United States Food and Drug Administration (U.S. FDA) estimated the daily dietary intake of copper and other essential trace elements for eight groups of the U.S. population by sex and age. These estimates were based on composite samples of 234 foods purchased in 24 U.S. cities, together with earlier estimates of dietary intakes of these foods by both males and females per age groups. Table 1 displays the results of this study for copper.

Gibson (1994) compiled several studies and found that copper intakes in adults were approximately 1.0 to 1.5 mg/day from omnivore diets, and 2.1 to 3.9 mg/day in vegetarian diets. Copper intakes for children were 0.8 to 1.9 mg/day, with most of the higher intakes from vegetarian diets (Gibson, 1994). Davies and Bennett (1985) used a value of 2 mg/day in their copper exposure assessment. These estimated dietary intakes of copper are well over the estimated average requirements for copper established by the Food and Nutrition Board of the U.S. Institute of Medicine (IOM) (0.9 mg/day for adults), but well below the estimated adult tolerated upper intake level (10 mg/day) (IOM, 2001).

Table 1. Dietary Copper Intakes for Females and Males per Age Group

Age Group	Sex	Dietary Copper Intake (mg/day)
6-11 months	F/M	0.47
2 years	F/M	0.58
14 to 16 years	F M	0.77 1.18
25 to 30 years	F M	0.93 1.24
60 to 65 years	F M	0.86 1.17

Data from Pennington *et al*., 1986.

Data collected from the U.S. National Health and Nutrition Examination Survey (NHANES)(1988-1994) and from the Continuing Survey of Food Intakes by Individuals (1994-1996) indicated that the median intake of copper from the diet was 1.2 to 1.6 mg/day for adult males and 1.0 to 1.1 mg/day for adult females. The median intake for infants and young children (six months to three years) was 0.6 to 0.7 mg/day (IOM, 2001).

The Food and Nutrition Board (FNB) recently established RDAs for copper in adults and children (IOM, 2001). The RDA for adults is 900 µg Cu/day. Values for children are 340 µg/day for the first three years, 440 µg/day for ages four to eight, 700 µg/day for ages 9 to 13 and 890 µg/day for ages 14 to 18. RDAs of 1000 µg/day and 1300 µg/day, respectively, are recommended during pregnancy and lactation. The data were judged not sufficient to establish RDAs for infants. However, intakes for infants in the first year of life were estimated based on the copper concentration of human milk. A copper intake of 200 µg/day was deemed adequate for the first six months of life, and 220 µg/day for the second six months.

In 1973, WHO recommended 80 µg/kg of copper/day for infants, and set a value of 150 µg/kg per day as the upper limit of the safe range for infants. However, the FNB more recently concluded that an upper limit could not be established for infants (IOM, 2001). In a subsequent document, the WHO (1996) estimated that average copper requirements are 12.5 µg/kg of body weight per day for adults and about 50 µg/kg of body weight per day for infants. For infants, the WHO (1996) set 150 µg Cu/kg per day as the upper limit of the safe range. The IOM (2001) recommended 10 mg/day as a tolerable upper intake level for *adults* from food and supplements.

Breast milk copper concentration is low, containing approximately 0.2-0.3 mg Cu/L (Dewey *et al*., 1983; Vuori and Kuitunen, 1979), but copper from breast milk is well absorbed (Lonnerdal, 1998). The American Academy of Pediatrics (1985) has recommended 60 µg of copper per 100 kcal in infant formulas (infant formulas sold in the U.S. generally contain 75 µg of copper per 100 kcal). This would provide 0.5 mg of copper/day for an infant consuming 700 kcal/day. Term infant formulas generally

contain from 0.4-0.8 mg Cu/L, whereas formulas for pre-term infants may contain up to 2 mg Cu/L (Bauerly *et al.*, 2005).

Copper in the diet is contributed by a variety of foods. Potatoes and other vegetables make the largest contribution (approximately 30 percent). Meat, poultry, fish and bread contribute significantly (approximately 20 percent). Other food groups contribute lesser amounts (Solomons, 1985; Lonnerdal, 1996a). The food with the highest copper content is beef liver, which was reported to contain 61 ppm copper. In most foods, copper is present bound to macromolecules rather than as a free ion (IOM, 2001).

Surveys in the United States indicate that about 15 percent of the population uses a nutritional supplement containing copper (IOM, 2001). Vitamin/mineral preparations for children and adults typically contain 2 mg Cu per tablet or capsule, most often as copper oxide (Olivares and Uauy, 1996).

METABOLISM AND PHARMACOKINETICS

Copper probably occurs in drinking water in the form of cupric ion (Cu^{2+}) complexed with organic ligands (U.S. EPA, 1987). Copper ions may be more bioavailable in water than in food; various components in food can influence the metabolism, absorption and mobilization of copper in human diets. For example, high levels of vitamin C (ascorbic acid) adversely affect the absorption and metabolism of copper. Also, there appears to be an antagonistic relationship between copper and zinc absorption and transport (Cousins, 1985).

Absorption

In humans, dietary copper is absorbed from the stomach and small intestine and transferred into the interstitial fluid and blood (Linder and Hazegh-Azam, 1996). In humans about 65 percent of an oral dose of ^{64}Cu as copper acetate was absorbed from the gastrointestinal (GI) tract (range 15 – 97 percent) (Weber *et al*., 1969; Strickland *et al*., 1972). Absorption efficiency appeared to be inversely correlated with copper level in the diet (Turnlund *et al.*, 1989, 1998). Turnlund *et al.* (1989) measured copper absorption in young men using ^{65}Cu retention; ^{65}Cu retention was found to change from 55.6 percent to 36.3 percent and to 12.4 percent when copper intake was 0.79, 1.68 and 7.53 mg/d, respectively. Orally administered ^{64}Cu rapidly appears in the plasma (Bearn and Kunkel, 1955).

Results from a number of studies suggest that the ability to regulate copper homeostasis is age-dependent. Mechanisms that control copper absorption from the GI tract are immature during early neonatal life in rats. Lonnerdal *et al.* (1985) found that copper absorption is very high during the neonatal period in rats, but that it decreases by the weaning period. Varada *et al.* (1993), using perfused rat intestines, found that copper absorption was linear and nonsaturable in infant and weanling rats; copper absorption was saturable in adolescent rats. Suckling rats had considerably higher tissue copper concentrations than weanling or adolescent rats. Several investigators have found significantly higher small-intestine and liver copper concentrations in copper-

supplemented suckling rats than in adult and/or control rats (Bauerly *et al*., 2004; Fuentealba *et al.*, 2000).

Dorner *et al.* (1989) found that full-term, breast-fed human infants, with a copper intake of 114 µg/kg-day, retained 88 µg/kg-day copper, representing an absorption value of ~77 percent. Copper retention decreased with age. At two weeks of age, 130 µg/kg-day was retained, and at age 16 weeks, 64 µg/kg-day was retained. In comparison, mean relative retention in infants fed copper-fortified formula was 52 percent. Copper absorption in infant rhesus monkeys using ^{67}Cu ranged from 50 to 70 percent, similar to the values found for full-term human infants (Lonnerdal *et al.,* 1996b). Citrate, a dietary ligand found in human and cow milk, has been shown to have a positive effect on copper absorption in animal models (Shah, 1981).

Olivares *et al*. (2002) administered an oral supplementation of 80 µg Cu (as copper sulfate solution)/kg daily for 15 days to Chilean infants aged one to three months (n=20); one half of the group (n=19) received no supplementation. At the end of the trial, copper absorption was measured by using orally administered ^{65}Cu as a tracer and fecal monitoring of recovered ^{63}Cu. No major difference in the percentage of copper absorbed was observed between the two groups. Mean ($\pm$SD) copper absorption at one month of age was 83.6 $\pm$ 5.8 percent and 74.8 $\pm$ 15.2 percent for the unsupplemented and supplemented infants, respectively. The authors concluded that the experimental design of the study was inadequate because copper intakes were too low to "trigger homeostatic adaptation of intestinal absorption."

Araya *et al.* (2005) fed infant rhesus monkeys, from birth to five months of age, a formula supplemented with a high copper load (6.6 mg Cu/L). Controls were fed a commercially available infant formula that contained 0.6 mg Cu/L. Copper retention was measured after radioisotope administration. Retention of ^{67}Cu at ages one and five months was 19.8 $\pm$ 2.6 percent and 10.9 $\pm$ 2.0 percent, respectively, in the copper-treated animals (n = 4). ^{67}Cu retention was not measured in control animals in this study. Data from previous studies by some of the same authors showed that ~75 percent of ^{67}Cu is retained in control animals at ages one and five months (Lonnerdal *et al.,* 1999). At age eight months (i.e., three months after cessation of copper supplementation) there was no significant effect of prior copper treatment on ^{67}Cu retention (copper-treated animals: 22.9 $\pm$ 5.6 percent; control animals: 31.5 $\pm$ 13 percent).

Copper absorption in the GI tract has been studied in rats and hamsters. Absorption takes place from the stomach and duodenum in rats (Van Campen and Mitchell, 1965) and from the lower small intestine in hamsters (Crampton *et al.*, 1965). Copper absorbed from the GI tract may be bound to amino acids or in the form of ionic copper. Copper becomes bound to metallothionein (MT) in the intestine and is released into the bloodstream as metallothionein-copper (Marceau *et al.*, 1970). It does not appear that MT is the protein binding Cu in the small intestine during early life because induction of MT is much higher in adolescent rats than in younger rats (Varada *et al.,* 1993).

Intragastric administration of a copper sulfate solution into the stomach and duodenum of ferrets with ligated pyloric sphincters showed the stomach to be the primary site of the emetic response to copper sulfate in this species (Makale and King, 1992). Studies conducted by other authors in both dogs and ferrets (Andrews *et al*., 1990; Bahandari and

Andrews, 1991; Fukui *et al*., 1994) confirm the importance of GI neural pathways and receptors in copper sulfate-induced emesis. In beagle dogs, the vomiting response to 100 mg of copper sulfate per kg of body weight was reduced or eliminated by high doses of a chemical blocker of receptors for serotonin as well as severing the vagus and splanchnic nerves. Serotonin is a neuroactive compound that may activate or sensitize abdominal gastric nerves involved in the emetic response (Fukui *et al.*, 1994).

Protein source (plant or animal protein), amino acids, carbohydrates and ascorbic acid can affect copper availability (Gibson, 1994; Lonnerdal, 1996). Competition with zinc and cadmium affects copper absorption from both diet and drinking water (Davies and Campbell, 1977; Hall *et al.*, 1979). Ascorbic acid may alter the metallothionein binding site. High dietary ascorbic acid has been shown to interfere with absorption of copper in guinea pigs (Smith and Bidlack, 1980), but this does not appear to be a factor at the usual ascorbic acid doses in humans (Jacob *et al.*, 1987). Phytates and fiber have been postulated to interfere with copper absorption by forming complexes with copper (Gibson, 1994). However, phytic acid reportedly affects human absorption of iron, calcium, and zinc but not copper (Hurrel, 2003; Egli *et al*., 2004). Relative absorption of copper from a vegetarian diet was reported to be slightly less than that from a non-vegetarian diet (33 versus 42 percent) (Hunt and Vanderpool, 2001). The endogenous copper in pinto beans was recently reported to be as bioavailable in rats as copper from copper sulfate (Saari *et al.*, 2006). The amount of stored copper in humans (mainly in the liver) does not appear to affect copper absorption (Strickland *et al.*, 1972). There do not appear to be any available studies of copper absorption in humans by inhalation.

Batsura (1969) observed copper oxide in alveolar capillaries after rats were exposed to welding dust from a pure copper wire. No studies of the rate or extent of absorption of copper through intact human skin were found, but as copper can cause contact dermatitis, some absorption must occur (ATSDR, 1990). Pirot *et al.* (1996) studied the absorption of copper and zinc through human skin *in vitro*. Skin absorption is not likely to contribute significantly to total copper absorption.

Distribution

Copper in the portal blood and general circulation is transported in the plasma bound to ceruloplasmin, albumin and transcuprein (Cousins, 1985). Most of this bound copper is then rapidly deposited in the liver. Ceruloplasmin is a cysteine-rich glycoprotein with many free sulfhydryl groups that serve as binding points for metals; it can bind copper or zinc, but has a stronger affinity for copper (Cousins, 1985). Ceruloplasmin is synthesized on membrane-bound polyribosomes of liver parenchymal cells and secreted into the plasma. Copper that enters the portal circulation from the intestine is transported directly to the liver. Copper released from the liver is transported in the bloodstream to other organs including the kidney and brain. The synthesis of ceruloplasmin is controlled by interleukin-I via glucagon or glucocorticoid (Cousins, 1985). Circulating copper levels are elevated in pregnant women because hormonal changes associated with pregnancy stimulate ceruloplasmin synthesis (Solomons, 1985). Ceruloplasmin levels may be useful as an indicator of copper status (Mendez *et al.*, 2004).

Recently, several copper transporters involved in copper uptake and transport by cells have been identified (Bauerly *et al.,* 2005). Copper transporter-1 (Crt1) is a copper import protein that is copper specific, and is believed to mediate copper uptake into the small intestine (Lee *et al*., 2002). Crt1 is expressed in the enterocytes of the small intestine and in enterocyte-like Caco-2 cells in culture (Klomp *et al*., 2002; Kuo *et al*., 2001). The copper efflux protein, ATPase7A, is thought to mediate copper efflux across the plasma membrane during copper excess in transfected cells (Petris *et al.,* 1996). Menkes disease, characterized by excessive copper accumulation in the intestine and systemic copper deficiency, is a consequence of a defect in ATPase7A (Schaefer and Gitlin, 1999). ATPase7B, with functional similarity to ATPase7A, exports copper into bile for excretion (Roelofsen *et al.,* 2000); ATPase7B is localized primarily in the liver with lower expression found in the intestine, kidney and placenta (Lockhart *et al*., 2000). A defect in ATPase7B results in Wilson's disease, characterized by copper accumulation as a result of impaired biliary copper excretion, and liver and brain damage.

Metabolism/Excretion

The liver and intestine play key roles in copper metabolism. Copper is taken up by hepatocytes from the portal circulation. The mechanism by which copper enters hepatocytes from transcuprein and albumin has not yet been elucidated. After uptake by the hepatocyte, a portion of copper is incorporated into ceruloplasmin. Much of the incoming copper binds to several macromolecules, including metallothionein, a protein that also binds zinc, iron and mercury (Linder, 1991). Copper can be released from hepatocytes into the general circulation to be transported to other tissues, or it can be excreted from the liver in bile (Cousins, 1985). Copper is excreted from the body in bile, feces, sweat, hair, menses and urine (Luza and Speisky, 1996; Cox, 1999). Only a small amount of copper is excreted in the urine (Cousins, 1985); the major route of excretion is in the bile. Biliary copper is discharged to the intestine, where, after minimal reabsorption, it is eliminated in feces. Biliary export seems to involve glutathione-dependent and glutathione-independent processes (NRC, 2000). Biliary excretion in human infants is immature at birth, and the lack of an effective excretion mechanism may place infants at increased risk for copper toxicity. It has been suggested that sheep susceptibility to copper toxicity is due to a reduction in biliary copper excretion (Weber *et al.*, 1980). Age-related differences in susceptibility to copper-induced liver damage have also been observed in studies with young animals (Bauerly *et al.*, 2005; Fuentealba *et al.,* 2000). Excretion of copper in bile may be even more important than absorption in regulating total body level of copper (Turnlund *et al*., 1998).

Physiological/Nutritional Role

Because copper is an essential nutrient that has numerous physiological roles in the body, an understanding of these roles is essential for understanding the deleterious effects of copper deficiency or excess. Copper is essential for hemoglobin synthesis and erythropoiesis (Solomons, 1985; Harris, 1997). Copper deficiency can therefore lead to anemia. Copper deficiency can likewise lead to abnormalities of myelin formation, with attendant effects on the nervous system (Solomons, 1985; Harris, 1997). Nervous system

effects, including dementia, have been observed in individuals with copper deficiency or excess (Solomons, 1985; Harris, 1997). Effects on catecholamine metabolism likewise are involved in the nervous system abnormalities. Other physiological functions that involve copper include: leukopoiesis, skeletal mineralization, connective tissue synthesis, melanin synthesis, oxidative phosphorylation, thermal regulation, antioxidant protection, cholesterol metabolism, immune and cardiac function, and regulation of glucose metabolism. Since all of these physiological processes involve copper, any of them can be affected by the availability of copper in the body or in specific tissues. In general, deleterious effects may occur in any of these physiological processes due to either deficiency or excess of copper in the systems affected (Solomons, 1985; Harris, 1997). Clinical dietary copper deficiencies are rare, especially in developed countries (Olivares *et al.*, 1999; IOM, 2001).

In infants, copper is an essential mineral that is required for normal growth, and the development of bone, brain, immune system, and red blood cells (Hurley *et al*., 1980). Fetal copper accumulation occurs primarily during the third trimester (Widdowson, 1977). Full-term infants are believed to possess adequate copper stores at birth to last through weaning, but premature infants, prone to copper deficiency, must be given higher provisions of copper to compensate for inadequate copper stores (Lonnerdal, 1998). Preterm infants appear to have limited capacity to utilize copper from the diet. Studies in human infants suggest that either the absorption mechanisms of preterm infants are ineffective or the capacity of preterm infants to retain copper is poor (Cavell and Widdowson, 1964; Dauncey *et al.,* 1977). Copper deficiency has been described in premature infants, and is characterized by edema, anemia, leucopenia, neutropenia, and osteoporosis (Sutton *et al.*, 1985).

Recommended Daily Allowances for copper were not provided in the early RDA compilations because of difficulty in determining the values (NAS, 1989). Homeostatic mechanisms result in variable absorption and excretion of copper as dietary intake is manipulated, complicating mass balance calculations in dietary studies. However, in the most recent publication of recommended allowances (IOM, 2001), copper nutritional requirements have at last been established for adults.

Table 2 shows the Dietary Reference Intake (DRI) values for copper for various age groups, broken down into Estimated Average Requirements (EAR), RDAs, and Tolerable Upper Intake Levels (UL) (IOM, 2001). The data were not sufficient to establish RDAs for infants. Values for infants were provided only as Adequate Intake (AI) values, based primarily on content of copper in human milk. The AI values are 200 μg/day for infants 0-6 months of age, and 220 μg/day for infants at 7-12 months; an estimated UL for infants could not be established (IOM, 2001). The IOM (2001) recommended 10 mg/day as a UL for adults from foods and supplements. The WHO (1996) estimated that average copper requirements are about 50 μg/kg of body weight/day for infants.

Copper intake values from food and supplements, developed from the NHANES III nationwide survey (1988-1994) (NHANES, 1996) are shown in Table 3. The NHANES III table and Continuing Survey of Food Intakes of Individuals (CSFII) indicate that intake of copper is adequate for the great majority of the population in all age and sex groups. However, results for some younger sex/age groups indicate as much as 10 percent of the population consuming less than the RDA of copper. On the other hand,

considering the tendency for underreporting of food intakes, particularly for teenagers (Champagne *et al.*, 1998), the lower end of the distribution curve is likely to be inaccurate.

Table 2. Recommended Daily Copper Dietary Reference Intakes by Sex/Age

Age (years)	Sex	Estimated Average Requirement μg/day	Recommended Dietary Allowance μg/day	Tolerable Upper Intake Level μg/day
1-3	F/M	260	340	1,000
4-8	F/M	340	440	3,000
9-13s	F/M	540	700	5,000
14-18	F/M	685	890	8,000
19+	F/M	700	900	10,000
Pregnant, 14-18 19+	F	785 800	1,000 1,000	8,000 10,000
Lactating, 14-18 19+	F	985 1,000	1,300 1,300	8,000 10,000

Values from IOM, 2001.

Table 3. Copper Intake (mg/day) from Food and Supplements[a] Versus the RDA[b,c]

Age and Sex		Intake Percentiles								RDA (mg/day)
		5	10	25	50	75	90	95	99	
2-6 mo	M/F	0.3	0.4	0.5	0.7	0.9	1.1	1.2	1.6	0.20
7-11 mo	M/F	0.3	0.4	0.5	0.7	0.9	1.2	1.3	1.7	0.22
1-3 yr	M/F	0.3	0.4	0.5	0.7	1.0	1.3	1.7	2.9	0.34
4-8 yr	M/F	0.59	0.67	0.80	0.95	1.14	1.36	1.61	3.06	0.44
9-13 yr	F	0.64	0.72	0.86	1.04	1.26	1.54	1.84	3.23	0.70
	M	0.88	0.94	1.05	1.21	1.41	1.61	1.78	3.13	0.70
14-18 yr	F	0.64	0.75	0.89	1.08	1.32	1.64	1.96	3.32	0.89
	M	0.79	0.89	1.11	1.42	1.80	2.28	2.71	3.56	0.89
19-30 yr	F	0.77	0.83	0.95	1.12	1.38	1.82	3.03	3.84	0.90
	M	1.37	1.43	1.56	1.69	1.86	2.12	3.55	4.44	0.90
31-50 yr	F	0.72	0.81	0.95	1.17	1.52	2.32	3.09	4.19	0.90
	M	0.89	1.03	1.29	1.61	2.09	2.93	3.67	4.87	0.90
51-70 yr	F	0.61	0.68	0.84	1.07	1.48	2.92	3.25	4.22	0.90
	M	0.75	0.87	1.09	1.43	1.98	3.00	3.65	5.02	0.90
71+ yr	F	0.58	0.65	0.80	1.02	1.37	2.94	3.21	3.79	0.90

Age and Sex		Intake Percentiles								RDA (mg/day)
		5	10	25	50	75	90	95	99	
	M	0.72	0.83	0.99	1.26	1.66	2.89	3.41	4.61	0.90
Pregnant	F	0.71	0.82	1.07	1.62	3.11	4.03	4.39	5.56	1.0

[a] Food and supplement values from NHANES, 1996.
[b] RDA values from IOM, 2001.
[c] Breast-feeding infants and children, and eight individuals reporting greater than 150 mg/day of copper from supplements excluded from the analysis.

TOXICOLOGY

Toxicological Effects in Animals

Mechanism of Action

Manifestations of acute copper toxicity include abdominal pain, nausea, vomiting and diarrhea. Copper can produce GI symptoms by irritating the gut mucosa and/or by altering the microbial flora of the colon. In animal studies, absorption of copper ions has been shown to have a direct effect on gastric mucosa nerve endings of the parasympathetic nervous system (Niijima *et al.*, 1987). Several studies indicate that vomiting induced by copper is mediated by serotonin gastric receptors. Intragastric administration of a copper sulfate solution into the stomach and duodenum of ferrets with ligated pyloric sphincters showed the stomach to be the primary site of the emetic response to copper sulfate in this species (Makale and King, 1992). Studies conducted by other authors in both dogs and ferrets (Andrews *et al*., 1990; Bahandari and Andrews, 1991; Fukui *et al*., 1994) confirm the importance of GI neural pathways and receptors in copper sulfate-induced emesis. In beagle dogs, the vomiting response to 100 mg of copper sulfate per kg of body weight was reduced or eliminated by high doses of a chemical blocker of receptors for serotonin as well as severing the vagus and splanchnic nerves. Serotonin is a neuroactive compound that may activate or sensitize abdominal gastric nerves involved in the emetic response (Fukui *et al.*, 1994).

Exposure to high levels of copper in the diet can lead to hepatocellular necrosis in the liver, multifocal hepatitis, apoptosis, and structural damage to proximal convoluted tubules in the kidneys (Haywood, 1985; Fuentealba *et al*., 2000). Apoptosis has been consistently described in copper-induced liver damage (Deng *et al.,* 1998; Fuentealba and Haywood, 1988; Haywood *et al.*, 1996; King and Bremner, 1979). Many of the toxic effects of copper, such as increased lipid peroxidation in cell membranes and DNA damage, are related to its role in the generation of free radicals.

Young animals may be at greater risk for the adverse effects of high copper exposure than adults. Neonates naturally have a high liver copper concentration at birth, and mechanisms that control intestinal copper absorption are immature during early neonatal life in rats (Varada *et al.,* 1993). A number of studies in young animals have observed

age-related differences in susceptibility to copper-induced liver damage (Araya *et al*., 2005; Bauerly *et al.*, 2005; Fuentealba *et al.,* 2000).

Acute Toxicity

An oral LD_{50} of 300 mg cupric sulfate/kg in rats has been reported (Siegel and Sisler, 1977). Details of the toxic effects on the rats were not reported.

Acute toxicity values are available for a wide variety of aquatic animal and plant species, because of the high sensitivity of aquatic organisms (especially invertebrates) to copper, the use of copper in pesticides, and frequent contamination of waterways with copper. Toxicity to aquatic organisms is well summarized in the U.S. EPA ambient water quality criteria documents for copper (U.S. EPA, 2003, 2007), and will not be further discussed here.

Subchronic Toxicity

Bauerly *et al.* (2005) exposed suckling rat pups to amounts of copper that bottle-fed infants may receive to determine the effects of copper supplementation on tissue copper distribution, copper transport and copper transporter levels in early and late infancy. Newborn rat pups were given a daily dose of 0, 10 or 25 µg Cu/day as $CuSO_4$ in a 10 percent sucrose solution by oral gavage during the suckling period, and weaned to a standard diet containing 13 µg/g of Cu. Since the development of copper transporters is age-dependent, pups were killed on postnatal days 10 and 20, when copper transport mechanisms were "immature," and "mature," respectively. Small intestine, liver, kidney, brain and spleen were collected for mineral analysis. Copper concentration, copper transporter-1 (Crt1), Atp7B, and MT mRNA and protein levels were measured. ^{67}Cu absorption was measured in control and copper-supplemented pups on day 10 and day 20. There was no significant effect of copper supplementation on body weight, serum copper or ceruloplasmin activity, despite increased tissue copper concentration in day 10 pups. Copper supplementation and age had a significant effect on intestine copper concentration; at day 10, intestine copper concentration was significantly higher in pups supplemented with 25 µg/day of Cu ($P<0.0001$), while no significant effect of copper supplementation was observed at day 20 ($P=0.6$). At day 10, supplemented pups retained 149.9 µg/g Cu in the intestine, compared to 34.6 µg/g Cu in controls, while at day 20, no difference in copper concentration was observed between groups. Copper supplementation resulted in elevated plasma alanine aminotransferase (ALT) levels, "suggesting a risk of copper toxicity with supplementation during infancy." There was no significant effect of copper supplementation, age or interaction on total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (AP), bilirubin, or glutamate dehydrogenase (GD) activity. With increasing copper intake, total copper absorption decreased and intestinal copper retention increased. At day 10, intestinal copper concentration, MT mRNA and Crt1 protein levels increased with supplementation. At day 20, Ctr1 protein and Atp7A mRNA and protein levels were higher than controls. Despite these adaptive changes, copper accumulated in the liver of exposed animals, and liver enzymes were elevated, indicating that liver copper accumulation had adverse effects. The copper levels used in this study were chosen to

simulate the copper intake of formula-fed infants. The authors state that the supplementation level of 10 µg/day Cu is, on a body weight basis, similar to the copper intake of a formula-fed infant. This level of copper supplementation did not affect small intestine and liver copper concentration. However, rat pups supplemented with 25 µg/day Cu, a level similar to the copper intake of infants fed formula made with copper-contaminated water, retained copper in their liver and small intestine. The authors suggest that infants exposed at this level may be at risk for copper toxicity.

Araya *et al.* (2005) bottlefed *ad libitum* infant rhesus monkeys from birth to age five months with a standard infant formula containing 0.6 mg Cu/L (n=4) or the same formula supplemented with $CuSO_4$ (an additional 6.0 mg Cu/L) (n=5). (The copper load was based on estimations of the copper intake needed to induce Indian childhood cirrhosis). The objective of the study was to assess copper retention, liver copper content and liver function. Food intake was monitored daily. Blood samples were drawn every month during treatment, and at ages six, eight, and twelve months. At ages one, five, and eight months, copper absorption was measured following radioisotope administration (^{67}Cu). One biopsy of liver tissue was taken for each control animal at two months of age; tissue samples for copper-supplemented animals were taken at one and five months of age. Liver function and histology was monitored up to eight months of age.

Food intake did not differ significantly between copper-treated and control animals (data not provided). No clinical evidence of copper toxicity was observed, however, copper treatment induced detectable changes in the liver. Fine, small cytoplasmic granules were seen in some areas in copper-treated animals at five months of age whereas, in the control animals, rhodamine staining was negative at all times. (In children with biliary atresia, Wilson's disease, and ICC, rhodamine staining is intensely positive (Tanner *et al.*, 1979, Suchy *et al.,* 1981).) The copper-treated animals showed a marked increase in liver macrophages (Kuppfer cells) over control animals; 70 and 49 percent more Kupffer cells at ages one and five months, respectively, than controls. (Kuppfer cells are known to migrate rapidly and proliferate locally in response to various stimuli.) At five months of age, treated animals had twice as many apoptotic nuclei as controls, and this effect was significantly greater at age five months than at age one month. (Similar findings have been observed in rats due to excess dietary copper) (Fuentealba *et al.*, 2000). At age eight months, all indicators measured were normal and no significant differences were seen between groups. Copper treated animals had significantly higher liver copper concentrations at ages one, five, and eight months compared to control animals. Copper-supplemented animals had significantly lower plasma zinc concentrations than did control animals during the supplementation period; plasma zinc concentrations returned to normal after discontinuation of copper supplementation. Changes in cell ultrastructure, suggestive of early tissue damage, were seen one month after copper treatment: irregularly shaped nuclei containing condensed chromatin were often seen on electron microscopy, mitochondria displayed visible cristae, hepatocytes contained numerous secondary lysosomes which were filled with electron dense material, many cells had scanty glycogen rosettes and numerous small vesicles, the rough endoplasmic reticulum was somewhat distorted in certain cells. At five months of age, the cell ultrastructure of hepatocytes was normal in all animals.

Excess dietary copper caused substantial liver injury, as evidenced by morphologic changes and increased activity of liver enzymes in both adult and young rats fed a copper-loaded diet, though the young rats were more susceptible than adults to copper-induced liver injury (Fuentealba *et al.,* 2000). Adult Fischer 344 male and female rats were administered a diet containing 1,500 ppm copper for 18 weeks. Young male and female rats were fed a similar diet from birth until 16 weeks of age. Age- and sex-matched control rats were fed a normal rodent diet (<10 ppm copper). After 12 weeks on the experimental diet, young Cu-loaded rats were pale and weighed 50 percent less than control animals, and the experiment was concluded for this age group (as per Guidelines of the Canadian Council on Animal Care). All copper-loaded rats had significantly ($p<0.05$) increased hepatic copper concentrations compared to controls. Young copper-loaded rats accumulated more hepatic copper, had more severe liver changes, and had higher serum liver enzyme activities than adult rats. Two out of eight Cu-loaded young female rats died during the experiment. Young female rats also accumulated almost 100 ppm more copper than young male rats (the authors state that sexual dimorphism with regard to hepatic copper accumulation in rats is highly dependent on the strain used). Adult male and female Cu-loaded rats showed significantly increased activity of alanine amine transferase (ALT) and sorbitol dehydrogenase (SDH) relative to controls. In young rats, ALT, SDH, alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) were significantly increased in both sexes relative to control animals. Increased levels of total bilirubin were also found in all Cu-loaded groups compared to controls. Histologic changes in copper-loaded rats consisted of multifocal hepatitis and widespread single-cell necrosis. Cytoplasmic copper was detected histochemically in centroacinar zone 1 (periportal) and mid-zone in copper-loaded rats. Intracytoplasmic immunoreactivity for MT was prominent in necrotic hepatocytes and within inflammatory foci in copper-loaded rats. (According to the authors, the presence of MT in these areas may be interpreted as an indication of free radical scavenging by MT in an effort to protect the liver from further damage.) Hepatic MT was significantly ($p \leq 0.05$) lower in male Cu-loaded rats compared to control male rats and compared to both Cu-loaded and control female rats. No differences in hepatic zinc concentrations were detected between adult copper-loaded and control rats.

In a National Toxicology Program (NTP) study (1993), rats and mice were exposed to cupric sulfate in drinking water (free drinking) at concentrations up to 30,000 ppm for 15 days. The only compound-related toxic effect observed was an increase in the size and number of cytoplasmic protein droplets in the epithelium of the renal proximal convoluted tubules in male rats of the 300 and 1,000 ppm groups (NTP, 1993). The absence of effects at the highest exposure level (30,000 ppm) may have been due to taste aversion.

The above-mentioned NTP study also included a two-week feeding study with concentrations of cupric sulfate in feed ranging from 1,000 to 16,000 ppm (NTP, 1993). In this study hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the forestomach was observed in rats and mice of both sexes in all dosage groups (NTP, 1993). Periportal to midzonal inflammation of the liver occurred in rats of the 8,000 and 16,000 ppm groups. Both male and female rats in the 8,000 and 16,000 ppm groups showed depletion of hematopoietic cells in the bone marrow and spleen. Male

and female rats in the 4,000, 8,000 and 16,000 ppm groups exhibited increased protein droplets in the epithelia of the renal cortical tubules (NTP, 1993).

Exposure to high levels of copper in the diet can lead to hepatocellular necrosis in the liver and structural damage to proximal convoluted tubules in the kidneys (Haywood, 1985). Rats administered 3,000 to 5,000 ppm of copper in the diet developed these pathological changes, but gradually adapted to the high copper diets after four to six weeks (Haywood, 1985). Adaptation involved changes in copper metabolism, and regeneration of damaged tubular epithelium in the kidneys. Regenerated epithelium is histologically different from undamaged epithelium. Rats exposed to 6,000 ppm of copper in the diet (300 mg/kg-day) were not able to adapt, and in some cases died from extensive centrilobular necrosis (Haywood, 1985).

Rats administered a diet containing 4,000 ppm of copper (approximately 133 mg/kg-day) for one week exhibited increased mortality from anorexia, possibly resulting from taste aversion (Boyden *et al.*, 1938).

Chronic Toxicity

Pigs administered up to 250 ppm copper in their diet had significantly reduced body weight gain, apparently resulting from reduced food consumption. They also exhibited reduced hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin and plasma and liver iron levels (Gipp *et al*., 1973). Sheep are more sensitive to copper toxicity than are pigs. As little as 10 to 15 ppm copper in the diet of sheep resulted in hemolytic anemia (Booth and McDonald, 1982). In the case of copper poisoning in sheep there is a long delay period of several months, during which copper accumulates in the liver lysosomes. When the capacity of the sheep liver to store copper is exceeded, the copper is released and brings about the toxic effects.

Genetic Toxicity

Dose-related mutagenesis in a reverse mutation assay in *Escherichia coli* exposed to 2 to 10 ppm cupric sulfate have been reported by Demerec *et al.* (1951). A more recent study by Moriya *et al.* (1983) resulted in no increase in mutations in *E. coli* or *Salmonella typhimurium* strains TA98, TA1535, TA1537 and TA1538 incubated with up to 5 mg copper quinolinate per plate or in *S. typhimurium* strains TA98 and TA100 incubated with up to 5 mg cupric sulfate per plate. Negative results were also obtained with cupric sulfate or cupric chloride in assays with *Saccharomyces cerevisiae* (Singh, 1983) and *Bacillus subtilis* (Nishioka, 1975; Matsui, 1980; Kanematsu *et al.*, 1980).

Sirover and Loeb (1976) investigated the effect of metal salts, including copper salts, on the fidelity of DNA transcription from poly(C) and other templates in an *in vitro* system that included DNA polymerase from avian myeloblastosis virus. They found that the copper salts tested decreased the fidelity of transcription by more than 30 percent. Induction of chromosomal aberrations has been reported in isolated rat hepatocytes incubated with cupric sulfate (Sina *et al.*, 1983).

Cuprous sulfide and cupric sulfate enhanced cell transformation in Syrian hamster embryo cells infected with simian adenovirus (Casto *et al.*, 1979). Kim *et al.* (1994) studied the mechanism of cellular copper toxicity in Long-Evans Cinnamon (LEC) mutant rats. They found that a cellular event required for the initiation of DNA synthesis upon growth stimulation is impaired by copper cytotoxicity.

Injection of inbred Swiss mice with doses of copper sulfate ranging from 5 to 20 mg/kg resulted in dose-dependent statistically significant increases in chromosomal aberrations, micronuclei and sperm abnormalities (Bhunya and Pati, 1987). Thus, there is *in vivo* as well as *in vitro* evidence for the genotoxicity of copper salts (ATSDR, 2004).

Neurotoxicity

Excess copper has been reported to disrupt a number of processes in the central nervous system (De Vries *et al.*, 1986). Copper administered to rats acted on brain synapses to inhibit uptake of monoamines including noradrenaline and dopamine (De Vries *et al.*, 1986).

Copper exposure has also been investigated as a factor in the pathogenesis of neurodegenerative diseases, especially Alzheimer's disease. One line of research has been directed toward the hypothesis that Alzheimer's disease is associated with excess copper in plasma and/or brain (Sparks, 2004; Puglielli *et al.*, 2005, Sparks *et al.*, 2006; Dai *et al.*, 2006; Lu *et al.*, 2006; Morris *et al*., 2006; Brewer, 2007), while another line has associated Alzheimer's disease with a copper deficit (Pajonk *et al.*, 2005; Kessler *et al.*, 2005, 2006; Schafer *et al.*, 2007). The common factor among these studies is the recognition that copper is a component of many important metalloenzymes, such as superoxide dismutase, and also can be involved in oxidation/reduction reactions (White *et al.*, 2004) and possibly, inflammation processes (Campbell, 2006; Becaria *et al*., 2006). Copper also binds to amyloid beta protein (Puglielli *et al.*, 2005), which forms plaques in the brains of Alzheimer's patients.

Some of the differences between the two hypotheses relate to different neurodegeneration models used in animal studies. Several of the studies implying a positive association between copper and neurodegeneration involve animals fed high-cholesterol diets, which are associated with brain pathological changes. These changes may be accelerated by copper administration in drinking water (Sparks, 2004, Lu *et al*., 2006; Sparks *et al.*, 2006). However, the low level of copper in drinking water (0.12 ppm) associated with this effect in the reports (Sparks, 2004, Sparks *et al.*, 2006) represents a very small fraction (less than 10 percent) of the contribution from food (copper is added to all animal diets because it is an essential nutrient). While it can be postulated that the difference is due to greater availability of copper from water than from food, numerous nutritional studies indicate substantial bioavailability of copper from normal dietary sources. A recent study reported that endogenous copper in pinto beans is equally as bioavailable as copper from copper sulfate (Saari *et al.*, 2006). Influence of copper carriers in food on disposition and effects of copper remains to be investigated.

Another line of research involves the demonstration that a copper and zinc chelator, clioquinol, can apparently decrease beta-amyloid deposition in a mouse model of Alzheimer's disease (Cherny *et al.*, 2001). This was taken as evidence that excess copper

might increase the risk of Alzheimer's disease. However, other studies show that clioquinol binding to copper can help transport copper into the brain, rather than decrease it (Treiber *et al.*, 2004; Schafer *et al.*, 2007). Coupled with other evidence that mouse models of Alzheimer's disease are associated with low copper in brain (Kessler *et al.*, 2005; Maynard *et al.*, 2006), as has also been reported for the human disease (see section on Neurotoxicity in humans), the evidence that elevated exposure to copper might have a causative influence on the development of Alzheimer's disease seems weak.

Developmental and Reproductive Toxicity

To investigate reproductive effects of copper, Haddad *et al.* (1991) administered copper acetate in drinking water to albino Wistar rats before and during pregnancy. The water was supplemented with copper acetate increasing stepwise to a concentration of 0.185 percent over a period of seven weeks (details not provided). Copper was deposited in the liver and kidneys of pregnant rats, leading to inflammation of those organs. In 11.5-day-old embryos, mean yolk sac diameter, crown–rump length and somite number were significantly reduced. Moderate retardation of growth and differentiation, especially of the neural tube, was also found. Older embryos (21.5 days) had markedly reduced numbers of ossification centers in the vertebrae, sternum and phalanges of the forelimbs and hindlimbs when compared to untreated controls. In newborn rats, minimal growth retardation was found; only the numbers of cervical and caudal vertebrae and hind-limb phalanges were significantly reduced. The authors concluded that loading maternal rats with copper at tissue levels approximately 10-fold above normal was toxic to the dams (inflammation of liver and kidneys) but resulted in only minor growth retardation to the offspring.

Bataineh *et al.* (1998) evaluated the effects of long-term ingestion of four metal salts on aggression, sexual behavior and fertility in adult male rats. Only the findings for the metal salt copper chloride are summarized here. Sprague-Dawley rats (n = 5) were exposed via drinking water for a period of 12 weeks to copper chloride [$CuCl_2 \cdot 2H_2O$] dissolved in tap water at a concentration of 1000 ppm/L. Control rats (n=10) were given tap water for the same period. No mortality or clinical signs of toxicity were observed in treated animals. Body weight, absolute and relative testes weight, and seminal vesicle weight was significantly decreased in copper chloride exposed males compared with controls. Ingestion of copper chloride resulted in marked suppression of sexual performance and aggression. The ingestion of copper chloride affected the initiation of copulatory behavior as evidenced by a significant latency in intromission and time to first mount ($P<0.001$), and significant prolongation of the post ejaculatory interval ($P<0.01$) compared to controls. Copulatory efficiency was also significantly reduced in male rats exposed to copper chloride ($P<0.001$) compared to control animals. Male rats exposed to copper chloride exhibited low aggression evident as significantly less lateralizations, boxing bouts and fights with a stud male. The authors concluded that the metal salts produced their effects on aggression and sexual behavior by acting directly or indirectly on the testes, and by influencing the androgen biosynthesis pathway. Preputial gland weights, which produce behavior-modulating pheromones that alter fighting and other behavior, were not affected by exposure to copper chloride.

Immunological Effects

Copper and copper complexes have anti-inflammatory, antiulcerogenic and anticarcinogenic effects. They are sometimes administered to patients for these effects (Sorenson, 1983). However, excess copper may have deleterious effects on the immune system as evidenced by increased severity of infections in chickens (Hill, 1980) and mice (Vaughn and Winberg, 1978).

To further investigate the effect of excess copper on the immune system, Pocino *et al.* (1991) investigated the proliferative response to T and B cell mitogens, and the delayed-type hypersensitivity (DTH) response in mice exposed to excess copper (50, 100, 200 or 300 ppm) in drinking water (Pocino *et al.*, 1991). They found the DTH response was significantly inhibited in mice exposed to 100 ppm copper; and the proliferative response to T and B cell mitogens was significantly inhibited in animals exposed to 200 ppm copper.

Carcinogenicity

In a study published in 1968, Bionetics Research Labs tested copper hydroxyquinoline for carcinogenic effect in $B6C3F_1$ and $B6AKF_1$ mice. Groups of 18 male and 18 female seven-day-old mice were given daily by gavage 1,000 mg copper hydroxyquinoline per kg of body weight (180.6 mg Cu/kg) suspended in 0.5 percent gelatin until they were 23 days old, after which they were given 2,800 ppm (505.6 ppm Cu) in their feed for 50 additional weeks. No statistically significant increases in tumor incidence were observed in the treated animals (U.S. EPA, 1987; IRIS, 2007).

In the same study, 28-day-old mice of both strains and sexes were given a single injection of 1,000 mg copper hydroxyquinoline/kg (180.6 mg Cu/kg) suspended in 0.5 percent gelatin. Control mice were given injections of only the gelatin. After 50 days, the male $B6C3F_1$ mice had an increased incidence of reticulum cell sarcomas compared with the controls. No tumors were observed in the treated male $B6AKF_1$ mice, and a low incidence of reticulum cell sarcomas was observed in treated female mice of both strains.

In experiments intended to determine the active agents in nickel refinery dust, Wistar rats (two and three months old) were injected intramuscularly in the thighs with 20 mg of cupric oxide (16 mg Cu), cupric sulfide (13.3 mg Cu), or cuprous sulfide (16 mg Cu) (Gilman, 1962). After 20 months, no injection site tumors were observed in the animals that had been injected with the copper compounds. "Miscellaneous tumors" (mammary fibroadenomas and a reticulocytoma) were detected at very low incidence in rats that received cupric sulfide (2/30) and cuprous sulfide (1/30).

Rats and mice exposed to copper in the diet at concentrations that yielded doses of 5 to 1,000 mg/kg exhibited no significant increases in tumor frequencies (Kamamoto *et al*. 1973; Green *et al*. 1987). Copper inhibited the carcinogenic effect of DL-ethionine in rat livers (Kamamoto *et al.*, 1973).

In an NTP study (1993) rats and mice were given 500 to 8,000 ppm cupric sulfate in the diet for 13 weeks. Rats in the three highest dose groups exhibited hyperplasia and hyperkeratosis of the forestomach, inflammation of the liver, and increases in the number

and size of protein droplets in the epithelium of the renal proximal convoluted tubules. Both sexes of mice receiving 4,000 ppm cupric sulfate and higher in the 13-week study exhibited increased hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge of the forestomach (NTP, 1993).

The U.S. EPA reviewed the published data and concluded that there is inadequate evidence to conclude that copper is carcinogenic in animals (IRIS, 2007). We concur with this conclusion.

Toxicological Effects in Humans

Acute Toxicity

Epigastric pain, headache, nausea, dizziness, vomiting and diarrhea, tachycardia, respiratory difficulty, hemolytic anemia, massive GI bleeding, liver and kidney failure, and death have resulted from copper toxicity (WHO, 1998). Death from ingestion of copper salts has been reported after as little as 2 grams of cupric sulfate (Stein *et al.*, 1976). Immediate deaths are caused by central nervous system (CNS) depression and shock. Later deaths (after 24 hours) are caused by hepatic and renal failure (Jantsch *et al.*, 1985). Deaths have also been reported as the result of the use of water with dissolved cupric sulfate in religious rituals (Akintowa *et al.*, 1989). The poisoned individuals ingested approximately 20 grams each of cupric sulfate dissolved in "spiritual water" at a concentration of 100 g/L. There were four fatal cases. The symptoms exhibited by these individuals included toxic psychosis, profound greenish vomiting, hemolytic anemia and jaundice. Death occurred within eight days after ingestion for all four victims.

A group of military nurses attending a party consumed a cocktail that had become contaminated with copper from the corroded vessel in which the beverage was prepared and stored (Wylie, 1957). Symptoms of acute copper intoxication (nausea, vomiting, dizziness and headache) were experienced by 10 of 15 women ½ to one hour after consuming the whiskey cocktails. The lowest amount of copper that gave rise to these symptoms was determined to be 5.3 mg (Wylie, 1957). This was used as a lowest observed adverse effect level (LOAEL) by U.S. EPA in setting the MCLG for copper (U.S. EPA, 1991b). U.S. EPA incorporated a safety factor of 2 so the calculation is:

$$\text{MCLG} = \frac{5.3 \text{ mg Cu}}{2 \times 2 \text{ L/day}} = 1.3 \text{ mg Cu/L}$$

Elevated copper concentrations in tap water were associated with GI illness in at least 43 people in a hotel (Kramer *et al.*, 1996). With the exception of one water sample that had a copper concentration of 156 mg/L, the highest copper concentration documented for the many other samples tested was 4.7 mg/L. The source of the copper was the building's plumbing system.

Twenty workers experienced GI distress and other symptoms of copper poisoning as a result of drinking morning tea prepared with water from an old "geyser" (gas-run water heater) made of sheet copper. The internal surfaces of the geyser were not lined with tin

as they usually are in this type of appliance. Leftover tea prepared in this geyser had a copper content of 30 ppm (30 mg/L). It is likely that the tea consumed by the workers had an even higher copper content (Nicholas, 1968).

To determine the nausea threshold for copper in water, Olivares *et al.* (2001) administered copper sulfate in purified water at concentrations of 0, 2, 4, 6, 8, 10, or 12 mg/L to 61 adult volunteers aged 18 to 50 years old (31 women, 30 men). Subjects (10/group) drank a fixed volume of 200 mL per test, once a week, for a maximum of 12 exposures (maximum of 2.3 mg per test and up to 23 mg of copper over a 22-week period). No responses were detected at 0 and 2 mg Cu/L. Mild nausea shortly after copper ingestion was the most prominent finding (33/61), starting at 4 mg/L; vomiting was observed in seven subjects, starting at 6 mg/L. No age or gender-related differences were found. In this study, the benchmark dose approach was used to derive the tolerable intake (TI) of copper in drinking water. The lower 95 percent confidence levels (LCLs) for copper concentration in water for the first 5 percent of the population responding to copper were 2 and 4.2 mg Cu/L for nausea and vomiting, respectively. For risk assessment purposes, these levels are considered equivalent to the NOAEL.

The same group also conducted a prospective, double-blind study in a population of 179 apparently healthy adults to determine the threshold for acute GI effects associated with drinking copper-containing water as the sulfate salt ($CuSO_4 \cdot 5H_2O$) (Araya *et al.*, 2001). Subjects were recruited from three different geographic locations to present a sample with broad cultural representation (USA, Ireland, and Chile). Age and sex distributions in the populations were similar. Subjects consumed 0, 2, 4, 6 or 8 mg/L copper as $CuSO_4$ in a bolus of 200 mL of water once weekly over a consecutive five week period; copper doses were equivalent to 0, 0.4, 0.8, 1.2, and 1.6 mg of elemental copper per trial, respectively. GI symptoms of nausea, abdominal pain, vomiting, or diarrhea were screened for a period of up to 24 hours. Nausea was the most prevalent symptom observed (average prevalence of nausea among all subjects, 27.3 percent) and was reported within the first 15 minutes of ingestion. For the combined three-site population, 8, 9, 14, 25, and 44 subjects responded positively to one or more GI symptoms at 0, 2, 4, 6, and 8 mg Cu/L, respectively. Analysis of the data showed a clear dose-response to the combined GI effects and to nausea alone. Statistically significant greater reporting of effects occurred at 6 and 8 mg Cu/L. As copper dose increased, female subjects reported significantly more occurrences of nausea and GI symptoms than male subjects. Although one or more GI effects were reported by at least one subject at each dose level, because there was no statistically significant increase in symptoms for either nausea or total GI symptoms at 4 mg Cu/L, the authors defined the NOAEL and LOAEL from this study as 4 (0.8 mg Cu) and 6 (1.2 mg Cu) mg Cu/L, respectively, for the combined study population. From the dose-response curve, the 95 percent lower confidence level for response of the first 5 percent of the population was 3.5-4 mg Cu/L. This study relied upon a laboratory-generated source of drinking water, and subjects were only exposed 1x/week to 200 mL, which is one tenth of the default volume of daily water consumption of 2 L/day for an adult.

In an attempt to corroborate the dose-response seen in the prior study (Araya *et al.*, 2001), Araya *et al.* (2003a) conducted a second controlled trial in adult human volunteers using only female subjects, and bottled water in place of a laboratory-generated water

source used previously. Results from the prior study, Araya *et al.* (2001), showed that female subjects were more sensitive than males to GI effects, primarily nausea. In this study (Araya *et al*., 2003a), 70 adult females (ages 18-60 years) at four different international sites (potentially 280 total) were administered a single bolus of 100, 150, or 200 mL of bottled drinking water with 0.4, 0.8, or 1.2 mg Cu as the sulfate salt once each week for 11 successive weeks, or until all 11 administrations were completed. Two additional doses (0 and 1.6 mg Cu) were added at the 200 mL volume in an attempt to corroborate the results of the earlier Araya *et al.* (2001) study. Only the 200 mL dosing volume included a control group. All subjects completed a questionnaire at 0, 0.25, and one hour post-dosing that screened for GI effects (nausea, vomiting, abdominal pain and diarrhea). Over the course of the study period, the subjects appear to have received varying water volumes/copper concentrations, but the results tabulated and discussed include only outcomes for the 200 mL volume. The number of exposures (doses) giving rise to this data are not provided. According to the authors, nausea was the earliest and most prevalent symptom reported. Within 15 minutes post-dosing, 24.3, 41.1, 25.9 and 50.0 percent of the subjects at each of the four sites, Santiago (Chile), Shanghai (China), Coleraine (Ireland), and Grand Forks (N. Dakota), respectively, reported at least one occurrence of nausea at any dose (copper concentrations ranged from 2 to 12 mg/L). Incidence and generalized linear model results data were provided for nausea, but not for the outcome variables of vomiting, diarrhea and abdominal pain. A separate analysis for the outcome, "GI symptoms," defined as the occurrence of one or more outcome symptoms (nausea, vomiting, diarrhea or abdominal pain), was conducted. The study authors concluded that at a dosing volume of 200 mL, a NOAEL for nausea in adult females occurs at 0.8 mg Cu (4 mg Cu/L) and a LOAEL at 1.2 mg Cu (6 mg Cu/L). NOAELs/LOAELs for the 100 and 150 mL dosing volumes could not be identified, as there were no control groups. In light of the fact that the probability of experiencing nausea increased as water volume decreased (or copper dose increased), it would have been helpful to have had this information. Separate NOAELs/LOAELs were not identified for each location, but rather were identified based on the *combined* group. However, location was shown to be a significant variable; the probability of a significant response (across all doses) was greater in Grand Forks than Santiago at 15 minutes. At 60 minutes, the probability of a significant response was greater in Grand Forks than in Santiago and Coleraine. The International Copper Association provided funding for this work.

Other authors similarly have found geographic location to be a significant variable. Sharrett *et al*. (1982) reported that the median copper concentration of tap water in Seattle, Washington exceeded comparable values in approximately 80 percent of Canadian cities. The median measured copper concentration in Seattle homes was 760 µg Cu/L compared with 205 µg Cu/L in Canada. Seattle residents in this study whose homes had copper plumbing consumed a substantial proportion of their daily required copper from their drinking water. In cities with corrosive water, significant differences in everyday copper intake from tap water may result.

Copper has been shown to be involved in the metabolism of vasoneuractive amines such as serotonin, tyramine and the catecholamines (Harrison, 1986). Harrison presents evidence that the ingestion of foods with high copper content (e.g., chocolate) or which facilitate absorption of copper (e.g., citrus fruits) may trigger migraine headaches,

particularly in individuals with abnormal copper metabolism due to low levels of ceruloplasmin, transferrin, or albumin. This paper presents no data that could be used to estimate a dose-response relationship, but the author recommends that individuals subject to migraines avoid foods high in copper.

Subchronic Toxicity

Pratt *et al.* (1985) studied oral administration of copper gluconate capsules in seven adults who were administered 10 mg/day of copper (0.14 mg/kg-day) for 12 weeks. No changes were found in the biomarkers of liver damage, serum aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, and lactate dehydrogenase. Similarly, no changes in serum indicators of liver damage were found by Araya *et al.* (2003) in adults administered 0.17 mg/kg-day of copper as copper sulfate for eight weeks, although there were acute gastrointestinal symptoms at this dose and concentration (6 mg/L). However, O'Donohue *et al*. (1993) reported jaundice and hepatomegaly in an adult who had consumed dietary supplements containing 30 mg/day of copper for two years, followed by 60 mg/day for one year. The subchronic NOAEL for copper to avoid liver toxicity thus appears to be about 10 mg/day. Liver toxicity was used as the critical endpoint in the evaluation of the Food and Nutrition Board (IOM, 2001), and was cited, along with the acute GI effects, as a critical endpoint in the evaluation by the National Research Council (NRC) Committee on Copper in Drinking Water (NRC, 2000).

Olivares *et al.* (1998) found no differences in growth and morbidity (diarrhea and respiratory infections) among 128 Chilean infants who were randomly assigned to receive daily, from 3 to 12 months of age, water (and bottles) with either <0.1 mg Cu/L or 2 mg Cu/L. The formula and breast-fed groups who received water and/or formula with <0.1 mg Cu/L served as the controls. The average copper content in Santiago, Chile's tap water is reported in the study as < 0.1 mg/L. The 2 mg Cu/L concentration was chosen to confirm the safety of the WHO value for copper in drinking water during infancy. The study was comprised of four groups: 56 formula-fed infants who received water with a copper content of 2 mg Cu/L, 27 formula-fed infants who received water with a copper content of less than 0.1 mg Cu/L, 24 breast-fed infants who received water with a copper content of 2 mg Cu/L, and 21 breast-fed infants who received water with a copper content of less than 0.1 mg Cu/L. Water ingested by mothers of breast-fed infants also contained the specified copper concentration. A standard copper sulfate solution of 2 mg Cu/L, or an equal volume of "placebo" (<0.1 mg Cu/L) was given to the mothers to use in preparing the daily water to be consumed as drinking water, in formula, in cow's milk preparations, and in the preparation of meals. The copper content of the water was monitored weekly during the study period, and field workers visited the homes weekly to record water intake and GI, respiratory, and other illnesses.

The authors reported that there were no differences in growth and morbidity episodes among the four groups of infants studied. However, breast-fed infants had a significantly lower incidence of diarrheal episodes than did formula-fed infants during the nine-month observation period. There were no differences in copper status among the four groups of infants at six, nine, and 12 months of age. Significant differences were observed in serum copper concentrations between formula-fed and breast-fed groups at six months of

age (28.3 ± 7.2 μmol/L versus 24.9 ± 7.9 μmol/L, respectively), and in erythrocyte metallothionein levels at 12 months of age, 21.9 ± 7.0 U/g Hb versus 26.8 ± 7.5 U/g Hb, respectively. A significant difference in ceruloplasmin activity at nine months was found between subjects who received drinking water with high vs. low copper content, 350 ± 85 mg/L versus 322 ± 75 mg/L, respectively. In addition, there were significant differences for this parameter in the breast-fed groups between infants who received drinking water with high and low copper content ($p = 0.0032$). At six, nine, and 12 months of age, the four groups of infants did not have significantly different findings in liver function tests; liver function tests in formula-fed infants differed significantly from breast-fed infants in total bilirubin at six months of age (2.22 ± 1.18 μmol/L versus 2.8 ± 1.23 μmol/L, respectively) and in serum glutamic oxaloacetic transaminase at nine months of age (0.29 ± 0.09 μkat/L versus 0.35 ± 0.14 μkat/L, respectively). These differences in biochemical indexes of copper nutrition and liver function (between formula vs. breast-fed infants) are summarized in Table 4. MT does not appear to be the protein that binds copper in the small intestine during early life because MT induction has been shown to be much higher in adolescent rats than in younger rats (Varada *et al.,* 1993).

Table 4. Differences in Biochemical Indexes[a] and Liver Function in Infants exposed to Varying Levels of Copper in Drinking Water (from Olivares *et al.,* 1998).

Nutrition Source	Serum Copper Concentration (μmol/L)*	Ceruloplasmin Activity (mg/L)	Erythrocyte Metallothionein (U/g Hb)*	Total bilirubin (μmol/L)*	SGOT (μkat/L)*
Formula-fed	28.3±7.2 (at age 6 mo.)	Higher at 9 mo. in the added Cu group (350±85) than in the non-added group (322±75)	21.9±7.0 (at age 12 mo.)	2.22±1.18 (at age 6 mo.)	0.29±0.09 (at age 9 mo.)
Breast-fed	24.9±7.9 (at age 6 mo.)		26.8±7.5 (at age 12 mo.)	2.8±1.23 (at age 6 mo.)	0.35±0.14 (at age 9 mo.)

*Indicates significant difference
Hb = hemoglobin; SGOT = Serum Glutamic Oxaloacetic Transaminase
[a]Serum copper and ceruloplasmin concentrations are typically used to assess copper status; they are not currently used to evaluate copper overload, and may not be the best markers for excess copper. Erythrocyte Metallothionein = possible indicator of copper burden. Most hepatocellular copper is bound to metallothionein, and copper overload induces metallothionein (see text).

Both of the high copper exposure groups (2 mg Cu/L) had higher drop-out rates than the low copper (<0.1 mg Cu/L) groups (refer to Table 5). In the case of group I (high copper), the number of infants withdrawn from follow-up was three times the rate of group II (low copper). The dropout rate of group III (high copper) was 1.9 times the dropout rate of group IV (low copper). The primary reason given for subjects lost to follow-up was due to refusal of venous blood sampling, though the authors did state that the higher withdrawal rate of infants in the high copper content groups "could be the consequence of a higher prevalence of unreported symptoms of intolerance." This study was funded in part by the International Copper Association Research Program, Santiago,

Chile. The study protocol was approved by the Ethics of Human Research Committee of the Institute of Nutrition and Food Technology of the University of Chile, and parental consent was obtained for inclusion of the infants in the study.

Table 5. Number of Infants Lost to Follow-up in the Olivares *et al.* (1998) Copper Drinking Water Study.

Group #	Copper Exposure Level	Initial # Subjects	# Subjects Withdrawn	Drop-out Rate	Formula vs. Breast-fed
Group I	High (2 mg/L)	56	17	30.4%	Formula-fed
Group II	Low (<0.1 mg/L)	27	3	11.1%	Formula-fed
Group III	High (2 mg/L)	24	13	54.2%	Breast-fed
Group IV	Low (<0.1 mg/L)	21	6	28.6%	Breast-fed

The stated reason subjects were lost to follow-up included blood sampling refusal, protocol transgression, and change of address.

Pizarro *et al.* (1999b) exposed 60 healthy adult Chilean women to drinking water containing copper (as copper sulfate) for a two-week period. Each group received tap water with no added copper, 1, 3, or 5 mg Cu/L for two-week study periods, followed by one week of standard tap water after each test concentration. The average daily consumption of study water was about 1.6 L per subject. Thirty-five percent of the subjects recorded GI disturbances sometime during the study: 15 percent had diarrhea, some with abdominal pain and vomiting, and 20 percent presented with abdominal pain, nausea or vomiting. Consumption of drinking water containing $\geq$ 3 mg/L ionized copper was associated with a significant increase ($p < 0.05$) in nausea, abdominal pain, or vomiting. Thus, this study indicates that acute, reversible GI symptoms occur below the WHO Tolerable Daily Intake limit of 0.5 mg/kg-day provisionally established as safe in terms of chronic effects (NRC, 1989). Throughout the study, levels of serum copper, ceruloplasmin, and liver enzymes remained stable and within normal ranges. The threshold for specific GI symptoms could not be established because of the study design used, but results suggest that nausea may be an adequate indicator of acute GI effects.

In a subsequent study, Pizarro *et al.* (2001) found that both insoluble copper (copper oxide) and soluble copper (copper sulfate) have comparable results on the induction of GI effects. In this study, 45 healthy adult women (18-55 years of age) ingested tap water containing 5 mg/L of added copper (in different proportion of copper sulfate:copper oxide) over a nine-week period (not continuous). The different proportions of copper sulfate (soluble) to copper oxide (insoluble) were 0:5, 1:4, 2:3, 3:2, and 5:0 mg/L. Test subjects served as their own controls; during break weeks, subjects ingested plain tap water (the copper content of water in Santiago, Chile was measured as <0.1 mg/L). The authors reported that houses shared similar characteristics and that all had copper piping systems. Study subjects were responsible for mixing the test solution with tap water in a 2 liter container at home. Subjects recorded their water consumption and GI symptoms

daily. The authors reported that mean water consumption was similar among groups; 70 percent of the subjects reported that they consumed > 1.0 L daily, and three percent ingested < 0.5 L water/day. Blood samples were taken one week prior to the start of the study and again at the end of the protocol. The incidence of total GI symptoms in subjects who consumed tap water with 5 mg/L added copper was significantly higher (p <0.01) than controls. Twenty subjects experienced GI disturbances at least once during the study, nine suffered diarrhea (with or without abdominal pain and vomiting), and the other 11 subjects reported abdominal pain, nausea, or vomiting. No differences were found in incidence of abdominal pain, nausea, vomiting and diarrhea regardless of the ratio of copper sulfate to copper oxide. A high percentage of copper was ionic (Cu^{2+}) regardless of the proportion of salts present in the drinking water; percentages of Cu^{2+} ranged from 90 to 100 percent for all of the copper solutions studied. Serum copper levels, ceruloplasmin, and activities of liver enzymes were within the normal limits, but seven women were anemic (Hb < 120 g/L).

In a randomized, controlled, double-blind study designed to assess acute GI effects and blood markers of copper status, Araya *et al.* (2003b, 2004) exposed 1,365 healthy adults in Santiago, Chile to <0.01, 2, 4 or 6 mg Cu/L daily as copper sulfate for two months. Families participating in the study prepared the water at home on a daily basis using tap water and a stock solution provided by the researchers; final concentrations were verified by atomic absorption spectrometry in a weekly sample from each household. Tap water in Santiago provides a mean of 0.01 mg Cu/L. During the survey, individual mean fluid consumption was 1.5 L. A total of 240 people (60 from each group) provided a blood sample. GI symptoms were analyzed by treatment group. Background incidence of the target symptoms (nausea, vomiting, diarrhea, and abdominal pain) was determined to be about five percent in a pilot study; over the two-month study duration, the gross incidence of symptoms in *control* subjects varied from 0 (vomiting) to 60 percent (abdominal pain). Analysis of symptoms at each copper exposure level by week showed highest incidences, directly related to copper level, in the first week, which tapered off markedly during the subsequent weeks. In week one the risk became significant in women at 4 mg/L (RR 1.53, 95 percent CI 1.02-2.05) and in men at 6 mg Cu/L (RR 1.9, 95 percent CI 1.02-2.79). Reported symptom incidence was higher in women than in men during all weeks, though this difference was apparently not significant after the first week, when symptom levels were highest. The authors interpreted the results as indicating "an adaptive response to repeated Cu exposure." No detectable changes were observed in indicators of copper status (serum copper, ceruloplasmin, superoxide dismutase), which may suggest competent homeostatic regulation. Liver function tests remained normal in all subjects.

Chronic Toxicity

Chronic effects of copper poisoning include respiratory symptoms, gastrointestinal disturbances, nervous dysfunction, dermal and hematological changes, hepatomegaly and cirrhosis of the liver. Atrophic changes in the mucous membranes of the nose have also been noted in those chronically exposed to copper dust in the air.

A number of cases of Non-Indian Childhood Cirrhosis (NICC), in which an excessive accumulation of copper in the liver leads to liver cirrhosis, have been reported in Germany (Dieter *et al.*, 1999; Eife *et al*., 1991; Schafer and Schumman, 1991), purportedly as a result of high copper levels in the tap water of households with copper pipes and from the use of this water in the preparation of the infants' food. Out of a total of 103 cases of childhood cirrhosis reported between 1982 and 1994 in Germany, Dieter *et al.* (1999) found that five were likely the result of excessive copper intakes, based on elevated copper concentrations in liver. Three additional cases lacked data on copper levels in liver, but were plausibly linked to copper because of the use of acidic well water from homes with copper pipes in preparing formula. A comparison of all published cases of NICC revealed that all of the households in question used drinking water from wells and that this drinking water did not correspond to drinking water guidelines (*i.e.*, pH< 6.5 in these cases).

Zietz *et al.* (2003) conducted an epidemiological investigation of liver damage in infants exposed to elevated copper concentrations through drinking water from public water supplies in Berlin, Germany. The coverage of neighborhoods in Berlin included different ethnic backgrounds and economic strata. Families were asked to collect two different composite samples of drinking water and to return them by mail. In total, water samples from 2,944 households with copper plumbing (self-reported) and who had infants (up to the age of 18 months) were analyzed for copper. Households with a copper concentration equal to or greater than 0.8 mg/L copper (29.9 percent of all sampled households), and whose infants consumed 200 mL tap water or more per day (for at least 6 weeks), were recommended to undergo a pediatric examination. The copper limit of 0.8 mg/L was chosen for the study because it approximates the average copper content in human milk. Approximately 541 recommended infants were examined by a pediatrician, and of these, 183 also received a blood serum analysis. For ethical and practical reasons, a control group for serum values was not suitable. No significant correlation of glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma-glutamyl transpeptidase (GGT), total bilirubin, serum copper, or ceruloplasmin and estimated copper intake through tap water could be found, except one. None of the examined infants showed any symptom of liver damage such as icterus or frequent vomiting. Results of the liver palpitation were only evaluated by the local pediatricians (no external standardization). Eight infants were found to have outlying values of GOT, GPT, GGT, or serum copper; six of these infants had clinically diagnosed infections at the time of serum analysis, and only in one case was there no hint of a disease that might be associated with the outlying serum parameter. Serum copper is typically elevated by infective diseases (Beshgetoor and Hambidge, 1998). In five cases, unusual ultrasound images of the liver or spleen were found; the three cases of splenomegaly were thought due to infective disease, one 11-month old girl had a slight hepatomegaly (serum copper, total bilirubin, GOT, GPT, GGT and ceruloplasmin were within the reference ranges), another 11-month old girl showed a slightly enhanced echogenicity throughout the liver with ultrasound imaging. Additionally, in this latter case, the bile ducts in the liver were slightly rarefied. Serum values of copper, GOT, GPT, GGT were in the reference ranges and the total bilirubin level (1.0 mg/dL) was at the upper reference limit.

Spitalny *et al.* (1984) reported on a Vermont family who consumed water contaminated with 7.8 mg/L copper. Three of four members of this family reported recurrent episodes

of gastrointestinal problems including vomiting and abdominal pain. The seven-year-old girl experienced periumbilical abdominal pains five to 10 minutes after drinking water and orange juice in the morning. The five-year-old girl had vomiting episodes with abdominal pain after drinking the water. The father also experienced periods of emesis and abdominal pain after drinking water drawn from the kitchen faucet. This family was exposed to excess copper in their drinking water in addition to dietary exposure as described previously under 'Environmental Occurrence and Human Exposure." The investigators did not attempt to estimate the amount of copper this family received in their diet. In the absence of specific data on this family, the simplest assumption would be that their dietary exposure was not unusual. In deriving an LOAEL from this report, it should be considered that the drinking water exposure is in addition to dietary exposure and the toxicological effects might have been cumulative; no data are available to quantify any cumulative exposure or toxicity. Therefore, any LOAEL derived from this report would be for the drinking water exposure added to a baseline dietary exposure.

Stenhammar (1999) attributed prolonged diarrhea and weight loss in three infants to copper in drinking water, ranging from 0.22 to 1.0 mg/L. Two of the infant's homes had recently been built, whereas the third was an old house that had just had its copper pipes replaced. The children had normal serum ceruloplasmin concentrations but moderately increased serum copper levels (23-36 μmol Cu/L); one child had a substantially elevated urinary copper concentration of 6.1 μmol/L (the reference range is: 0-1.6 μmol/L). The diarrhea promptly disappeared when the children were given drinking water of low copper concentration in the hospital, but reappeared when they were sent home and drank their home water.

Berg *et al.* (1981) described diarrheal illness in children attending seven newly-built kindergartens in Sweden. The symptoms disappeared immediately after the children went home for a few days but reappeared as soon as they returned to kindergarten. The Public Health Administration in Sweden conducted a study that showed a correlation between the copper content in the drinking water of the new establishments (1.0 to 6.5 mg Cu/L) and the appearance of diarrhea in children under three years of age.

The Wisconsin Division of Health reported investigations of five cases of individuals who ingested drinking water with copper above the federal action level of 1.3 mg/L (Knobeloch *et al.*, 1994). Based on these cases they concluded that drinking water with elevated copper levels may be a relatively common cause of diarrhea, abdominal cramps and nausea.

A 26-year-old male presented with symptoms of cirrhosis, liver failure and Kayser-Fleischer rings (greenish rings at the edge of the cornea) after more than two years of self-prescribed use of copper supplements (O'Donohue *et al.*, 1993). The patient ingested 30 mg of supplemental copper per day for two years and 60 mg/day for a poorly defined period of up to a year. Liver damage was extensive, and a transplant was required. The diseased liver had an average copper concentration of 3,230 μg/g dry weight (normal 20 to 50 μg/g); tissue histopathology was similar to that seen in Indian childhood cirrhosis and Wilson's disease. Based on an evaluation of the patient's family medical history and the copper excretion of his parents and sisters, he did not appear to carry the Wilson's disease gene. Liver damage apparently resulted from the prolonged daily exposure to three to six times the recommended upper limit for dietary copper.

Table 6. A Comparison of NOAELs/LOAELs from Oral Copper Exposure Studies in Humans

Study	Adults/Children (sex)	No. of Subjects	Exposure Duration	Adverse Effects	Copper Conc. in Drinking Water	NOAEL/LOAEL (endpoint):
[a]Wylie, 1957	Adults (F)	10-15	Acute poisoning	nausea, vomiting, dizziness, headache	5.3 mg	LOAEL (GI effects): 5.3 mg Cu
Spitalny *et al.,* 1984	One adult and two children, aged 5 and 7 yrs	3	Subchronic	Vomiting, abdominal pain	7.8 mg/L	LOAEL (vomiting, abdominal pain): 7.8 mg/L
Pratt *et al.,* 1985	Adults	7	12 wks	None	10 mg Cu/day for 12 wks (as copper gluconate capsules)	NOAEL (liver toxicity): 10 mg/day
Akintowa *et al.*, 1989	Unknown	4	Acute poisoning	vomiting, hemolytic anemia, jaundice, death	100 g/L cupric sulfate	LOAEL (death): 100 g/L cupric sulfate
Olivares *et al.,* 1998	Infants 3-12 mo. (M/F)	128	9 months	[b]Differences in ceruloplasmin levels btw. exp. groups	<0.1 or 2 mg/L	NOAEL: 2 mg/L
Pizarro *et al.,* 1999b	Adults (F)	60	2 wks	Nausea, abdominal pain, vomiting	0, 1, 3, or 5 mg Cu/L	[c]LOAEL(nausea, abdominal pain, or vomiting): 3 mg Cu/L
[d]Olivares *et al.,* 2001	Adults 18-50 yrs. of age (M/F)	61	200 mL 1x/week for 12 weeks	Nausea, vomiting	0, 2, 4, 6, 8, 10 and 12 mg/L	NOAEL: 2mg/L; LOAEL (nausea): 4 mg/L
[e]Araya *et al.,* 2001	Adults (M/F)	179	200 mL 1x/week for 5 wks	Nausea, abdominal pain, vomiting, diarrhea	0, 2, 4, 6 or 8 mg/L copper as $CuSO_4$	NOAEL: 4 mg/L LOAEL (nausea, GI disturbances): 6 mg/L
Araya *et al.,* 2003a	Adults 18-60 yrs (F)	70	100, 150 , or 200 mL 1x/wk for	Nausea, abdominal pain, vomiting,	0.4, 0.8, or 1.2 mg Cu as the	NOAEL: 4 mg Cu/L (0.8 mg Cu)

Study	Adults/Children (sex)	No. of Subjects	Exposure Duration	Adverse Effects	Copper Conc. in Drinking Water	NOAEL/LOAEL (endpoint):
			11 wks	diarrhea	sulfate salt	LOAEL (nausea): 6 mg Cu (1.2 mg Cu)
Araya *et al.,* 2003b, 2004	Adults (M/F)	240	~ 1.5 L/day for 2 months	Nausea, abdominal pain, vomiting, diarrhea	<0.01, 2, 4 or 6 mg Cu/L	NOAEL: 2 mg/L [f]LOAEL (abdominal pain): 4 mg/L

[a] Used by U.S. EPA in setting the copper Maximum Contaminant Level Goal (MCLG) of 1.3 mg/L.

[b] Not considered a frank effect. Ceruloplasmin concentrations are typically used to assess copper status; they are not currently used to evaluate copper overload, and may not be the best markers for excess copper.

[c] Consumption of drinking water containing $\geq$ 3 mg/L ionized copper was associated with a significant increase ($p < 0.05$) in nausea, abdominal pain, or vomiting. The threshold for specific GI symptoms could not be established because of the study design used.

[d] In this study, the benchmark dose approach was used to derive the tolerable intake (TI) of copper in drinking water. The lower 95 percent confidence levels (LCLs) for copper concentration in water for the first 5 percent of the population responding to copper were 2 and 4.2 mg Cu/L for nausea and vomiting, respectively. For risk assessment purposes, these levels are considered equivalent to the NOAEL.

[e] The number of individuals reporting symptoms for the 4 mg/L concentration was about twice the numbers for the control and 2 mg/L groups, but because there was no statistically significant increase in symptoms for either nausea or total GI symptoms at 4 mg Cu/L, the authors defined the NOAEL and LOAEL from this study as 4 (0.8 mg Cu) and 6 (1.2 mg Cu) mg Cu/L, respectively, for the combined study population.

[f] Although one or more GI effects were reported by at least one subject at each dose level, the risk for abdominal pain became statistically significant (in women) at 4 mg Cu/L.

Genotoxicity

Massive DNA damage was observed in hepatocytes from patients with Indian Childhood Cirrhosis (ICC) and was postulated to result from excessive accumulation of copper in the nucleus, leading to the production of free radicals that cause DNA strand breakage (Prasad *et al.*, 1996). Similarly, distinct bulky DNA adducts but no increases in 8-hydroxydeoxyguanosine were seen in the livers of six out of eight patients with Wilson's disease. The adduct levels of one patient were elevated 100-fold over background adduct levels in control patients (Carmichael *et al.,* 1995). In LEC rats, which abnormally metabolize copper, the formation of etheno–DNA adducts was positively correlated with age-dependent elevated levels of hepatic copper (Nair *et al.*, 1996).

Neurotoxicity

Copper has been investigated as a factor in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease. Both positive and negative associations have been postulated, and animal studies are available to support both interpretations. Initial reports of increased metals in brain plaques and deposits of Alzheimer's disease have generally not been substantiated. Blood copper levels were reported to be increased in Alzheimer's disease (Squitti *et al.,* 2002, 2005, 2006), while other studies have reported blood and brain levels of copper to be unchanged or decreased in Alzheimer's patients (Snaedal *et al.,* 1998; Torsdottir *et al.*, 1999; Pajonk *et al.*, 2005; Kessler *et al.*, 2005, 2006).

Morris *et al.* (2006) reported that a high intake of copper in people over 65 years of age whose diets are high in saturated and trans fats was associated with a faster rate of cognitive decline, but found no relationship in people whose diets were not high in these fats. However, Pajonk *et al.* (2005) reported that rate of cognitive decline in Alzheimer's disease appears to be associated with low plasma copper levels.

Human studies and the weight-of-evidence from both animal and human data thus far appear to be equivocal in terms of a direct pathogenic influence of copper on the Alzheimer's disease process (Richie *et al*., 2003; Adlard and Bush, 2006; Morris *et al.*, 2006; Solfrizzi *et al*., 2006; Brewer, 2007).

Sensitive Subpopulations

Several population subgroups may be considered more susceptible to the toxic effects of copper exposure (adapted from ATSDR, 1990, 2000):

1. Individuals with Wilson's Disease (McClain and Shedlofsky, 1988; Lee *et al.*, 1989), an autosomal recessive disorder caused by an impaired biliary copper excretion, leading to excess copper retention and liver, brain, and eye damage. This occurs in about one in 40,000 to one in 50,000 people in the U.S. (NRC, 2000; Olivarez *et al.*, 2001).
2. Infants and children. Infants and children up to age 10 are susceptible to the toxic effects of copper as evidenced by the incidence of ICC and reports of adverse effects in children drinking water containing low levels of copper (Spitalny *et al.*, 1984; Mueller-Hoecker *et al.*, 1988; ATSDR, 2004). This may be because the fetus and newborn have elevated hepatic copper levels and since their homeostatic mechanisms are not fully developed at birth, they may not be able to cope with excess copper in the diet (Klein *et al.*, 1991). There is also an indication of a genetic susceptibility in the ICC data, because the incidence appears to be familial. Conversely, infants (especially when premature) may be at risk for copper deficiencies because of "low prenatal stores" and because breast milk is low in copper (Solomons, 1985; Lonnerdal, 1996). As with other metals, copper intake in the infant should be adequate but not excessive (Solomons, 1985; Lonnerdal, 1996, IOM, 2001).
3. Extracorporeal dialysis patients. Kidney dialysis patients exposed to excess copper in the dialysate can suffer acute hemolytic anemia (Williams, 1982).

4. Individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Beutler, 1991). It has been postulated that these individuals would be more susceptible to the toxic effects of oxidative stressors such as copper, but no epidemiological or clinical data exists that clearly links this genetic variation with copper sensitivity.

Carcinogenicity

Epidemiological studies have not established a positive correlation between high copper exposure and cancer. Although an increased incidence of lung cancer has been reported among workers in copper ore mines, this was probably due to contaminating arsenic compounds (U.S. EPA, 1987). There have been some geographical studies comparing cancer incidences in areas with high or low copper, but these studies considered together are inconclusive (U.S. EPA, 1987). Higher copper levels have been found in tumor tissues at many sites. However, this may be a consequence rather than a cause of the disease; cancer may increase copper absorption into the tissue. The U.S. EPA (1991) classifies copper as Group D, not classifiable as to human carcinogenicity.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

It is important to (re)emphasize that copper in drinking water is not a required (nor necessarily a desired) source of copper. The NHANES III nationwide survey (1988-1994) and CSFII (1994-1996) indicate that intake of copper (from food and supplements) is adequate for the great majority of the population in all age and sex groups. For children aged six months to three years, the median copper intake from the diet is 0.6 to 0.7 mg Cu/day. The IOM (2001) has estimated Adequate Intake (AI) values of 200 μg/day for infants zero to six months of age, and 220 μg/day for infants aged seven to twelve months. The WHO (1996) estimated that average copper requirements are about 50 μg/kg of body weight/day for infants. The average value received by an infant who consumes formula supplemented with copper has been estimated at about 400 μg Cu/day for an infant consuming 700 kcal/day, or about 115 μg Cu/kg-day. For a formula-fed infant <six months (time-averaged bodyweight ~ 5-6 kg; CDC, 2007), this would result in an intake of 690 μg Cu/day from food alone, which is more than three-fold in excess of the estimated AI of 200 μg Cu/day for infants aged zero to six months as outlined above. Thus, copper deficiencies would not be anticipated to occur in normal, healthy infants who were adequately nourished.

Estimates of the upper limit for the acceptable daily dose have been acknowledged as uncertain. The FNB recently concluded that an upper limit could not be established for infants (IOM, 2001). The IOM (2001) recommended 10 mg/day as a tolerable upper intake level for *adults* from food and supplements (liver toxicity was used as the critical endpoint). The WHO has set a value of 150 μg/kg-day as the upper limit of the safe range for *infants* (WHO, 1996).

It has been shown that in communities with corrosive water whose residents have copper plumbing, residents consume an amount equivalent to a "substantial" portion of their daily required copper from drinking water (Sharrett *et al.,* 1982). The WHO (2004) document states that, "Consumption of standing or partially flushed water from a distribution system that includes copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap water." Thus, for infants who consume a mostly liquid diet in the first six months of life, and whose formula may be constituted with household drinking water, the potential exists for high copper consumption (far in excess of the AI).

The most sensitive adverse endpoint for excess copper appears to be GI effects in children, particularly infants. The data are somewhat limited for assessing the dose-response relationship for this effect. Copper ions are generally more bioavailable in water than in food, and because acute irritation of the GI tract is caused by the ionic form of copper, it is reasonable to assume that GI irritation is more likely to be produced by drinking water than by eating food. The majority of reports on copper-induced GI irritation concern the ingestion of fluids high in this element. However, Pizarro *et al.* (2001) reported that insoluble and soluble copper had similar GI effects. Two case studies have reported weight loss and gastrointestinal effects in infants and children at low levels of copper in the drinking water, from 0.22 to 1.0 mg Cu/L (Stenhammar, 1999) and 1.0 to 6.5 mg Cu/L (Berg *et al.*, 1981). However, the copper concentrations in the drinking water were measured retrospectively and the duration of exposure is unknown.

In the study by Olivares *et al.* (1998), 128 infants were given water (and bottles) with either <0.1 mg Cu/L or 2 mg Cu/L from the third to the twelfth month of life. No differences in growth and morbidity (diarrhea and respiratory infections) were observed between the exposed groups. Breast-fed infants had a significantly lower incidence of diarrheal episodes than did formula-fed infants during the nine-month observation period. It is not clear whether this difference may be at least partly attributable to the differences in copper consumption levels. Subclinical differences were observed between some of the exposure groups. A significant difference in ceruloplasmin activity at nine months was found between subjects who received drinking water with high vs. low copper content, 350 $\pm$ 85 mg/L versus 322 $\pm$ 75 mg/L, respectively. In addition, there were significant differences for this parameter in the breast-fed groups between infants who received drinking water with high and low copper content. Most of the differences, however, were observed between breast-fed and formula-fed infants and not between copper-supplemented and unsupplemented infants.

The assessment of copper status in infants is complicated. Traditionally, measures of serum copper and of ceruloplasmin, the major copper-binding protein in serum, have been used to assess copper status. Serum copper concentrations are low in newborn infants, and both serum copper and ceruloplasmin increase rapidly during the first six months of life (Lonnerdal, 1998). Studies in adults have shown that neither serum copper nor ceruloplasmin concentrations are a sensitive indicator of marginal changes in copper status (Turnlund *et al*., 1990). Even at levels three to 10 times the customary copper intake (up to 9 mg Cu/day), traditional indicators of copper status have not shown significant changes (Araya *et al.* 2003b, 2003c; Pizarro *et al.*, 2001). Ceruloplasmin

concentration in infants has likewise not been shown to correlate well with copper intake (Salmenpera *et al*., 1989; Olivares *et al.*, 2002). Furthermore, it does not appear that MT is the protein binding Cu in the small intestine during early life because induction of MT is much higher in adolescent rats than in younger rats (Varada *et al.,* 1993).

Although no potential early markers of copper *excess* have been identified to date, the limits of homeostatic regulation are not known. Full-term infants and children up to age 10 are susceptible to the toxic effects of copper, as evidenced by the incidence of ICC and reports of adverse effects in children drinking water containing low levels of copper (Spitalny *et al.*, 1984; Mueller-Hoecker *et al.*, 1988; ATSDR, 2004). This appears to be because newborns have elevated hepatic copper levels, and since their homeostatic mechanisms are not fully developed at birth (e.g. immature biliary excretion), they may not be able to cope with excess copper exposure (Klein *et al.*, 1991; Bauerly *et al.,* 2005). Studies in young rats, which show copper accumulation and liver injury in response to copper supplementation, suggest that infants may be unusually susceptible to copper toxicity (Bauerly *et al.,* 2005; Fuentealba *et al.*, 2000; Varada *et al.*, 1993). Excretion of copper in bile may be even more important than absorption in regulating total body level of copper (Turnlund *et al*., 1998). In the case of ICC, poor biliary excretion of copper may play a role in the etiology of the disease. Studies in animals have suggested that susceptibility to copper toxicity is due to reduction in biliary copper excretion (Weber *et al.*, 1980).

The mechanism of copper-induced liver damage caused by excess dietary copper differs from that seen in diseases due to genetic defects of copper metabolism. Individuals with Wilson's disease, as well as Long-Evans cinnamon rats, which have a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene, have an impaired ability both to excrete copper in bile and to synthesize ceruloplasmin (Wu *et al.,* 1994). In Wilson's disease, the affected *ATP7B* gene encodes a copper transporter (ATPase7B), which is responsible for biliary transfer of copper, as well as transfer of copper into endoplasmic reticulum and Golgi channels for incorporation into ceruloplasmin (Fuentealba *et al.*, 2000). The resultant accumulation of copper in the hepatocytes leads to liver damage, and eventually, toxic effects in several other organs (brain, eyes, kidneys). In contrast, a number of studies in animals have found that most excess copper accumulated as a result of excess dietary copper intake is found within lysosomes (Evering *et al.*, 1991; Haywood *et al.*, 1996; Kumaratilake and Howell, 1989). Liver injury results when the extra copper can no longer be accommodated or specific stimuli result in release of copper from the lysosomes into the cytoplasm (Haywood *et al.*, 1996). Different patterns of copper distribution have also been observed in different copper-associated diseases. In copper-loaded rats, copper was found in the centroacinar zone 1 (periportal areas) and the midzone 2 (Fuentealba *et al.*, 2000). Copper has also been shown to be preferentially deposited in liver cells of acinar zone 1 (periportal area) in human newborn livers and in some human diseases, including primary biliary cirrhosis, precirrhotic stages of Wilson's disease, and ICC (Faa *et al.,* 1987; Goldfischer *et al.*, 1980; Kanel and Korula, 1992), whereas copper accumulation in advanced stages of Wilson's disease is diffuse with no particular acinar pattern.

The consequences of high copper intake on copper regulatory mechanisms in the liver and intestine of infants have not been well characterized. The liver of newborn infants

contains 50 to 60 percent of the body's copper, compared with 8 to 10 percent in liver of healthy adults (Luza and Speisky, 1996). Strickland *et al.* (1972) found that the amount of copper stored in human liver does not affect copper absorption. Conversely, preterm infants are born with very low copper stores due to the fact that fetal copper accumulates largely during the third trimester (Widdowson, 1974). The copper balance in preterm infants may be negative for several months after birth (Dauncey *et al.,* 1977; Tyrala, 1986). For this reason, preterm infants are often given copper-fortified formulas that may contain as much as 2 mg Cu/L (Lonnerdal, 1998).

Experimental studies using adult human subjects have reported acute NOAELs for copper in drinking water ranging from 2 to 4 mg Cu/L for GI effects (Araya *et al*., 2001, 2003, 2004; Olivares *et al*., 2001). In the Araya *et al*. (2001) study, the dose response curve shows that the first 5 percent of the population would respond at 3.5 to 4 mg Cu/L; subsequent studies by this group indicate that women appear to be slightly more sensitive to the gastrointestinal effects than men (Araya *et al*., 2004). Olivares *et al*. (2001), using the benchmark dose approach, calculated 5 percent response levels of 2 and 4.2 mg Cu/L for nausea and vomiting, respectively, in adult volunteers given varying concentrations of copper sulfate solutions once a week for up to 12 weeks.

Because the study by Olivares *et al.* (1998) used human infants, the sensitive population of greatest concern for this chemical, and because the exposure was continuous over a nine month period, the Olivares *et al*. (1998) study was chosen for calculation of the copper PHG. In addition, the Olivares *et al.* (1998) study is based on a large number of subjects, as opposed to the case studies, which involve only a few individuals.

The following assumptions are made in the calculation of the NOAEL:

1. The NOAEL was calculated using the data for the formula-fed infants (n=56) from four to six months of age (Group I). According to the authors, this group given drinking water with a high copper content received 2.3 $\pm$ 0.8 mg/day (318.7 $\pm$ 107.3 µg/kg-day) of copper from water (range 1.5 – 3.1 mg Cu/day). According to the local practice, solid food consisted of fruit at three months of age; vegetable soup, legumes, and eggs at six months; and regular table food at 12 months.
2. The average copper content of the tap water in Santiago, Chile is <0.1 mg/L.
3. The milk formula used in the study was fortified with 7.87 µmol/L of copper (when prepared with copper-free water).

The Olivares *et al.* (1998) study has some major weaknesses that make it difficult to draw conclusions on a safe copper concentration in drinking water, or to identify/quantify any real differences in copper status among the various study groups. The copper drinking water solutions were prepared by the test subjects' families in the home. Information about infant meals, formula preparation, and illnesses (e.g., diarrhea) were reported by the mothers, and are therefore subject to recall bias. Breast milk was not assayed for copper. Formula-fed infants (groups I and II) were "partially or totally" weaned from the breast by three months of age. This makes it difficult to group all formula-fed infants as a separate group, as consumption of the copper drinking water may have varied considerably among individuals within the same group. The authors stated that breast-

fed infants were "exclusively" breast-fed until six months of age; however, breast-fed infants who began weaning were fed powdered (unfortified) cow's milk and solids, according to local practice. The age(s) at which this occurred, and the number of subjects affected by this, were not provided. This may also have had an impact on copper status. A number of the laboratory parameters measured in the study (e.g. ceruloplasmin and serum copper concentrations, superoxide dismutase activity) are not currently used to evaluate copper overload, and may not be the most suitable or sensitive markers to assess the presence of excess copper. The most sensitive (clinical) endpoints for copper excess appear to be GI distress (epigastric pain), diarrhea, vomiting and nausea. Infants are unable to point to the source of their pain and/or distress, and diarrhea and colic can be common effects in young children (and potentially have life-threatening consequences). The 2 mg Cu/L concentration utilized as drinking water in the study is the limit for copper concentration of drinking water proposed by the WHO (2004). The WHO committee also stated that no more than 10 percent of copper intake should come from drinking water.

In the high-copper exposure groups of the Olivares *et al.* (1998) study, water with 2 mg/L of copper was used exclusively for all formula and meal preparation, and for drinking water in these infants. As formula-fed infants less than six months are nearly exclusively bottle-fed, and fed a formula fortified with copper (7.87 µmol Cu/L) at that, they received far in excess of 10 percent of their copper intake from drinking water. According to the study authors, the formula-fed high content group received as much as 426 µg Cu/kg-day from water alone. It should be noted that 426 µg Cu/kg-day exceeds the WHO recommended daily intake of 80 µg Cu/kg by more than five-fold, and exceeds the FNB Adequate Intake value of 30 µg Cu/kg-day (IOM, 2001) by nearly 15-fold. Bauerly *et al.* (2005) reported that infant rat pups supplemented with 25 µg/Cu-day retained copper in their liver and small intestine, suggesting that they may be at risk for copper toxicity. Previously, the WHO 2 mg/L provisional guideline had come under criticism for lacking a strong scientific basis (Fewtrell and Kay, 1995; Fitzgerald, 1995, 1998; Fewtrell *et al.*, 2001).

At higher doses, longer-term exposure to copper will cause liver and kidney toxicity. A NOAEL of 10 mg/day (0.14 mg Cu/kg-day) was established for liver toxicity by the FNB (IOM, 2001), based on absence of serum enzyme changes indicative of liver effects.

CALCULATION OF PHG

Noncarcinogenic Effects

The calculation for copper in drinking water is based on infants as a sensitive group, and absence of an adverse effect in the principal study selected (Olivares *et al.*, 1998), with corroborative data on GI effects from other studies as the adverse effect endpoint of concern (Berg *et al*., 1981; Stenhammar, 1999; Pizarro *et al.,* 1999b; Araya *et al*., 2001, 2003, 2004; Olivares *et al*., 2001). This does not include infants or children with the inherited abnormality in copper metabolism that results in Wilson's disease, because such individuals are rare (about one in 40,000-50,000 individuals; NRC, 2000; Olivarez *et al*., 2001), and they must be under a physician's care to control accumulation of copper from

food alone. Attempting to protect such individuals by means of a water standard would be impractical. Heterozygotes for the genetic abnormality, who are estimated to comprise about one percent of the population (NRC, 2000), may also be more susceptible to copper. This fraction of the population may be possible to protect in establishment of a drinking water standard for copper, but the magnitude of their potential susceptibility to copper is unknown.

The highest NOAEL for GI effects identified in the Olivares *et al*. (1998) study in formula-fed infants, 426 µg Cu/kg-day (318.7 $\pm$ 107.3 µg/kg-day from water alone), was used as the point of departure. An appropriate uncertainty factor, based on intra-species variability and strength of this critical study is applied to this value. Although the exposure duration in the Olivares *et al.* (1998) study (from three to 12 months of life), was not over a lifetime, use of less than chronic data is judged not to require an additional uncertainty factor because the most sensitive effect, gastric irritation, is an acute effect. Moreover, with increasing postnatal age, tissue copper concentrations decrease (Lonnerdal *et al.*, 1985; Varada *et al.,* 1993), which would suggest that the risk of copper toxicity due to excess dietary copper decreases as children get older. However, studies in young animals, including primates, show that neonates are particularly susceptible to the effects of excess dietary copper (Araya *et al.,* 2005; Bauerly *et al.,* 2005; Fuentealba *et al.,* 2000). The infants admitted to this study were three months of age at start-up, and thus, the Olivares *et al.* (1998) study does not comprise exposure information about the early neonatal period from zero to three months.

With regard to human variability, infants appear to represent a sensitive sub-population, which therefore accounts for some aspects of intra-species variability. However, the infants admitted to the Olivares *et al.* (1998) study (n =128) were healthy and had body weights higher than 2,000 g at the time they entered the study at three months of age. Since this may not be the case with all infants, the application of an uncertainty factor is appropriate to address the issue of variability among individuals with respect to sensitivity to the GI and liver effects of copper; it also should be noted that infants cannot readily complain about gastric distress, so the incidence of this effect may be underreported. Both of the high copper exposure groups that received drinking water containing 2 mg Cu/L had higher drop-out rates than the low copper (<0.1 mg Cu/L) groups. In the formula-fed, copper-supplemented group the number of infants withdrawn from follow-up was three times the rate of unsupplemented formula-fed infants. The authors stated that the higher withdrawal rate of infants in the high copper content groups "could be the consequence of a higher prevalence of unreported symptoms of intolerance." For this reason, as well as other study weaknesses, the conclusion that the highest dose administered in the study, 2 mg Cu/L, represents a true NOAEL is uncertain. An uncertainty factor of three is utilized in this risk assessment to account for uncertainties in the study data.

A public health-protective concentration (C, in mg/L) for copper in drinking water can be calculated using the following equation for noncarcinogenic endpoints:

$$C = \frac{\text{NOAEL µg/kg-day x RSC}}{\text{UF x L/kg-day}}$$

where,

NOAEL	=	no-observed-adverse-effect level;
RSC	=	relative source contribution (usually 20 to 80 percent, entered as 0.20 to 0.80);
UF	=	uncertainty factor;
L/kg-day	=	daily water consumption volume, which is based on the upper 95 percent confidence level of municipal water supply consumption for the most relevant exposed population (U.S. EPA, 2004).

The NOAEL derived from Olivares *et al.* (1998) is for the drinking water component of the total copper exposure only. For infants under six months of age, formula, breast milk, and water comprise essentially the total diet. Full-term infant formulas generally contain from 0.4-0.8 mg Cu/L, which would provide about 0.4 mg of copper/day for an infant consuming 700 kcal/day. For powdered formula, the copper derived from the water used to make up the formula would be added. For children older than six months, total copper exposure would include exposure from other dietary components (see Table 1). Therefore, the appropriate relative source contribution depends on which age group is judged to be most relevant for the effect in question, and the relative proportions of copper assumed to be derived from the diet versus from the municipal water supply.

On a body-weight basis, infants drink considerably more water than adults, particularly those that are formula fed. Mean water intake for non-breastfed infants less than six months old was estimated as 0.095 L/kg-day, with an upper 95th percentile of 0.221 L/kg-day (U.S. EPA, 2004). The time-averaged mean body weight of infants <six months old is about 6 kg (CDC, 2007). To provide adequate protection to this sensitive population, we recommend that the upper 95th percentile water consumption value be used, rather than the mean. With an estimated relative source contribution of 0.5 (50 percent of the total copper derived from municipal water), the resultant calculation for infants less than six months old is as follows:

$$C = \frac{426\ \mu g/kg\text{-}day \times 0.5}{3 \times 0.221\ L/kg\text{-}day} = 321\ \mu g/L = 300\ ppb\ (rounded)$$

The PHG for copper in drinking water is therefore set at 300 ppb. This value is judged adequately protective of sensitive subpopulations, including infants, children, pregnant women and their fetuses, the elderly, and other subgroups that are identifiable as being at greater risk of adverse health effects than the general population, in accordance with Health and Safety Code Section 116365(c)(C)(ii).

Carcinogenic Effects

There is inadequate evidence to conclude that copper is carcinogenic in animals (IRIS, 2007). Epidemiological studies of potential carcinogenic effects in humans are inconclusive (U.S. EPA, 1987). The U.S. EPA (1991) classifies copper as Group D, not

classifiable as to human carcinogenicity. Given the lack of data, no cancer dose-response assessment can be made.

RISK CHARACTERIZATION

This PHG for copper is based on a drinking water study by Olivares *et al.* (1998) using human infants, supported by several other studies in adults and infants. The NOAELs (for GI effects and/or significant changes in liver function) from Olivares *et al.* (1998) ranged from 1.5 to 3.1 mg/day (211-426 µg/kg-day) of copper. This was the highest copper content administered in the study. Another study of subchronic exposure in adult humans (Olivares *et al*., 2001) yielded a NOAEL of 2 mg Cu/L (4 mg Cu/day). Considering the difference in body weight (and other factors) between adults and children, the two reports produced comparable estimates of the NOAEL for gastrointestinal effects.

The Olivares *et al*. (1998) study was chosen because it is the best and most directly applicable report on human exposures. It directly addresses the sensitive population group (children under ten years of age). However, there are several areas of uncertainty that should be considered in using these data to derive a PHG.

1. The purpose of the Olivares *et al.* (1998) study was not to identify the toxic limit of copper exposure in drinking water but to verify the tolerance and safety of the WHO provisional guideline of 2 mg Cu/L for infancy.
2. The biochemical and GI effects observed are not classifiable as "frank toxicity," and therefore it may be argued that the true NOAEL is higher.
3. A high number of infants (30.4 percent) in the formula-fed high copper content exposure group (2 mg/L) were withdrawn during follow-up. The higher withdrawal rate of infants in this group could be the consequence of a higher prevalence of unreported symptoms of intolerance.
4. It was not possible to include copper provided by breast milk because breast milk was not assayed for copper. Several authors have reported that breast milk is low in copper (Solomons, 1985; Lonnerdal, 1996).
5. The dose calculations were not clearly shown.

The study does not provide complete information about dietary exposure to copper in these infants. For infants eating solid foods, we can only assume that dietary exposure was normal (i.e., in the range shown in Table 1). According to Table 1, infants six to 11 months old receive 0.47 mg/day of copper from their diet, compared to a nutritional requirement (Adequate Intake) of about 0.22 mg/day (IOM, 2001). For children, drinking water can contribute an amount equivalent to a large fraction of the daily nutritional requirement for copper, but this is not a required source of copper, considering the copper content of food/formula. The PHG of 300 ppb would allow up to 0.4 mg/day from drinking water for infants in this age range at the upper 95 percent confidence limit of drinking water consumption (0.221 L/kg-day for infants <six months of age or 0.185 L/kg-day for infants <12 months; U.S. EPA, 2004). Drinking water at this level would thus provide nearly 50 percent of the total average daily copper consumption, which

substantiates the relative source contribution used for the PHG calculation. This potential copper exposure from water is also well over the total nutritional requirement for copper for this age group. The PHG is low enough to protect against the toxic effects of copper (with a margin of safety), but has no effect on copper nutritional status.

A study of households in Seattle, Washington found that in those homes with copper pipe, the 50th percentile for copper concentrations in standing and running water were 760 µg/L and 353 µg/L, respectively; the 75th percentile concentrations were 1,303 µg Cu/L and 758 µg Cu/L, respectively. For Seattle city employees (males only) chosen as the study subjects, the mean daily copper consumption was 2.2 mg Cu from standing and 1.3 mg Cu from running water. Running water appeared to provide more than half the daily copper requirement for their wives and children (Sharrett *et al.*, 1982). These data show that it is possible to limit copper concentrations in drinking water to about the PHG level merely by running the water before drawing the water for consumption. It should be noted that the RSC could be much higher than 50 percent if water standing in copper pipes is consumed, especially for infants.

The NRC Committee on Copper in Drinking Water recently reviewed the adequacy of the U.S. EPA MCLG of 1.3 mg/L for copper (NRC, 2000). The NRC Committee, apparently referring to heterozygotes for Wilson's disease, concluded "Given the potential risk for liver toxicity in individuals with polymorphisms in genes involved in copper homeostasis, the committee recommends that the MCLG for copper not be increased at this time." U.S. EPA acknowledges that the MCLG is not protective for individuals with Wilson's Disease. Both consumer confidence and public notification language recommend consultation with a personal physician for this population. The NRC Committee declined to base recommended levels on acute GI effects with the reasoning that "the GI effects are not severe or life-threatening." OEHHA disagrees with this conclusion because it believes that the acute GI symptoms, including nausea, vomiting, and diarrhea, are indeed a cause for concern in infants, and in some cases may be life-threatening. California law (Health and Safety Code Section 116365(c)(1)) requires OEHHA to set the PHG at a level that is "not anticipated to cause or contribute to adverse health effects, or that does not pose any significant risk to health."

The Food and Nutrition Board also based their evaluation of the appropriate upper limit of (chronic) copper administration of 10 mg/day on the potential for liver damage (IOM, 2001) rather than the GI effects. The Board reasoned that "in the United States and Canada, liver damage is a much more relevant endpoint because of the potential for excess intake from food and supplements. Furthermore, extensive evidence from studies in humans and experimental animals indicates that liver damage is the critical endpoint resulting from daily intake of high levels of copper salts." In this context, it would appear that the potential for excessive exposure to copper from drinking water was a subset of their concerns, and that total exposure (from both food and water) was clearly the more relevant concern.

The ATSDR in its recent updated Toxicological Profile for copper (ATSDR, 2004) set acute and subchronic Minimal Risk Levels (MRLs) for copper of 0.01 mg/kg-day, based on the study of Pizarro *et al*. (1999b) showing acute GI symptoms in adult women consuming copper in drinking water. The NOAEL for this effect was 0.0272 mg Cu/kg-day, i.e., 1 mg/L, and the LOAEL was 3 mg/L (estimated as 0.091 mg/kg-day). The

MRL was estimated from this value by dividing the NOAEL by an uncertainty factor of three, then rounding to the value of 0.01 mg/kg-day. ATSDR chose an uncertainty factor of three for this effect, based on the reasoning that "a partial uncertainty factor was used because toxicokinetic differences among individuals should not affect the sensitivity of this direct contact effect." It should be noted that a total exposure limited to the MRL of 0.01 mg/kg-day would provide copper consumption less than the RDA (see Table 2), which would be inappropriate. OEHHA concurs with ATSDR that direct irritation of the stomach lining is not subject to toxicokinetic variation, so that an uncertainty factor of three from the drinking water exposure level in the study of Pizarro *et al*. could be justified. This would result in an acceptable drinking water level of about 0.33 mg/L, which is consistent with the PHG.

The PHG level of 300 ppb (0.3 mg/L) is about one-seventh of the WHO (2004) limit of 2 mg/L for copper in tap water, and incorporates drinking water consumption and RSC values appropriate to human infants. As of 2004, the 2 mg/L WHO guideline value for copper is no longer considered provisional. While the 2 mg/L limit remains the same as in earlier version of the document, the scientific basis for the guideline has changed. The initial basis for the 2 mg/L guideline value was a lack of adverse effects in animals, a NOAEL from a small-scale unpublished study conducted in dogs (Shanaman *et al*., 1972). (The calculations showing the initial derivation of the 2 mg/L guideline are shown below). The International Program for Chemical Safety reviewed the evidence provided in the WHO *provisional* guideline for copper and concluded that "the available data on toxicity in animals is unhelpful because of uncertainty about an appropriate model for humans" (WHO, 1998). The European Commission also reassessed the evidence for copper toxicity and stated that the animal data were insufficient; it recommended an amended value as low as 1 mg Cu/L (CEC, 1996). The current basis for the WHO guideline of 2 mg Cu/L is the human studies by Araya *et al.* (2001, 2003), Olivares *et al.* (1998, 2001), Pizarro *et al.* (1999, 2001) and Zeitz *et al.* (2003). According to WHO (2004), the 2 mg Cu/L value "provides an adequate margin of safety in populations with normal copper homeostasis," and "should permit consumption of 2 or 3 litres of water per day, use of a nutritional supplement and copper from foods without exceeding the tolerable upper intake level of 10 mg/day (IOM, 2001) or eliciting an adverse gastrointestinal response." The 2 mg/L guideline is not intended to be health protective for certain sensitive populations, "such as those with defects in the gene for Wilson's disease and other metabolic disorders of copper homeostasis" (WHO, 2004).

In the dog study used for the initial derivation of the provisional WHO guideline, three dose levels of copper gluconate were used (3, 15 and 60 mg/kg-day) and elevated liver serum glutamic pyruvic transaminase (SGPT) was observed in one of 12 dogs at the highest exposure level. Thus, the stated no-effect level from the Shanaman *et al.* (1972) study is 15 mg/kg-day, although the WHO (1982) summary of this study erroneously states that the NOAEL was 5 mg/kg-day. In the WHO tolerable daily intake (TDI) calculation, a 10-fold reduction for interspecies variation was adopted, resulting in a provisional maximum TDI of 0.5 mg/kg-day (the WHO 5 mg/kg-day transcription error divided by an uncertainty factor of 10). A 10 percent allocation of the TDI was made to water, and based on a standard body weight of 60 kg and a water consumption of 2 L/day, a figure of 1.5 mg/L was derived. This was rounded up to 2 mg/L to reflect uncertainty in the data and assumptions. In fact, the doses reported in the Shanaman *et*

al. (1972) study refer to the copper salt (copper comprises 14 percent by weight of the gluconate salt) and not elemental copper. The copper-equivalent doses used in this study are 0.42, 2.1, and 8.4 mg Cu/kg-day. The no-effect level should therefore be 2.1 mg Cu/kg-day, and allocation of a 10-fold safety factor (as was done in the initial WHO calculation) yields a TDI of 0.21 mg/kg-day. This would result in a copper guideline of 0.63 mg/L, as follows:

$$\frac{\text{TDI x body weight x RSC}}{\text{water volume/day}} = \frac{\text{0.21 mg Cu/kg-day x 60 kg x 0.10}}{\text{2 L/day}} = 0.63 \text{ mg/L}$$

This recalculated value is lower by a factor of three than the current WHO limit of 2 mg Cu/L, and represents approximately a two-fold difference from the revised copper PHG. Also, in at least one drinking water study, acute GI symptoms (diarrhea, nausea, abdominal pain, vomiting) appeared to occur in adults at copper intake levels below the current WHO TDI limit of 0.5 mg/kg-day of copper (Pizarro *et al*., 1999b).

Although copper is an essential element, copper in water is generally not needed to fulfill dietary copper requirements. The current MCLG requires that 90 percent of the first draw tap samples collected after a period of at least six hour stagnation be less than 1.3 mg/L, the federal (and California) Action Level. At this level, the copper concentration in tap water could increase the copper intake of formula-fed infants by as much as 1.5 mg/day or about 500 µg Cu/kg-day. This would result in tap water making a sizeable – and, we believe, excessive – contribution to daily copper intake, considering the estimated dietary intake of about 0.5 mg/day in infants six to 11 months (Table 1), or the recommended dietary intake for infants less than one year old of about 220 µg/day (IOM, 2001). Given copper's narrow safety margin, the knowledge that copper metabolism in human infants is not well developed, that the liver of the newborn infant contains 90 percent of the body burden, with much higher levels than in adults (WHO, 1993), and that the parameters of homeostatic regulation in infants are not known, OEHHA has set the PHG for copper in drinking water at 300 ppb, which is well below the Action Level for copper of 1.3 mg/L. This value is increased from the existing PHG of 170 ppb, set in 1997, which was also based on gastrointestinal distress in children. The earlier calculation used a higher uncertainty factor (10-fold) and a lower water consumption value. We believe the additional studies published since 1997 provide a better documentation of the NOAEL for infants and children, thus justifying the lower uncertainty factor used in the revised PHG. The recent U.S. EPA (2004) analysis of drinking water consumption rates also provides an improved basis for our drinking water consumption values.

OTHER REGULATORY STANDARDS

ATSDR has suggested an acute and intermediate-duration MRL of copper of 0.01 mg/kg-day. Although ATSDR does not develop guidelines for concentrations of chemicals in drinking water, application of default parameters for adult women of 60 kg body weight and 2 L/day of drinking water consumption would yield a value of 300 µg/L. Calculation using default values for infants of 10 kg body weight and 1 L/day would yield a value of 100 µg/L. Neither of these values considers additional exposures from food nor

incorporates a relative source contribution, which is appropriate since this copper exposure level is below the RDA for both adult women and infants.

In 1993, the WHO (1993) set a provisional limit of 2 mg/L (31.48 μmol/L) for copper in tap water, on the basis of the level that produced no adverse effects in animals. The WHO committee suggested that copper intake should be limited to 0.5 mg/kg body weight per day. It also stated that no more than 10 percent of copper intake should come from drinking water. As of 2004, the 2 mg Cu/L guideline value is no longer considered provisional (WHO, 2004). It must be stated that the 2 mg/L limit is intended to provide an adequate margin of safety in populations with *normal* copper homeostasis. According to WHO (2004), "There is still some uncertainty regarding the long-term effects of copper on sensitive populations, such as those with defects in the gene for Wilson's disease and other metabolic disorders of copper homeostasis."

In 1991, the U.S. EPA established an MCLG for copper in drinking water of 1.3 mg/L (U.S. EPA, 1991b). The MCLG is based on a report (Wylie, 1957) of an episode of acute GI symptoms in humans resulting from mixing alcoholic drinks in a copper-contaminated cocktail shaker. From a dose reconstruction, Wylie (1957) estimated that the lowest dose resulting in symptoms was 5.3 mg Cu. The 1.3 mg/L MCLG level was recommended by U.S. EPA because it satisfied the nutritional requirements (noted by U.S. EPA [1987] as 2-3 mg/d for adults and 1.5-2.5 mg/d for children) and because consumption of 2 L/day would result in intakes below the LOAEL (by a factor of two).

At the MCLG of 1.3 mg/L, average daily copper intake during the first six months of life (from water alone) is estimated to be 288 μg/kg day for the *average* formula-fed infant (infant formulas sold in the U.S. generally contain 75 μg Cu per 100 kcal), which exceeds the value of 150 μg/kg per day set by WHO (1996) as the upper limit of the safe range for mean copper intake for infants by almost two-fold. More voracious infants can consume at rates 30 to 50 percent higher than the average (Prentice *et al*., 1988; Whitehead, 1995; U.S. EPA, 2004). U.S. EPA set a secondary maximum contaminant level (SMCL) for copper in drinking water of 1.0 mg/L (40 CFR 143) and a copper action level of 1,300 ppb (Title 22 CCR section 64672.3). Both the U.S. EPA and the WHO have proposed an aesthetics guideline of 1.0 mg Cu/L based on consideration of taste and the staining of sinks and bathtubs. This is the principal regulatory guideline in many countries. The taste threshold for copper is 1 to 5 mg/L (Cohen *et al*., 1960; McKee and Wolf, 1971).

Copper in drinking water is regulated by the lead and copper rule, a federal and state drinking water standard (Title 22 CCR section 64672.3) that specifies requirements for copper in drinking water systems, measured at the customers' taps. The action level refers to a concentration measured at the tap rather in the municipal water supply system because much of the copper in drinking water is derived from household plumbing. The concentration at the tap is affected by water chemistry (pH and various dissolved constituents), which affects the corrosivity of the water. The action level for copper is exceeded if the concentration of copper in more than 10 percent of the tap water samples collected during any monitoring period (conducted in accordance with 22 CCR sections 64682 to 64685) is greater than 1,300 ppb. Failure to comply with the applicable requirements for lead and copper is a violation of primary drinking water standards for these substances (22 CCR Chapter 17.5). Therefore, for all practical purposes the standard described in the lead and copper rule is equivalent to an MCL.

Aquatic life criteria for ambient (surface) water have also been established for copper, based on the high toxicity of copper to aquatic organisms such as daphnia (U.S. EPA, 2003). The acute and chronic toxicity vary with water hardness. The draft criterion for fresh water is based on a biotic ligand model (BLM) that accounts for bioavailability under different conditions of water hardness and sediment concentrations. Thus there is no specific freshwater value, although calculated values would tend to be below 10 ppb. The saltwater criterion does not yet use the BLM. The draft criterion indicates that saltwater aquatic organisms should not be affected unacceptably if the four-day average concentration of dissolved copper does not exceed 1.9 ppb more than once every three years, and if the 24-hour average does not exceed 3.1 ppb more than once every three years (U.S. EPA, 2003). U.S. EPA has just released an update of this ambient water quality criteria document (U.S. EPA, 2007).

The American College of Government and Industrial Hygienists (ACGIH) has set air standards (time-weighted average, threshold limit value) for copper fume of 0.2 mg/m^3 and 1.0 mg/m^3 for dusts and mists (ACGIH, 1988). The National Institute of Occupational Safety and Health (NIOSH) has set occupational exposure limits of 0.1 mg/m^3 for copper fume and 1.0 mg/m^3 for dust and mists (NIOSH, 2003).

A group of state toxicologists (Sidhu *et al.*, 1995) have proposed a drinking water standard for copper of 0.3 mg/L, based on the same human study on which U.S. EPA based their standard, but employing a larger uncertainty factor. They argued that a more protective standard is needed because of the susceptibility of children under 10 years of age. We have chosen the report of Olivares *et al.* (1998) because it represents data on the sensitive subgroup that we are trying to protect, and because the data appear to be more reliable. The standard we have calculated utilizes the same three-fold margin of safety recommended by Sidhu *et al.* (1995) and a different method of calculating drinking water exposure, and yields effectively the same value. Only a few states other than California have set guidelines for drinking water concentrations of copper, i.e., Arizona, 1.3 mg/L; Kansas, 1.0 mg/L; Minnesota, 1.3 mg/L; Rhode Island, 1.0 mg/L (ATSDR, 1990, 2004).

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