COPPER AND HUMAN HEALTH: BIOCHEMISTRY, GENETICS, AND STRATEGIES FOR MODELING DOSE-RESPONSE RELATIONSHIPS

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Copper (Cu) and its alloys are used extensively in domestic and industrial applications. Cu is also an essential element in mammalian nutrition. Since both copper deficiency and copper excess produce adverse health effects, the dose-response curve is U-shaped, although the precise form has not yet been well characterized. Many animal and human studies were conducted on copper to provide a rich database from which data suitable for modeling the dose-response relationship for copper may be extracted. Possible dose-response modeling strategies are considered in this review, including those based on the benchmark dose and categorical regression. The usefulness of biologically based dose-response modeling techniques in understanding copper toxicity was difficult to assess at this time since the mechanisms underlying copper-induced toxicity have yet to be fully elucidated. A dose-response modeling strategy for copper toxicity was proposed associated with both deficiency and excess. This modeling strategy was applied to multiple studies of copper-induced toxicity, standardized with respect to severity of adverse health outcomes and selected on the basis of criteria reflecting the quality and relevance of individual studies. The use of a comprehensive database on copper-induced toxicity is essential for dose-response modeling since there is insufficient information in any single study to adequately characterize copper dose-response relationships. The dose-response modeling strategy envisioned here is designed to determine whether the existing toxicity data for copper excess or deficiency may be effectively utilized in defining the limits of the homeostatic range in humans and other species. By considering alternative techniques for determining a point of departure and low-dose extrapolation (including categorical regression, the benchmark dose, and identification of observed no-effect levels) this strategy will identify which techniques are most suitable for this purpose. This analysis also serves to identify areas in which additional data are needed to better define the characteristics of dose-response relationships for copper-induced toxicity in relation to excess or deficiency.

Copper occurs in nature in its metallic form and in ores and minerals, and was one of the first metals used by humans. The use of copper has been traced back to approximately 5000 BC in the Aegean region, where it was employed for creating valuable art objects. Cyprus, which draws its name from the Latin word cuprum, was a major source of copper, as were regions in Anatolia and Spain. Copper mixed with tin in a 9:1 ratio comprises bronze, and the ability to...
form this alloy marked the end of the Stone Age and the beginning of the Bronze Age. Copper and its alloys are now used extensively in domestic and other plumbing systems and to make cooking utensils. Copper is also used in the production of electrical wire and microelectronic applications, in electroplating and photography, as a roofing material, and as a catalyst in the chemical industry.

Copper is an essential element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor. Conversely, exposure to high levels of copper can result in a number of adverse health effects (Bremner, 1998). Acute copper toxicity is generally associated with accidental ingestion; however, some members of the population may be more susceptible to the adverse effects of high copper intake due to genetic predisposition or disease (International Programme on Chemical Safety, 1998). Copper status has also been associated indirectly with a number of neurological disorders, including Alzheimer’s disease and prion diseases, including bovine spongiform encephalopathy (BSE) (Llanos & Mercer, 2002).

Exposure of humans to copper occurs primarily from the consumption of food and drinking water. The relative copper intake from food versus water depends on geographical location; generally, about 20–25% of copper intake comes from drinking water. Georgopoulos et al. (2001) conducted a review of issues that affect the ability to assess and quantify human exposures to copper from environmental media with a primary consideration being exposure to copper from potable water supplies.

The focus of this review paper are the health issues surrounding copper-induced toxicity and deficiency, which raise some interesting challenges in terms of defining public health protection strategies and policies. For example, there is a lack of public information on the nutritional deficiencies of copper, which many nutritionists believe is much more widespread than toxicity due to copper excess, and thus more of a public health concern (Milne et al., 1990; Strain, 1994; Aggett, 1998; Ralph & McArdle, 2001). According to a U.S. Institute of Medicine report (2001), the intake levels of copper for a significant percentage of the population are lower than recommended levels. On the other hand, the U.S. National Research Council (2000) concluded in its report *Copper in Drinking Water* that there is concern for copper toxicity in susceptible populations and recommended that additional research be conducted to identify and characterize copper-sensitive populations. Further, emerging life-cycle assessments on ecological receptors have suggested that elevated copper levels may also be toxic to some environmental plant and animal species, depending on bioavailability, species sensitivity, and life stage (Preston & Snell, 2001; International Programme on Chemical Safety, 1998).

From the industry’s perspective, competition for copper markets is affected by the direction taken by regulatory initiatives and any indications that copper could be harmful to human health. One of the critical aspects of developing health protection programs for exposure to chemical substances is defining the relationship between exposure to the substance and the effects that exposures exert on health; hence, defining this dose-response relationship is an essential step in identifying what level of exposure can be tolerated and, in the case of nutritionally beneficial substances, what level is an optimum level of exposure.

The approaches that should be taken to characterize the dose-response curve for an essential trace element (ETE) such as copper are very different from those for chemical compounds that have neither biological essentiality nor utility. In conventional risk assessment approaches, it is assumed that exposures occur to substances with no desirable or essential physiological roles, that detoxification pathways are not likely to be chemical-specific, and that the dose-response curves are never negative (i.e., that an increase in adverse response does not occur with a decrease in dose). A qualitative evaluation of adverse effects (hazard identification) is first conducted, followed by quantitative estimation of risk (dose-response assessment). Assessment of dose-response includes extrapolation from high to low doses and, usually, the application of uncertainty factors to account for interspecies and intraspecies differences and the suitability and quality of the data for human health risk assessment. In contrast, ETEs have a requisite physiological role, and therefore biological organisms have evolved a large number of conserved, homeostatic control mechanisms for regulating ETE levels in the body and responding to
correct any excursions above or below physiological requirements. Qualitative evaluation of adverse effects includes toxicity due to excess and toxicity, (or impairment), due to deficiency. The combined dose-response slope is likely to be zero in the homeostatic range. Quantitative estimation of homeostatic bounds may be assessed on the molecular level or in the whole animal.

In the case of copper, both deficiency and excess can produce adverse health effects and the combined dose-response curve is therefore likely to be U-shaped. However, the slopes of the copper dose-response curve are not well characterized and, in reality, the shape of the overall curve is likely to be asymmetrical, with considerable variation in slope for toxicity versus that for deficiency. There is also considerable uncertainty regarding the nature of these slopes outside the range of experimental observations. Further, application of conventional uncertainty factors for toxicity due to exposures to high levels of ETE is likely to yield a reference dose that is in the deficiency range.

The commercial importance of copper and the challenges in defining the dose-response relationship, and hence an appropriate health protection strategy for copper, led the R. Samuel McLaughlin Centre for Population Health Risk Assessment, in collaboration with the International Copper Association, to undertake a detailed study of these issues. The availability of a rich database of both animal and human studies was considered to provide an excellent opportunity to model the dose-response relationship. Two critical levels of copper need to be specified: (1) a level required for essentiality and (2) a level not to be exceeded in order to protect health from excessive exposures. It was considered that there might be wide interest in such an analysis by members of the general public as well as government and industry.

COPPER HOMEOSTASIS AND TOXICITY: IMPLICATIONS FOR DOSE-RESPONSE ASSESSMENT

Copper is a member of the third transition series of elements, with atomic number and atomic weight being 29 and 63.546, respectively. Other members of this transition series include chromium, iron, cobalt, manganese, nickel, and zinc. With the exception of zinc, all elements of this series have a partially filled third orbital shell. There are two stable copper isotopes, $^{63}\text{Cu}$ and $^{65}\text{Cu}$, and these have natural abundances of 69.2 and 30.8%, respectively. Radioactive isotopes include $^{64}\text{Cu} (t_{1/2} = 12.7 \text{ h})$ and $^{67}\text{Cu} (t_{1/2} = 61.9 \text{ h})$. The usual oxidation states of copper are:

- Copper(0): metallic copper; Cu°.
- Copper(I): cuprous ion; Cu+, unstable at neutral pH, oxidized to Cu$^{2+}$ by air.
- Copper(II): cupric ion; Cu$^{2+}$, stable, forms Cu(OH)$_2$ in water at alkaline pH.

Copper also exists in the trivalent [copper(III)], but this is rarely the case. The ability of copper to cycle between Cu(I) and Cu(II) accounts for its importance in biological systems. Copper functions as an electron acceptor/donor in key redox reactions, such as in mitochondrial respiration, synthesis of melanin, and cross-linking of collagen (International Programme on Chemical Safety, 1998). The oxidation potential of copper may also be responsible for some of its toxicity. At high concentrations copper produces oxidative damage to biological systems, including peroxidation of lipids or other macromolecules (Bremner, 1998). However, copper is also an integral part of the antioxidant enzyme, copper-zinc superoxide dismutase, and has a role in iron homeostasis as a cofactor in ceruloplasmin (Ralph & McArdle, 2001). Copper deficiency alters the role of other cellular constituents involved in antioxidant activities, such as iron, selenium, and glutathione, and thus plays an important role in diseases in which oxidant stress is elevated (Johnson et al., 1992). For example, although the exact nature of interactions between copper and selenium are not fully understood, the antioxidant properties of copper superoxide dismutase and selenium-containing glutathione peroxidase are complementary in rats (Fischer et al., 1992; Olin et al., 1994). The results of a recent in vitro study (Zeng & Botnen, 2004) suggest that copper may interact with selenite extracellularly, thereby blocking its intracellular uptake in mammalian cells, and reducing or inhibiting selenite-induced cytotoxicity.
COPPER ESSENTIALITY

Copper is an essential trace element (ETE) for all biological organisms, from bacterial cells to humans. Depending on the source of the biological material, copper content ranges from parts per billion (ppb) to parts per million (ppm). Copper’s essentiality was first discovered in 1928 when Hart et al. (1928) demonstrated that rats fed a copper-deficient milk diet were unable to produce sufficient red blood cells. The anemia was corrected by the addition of ash from vegetable or animal sources. Hart et al. (1928) further showed that the hydrogen sulfide precipitate from the ash, which contains copper sulfide, was responsible for the recovery of the copper deficient animals. Similar observations regarding essentiality were seen in humans during the early 1930s.

Copper is found in much of the natural environment, including water, soils, and fugitive dusts. The amounts vary from location to location depending on specific conditions. Anthropogenic sources include smelters, power stations, municipal incinerators, and environmental residues from agricultural uses of copper as a pesticide. Dietary sources of copper are mushrooms, vegetables such as potatoes, and legumes such as beans and peas. Nuts, including peanuts and pecans, are especially rich in copper, as are certain grains such as wheat and rye, and several fruits including lemons and raisins. Some mollusks are exceptionally rich in copper with levels reaching as high as 5 mg/kg wet weight. Other significant dietary copper sources include organ meats such as liver and shellfish with hemocyanin as a respiratory pigment.

Copper physiological roles serve to provide the following functions:

- Essential component of metalloenzymes, where copper participates in redox reactions by cycling between the copper(I) and copper(II) oxidation states.
- Essential structural component of macromolecules, by providing the appropriate coordination chemistry to maintain higher order structure

Examples of copper-containing proteins are listed in Appendix A. The molecular roles of copper are manifested in its physiological actions where it is most critical for fetal/infant development and growth, brain development and function, immune function, bone strength, cholesterol and glucose metabolism, myocardial contractility, maintenance of hair and skin, and the formation of pigments.

OVERVIEW OF COPPER UPTAKE, DISTRIBUTION, AND EFFLUX

Copper, an essential element in every organism in which it has been studied, is needed for homeostatic maintenance. Homeostasis consists of the maintenance of a constant internal environment in response to changes in internal and external environments. Homeostatic maintenance requires tight coordinated orchestration of copper uptake, distribution, and efflux in cells and the organism as a whole. Many aspects of copper homeostasis are understood at the molecular level. Much of our current knowledge derives from the study of prokaryotes and unicellular eukaryotes such as yeast. Due to their high conservation of the copper homeostatic machinery, unicellular eukaryotes serve as excellent model systems for higher eukaryotes. These systems will be discussed in this review only in the context of human copper homeostasis (Vulpe & Packman, 1995; Linder & Hazegh Azam, 1996; Agranoff & Krishna, 1998; Landner & Lindström, 1999; Andrews, 2002; Hellman & Gitlin, 2002; Llanos & Mercer, 2002; Mercer et al., 2003; Lu et al., 2003; Ralph & McArdle, 2001; Strausak et al., 2001).

Absorption

In mammals, copper is absorbed in the stomach and small intestine, although there appear to be differences among species with respect to the site of maximal absorption. Copper is absorbed from the stomach and duodenum in rats (van Campen & Mitchell, 1965) and from the lower small intestine in hamsters (Crampton et al., 1965). The site of maximal copper absorption
is not known for humans, but is assumed to be the stomach and upper intestine because of the rapid appearance of $^{64}$Cu in the plasma after oral administration (Bearn & Kunkel, 1955). Absorption ranges from 15–97%, depending on copper content and composition of the diet (Strickland et al., 1972a, 1972b; Turnlund et al., 1989; Turnlund, 1998; Ehrenkranz et al., 1989).

Fractional absorption appears to be a function of the amount of copper in the diet and individual copper stores. Using stable isotope ($^{65}$Cu) methodology, Turnlund et al. (1989) reported a mean copper absorption of 56% in adult humans when a low-copper diet (0.78 mg Cu/d) was fed, 36% when the dietary level was 1.68 mg Cu/d, and 12% when the diet contained 7.53 mg Cu/d. In an update of this study, Turnlund (1998) estimated copper retention from measurement of fecal copper excretion for 12 d following oral or iv dosing of human volunteers; the results indicated that 67% of the ingested copper was retained at a dose of 0.38 mg/d, 54% at 0.66 mg/d, and 44% at 2.49 mg/d. The estimated total percentage actually absorbed prior to endogenous biliary excretion was 77, 73, and 66%, respectively, for the 3 doses. A similar variation in absorption was seen in human infants fed formula containing differing levels of copper (Ehrenkranz et al., 1989), although the fraction absorbed was generally higher than in adults for the same mg/kg dose. Thus, it appears that the percent of copper absorbed decreases with increasing level of dietary copper, although changes in endogenous excretion (via the bile) rather than gastrointestinal absorption appear to be the primary mechanisms for regulation of total body copper. Urinary losses are less than 3% of daily intake and do not contribute to the regulation of copper stores or to overall copper balance.

Factors that influence dietary copper absorption include competition by zinc, iron, molybdenum, lead, or cadmium (Cousins, 1985; Oestreicher & Cousins, 1985). Zinc and cadmium appear to be the most potent inhibitors of copper absorption, possibly by competing with copper for transport and/or by increasing intestinal metallothionein concentrations. Metallothioneins are a group of small, heavy-metal binding proteins that serve in detoxification and metal buffering (Suzuki et al., 2002; Sturniolo et al., 1999). Fructose and other carbohydrates, dietary cellulose fiber, and phytate were found to reduce the bioavailability of copper (Lee et al., 1984; Greger & Mulvaney, 1985; Werman & Bhathena, 1995; Wapnir, 1998). Soybean products used in infant formula are very rich in phytyte, which may be of concern in infant nutrition. Animal protein in the diet enhanced copper absorption relative to plant protein in pregnant women (Turnlund et al., 1983), possibly due to the presence of certain flavonoids in some plant foods that inhibit copper uptake (Kuo et al., 1998). Citrate, phosphate, and glutamate form copper complexes that increase absorption. The effects of ascorbic acid on copper absorption was studied in rats. Doses of 10 g ascorbic acid/kg diet led to an initial reduction in copper absorption, followed by a stimulation following ascorbate feeding for several weeks (van den Berg & McArdle, 1994). The relevance of these studies to humans remains unclear. Clinical studies suggest that ascorbic acid administered at high doses to human volunteers may affect absorption, as well as levels of serum copper and ceruloplasmin; however, these study designs varied and results were not consistent across studies (Jacob et al., 1987; Milne & Omaye, 1980; Milne et al., 1981; Finley & Cerklewski, 1983).

Early studies on isolated segments of the duodenum suggested that copper ions enter mucosal cells lining the intestine by simple diffusion (Linder & Hazegh Azam, 1996). However, recent work has led to the identification of several putative copper transporters that can move copper across cell membranes (see “Copper-Transporting ATPases” later for molecular details on transport) (Lutsenko & Kaplan, 1995; Solioz & Vulpe, 1996).

A divalent metal ion transporter, DMT1 (earlier called DCT1), was identified by expression cloning in Xenopus laevis oocytes (Gunshin et al., 1997). It is significantly expressed in the proximal duodenum and kidneys, but was found in all tissues. This transporter may be the key mediator for intestinal iron absorption, but it also has a function in peripheral tissues.

There may be other intestinal copper transporters. It was suggested that intestinal copper uptake is catalyzed by Ctr1, which was first identified in yeast (Dancis et al., 1994). Ctr1 is expressed in all cell types so far investigated, including enterocytes, and catalyzes the transport of Cu(I) across the cell membrane (Lee et al., 2002b).
Excess copper (as well as other heavy metal ions like zinc or cadmium) is sequestered in enterocyte metallothionein. Metallothionein expression is induced by heavy metals and thus acts as a buffering system in enterocytes and other cell types (Kägi & Schaffer, 1988; Suzuki et al., 2002).

**Distribution**

Copper released from intestinal cells moves to the serosal capillaries, where it binds to albumin, glutathione, and amino acids in the portal blood (Marceau et al., 1970; Bligh et al., 1992). There is also evidence for a small protein, transcuprein, with a specific role in plasma copper transport (Linder & Hazegh Azam, 1996). Several or all of these copper-binding molecules may participate in serum copper transport, as would be expected for an ETE.

Copper from portal circulation is primarily taken up by the liver. Once in the liver, copper is either incorporated into copper-requiring proteins, which are subsequently secreted into the blood, transported to extra-hepatic tissues by albumin, amino acids, or mostly (estimates vary from 70–80%) by ceruloplasmin, or excreted into the bile (Ralph & McArdle, 2001). By regulating copper release, the liver exerts homeostatic control over extrahepatic copper (Harris, 2000).

Ceruloplasmin is a serum ferroxidase that contains greater than 95% of the copper found in plasma. The protein is a sialoglycosylated oxidase containing seven copper atoms. Ceruloplasmin functions in the release of iron from cells with mobilizable iron stores. In aceruloplasminemia, copper homeostasis is normal (Gitlin, 1998). Specific cell membrane receptors for ceruloplasmin have been identified in heart, brain, liver, kidney, and lymphocytes (Linder & Hazegh Azam, 1996) from a number of different species. Liver endothelial cells can remove sialic acid residues from ceruloplasmin. The hepatocytes that lie under the endothelium are then capable of absorbing the deglycosylated ceruloplasmin via a receptor and subsequently digesting the protein. While cells can apparently acquire copper by this route, it represents only an accessory mechanism (Hellman & Gitlin, 2002).

How the body senses and retains copper it is still imperfectly understood. Maintenance of homeostasis in the face of a copper deficit (i.e., low copper intake levels) requires that tissues preserve copper stores. Continuously feeding rats a copper-restricted diet for 8 weeks resulted in an organ-specific pattern of copper retention, with the brain and heart losing as little as 3 and 1%, respectively (Levenson & Janghorbani, 1994; Levenson, 1998). Copper conservation in the liver occurred only after 55–65% of total liver copper content was lost, at which point no further copper was exported to the plasma; serum ceruloplasmin activity also decreased at this point. Skeletal muscle does not appear to conserve copper significantly, and it is thought (Ralph & McArdle, 2001) that it may serve as a source of copper for other organs during prolonged restriction.

**Excretion**

Bile is the major pathway for the excretion of copper and is vitally important in the control of liver copper levels (Cousins, 1985; Winge & Mehra, 1990; Turnlund, 1998). After the oral administration of radioactive copper as copper acetate in healthy humans, 72% was excreted in the feces (Bush et al., 1955). Copper in bile is associated with low-molecular-weight copper-binding components as well as macromolecular binding species (Gollan & Deller, 1973); resorption of biliary copper is negligible (Farrer & Mistilis, 1967). Most fecal copper results from biliary excretion; the remainder is derived from unabsorbed copper and copper from desquamated mucosal cells. Biliary excretion of copper following iv administration does not increase proportionally with dosage, suggesting that the hepatobiliary transport of copper is saturable (Gregus & Klaassen, 1986).

Indices of copper status remain stable except under extreme dietary deficiency conditions. Turnlund et al. (1990) evaluated plasma levels of copper and ceruloplasmin, erythrocyte superoxide dismutase activity, and urinary copper levels in human volunteers over the dietary intake range from 0.8 to 7.5 mg/d and observed no significant changes. When dietary copper intake was decreased to only 0.38 mg/d, all of these indices declined significantly, but increased on repletion of copper in the diet. A Foodcue study in volunteers who consumed diets containing copper at concentrations ranging from 0.7 to 6 mg/d for 6 wk showed no significant effects on low-density lipoprotein (LDL) levels, on susceptibility to in vitro induced oxidation, and on ceruloplasmin oxidase
activity (Turley et al., 2000). Studies on copper deficit are summarized in this Table B-3 and B-4 of Appendix B.

**MOLECULAR MECHANISMS INVOLVED IN COPPER HOMEOSTASIS**

The transport and cellular metabolism of copper is the subject of active research. Model systems, including prokaryotes and unicellular eukaryotes, provided invaluable guides to our understanding of the molecular mechanisms involved in copper homeostasis in mammals including humans. Maintaining appropriate copper homeostasis involves coordination between copper uptake, distribution, storage, and efflux. The existence of Menkes disease (MD) and Wilson disease (WD), diseases resulting from inborn errors of copper metabolism (discussed later in this review), provided a key to understanding copper influx and efflux in eukaryotic cells. Copper transport across cell membranes and within the cell evolved early in evolution, and thus the basic mechanisms of copper transport have been conserved from bacteria to humans (Solioz et al., 1994; Lu et al., 2003). Pioneering studies conducted in prokaryotic organisms provided valuable insight into the molecular mechanisms by which copper is transported vectorially (Odermatt et al., 1992, 1993; Solioz et al., 1994; Solioz & Stoyanov, 2003; Rensing & Grass, 2003) and led to the identification and understanding of functional homologues in eukaryotic cells.

In broad terms, copper transport at the cellular level involves transport of extracellular copper across the cell membrane by specialized pumps. Intracellular copper is routed to copper-requiring enzymes and to organelles by specialized proteins called metallochaperones (Camakaris et al., 1999; Harris, 2000). Another set of these transporters pumps copper into subcellular compartments (Arnesano et al., 2002; Bertinato & L’Abbe, 2004; Harris, 2001). Finally, specific mechanisms exist to release copper from the cell (Camakaris et al., 1999; Harris, 2000; Petris et al., 2003). The details of how all these steps are accomplished are being revealed at a rapid rate but our information is still incomplete. Figure 1 depicts the key elements of copper circulation in humans to the extent that it is currently understood. It is discussed in some detail in the following subsections.

**Transfer of Copper Across Cell Membranes**

In mammals, dietary copper is absorbed across the apical brush border membrane of the mucosal cells lining the intestinal villi. Transport into these cells has long been thought to be due to passive diffusion (Goode et al., 1989). Today, several transport proteins that may be involved in this process have been identified.

Dietary copper in the intestine is taken up by the divalent metal transporter 1 (DMT1, earlier called DCT1). DMT1 expressed in *Xenopus laevis* catalyzes proton-coupled transport of a variety of divalent metal ions, including manganese, iron, cobalt, nickel, and copper (Sacher et al., 2001; Knöpfel et al., 2000). The role of DMT1 in copper uptake is further supported by the observation that treatment of cells with a DMT1 antisense oligonucleotide resulted in a 48% inhibition of copper uptake (Arredondo et al., 2003). However, the affinity of DMT1 for copper is low and its major function appears to be transferring-independent iron uptake (Gunshin et al., 1997; Forbes & Gros, 2001). Recent evidence suggests the existence of an ATP-driven high-affinity copper uptake system in the brush border membrane, but it has not yet been identified at the molecular level (Knöpfel et al., 2005). A ubiquitous high-affinity copper-transport protein, CTR1, was identified, but it remains unclear whether this functions at the brush border membrane (Zhou & Gitschier, 1997). Clearly, the important question of how copper is taken up by intestinal cells requires more research.

Experiments in the yeast *Saccharomyces cerevisiae* indicated that CTR1 transports Cu(I) across the plasma membrane (Dancis et al., 1994). The human homologue, hCTR1, was identified by complementation of a CTR1 “knock-out” yeast strain (Zhou & Gitschier, 1997). hCTR1 was expressed in all organs and tissues examined, with liver, heart, and pancreas exhibiting the highest levels, brain and muscle the lowest, and the intestine expressing intermediate levels. This transporter is likely to be the major copper uptake system of all cells, except perhaps enterocytes (Lee et al., 2002a; Puig et al., 2002). A second human copper transport protein, hCTR2, was also identified (Zhou & Gitschier, 1997), but its function remains unclear at this time. CTR1 does not appear to...
require ATP for copper transport, but the driving force for copper uptake into cells remains unclear (Lee et al., 2002a). More recent findings suggest that hCtr1-mediated copper uptake into mammalian cells is regulated by a posttranslational mechanism involving copper-stimulated endocytosis and degradation of the transporter (Petris et al., 2003; Thiele, 2003).

Mice heterozygous for CTR1 exhibit tissue-specific defects in copper accumulation and reduced cytochrome c oxidase activity (Lee et al., 2002b). Mice completely deficient for CTR1 exhibit profound growth and developmental defects and die in utero in midgestation (Lee et al., 2001). This demonstrates a crucial role for Cu acquisition through the CTR1 transporter for mammalian cells and embryonic development (Lee et al., 2001, 2002b).

**Copper Reduction**

The substrate for CTR1 and other copper transporters is Cu(I). Most extracellular copper and copper in the gut are in the form of Cu(II), requiring the presence of a reductase to convert Cu(II) to Cu(I). Such an activity was described in rat liver cells (van den Berg & McArdle, 1994), but the protein has not been identified.

A gene that appears to encode an iron reductase was recently cloned from mouse (McKie et al., 2001). The gene encodes a duodenal cytochrome b, Dcytb, which shares 45 to 50% sequence similarity to the cytochrome b$_{561}$ family of membrane reductases. It was shown that this, or a closely related protein, exhibits ascorbate-stimulated iron and copper reductase activity and may thus also be involved in copper reduction and uptake (Knöpfel & Solioz, 2002).

Extracellular copper reduction was most extensively characterized in yeast. It was shown that copper uptake in yeast is facilitated by the plasma membrane reductases Fre1p and Fre2p (Hassett & Kosman, 1995; Georgatsou et al., 1997), but as many as nine genes may play a role in iron and copper reduction for uptake (Georgatsou & Alexandraki, 1999). Of these, FRE1 and FRE7 are markedly induced by copper chelators, such as bathocuproine disulfonic acid, suggesting a direct role in
copper reduction for uptake. Fre1p is a b-type cytochrome that uses NADPH as an electron donor (Shatwell et al., 1996).

While it is clear that copper reduction plays an important role in uptake and distribution, the process and the underlying enzyme functions are still largely unknown in humans. Further work in this area will be necessary to understand copper homeostasis, including intestinal copper absorption and bioavailability.

**Copper-Transporting ATPases**

Two key enzymes responsible for copper translocation across membranes in eukaryotic cells appear to have evolved from bacterial copper ATPases (Solioz et al., 1994). In humans, these two copper ATPases were designated Menkes ATPase (also MNK or ATP7A) and Wilson ATPases (also WND or ATP7B) based on the associated diseases (see later discussion). The main difference between the two enzymes is tissue distribution (Camakaris et al., 1999; Bull et al., 1993; Petrukin et al., 1993).

The copper ATPases are transmembrane proteins containing a single subunit with a molecular mass of approximately 170 kD, which is further increased by glycosylation. The N- and C-terminal regions are cytoplasmic. The pumps feature distinct domains (see Figure 2): six repeat metal binding domains, a transduction domain of motif TGE (threonine/glycine/glutamate based on the one-letter amino acid code), a channel probably formed by all 8 transmembrane helices, a CPx motif in helix 6, probably forming the gate in the copper channel, a phosphorylation domain with the motif DKTGT, and an ATP binding domain of motif GDG (Lutsenko & Kaplan, 1995). The number and location of membrane helices, the presence of metal binding domains in the N-terminus and the conserved CPx-motif in helix 6 are unique features of heavy metal ATPases (Lutsenko & Kaplan, 1995). Copper ATPases are members of the class of P-type ATPases that includes Na,K-ATPases, Ca-ATPases, and related enzymes. Copper ATPases form a distinct subgroup, and it was proposed that these be termed P- or CPx-type ATPases (Lutsenko & Kaplan, 1995; Solioz & Vulpe, 1996). The function of copper ATPases is to translocate copper across different biological membranes as detailed next.

The Menkes copper ATPase gene was cloned as the gene defective in Menkes disease (Chelly et al., 1993; Mercer et al., 1993; Vulpe et al., 1993). The Menkes ATPase is a membrane-associated copper ATPase that (1) is found in most cell types except in the liver (Camakaris et al., 1999), (2) is highly expressed in intestinal epithelial cells (Murata et al., 1997), and (3) appears to be essential for

**FIGURE 2.** Comparison of heavy metal and non-heavy-metal ATPases. (A) The human Wilson ATPase. (B) The Ca$^{2+}$-ATPase of sarcoplasmic reticulum. Membrane helices are numbered M1 to M10. Helices common to both types of ATPases are in gray and helices unique to one type of ATPase are in black. Key sequence motifs are indicated in the one-letter amino acid code. In the center of the figure, the approximate locations of the three cytoplasmic domains A, P, and N are indicated. MBD, metal binding domains; TGE, conserved site in transduction domain (A); CPx, putative copper channel; DKTGT, phosphorylation site in domain P; HP, motif of unknown function, probably in domain N; GDG, nucleotide binding site residues in domain N. The upper sides of the proteins are cytoplasmic.
copper transport across the basolateral cell membrane into the portal circulation for delivery to the liver. Clinical data showing the accumulation of copper in the intestinal mucosal cells of Menkes patients provide supporting evidence for the basolateral transport role for this protein (Pena et al., 2000). The Menkes ATPase undergoes copper-regulated trafficking. Under conditions of low copper concentrations, the ATPase is localized in the trans-Golgi network, where it operates to deliver copper to the secretory pathway for incorporation into secretory proteins; under conditions of high intracellular copper concentrations, it moves from the trans-Golgi network to the plasma membrane, where it appears to be involved in the efflux of excess copper from the cell (Petris et al., 1996, 2003).

The molecular characterization of Wilson disease led to the identification of the Wilson ATPase. It shares 65% sequence similarity with the Menkes ATPase but is present mainly in hepatic cells (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). It is primarily responsible for the delivery of copper to cuproenzymes and for biliary copper efflux (Terada et al., 1998; Dijkstra et al., 1996). In Wilson disease patients, a defect in the gene encoding this protein interferes with these functions, resulting in (1) faulty excretion, (2) hepatic copper accumulation, and (3) if untreated, severe copper toxicosis. Similar to the Menkes ATPase, the Wilson ATPase undergoes copper-dependent trafficking: Under low copper conditions, it is localized in the trans-Golgi network in the liver and brain, where its role appears to be transport of copper to the secretory compartment for incorporation into ceruloplasmin and other cuproenzymes (Nagano et al., 1998). When cell copper concentrations are elevated, the Wilson ATPase moves from the trans-Golgi network to a cytoplasmic vesicular compartment (Hung et al., 1997) that is not well characterized but is postulated to be associated with the transport of copper to the bile canalicular membrane for excretion (Dijkstra et al., 1996; Roelofsen et al., 2000).

Recently, it was shown that the toxic milk (tx) mouse is also a model for Wilson disease. These mice harbor a mutation that changes methionine at position 1386 to valine in the Wilson ATPase, which abolishes the secretion of copper into milk, producing “toxic” milk (Voskoboinik et al., 2001; Michalczyk et al., 2000). These findings demonstrate a role of the Wilson ATPase in copper secretion into milk. No relevant observations have been reported for humans in this regard.

**Metallothioneins and Metallochaperones**

The accumulation of copper in the cytoplasm poses a risk for oxidative damage; to minimize this possibility, two protective systems may participate in detoxification. One of these systems is thought to be a relatively nonspecific sequestration pathway in which glutathione rapidly binds Cu(I) and conveys Cu to metallothioneins or other copper-binding proteins (Winge, 1991; Winge & Mehra, 1990). Mammalian metallothioneins are cysteine-rich polypeptides containing two polynuclear clusters of cysteinyl thiolates that bind metal ions, including Cu(I) (Winge, 1991). Under conditions of elevated intracellular copper concentrations, copper is sequestered by metallothioneins in the cytosol and, if excessive, transported to lysosomes. Metallothioneins are inducible and their expression is enhanced in response to elevated metal-ion levels. However, they appear to function only as a transient store of copper and other heavy metal ions present in excess (Sturniolo et al., 1999; Suzuki et al., 2002).

Metallochaperones are cytosolic proteins that bind heavy metal ions in labile complexes. During the past few years, a number of copper “chaperones,” first identified in yeast and subsequently in mammalian cells, were identified (Huffman & O’Halloran, 2001). They enable copper exchange when docking to a target protein (Huffman & O’Halloran, 2001). Thus, the chaperones function to deliver copper to precise intracellular locations and compartments.

At least six copper chaperones were identified in eukaryotes:

- Atox1 (HAH1) directly interacts with the Menkes/Wilson copper ATPase to donate copper to the N-terminal metal binding domains; whether this is an essential step in the transport of copper by ATPase or exerts a regulatory function remains to be established (Arnesano et al., 2001; Multhaup et al., 2001; Walker et al., 2004).
• COX17 was shown in yeast to deliver copper to the mitochondria for incorporation into cytochrome c oxidase (Srinivasan et al., 1998); COX17 binds three Cu(I) and was detected in the cytoplasm and mitochondrial intermembrane space and may serve as a copper shuttle (Heaton et al., 2000).

• Sco1p and Sco2p of yeast seem to participate in copper delivery from COX17 to cytochrome c oxidase (Glerum et al., 1996). The C-terminal domain of Sco1 was found to bind one Cu(I) (Nittis et al., 2001), which it may deliver specifically to the CuA site of cytochrome c oxidase subunit II (Mattatall et al., 2000).

• Cox11 encodes a protein of the inner mitochondrial membrane. According to studies in yeast, Cox11 is a heme A biosynthetic enzyme (Tzagoloff et al., 1993) but, according to studies in Rhodobacter sphaeroides, it is required for stable formation of the CuB and magnesium centers of cytochrome c oxidase (Hiser et al., 2000).

• CCS (chopper chaperone for superoxide dismutase) delivers copper specifically to the cytosol for incorporation into Cu,Zn superoxide dismutase (Culotta et al., 1997). CCS possesses an N-terminal, Atox1-like copper binding domain, which is fused to a sequence similar to its SOD1 target (Huffman & O’Halloran, 2001). Although the mechanisms of copper loading of CCS and transfer of copper from CCS to SOD1 are still unknown, it is clear that the process involves the formation of a CCS–SOD1 heterodimer (Lamb et al., 2000).

• NML45 (nuclear Menkes-like protein), which may transport copper into the nucleus of human cells, has not yet been well characterized but may be a splice variant for the MNK gene (Reddy et al., 2000).

INBORN ERRORS OF COPPER METABOLISM

Much of our knowledge of the molecular mechanisms by which copper homeostasis is achieved comes either from studies in model systems or from two diseases of copper metabolism: Menkes disease and Wilson disease (Andrews, 2002; Llanos & Mercer, 2002; Mercer, 2001; Strausak et al., 2001; Harris, 2000). Menkes disease manifests as an apparent copper deficiency, while Wilson disease manifests as a copper excess toxicosis. These two very different diseases arise from defects in the two similar copper pumps, the Menkes and the Wilson Cu-ATPases. The Menkes ATPase is expressed in tissues like skin-building fibroblasts, kidneys, placenta, brain, gut and vascular system, while the Wilson ATPase is expressed mainly in the liver, but also in mammary glands and possibly in other specialized tissues (International Programme on Chemical Safety, 1998).

Menkes Disease

Presentation and Prognosis  Menkes disease, first described by physician John Menkes in 1962, is an X-linked syndrome occurring at a frequency of approximately 1/200,000 live births. Although the disease primarily occurs in boys, it was also reported in eight females (Kodama & Murata, 1999). Menkes patients show a profound systemic copper deficiency that is often fatal in early childhood and accompanied by severe neurological abnormalities, apparently due to the lack of several copper-dependent enzymes required for brain development (Kaler, 1994). Affected individuals present with hypopigmented hair due to tyrosinase deficiency, resulting in lack of melanin synthesis. The hair of steely appearance is also brittle and kinky because of a deficiency in an unidentified cuproenzyme required for cross-linking keratin. This has led to the alternative designation “kinky hair disease.” Reduced lysyl oxidase activity results in defective collagen and elastin polymerization and corresponding connective-tissue abnormalities including aortic aneurisms, loose skin, and fragile bones. The severe neurological defects are thought to be due to reduced cytochrome c oxidase activity (Kaler, 1998). With early diagnosis and treatment, which consists of daily injections of copper histidine intraperitoneally and intrathecally to the central nervous system, some of the severe neurological problems may be avoided and survival prolonged. However, Menkes disease patients retain abnormal bone and connective-tissue disorders and show mild to severe mental retardation (Kaler, 1996). Even with early diagnosis and treatment, Menkes disease is usually
fatal; most affected individuals die before the age of 10 yr, although several have survived into their teens and early 20s (Kaler, 1998).

Individuals with Menkes disease absorb copper from the small intestine, but this copper cannot be pumped out of the intestinal cells into the blood for transport to the liver and consequently to the rest of the body (Kaler, 1996, 1998). The disease thus functionally resembles severe nutritional copper deficiency.

Milder clinical variants of Menkes disease exist, of which the best described is the very rare (about 100 cases reported) occipital horn syndrome. These male patients present with severe connective tissue disorders, congenital X-linked cutis laxa (inelastic skin that hangs in folds, usually on the face, resulting in an aged appearance), hyperextensible and hypermobile digits, and bony protruberances of the occiput, the back portion of the cranium. Low normal mental function, or mild mental retardation, is also present and there may be late-onset seizures (Ralph & McArdle, 2001). Occipital horn syndrome patients survive to adulthood but there are no reports to date of treatments to manage or improve the disease (Kodama et al., 1999).

Molecular Characterization of Defect Genetic analyses of affected individuals and model animal systems demonstrated that almost 20% of the mutations that produce Menkes disease are deletions in the Menkes ATPase gene (Tumer & Horn, 1997). Single base-pair changes, splice mutants, nonsense, missense, and duplications were also identified (Tumer & Horn, 1997). Severe Menkes disease results from the absence or very low levels of ATPase function. Clinical phenotypes associated with mutations are thus a function of the degree to which protein function is decreased, thereby reducing copper transport and altering copper intracellular location and trafficking. However, it remains difficult to associate particular mutations with severe or milder forms of the disease (Moller et al., 2000).

Mottled mice are a model for Menkes disease. These mice have mutations similar to those observed in humans, affecting the function of the Menkes homologous copper ATPase gene. The dappled mouse, brindled mouse, macular mouse, and blotchy mouse have different clinical phenotypes, which depend on the type of mutation. The overall presentation of the disease in mice and humans is similar, involving, among others, (1) abnormal, kinky hair, (2) reduced growth, (3) neurological damage, and (4) premature death. It has not been possible to establish clear genotype–phenotype relationships either in mice or humans (Levinson et al., 1994, 1997; Murata et al., 1998; Cecchi et al., 1997; Reed & Boyd, 1997).

Wilson Disease

Presentation and Prognosis Wilson disease, also referred to as hepatolenticular degeneration, is an autosomal (chromosome 13) recessive inherited disorder of copper transport (Mercer, 2001; Llanos & Mercer, 2002; Gitlin, 2003), which (1) involves poor incorporation of copper into ceruloplasmin and impaired biliary copper excretion and (2) is usually induced by mutations impairing the function of the Wilson copper ATPase. These mutations produce copper toxicosis due to excessive copper accumulation, predominantly in liver and brain and, to a lesser extent, in kidneys, eyes, and other organs. The incidence of Wilson disease was estimated to be 1/30,000, resulting in an estimated heterozygotic frequency of 1/90 in the overall population (International Programme of Chemical Safety, 1998).

The age on onset of Wilson disease ranges from 3 to 50 yr and the clinical outcomes vary widely. Initial presentation of patients with Wilson disease involves hepatic, neurologic, or psychiatric manifestations and, rarely, renal, skeletal, or endocrine symptomatology. Hepatic symptoms may be acute and self-limited, mimicking acute hepatitis, or may progress rapidly, suggesting fulminant hepatitis. Alternatively, onset may resemble chronic active hepatitis or cirrhosis with hepatic insufficiency. In adolescents and younger adults, Wilson disease may mimic fulminant hepatitis, with initial severe hepatitis, frequently complicated by profound, Coombs-negative, hemolytic anemia (Ferenci et al., 2003). Standard marker enzymes of liver damage are unreliable diagnostic markers in Wilson disease; aminotransferase activity levels are not markedly elevated and serum alkaline phosphatase activity values are almost always inappropriately low, despite severe hepatic insufficiency (Ferenci et al., 2003). The disease progresses with deepening jaundice and the
development of encephalopathy, severe clotting abnormalities, occasionally associated with intravascular coagulation, and terminal renal insufficiency. Almost always, death occurs if the disease is untreated.

Copper accumulates in hepatocytes, and lysis occurs when their capacity is exceeded. The released metal then diffuses into the blood and is accumulated in extrahepatic tissues. Neurological damage primarily occurs in the putamen and globus pallidus, collectively known as the lenticular nucleus (hence the nomenclature of hepatolenticular degeneration). Symptoms of the disease include a peculiar type of tremor in the upper extremities, slowness of movement, and changes in temperament. Persons may become exceptionally argumentative, overly emotional, or may experience a decrease in mental capabilities. Kayser-Fleischer rings, a rusty brown discoloration at the outer rims of the iris due to copper deposition, become evident as copper begins to accumulate in and affect the nervous system (Ferenci et al., 2003). The rings are noted in 90% of patients with Wilson disease and, occasionally, in patients with prolonged cholestasis and cryptogenic cirrhosis (Ferenci et al., 2003).

A number of biochemical laboratory screening tests may be used to help in the diagnosis and may reveal the underlying physiological and molecular defects of the disease (Baerlocher & Solioz, 2003). For example, low serum ceruloplasmin levels, elevated serum copper and urine copper excretion values, both before and after exposure to a copper challenge, are indicative of Wilson disease. The kinetics of radiolabeled copper binding to ceruloplasmin is also diagnostic for Wilson disease; following oral administration of radiolabeled copper, serum levels of radioactivity are measured at 1 to 2 and 48 h. Both Wilson disease and normal patients show an increase in blood copper levels at the early time measurement. In normal individuals, there is a secondary rise in plasma radioactivity at 48 h due to ceruloplasmin-bound copper reappearing in the blood. In Wilson disease patients, where hepatic incorporation of copper into ceruloplasmin is defective, there is only a minimal secondary rise in plasma radioactivity at 48 h (Baerlocher & Solioz, 2003). Identification of the genetic defect underlying most cases of Wilson disease (see later discussion) has made DNA testing for diagnosis possible.

Treatment of Wilson disease includes chelation therapy with chelating agents such as D-penicillamine or zinc therapy using either zinc sulfate or zinc acetate. Zinc therapy is now the treatment of choice. Zn produces a mucosal block by inducing metallothionein, which binds copper in mucosal cells until they slough off and are eliminated in the feces (Brewer et al., 1998). More recently, experimental treatments with tetrathiomolybdate showed promising results. Tetrathiomolybdate appears to be an excellent form of initial treatment in patients who present with neurologic symptoms. In contrast to penicillamine therapy, initial treatment with tetrathiomolybdate rarely allows further, often irreversible, neurologic deterioration (Brewer et al., 1996). Dietary restrictions in Wilson disease involve the elimination of foods high in copper, such as chocolate, oysters, and mushrooms. If diagnosed and treated early enough, patients with Wilson disease may live normal lives.

Molecular Characterization of Defect In most cases, molecular biological techniques allow for the identification of the mutations in the Wilson ATPase gene that produce Wilson disease. Phenotypic variability in manifestations of Wilson disease are likely due to the nature of the relevant mutations but a clear correlation of mutation and severity (genotype/phenotype) of the disease is not apparent (Baerlocher & Solioz, 2003; Ferenci et al., 2003). Over 100 different genetic defects leading to Wilson disease have been described and are available on the Internet at http://www.uofa-medical-genetics.org/wilson/index.php. Some of the mutations have geographic clustering; 38% of Wilson disease mutations in North America are a histidine-1069 to glutamine mutation, whereas other populations have different common mutations (Garcia-Villarreal et al., 2000). Because many Wilson patients carry different mutations on each chromosome 13 (i.e., they are compound heterozygotes), it is difficult to correlate genotype and phenotype. Even in individuals who are homozygous for a mutation, onset and severity of the disease may vary (Duc et al., 1998; Ferenci et al., 2003). Individuals homozygous for severe mutations (e.g., those truncating the protein) have earlier disease onset. Disease severity may also be a function of environmental factors, including the amount of copper in the diet or variability in the function of other proteins that influence copper homeostasis.
There are two animal models for Wilson disease. The Long-Evans cinnamon (LEC) rat, which has a deletion in the gene of the Wilson homologue resulting in undetectable expression of the gene (Yamaguchi et al., 1994). The toxic milk mouse has a missense mutation in the homologous gene, which is expected to change methionine 1356 in the eighth transmembrane helix to valine (Theophilos et al., 1996). Both rodent strains develop liver diseases that resemble Wilson disease (Coronado et al., 2001). The Bedlington terrier exhibits clinical symptoms similar to those occurring in patients with Wilson disease and was long considered to be a canine model of the disease. However, the genetic defect in these dogs was recently localized to the MURR1, a gene of unknown function (van De et al., 2002).

Other Copper-Related Hereditary Syndromes

Other diseases in which abnormalities in copper metabolism appear to be involved include Indian childhood cirrhosis (ICC) and idiopathic copper toxicosis (ICT), or non-Indian childhood cirrhosis. These are infancy syndromes that are similar in their apparent etiology and presentation (Wijmenga et al., 1998). Both appear to have a genetic component and a contribution from elevated copper intake. In cases of ICC, the elevated copper intake is due to heating and/or storing milk in copper or brass vessels. ICT cases, on the other hand, are due to elevated copper concentrations in water supplies (Muller et al., 1998; International Programme on Chemical Safety, 1998). Although exposure to elevated concentrations of copper is commonly found in both diseases, some cases appear to develop in children who are exclusively breast fed or who receive only low levels of copper in the water supply (Muller et al., 1998). The currently prevailing hypothesis is that ICT is due to a genetic lesion resulting in impaired copper metabolism combined with high copper intake. This hypothesis was supported by the frequency of occurrence of parental consanguinity in most of these cases, which is absent in areas with elevated copper in drinking water and in which these syndromes do not occur (Muller et al., 1998).

UNRESOLVED ISSUES IN COPPER HOMEOSTASIS

Although our knowledge of the molecular mechanisms involved in copper homeostasis has increased exponentially in the past decade, many questions remain that are important for understanding copper homeostasis and for risk assessment, particularly in view of identifying potentially susceptible subpopulations. Such questions include:

- How is copper taken up from the gastrointestinal tract?
- How does copper cross the blood–brain barrier?
- What is the nature and role of copper reductases?
- How do copper ATPases work and how are they regulated?
- How does trafficking of Menkes and Wilson ATPase work and how is it regulated?
- How does CTR1 work in delivering copper to cells?
- What is the function of the MURR1 gene defective in Bedlington terriers?
- What is the genetic defect in idiopathic copper toxicosis (e.g., Indian childhood cirrhosis)?
- Are there additional copper homeostatic genes?

BIOLOGICAL EFFECTS OF COPPER: HAZARD IDENTIFICATION

Copper Toxicity from Excess Exposures

In humans, the liver is the primary organ of copper-induced toxicity (International Programme on Chemical Safety, 1998). In animals, adverse effects were observed mainly in organs involved in the absorption and excretion of copper (liver, kidneys, rodent forestomach) (Hebert et al., 1993; International Programme on Chemical Safety, 1998). Other target organs include bone and the central nervous and immune systems (International Programme on Chemical Safety, 1998). Excessive copper intake also induces toxicity indirectly by interacting with other nutrients; for example,
excess copper intake produces anemia by interfering with iron transport and/or metabolism (International Programme on Chemical Safety, 1998; Ralph & McArdle, 2001). A summary of the toxicity observed in human populations is found in Table B-2 of Appendix B.

**Humans** Copper toxicity in the general population was not a public health concern until recently, mainly because of a lack of reported toxicity, despite centuries of copper use in a wide variety of applications. The identification of genetic disorders of copper metabolism leading to severe copper toxicity (Wilson disease) or copper deficiency (Menkes disease) has not only spurred research into the molecular genetics and biology of copper homeostasis but also focused attention on the potential consequences of copper toxicity in normal and potentially susceptible populations. Potentially susceptible subpopulations include hemodialysis patients and individuals with chronic liver disease. Recently, concern was expressed about the potential sensitivity to liver disease of individuals who are heterozygote carriers of Wilson disease genetic defects (i.e., those having one normal and one mutated Wilson copper ATPase gene) but do not have the disease (which requires defects in both relevant genes) (Brewer, 2000). However, to date, no data are available that either support or refute this hypothesis.

**Case Reports** In case reports of humans intentionally or accidentally ingesting high concentrations of copper salts (doses usually not known but reported to be 20–70 g copper), a progression of symptoms was observed including abdominal pain, headache, nausea, dizziness, vomiting and diarrhea, tachycardia, respiratory difficulty, hemolytic anemia, hematuria, massive gastrointestinal bleeding, liver and kidney failure, and death.

**Acute Exposures** Episodes of acute gastrointestinal upset following single or repeated ingestion of drinking water containing elevated levels of copper (generally above 3–6 mg/L) are characterized by nausea, vomiting, and stomach irritation; symptoms resolve when the drinking water source is changed. Most reported cases have not provided good estimates of the copper levels that induce these effects. Three experimental studies of high quality were conducted (Araya et al., 2001, 2003a; Pizarro et al., 1999a, 1999b) that demonstrate a threshold for acute gastrointestinal upset of approximately 4–5 mg/L in healthy adults, although it is not clear from these findings whether symptoms are due to acutely irritant effects of copper and/or to metallic, bitter, salty taste. In another experimental study with healthy adults, the average taste threshold for copper sulfate and chloride in tap water, deionized water, or mineral water was 2.5–3.5 mg/L (Zacarias et al., 2001), which is just below the experimental threshold for acute gastrointestinal upset.

**Chronic Exposures** The long-term toxicity of copper has not been well studied in humans, but it is infrequent in normal populations not having some hereditary defect in copper homeostasis (Olivares & Uauy, 1996). Chronic copper poisoning leading to liver failure was reported in a young adult male with no known genetic susceptibility who consumed 30–60 mg/d of copper as a mineral supplement for 3 yr (O’Donohue et al., 1999). Pratt et al. (1985) reported no evidence of liver damage or gastrointestinal effects in a double-blind study of 7 healthy subjects given 10 mg/d of copper gluconate in capsules for a period of 12 wk. Further, there is some evidence that individuals are able to adapt to elevated copper concentrations in drinking water. Exposures of children under the age of 6 yr, totaling 64,124 child-years, to drinking water containing 8 mg/L of copper produced no deaths associated with any form of liver disease (Scheinberg & Sternlieb, 1996). Similarly, individuals residing in U.S. households supplied with tap water containing >3 mg/L of copper exhibited no adverse health effects (Buchanan et al., 1999).

Dermal exposure has not been associated with systemic toxicity, but copper was reported to occasionally induce allergic responses in sensitive individuals (International Programme on Chemical Safety, 1998). Workers exposed to high air levels of copper (resulting in an estimated intake of 200 mg Cu/d) developed signs suggesting copper toxicity (e.g., elevated serum copper levels, hepatomegaly); however, other co-occurring exposures to pesticidal agents or in mining and smelting may contribute to these effects (International Programme on Chemical Safety, 1998).

**Animals** A summary of the results of studies of copper toxicity in laboratory animals is found in Table B-1 of Appendix B.

**Acute Toxicity** In animals, the acute toxicity of a single oral dose of copper varies widely among species; the LD₅₀ range is 15–857 mg Cu/kg body weight (International Programme on
Chemical Safety, 1998). The more soluble salts [e.g., copper(II) sulfate, copper(II) chloride] are generally more toxic than the less soluble copper compounds [e.g., copper(II) hydroxide, copper(II) oxide]. Death is preceded by gastric hemorrhage, tachycardia, hypotension, hemolytic crisis, convulsions, and paralysis. LD$_{50}$ values for dermal exposure were reported as >1124 and >2058 mg Cu/kg body weight in rats and rabbits, respectively. The inhalation LC$_{50}$ (exposure duration unspecified) was >1303 mg Cu/m$^3$ in rabbits, and respiratory function was impaired in guinea pigs exposed to 1.3 mg Cu/m$^3$ for 1 h (International Programme on Chemical Safety, 1998).

**Chronic Toxicity** Data from repeated-dose animal toxicity studies are generally consistent with those from human case reports. A number of studies were conducted with commercially important farmland animals (e.g., cattle, sheep, pigs) in which the essentiality of copper for normal development was first identified through cases of copper deficiency. Copper-induced toxicity was assessed in some repeated-dose studies of laboratory animals that vary widely in study design, study quality, and reporting of experimental details and results. Two studies following standard regulatory toxicological protocol were conducted: a 1-yr study in dogs and a 13-wk study in rats and mice (Shanaman, 1972; Hebert et al., 1993). No adverse effects were reported in dogs administered dietary copper gluconate at doses of up to 8.4 mg/kg/d for 6–12 mo. In rats exposed to dietary copper sulfate for 13 wk, forestomach, liver, and kidney lesions were observed at doses of approximately 17–34 mg/kg/d. In other rodent studies, considered to have limited usefulness for dose-response assessment due to study limitations such as the use of only one dose group, inappropriate route of compound administration (e.g., ip or iv), lack of statistical analysis, or unsuitable animal model (e.g., ruminants) for human hazard characterization, the no-observed-adverse-effect level (NOAEL) generally ranges from approximately 20 to greater than 100 mg/kg/d (International Programme on Chemical Safety, 1998).

**Reproduction/Development in Humans and Animals** Reproductive/developmental toxicity studies are few and generally inadequate to assess reproductive and developmental hazards of excess copper intake. Copper has, however, been used as a contraceptive agent in intrauterine contraceptive devices, and metal toxicity to sperm and ability to inhibit blastocyst implantation in utero or to otherwise inhibit embryonic development are well known (International Programme on Chemical Safety, 1998). Studies of copper intrauterine devices have not demonstrated any abnormalities in the offspring of rats, hamsters, rabbits, or guinea pigs exposed to high intrauterine copper environments (Chang & Tatum, 1975; Moo-Young & Tatum, 1974). Abnormalities in the offspring of mothers with untreated Wilson disease were not reported, although women with untreated Wilson disease apparently have a higher than average incidence of spontaneous abortions (Mustafa & Shamina, 1998). Copper increases the incidence of fetal resorptions and induces malformations in the offspring of pregnant hamsters administered high iv doses of copper (Ferm & Hanlon, 1974); however, this route of exposure is not relevant to human intake. Increased mortality was observed in the fetuses of pregnant mice fed 104 mg Cu/kg/d as copper sulfate during gestation, and developmental abnormalities were observed at 155 mg Cu/kg/d (Lecyk, 1980). An increased mortality rate in the older offspring of minks was attributed by Aulerich et al. (1982) to impaired lactation in females fed a diet supplemented with >6 mg Cu/kg/d as copper sulfate; however, neither the amounts of copper in the basal diet nor indices of maternal toxicity were reported. Maternal body weights and maternal symptoms were not reported in these studies, and developmental toxicity may have occurred secondary to adverse maternal effects.

**Measurements of Elevated Copper Status** Although a number of indicators are useful in diagnosing copper deficiency, there are no reliable biomarkers of copper excess resulting from dietary intake. Increased serum copper or ceruloplasmin levels are not reliably associated with copper toxicity. Ceruloplasmin is an acute-phase reactant, and elevations in serum copper and ceruloplasmin concentrations are induced by inflammation, infection, disease, malignancies, pregnancy, and other biological stressors (Milne, 1998). Levels of copper-containing enzymes, such as cytochrome c oxidase, superoxide dismutase, and diamine oxidase, vary not only in response to copper state but also in response to a variety of other physiological and biochemical factors and thus are inconsistent markers of excess copper status (Milne, 1998). The most reliable indicator of excess copper status is liver copper concentration; however, measurement of this endpoint in humans is intrusive and not generally conducted except in cases of suspected copper poisoning (Milne, 1998).
Copper Deficiency

In both humans and animals, the major target organs for copper deficiency are the blood and hematopoietic system, the cardiovascular system, connective tissue and bone, the nervous system, and the immune system (Danks, 1988; International Programme on Chemical Safety, 1998; Ralph & McArdle, 2001).

Humans

Chronic Deficiency Although the essentiality of copper for erythropoiesis in animals was first reported in 1928, the spectrum of copper-deficiency effects in humans was not well described until Cordano (1978) completed a series of clinical case studies of copper deficiency. These effects included anemia, neutropenia, and bone-marrow abnormalities that resolved when the infants were given copper supplementation. Additional studies showed the essentiality of copper in bone formation, cardiac function, lipid metabolism, immune function, neurological development, and maturation of blood cells (Danks, 1988).

Clinically evident copper deficiency is rare in humans and occurs most commonly in low-birth-weight neonates, and in infants and children on parenteral nutrition if the nutrient formula does not contain adequate copper (<0.1 mg Cu/kg body weight/day in otherwise healthy infants; <0.2 mg/kg/day in low-birth-weight infants or those suffering from protein energy malnutrition) (Cordano, 1978). A primary characteristic of copper deficiency is hypochromic, normocytic or macrocytic anemia that is refractory to iron, with low copper plasma levels. The anemia, which is readily reversed by copper supplementation, is apparently due to defective iron mobilization resulting from reduced ceruloplasmin (ferroxidase I) activity (International Programme on Chemical Safety, 1998). Bone abnormalities, mimicking the changes observed in scurvy, are also common in copper deficiency in low-birth-weight infants and young children, and symptoms include osteoporosis, bone fractures, and abnormal bone growth. Other symptoms in this population are impaired growth, increased incidence of infections, and hypopigmentation of the hair (International Programme on Chemical Safety, 1998).

In severely handicapped patients fed an enteral diet low in copper (0.15 mg/100 kcal) for 1–5 yr, symptoms were similar to those observed in copper-deficient infants and young children (Higuchi et al., 1988). Extrapolation of copper intakes for these patients to the normal adult population suggested that copper deficiency would be induced at dietary intakes of 0.44 mg/d for men at 0.29 mg/d for women. Turnlund et al. (1997) observed a decrease in measures of deficient copper status (serum copper and ceruloplasmin concentrations) in healthy adult males fed a diet of 0.38 mg/d of copper for 42 d; the concentrations of these parameters increased following copper repletion (Turnlund et al., 1997).

Although severe copper deficiency is infrequent in humans, unrecognized or marginal copper deficiency may be more widespread. No diagnostic criteria are available for the assessment of marginal copper status defined as <1 mg/day; however, there is increasing public health concern that individuals whose copper intake is low (or whose absorption of dietary copper is decreased by interactions with other nutrients such as zinc) may exhibit marginal deficiency resulting in (1) increased susceptibility to infection, (2) impaired neurological function, and (3) elevated risk for a range of diseases including coronary heart disease and osteoporosis (Klevay, 1980; Strain, 1994). Experimentally induced alterations in cholesterol and glucose metabolism as a result of copper induced toxicity were reported in adults. Total cholesterol and low-density lipoprotein (LDL) cholesterol levels were increased and high-density lipoprotein (HDL) cholesterol was decreased in a subject fed an experimental diet marginal in copper (0.83 mg/day) (Klevay et al., 1984). Marginal copper intake (Klevay, 1998) was also shown to reduce glucose tolerance, alter cardiac rhythm and electrocardiogram waves, and increase the hypertensive response to a hand-grip test (0.65 mg/d) (Lukaski et al., 1988). However, these findings have not been replicated in other experimental studies (Milne & Nielsen, 1996; Medeiros et al., 1991), though the studies varied in terms of design, testing protocol, age and gender of study population, and endpoints measured. Klevay (1980) hypothesized that the high prevalence of cardiovascular disease in human populations is linked to low copper and high zinc in the diet, but this hypothesis remains controversial. Copper deficiency
was associated with altered immunity in humans (Bala & Failla, 1993). In 11 subjects receiving a
low-copper diet (0.38 mg/d), a decrease in the proliferative response of peripheral blood mononuclear
cells was observed (Kelley et al., 1995). Other effects reported in human studies include
changes in blood clotting factors (copper intakes of 0.57 mg/d; Milne & Nielsen, 1996), markers of
bone metabolism (0.7 mg/d; Baker et al., 1999), and oxidant status (Johnson et al., 1992).

Sensitive Populations Some subpopulations may be more sensitive to copper due to genetic
defects. Individuals who are heterozygous for Wilson disease, (e.g., those who carry one wild-type
and one mutant gene for the Wilson disease protein) might be more sensitive to high copper intake;
however, there is currently no evidence in this regard. Some cases of early childhood cirrhoses have
been ascribed to the combination of an unknown inherited genetic defect and high copper intake.
The condition has been described as endemic Tyrolean infantile cirrhosis (ETIC), Indian childhood
cirrhosis (ICC), or idiopathic copper toxicosis (ICT) (Muller et al., 1998). Although the condition
resembles Wilson disease, no defect in the corresponding genetic locus could be identified
(Wijmenga et al., 1998) and the underlying genetic defect awaits identification.

Single-nucleotide polymorphisms (SNPs) are receiving increasing attention as a sporadic cause
disease. SNPs are random DNA mutations, involving one or a few nucleotides. They occur anywhere
in the genome and may impair vital function, thus producing disease (Salisbury et al., 2003;
Chen & Sullivan, 2003). This research area is still in its infancy and no correlations between SNPs
and copper susceptibility have yet been made.

In addition to genetically sensitive populations, other populations susceptible to copper defi-
ciency include low-birth-weight infants, infants fed cow’s milk instead of breast milk or fortified for-
medula, pregnant and lactating mothers, patients receiving total parenteral nutrition, individuals with
“malabsorption syndrome” (impaired dietary absorption), diabetics, individuals with chronic dis-
eases that result in low food intake, such as alcoholics, and persons with eating disorders. The elderly
and athletes may also be at higher risk for copper deficiency due to special needs which
increase daily requirements (Wapnir, 1998). Vegetarians may have decreased copper intake due to
the consumption of plant foods in which copper bioavailability is low (Lonnerdal, 1996; Kelsay,
1987; Lee et al., 1984).

Animals

Chronic Studies Repeated-dose copper deficiency studies in animals generally support the
findings observed in clinically diagnosed cases of copper deficiency. More subtle effects are
observed in specialized laboratory studies targeting specific tissues or organ systems. Reported
adverse effects include anemia, decreased erythropoiesis and altered hematology, impaired
immune function and neurological development, altered cardiac function and lipid metabolism,
and reproductive/developmental toxicity (Danks, 1988).

Reproduction/Development in Humans and Animals “Failure to thrive” was reported in
low-birth-weight infants whose postnatal diets were deficient in copper, and in infants and children
receiving parenteral nutrition with low copper (Cordano, 1978). Although teratogenesis due to cop-
per deficiency occurs in Menkes disease, it is not clear to what extent hypocupremia contributes to
teratogenesis in human infants with no known genetic susceptibility to copper deficiency (Hurley
et al., 1982). Two cases were reported in which infants with congenital abnormalities in connective
tissue and bone were born to mothers taking large daily doses (2000 mg) of penicillamine (a cop-
per-chelating agent) during pregnancy for treatment of medical conditions unrelated to copper
(Hurley & Keen, 1979); however, other cases of copper-associated teratogenesis in humans have
not been reported.

In animals, copper deficiency is clearly teratogenic and also induces adverse developmental
and neurobehavioral effects. The nature and magnitude of these effects depend on (1) timing of
copper deficiency during reproduction and development, (2) extent of copper deficiency, and (3)

In female rats, severe copper deficiency during gestation induces fetal resorptions or stillbirths
(Hall & Howell, 1969). The offspring of pregnant rats given a copper-deficient diet during gestation
have increased postnatal mortality and a high incidence of structural and behavioral abnormalities,
including brain lesions, skeletal malformations, cardiovascular lesions, severe growth retardation, convulsions, and hyperirritability to noise (Hurley & Keen, 1979). Young rats or mice receiving sufficient copper during gestation and lactation but weaned onto a diet low in copper exhibit cardiovascular lesions, hematologic abnormalities, and alterations in lipid metabolism, lipid peroxidation, and hormone levels (Lynch & Klevay, 1994; Bode et al., 1992; Lear & Prohaska, 1997; Mao et al., 1999; Rasyssiguier et al., 1993; Rock et al., 1995; Nelson et al., 1992; Fields & Lewis, 1997).

**Measurement of Deficient Copper Status**  
Serum copper levels, superoxide dismutase activity, and ceruloplasmin concentration are considered to be reasonably reliable indicators of copper status in that they are significantly decreased in severely copper-deficient individuals (Louro et al., 2001). However, serum concentrations of these measures are typically increased during acute-phase injuries, in disease states, and under conditions of biological stress, such as infection, inflammation, myocardial infarction, chronic liver disease, malignancies, and pregnancy (Milne, 1998). Thus, copper deficiency might be masked with any of these conditions if these parameters are used as markers for copper-deficient status.

Other possible indicators of copper-deficient status suggested include erythrocyte superoxide dismutase activity, skin lysyl oxidase activity, leukocyte copper concentrations, a combination of platelet copper concentrations and platelet cytochrome c oxidase activity (Milne, 1998), and the copper chaperone for superoxide dismutase (CCS) (Bertinato & L’Abbe, 2004). None of these markers has been validated, and methods of analysis for these endpoints have not yet been standardized.

**DOSE-RESPONSE ASSESSMENT**

**Dose-Response Modeling**

Advances in toxicology and risk assessment in recent decades have greatly enhanced our abilities to assess dose-response and characterize risk for noncancer endpoints (Krewski et al., 1999). This research has enabled significant movement from crude guesses of a chemical’s “safe” dose, such as an acceptable daily intake (Lu, 1988), to the development of more sophisticated and refined methods for establishing “virtually safe doses” and characterizing human health risks. These advances have included incorporation of toxicokinetics and/or toxicodynamics into the judgment of chemical-specific adjustment factors for establishing tolerable intakes (TI) or RfDs (RfCs) (International Programme on Chemical Safety, 2001; Meek et al., 2001) and the evolution of more sophisticated statistical approaches (e.g., benchmark dose and categorical regression modeling) and biologically based dose-response models.

**Reference Dose**  
For most substances a dose or level of exposure is assumed to exist below which adverse effects will not occur. For example, the U.S. Environmental Protection Agency (EPA) defines the RfD (or RfC) as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily (or continuous) exposure for the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime” (Barnes & Dourson, 1988; U.S. Environmental Protection Agency, 1994). The RfD/RfC is developed based on division of the no-observed-adverse-effect level (NOAEL), the lowest-observed-adverse-effect level (LOAEL), or a NOAEL surrogate, such as a benchmark dose/concentration (BMD/BMC) for the critical effect, by the composite uncertainty factor.

**Benchmark Dose (BMD) Modeling**  
The benchmark dose is a modeled point in the dose-response curve of an adverse effect representing a predetermined level of change when compared with controls. The statistical lower confidence limit of the BMD (i.e., the BMDL), rather than the maximum likelihood estimate (the MLE, also referred to as the BMD), is generally used for developing a TI or RfD, and serves as a point of departure for developing an RfD (Crump, 1984, 1995; Dourson et al., 1985; U.S. Environmental Protection Agency, 1995, 1999; Slob, 2002). In estimating the BMD, different models are appropriate for different types of data (U.S. Environmental Protection Agency, 1995, 1999; Gaylor et al., 1998; Sand et al., 2002, 2003; Slob, 2002), including both empirical or biologically based dose-responsive models (Krewski et al., 2002).
**Categorical Regression**  Categorical regression is a method by which a dose-response model may be fitted to data where only ordinal severity ratings are available (e.g., mild necrosis at 2 mg/kg/d, and moderate necrosis at 10 mg/kg/d), without any quantitative response data (Haber et al., 2001). Categorical regression can also be used for modeling dose-response information from disparate endpoints and/or multiple studies by using a common severity metric (in terms of severity categories), although care needs to be taken in how the combination is done. Categorical regression can also model concentration (or dose)–time–severity responses for acute through chronic chemical exposures (Dourson et al., 1997; Haber et al., 2001).

**Biologically Based Dose Response (BBDR) Models for Copper** Ideally, a comprehensive dose-response model for copper toxicity would take into account the biological mechanisms underlying copper toxicity. Because both excess and deficiency are known to result in adverse health outcomes, although by different mechanisms, the population health impacts of copper excess and deficiency both need to be taken into account. Consideration also needs to be given to the identification of population subgroups at particular risk. Sensitive biomarkers of the adverse health effects of copper can also be valuable in delineating dose-response relationships for copper.

The usefulness of BBDR modeling techniques in understanding copper toxicity is difficult to assess at this time. A valid model requires information regarding the molecular mechanisms and pharmacodynamics of copper toxicity. Although there exists considerable information on the mechanism by which copper homeostasis may be disrupted, such mechanisms have yet to be fully elucidated. At this point in time there is insufficient information to develop a credible BBDR for copper toxicity. In the absence of a valid BBDR, the empirical approach proposed for adoption in this review appears to be the most appropriate methodology for gaining an understanding of the dose-response relationship for copper toxicity.

**MODELING EXCESS AND DEFICIENCY**

Table 1 shows the postulated dose-response spectrum of endpoints from severe deficiency through homeostasis to severe toxicity from excess. At the ends of the homeostatic range, subtle changes provide early evidence of perturbation of normal homeostasis. Usually, at these points, copper homeostasis is upregulated or downregulated to ensure that copper status is within bounds. The points of inflection occur at the points of failure of normal homeostasis.

The question of whether the dose-response relationships for copper excess and deficiency should and/or could be modeled separately or together received considerable attention from both toxicological and statistical perspectives. Some investigators were of the opinion that this issue should be resolved on the basis of biological mechanisms of toxicity. If the mechanisms of toxicity

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**TABLE 1. A Postulated Spectrum of Copper Metabolism (from Aggett, 1999)**

<table>
<thead>
<tr>
<th>Dose range</th>
<th>Approximate daily intakes</th>
<th>Health outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic</td>
<td>&gt;5.0 mg/kg body weight</td>
<td>Death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gross dysfunction and disturbance of metabolism of other nutrients; hepatic &quot;detoxification&quot; and homeostasis overwhelmed</td>
</tr>
<tr>
<td>Adequate</td>
<td>100 μg/kg body weight</td>
<td>Gastrointestinal metallothionein induced (possible differing effects of acute and chronic exposure);</td>
</tr>
<tr>
<td></td>
<td>34 μg/kg body weight</td>
<td>Plateau of absorption maintained; homeostatic mechanisms regulate absorption of copper</td>
</tr>
<tr>
<td></td>
<td>11 μg/kg body weight</td>
<td>Hepatic uptake, sequestration and excretion effect homeostasis; glutathione-dependent uptake of copper; binding to metallothionein; and lysosomal excretion of copper</td>
</tr>
<tr>
<td></td>
<td>9 μg/kg body weight</td>
<td>Biliary excretion and gastrointestinal uptake normal</td>
</tr>
<tr>
<td>Deficient</td>
<td>8.5 μg/kg body weight</td>
<td>Hepatic deposit(s) reduced; conservation of endogenous copper; gastrointestinal absorption increased</td>
</tr>
<tr>
<td></td>
<td>5.2 μg/kg body weight</td>
<td>Negative copper balance</td>
</tr>
<tr>
<td></td>
<td>2 μg/kg body weight</td>
<td>Peripheral pools disrupted; gross dysfunction and disturbance of metabolism of other nutrients; death</td>
</tr>
</tbody>
</table>
due to excess and deficiency are different, each curve could be modeled separately and then combined; however, if the mechanisms of toxicity are common to both deficiency and excess, then it might be feasible to model the curves jointly.

At present, incomplete understanding of the mechanisms underlying copper toxicity due to excess and deficiency limits our ability to develop biologically based combined dose-response models, even though some common mechanistic pathways exist. However, for statistical modeling, these mechanistic arguments against joint modeling of deficiency and excess do not apply. From an empirical rather than biologically based modeling perspective, the question of whether or not to attempt the construction of joint models for deficiency and excess depends on other factors, such as the availability of adequate data on both deficiency and excess, the goodness of fit of the joint models, and their ultimate utility in establishing human exposure guidelines.

The type, quantity, and quality of data to be modeled play a large role in determining the type of analysis that is appropriate. The fact that a meta-analysis of combined data from very disparate studies is being considered makes more difficult the use of detailed mechanistic models. To put this issue in perspective, it may be helpful to briefly discuss the types of data that are likely to be available for modeling. Ideally, it would be advantageous to have information on whether or not an individual (human subject) was adversely affected in any way (either from copper deficiency or excess) from a particular well-defined copper exposure. It is unlikely that such information will be available from any study. Instead, in a meta-analysis it is necessary to combine a wide variety of types of data from a diversity of study designs using a variety of response measures, including:

- Counts of subjects having a particular type of adverse response.
- Means and standard deviations of various biochemical measurements in various dose groups.
- Determination of or a judgment on whether or not a particular effect occurred in a dose group as a result of copper excess or deficiency.

Such data are available from a wide variety of experimental designs that include different species, different exposure routes, and different exposure time patterns. Combining such disparate data meaningfully into a common analysis is difficult at best, and the application of detailed mechanistic models, even if such models existed, is quite daunting.

In the absence of quantitative mechanistic models for deficiency and excess, the issue of whether or not to use a common dose-response model for both effects becomes of less importance. Lacking a mechanistic model, dose-response modeling needs to be implemented using an empirical approach. The adequacy of an empirical statistical model may be evaluated based on goodness of fit, perhaps supplemented with fairly general considerations of biological plausibility. Under these conditions, the advantages of a common model for both excess and deficiency seem more questionable. An important potential disadvantage of a common model is the difficulty in adequately fitting a nonmonotone dose response. If a single model is applied, the ability of the model to adequately describe the data needs to be given careful scrutiny. Further, results need to be compared with those obtained from individual modeling of excess and deficiency.

In view of the disparate nature of the data to be modeled, robust methods are needed. An initial analysis may simply involve graphing the data, showing the doses at which effects of either type did or did not occur. Even such a simple graphical approach may not be straightforward. Different methods of expressing dose (such as mg/kg or mg/kg/d) may yield different results. The effect of exposure duration should be incorporated in such a graph if possible. Since chronic exposures are of concern, the focus should be on estimating effects from long-term chronic exposures. Whether to incorporate data from acute exposures into this process and if so, how, need careful consideration.

A balance is needed in the approach for both deficiency and excess so that the result is not a fine line for deficiency and a gross measure for excess. The deficiency side of the U-shaped dose-response curve is of particular interest, since it is important to preserve essentiality. A BMD10 [or BMD(L)10] for toxicity due to excess may be used as a point of departure for the toxicity portion of the curve, with adjusted uncertainty factors applied to protect individuals from high-dose toxicity.
A BMD(L)10 for deficiency may be used as the point of departure for the deficiency portion of the curve, with the application of CSAF, derived from studies on low-dose toxicity from deficiency. Unlike for the toxicity portion, the BMD(L)10 for deficiency would be multiplied by an uncertainty or adjustment factor, rather than being divided by the factor.

In the context of determining a homeostatic and safe dose range for copper, using either BMD modeling or the NOAEL approach, the appropriate margin of safety and the appropriate adjustment factors to be applied need to be carefully examined, particularly if the range between the BMD(L)10 values for deficiency and excess is narrow. In this situation, the standard approach for the RfD [i.e., dividing a LOAEL, NOAEL or BMD(L) for excess copper by a default uncertainty factor] may put the daily intake value into the range of deficiency, where health is no longer being maintained. One can even envision a situation in which there would be a range of copper values at which deficiency and excess might occur in different individuals at the same copper level. In such a situation, there would be no dose that is safe for the entire population; ideally, one would like to estimate the copper dose at which the likelihood of adverse effects from either deficiency or excess is minimized in this population. Conceptually, this approach is consistent with the current “shifting target” in determining nutrient sufficiency. The definition of public health is no longer the absence of disease but the optimization of health and adverse health risk reduction. For example, supplements of copper in the range of 2–5 mg/d have been shown to be beneficial in reducing bone loss or increasing low bone mineral density associated with osteoporosis in postmenopausal women (Saltman & Stause, 1993; Lowe et al., 2002). In the older approach to nutrition, the development of a lower RDA for copper was driven by a lack of overt signs of deficiency. In the newer approach, the concern is to decrease the risk of chronic disease; this newer approach is being applied to multiple nutrients but has not yet been applied to copper.

Dose metrics are also important for the toxicokinetics and toxicodynamics of both excess and deficiency. Different dose metrics produce different results when extrapolating from animals to humans, and also affect the shape of the dose response curve in a single species. The effective copper dose for the synthesis of key copper-complexed molecules is likely to be important because it is expected that saturation/desaturation at the molecular level may be associated with changes in the slope in the dose-response curve.

A DOSE-RESPONSE MODELING STRATEGY FOR COPPER

Information Needs for Dose-Response Modeling

Ideally, detailed information regarding copper uptake, binding, distribution, metabolism, and excretion would be coupled with mechanistic models of how various organ systems respond to variations in their copper status. The dose-response curves for toxic endpoints that manifest in each of these organ systems would then be dictated directly by the underlying molecular biology of the prevailing mechanisms of toxicity in each system. However, our understanding of the molecular biology of copper remains far from complete, and is still essentially descriptive in nature. There are many gaps. Even the dose metric(s) that make up the primary causal agents for the various forms of excess- or deficiency-induced toxicity remain unidentified, with exposure in most studies being characterized solely in terms of the daily amount of copper that is administered via the diet.

It is known, however, that the metabolism and turnover of copper are highly organ specific. For example, in organs such as the brain and heart, copper conservation is highly efficient, and there is little loss of copper in response to even a pronounced dietary restriction. However, conservation of copper in the liver, the primary storage depot, is induced only after an appreciable amount of copper is lost from storage (Levenson, 1998). Thus, it is not unreasonable to expect that the dose-response curves for toxicity in different organ systems differ from one another temporally as well as with dose, in both scale and shape.

It is also clear that early responses to copper deficiency or excess are not simple, passive changes. Rather, they comprise a coordinated and adaptive suite of alterations that serve to modulate copper uptake, distribution, and storage, as well as metabolism and excretion. These active
responses serve to preserve homeostasis, and are sufficiently robust to maintain normal structure and function of the organism in the face of natural variations in copper intake. Turnlund (1998) found that when human dietary copper intake varies over a 10-fold range from about 0.8 to 8 mg/d, many indices of copper status, including plasma copper, erythrocyte superoxide dismutase, ceruloplasmin, and urinary copper excretion, are essentially unaffected. It is only when intake is reduced to about 0.4 mg/d that these parameters of copper status begin to change significantly (Turnlund et al., 1997).

While a mechanistically based approach to dose-response modeling provides a useful concept for organizing and integrating the large and diverse database for copper toxicity, the limitations of the toxicity database at the present time likely impede the successful implementation of such an approach. Most studies in the database are descriptive rather than mechanistic in nature. Most studies are limited in terms of experimental design, with only one or two nonzero dose groups, and observations recorded at a single time point, in a single species, representing toxicity in a single organ system arising from either copper excess or copper deficiency, but rarely both. There is insufficient information available on any endpoint, as assayed in any single study, to produce a well-defined dose-response curve for both copper deficiency and excess. Thus, there is a need to consider empirical approaches to modeling multiple studies and endpoints simultaneously using some form of common toxicity metric.

This might be accomplished by constructing a qualitative toxicity classification scheme with a limited number of “bins” or categories, say three or four, placed symmetrically about the central region of the homeostatic range of copper intake. The lowest category may be used to define the homeostatic range in terms of changes no more severe than those that are adaptive and rapidly reversible. On the side of copper excess, these would include decreased gastrointestinal absorption, increased hepatic deposition and storage, and increased biliary and urinary excretion. On the side of copper deficiency, the adaptive changes would include increased gastrointestinal absorption, mobilization of hepatic copper stores, and decreased biliary and urinary excretion. The next higher severity category may be comprised of nonadaptive but potentially reversible biochemical alterations, including, for example, decreases in the functionality of copper-dependent enzymes, changes in ceruloplasmin levels and superoxide dismutase activity in blood, and altered cholesterol and triglyceride levels in blood and liver.

Changes in the function of different organ systems as evidenced by altered metallothionein levels in various tissues, changes in the metabolism of other metals such as iron, altered immune function, cardiac hypertrophy, and anemia may be assigned to the next severity category of deficiency. Equivalent changes, such as the formation of kidney droplets, increased blood pressure and decreased weight gain being assigned to the next severity category of copper excess. In each case, these changes provide indications of early architectual, such as morphological, alterations in the various affected organ systems. Finally, the highest category of deficiency or excess may reflect gross histopathological changes and severe dysfunction of extrahepatic organ systems, potentially severe enough to be life-threatening.

After defining criteria for categories of toxicity, as discussed earlier, data from studies of copper toxicity due to both excess and deficiency need to be evaluated using the same criteria for determining study quality, and severity scores need to be assigned to those studies satisfying the inclusion criteria. (Note that it is possible that a single dose group could lead to more than one severity score, in the event that multiple adverse effects of varying severity are induced.) Once severity scores are assigned to all dose groups in the selected studies, the categorical toxicity database is then analyzed in relation to the intensity and duration of copper exposure. A hierarchical approach to empirical dose-response modeling could be taken. For example, the simplest analysis might simply involve a graphical display of the severity scores for each dose group at the appropriate point in the exposure intensity–duration plane. One might then coarsely identify the boundaries between adjacent severity categories, with the area including only those dose groups in the lowest severity categories of copper excess and deficiency empirically defining the homeostatic range.

Most toxicological studies of copper involve a limited number of dose groups, often only one control group with adequate copper intake and one experimental group with either highly deficient
or highly excessive copper intake. The lack of multiple doses corresponding to a continuum ranging from highly excessive to homeostatic to highly deficient doses limits our ability to describe dose-response relationships for copper. For the few individual studies having a sufficient number of dose groups to characterize the shape of the dose-response curve for copper, benchmark dose analyses might also be conducted. For this modeling approach, consideration might also be given to simultaneously analyzing multiple studies from the same laboratory or group of investigators; usually these studies have similar protocols, and differ mainly with respect to dose levels and endpoints examined. Finally, categorical regression methods may be utilized to analyze the toxicity database in its entirety. Analyses specific to different organ systems and species might first be undertaken to determine whether results were sufficiently similar to justify the pooling of data in more comprehensive analyses. For both the benchmark dose and categorical regression modeling approaches, data need to be evaluated carefully for quality and relevance to the overall goal of developing an accurate characterization of the homeostatic range. Especially important is achieving a clear understanding of the influence that high dose and high severity data have on dose-response estimates near the homeostatic range, and ensuring that such influence is not inappropriate.

Four principal outcomes of this kind of dose-response modeling strategy are envisioned:

- Determining whether the existing toxicity data for copper excess or deficiency can be utilized effectively in defining the limits of the homeostatic range in humans and other species.
- Establishing a rank ordering of the various analytical approaches that might be taken in terms of their utility in defining this range.
- Identifying critical data gaps that need to be filled by future research studies.
- Developing specific recommendations regarding experimental designs for future studies to ensure that their results will be maximally useful in determining the characteristics of dose-response relationships for copper toxicity in relation to excess or deficiency.

**Copper Toxicity Database**

Copper is an essential trace element, and a wide range of studies has been conducted on copper deficiency; far fewer studies have addressed copper induced toxicity. Copper-induced toxicity has rarely been observed in the general population, and cases are generally limited to individuals with genetic susceptibility to copper overload. The database on copper toxicity is described in the “Copper Homeostasis and Toxicity: Implications for Dose-Response Assessment” section and summarized in Appendix B of this review.

Studies of copper-induced toxicity or deficiency vary widely in terms of the animal model, study objectives, design, test protocol, statistical analysis, and degree of detail of experimental reporting and results. Most experimental studies in humans are of relatively short duration and thus do not provide sufficient information on the long-term health consequences of copper deficiency. In the majority of studies of copper toxicity, one or, at most, two doses have been employed and thus such studies are of limited usefulness for dose-response evaluation. Copper deficiency studies typically examine only one copper-deficient group, the results for which are compared with those from a concurrent control group of copper-adequate animals. More recently, some studies employed two doses of graded copper deficiency, one considered to be severely deficient and the other marginally deficient. The protocol for the study of nutritional deficiencies is fairly standardized across animal experiments, and thus, it might be possible to examine the results of several different copper deficiency studies in evaluation of dose-response. The major limitation of these studies is that similar doses for both copper deficiency and copper adequacy status are used across the studies, thereby generating a lot of hazard information but little data on dose response.

A depletion–repletion protocol was also used in human and animal studies in which subjects were administered a diet low in copper in order to induce copper depletion, and selected endpoints were measured during the copper depletion period. Subsequently, subjects were given increased dietary copper to replete diminished body stores, and changes in selected end points associated with copper repletion were monitored (Turnlund, 1998; Turnlund et al., 1990, 1997).
These studies provide evidence for a causal relationship between selected endpoints and changes in copper status.

**Selection of Studies for Categorical Regression Modeling**

The copper database summarized in Appendix B provides a comprehensive source of information that can be organized to do categorical regression and utilized in applying other types of modeling approaches. Data can be organized or “binned” for the categorical regression approach; for the NOAEL/LOAEL approach, it would be necessary to select the appropriate study (studies) based on considerations of the quality of the study and the use of the appropriate animal model. There is also a need to select a common dose metric in order to compare studies.

**Data Quality** Criteria for evaluating the quality and usefulness of animal studies for modeling included whether:

1. The animal species/strain was considered to be a suitable model for purposes of human risk assessment.
2. The route of exposure was relevant to human risk assessment (iv and ip exposures would be less relevant than dietary exposures).
3. The study design was adequate in terms of number of dose groups, number of animals per dose group (studies were considered to be limited if there was only one dose group or if the sample sizes were small).
4. The number and range of endpoints reported were informative about copper toxicity (studies involving a single narrowly focused endpoint may contribute little to our understanding of copper toxicity).
5. Data reporting was complete (for example, inclusion of indices of maternal toxicity in reproductive/developmental toxicity studies).
6. Data were subject to appropriate statistical analysis (studies were considered to be limited if there were no statistics presented, and it was not possible to estimate significance due to insufficient information or small magnitude in differences between control and treated animals).
7. There was sufficient information to adequately characterize dose response (or whether the data were sufficient for hazard evaluation only).

Separate, but similar, criteria were developed for assessing the quality of data from human studies. Specifically, consideration was given to whether:

1. The study included multiple doses and outcomes.
2. Copper balance studies provided for adequate repletion following the period of copper deprivation (and included appropriate measures to confirm repletion).
3. Human volunteers lived in a free-living environment in which their diets could be subject to unknown variation (rather than being completely controlled, as would be the case in a metabolic unit).
4. Accurate estimates of copper intake were available (particularly in ecologic studies).
5. Data were subject to adequate statistical analysis.
6. Dose response (rather than simply hazard) could be adequately characterized.
7. Individuals’ health outcomes could be reasonably associated with their copper intakes (taking into account factors such as temporality and specificity of exposure information).
8. Other variables in the diet or the general environment could have influenced the results.

**Dose Metrics** Although there are a large number of studies, they originate from different research laboratories and different environmental and exposure settings, and use different dose conversions. Doses are usually reported in terms of milligrams Cu per kilogram feed in animal studies, and not as daily intakes (mg/d) or daily dose per kilogram of body weight (mg/kg/d). In humans, the most commonly used metric in studies of copper toxicity is milligrams per day (mg/d). Since the most appropriate dose metric was considered to be milligrams per kilogram per day (mg/kg/day),
doses were converted to this metric wherever possible. Although not attempted here, pharmacokinetic modeling permits the use of additional dose measures of internal exposure, including tissue concentration, area under the concentration–time curve, and peak exposures. Although variation in exposure with time and life stage may also be an important determinant of risk (Krewski et al., 1995), there was generally insufficient information to describe the relative effectiveness of copper exposure at different ages.

**Establishing Categories of Copper Toxicity** Copper dose-response modeling using categorical regression requires that the data be grouped in severity categories prior to analysis. To accomplish this, a “binning team” was formed to review the relevant studies on copper toxicity and develop a database for subsequent analysis. Binning refers to the categorical assignment of a level of adverse effects along an ordered gradient representing the severity of the effect induced. (In addition to providing a convenient database for analysis, organization of the data in this fashion provides insight into copper toxicity pathways and mechanisms.) This database is then analyzed using categorical regression (as described in the “Dose-Response Assessment” section of this review), as well as more traditional approaches, including the BMD and NOAEL/LOAEL approaches. Regardless of the approach used, professional judgment needs to be used in identifying uncertainty factor(s) to extrapolate to a range of “safe doses.” The importance of careful judgement in the extrapolation from the modeled data is highlighted by the need to identify doses that result in neither deficiency nor excess.

An important consideration in such analyses is whether to model excess and deficiency separately or in a combined fashion. This decision involves consideration of the availability of suitable statistical techniques to describe the U-shaped dose-response curves arising from multiple mechanisms underlying copper toxicity (excess and deficiency). Consideration also needs to be given as to whether these mechanisms are independent or interrelated at the biological level, and whether these mechanisms also occur within the homeostatic range of copper intake. The paramount objective of dose-response modeling was to define this homeostatic range as precisely as possible, since it represents the optimum range of human intake of copper.

The use of binning also requires expert judgment in developing and ordering the binning categories and in allocating events and endpoints to the different categories. Further, since different dose metrics were used in studies involving disparate health effects, the most appropriate common dose metric to be used in conducting combined analyses of multiple studies requires careful consideration. While there is no clear evidence on which to select such a common dose metric, milligrams per kilogram body weight per day appears to be a reasonable choice. Another reasonable option is mg/kg0.75, a dose metric based on metabolic scaling originally proposed by Travis and White (1988), particularly when multiple species are included in the analyses; this is preferred by many investigators.

**SUMMARY**

Development of sound health protection strategies for exposure to chemical substances requires an understanding of the dose-response relationship, the construct of which is especially challenging in the case of essential trace elements such as copper. Since either copper deficiency or excess produces adverse health effects, the dose-response curve is U-shaped, although the precise form has not yet been well characterized. U-shaped dose-response curves can be constructed either by separate modeling of the dose-response relationships for copper deficiency and excess, or by joint modeling of these two effects. This article reviewed and summarized the extensive biological database for copper and evaluated alternate modeling strategies for conducting dose-response assessment for adverse health outcomes due to excess or deficiency. Individual studies have been critically assessed for quality and relevance, and adverse findings have been standardized with respect to severity (categories). The selection of the most appropriate modeling approach depends on knowledge of the mechanisms through which copper homeostasis can be disrupted, and the nature of the available data within and outside the region of homeostasis. Although copper has a rich human and animal database from which data suitable for dose-response modeling can be extracted, BBDR modeling techniques cannot be utilized at this time because the molecular mechanisms
and pharmacodynamics of copper excess or deficiency have yet to be sufficiently described. Thus, an empirical approach was deemed to be the most appropriate methodology for evaluating, organizing, and integrating the large and diverse database in order to gain an understanding of these dose-response relationships. Given the nature of the database, categorical regression appears to be a promising modeling approach, although benchmark dose may also be useful for examining individual studies with sufficient numbers of dose groups to describe a dose-response relationship, and the NOAEL/LOAEL approach may be more suitable for some studies or data subsets. The ultimate objective of these efforts will be to determine whether the existing biological data for copper excess or deficiency can be effectively utilized to define the limits of the homeostatic range in humans and other species, using alternative modeling techniques and applying uncertainty factors appropriate for this essential trace element to the observed no-effect levels. This analysis will also serve to identify key areas in which additional data are needed to better define the dose-response relationships for copper toxicity and essentiality. Some areas for further research have been proposed and include: (1) conduct of a detailed dose-response study with multiple doses in both excess and deficient ranges to serve as the basis for determining the homeostatic range as precisely as possible; (2) determination of whether the homeostatic range is comparable among different species; (3) development of a PBPK model for copper; and (4) conduct of a long-term intervention trial of copper deficiency and excess. Quantitative data analysis using alternate modeling strategies is currently in progress and will be reported in a future article.

REFERENCES


COPPER AND HUMAN HEALTH


COPPER AND HUMAN HEALTH


### APPENDIX A. Examples of Copper Enzymes With Redox Activity

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amine oxidases</td>
<td>Deamination of primary amines</td>
<td>Present in all eukaryotes; catalyze the oxidation of biogenic amines (e.g., tyramine, histidine, and polyamines) producing oxidized organic products, usually aldehydes, and generating NH₃ and H₂O₂ as by-products.</td>
</tr>
<tr>
<td>Benzylamine oxidase</td>
<td></td>
<td></td>
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<tr>
<td>Diamine oxidase</td>
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<tr>
<td>Indole 2,3-dioxygenase</td>
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<tr>
<td>Spermine oxidase</td>
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</tr>
<tr>
<td>Ascorbate oxidase</td>
<td>Terminal oxidase</td>
<td>Found in a wide variety of fruits and vegetables, including Valencia oranges, cucumbers, and zucchini. Couples one-electron oxidation of ascorbate to full molecular reduction of O₂ to water.</td>
</tr>
<tr>
<td>Ceramide glucosyltransferase</td>
<td>Myelin synthesis</td>
<td>Responsible for synthesis and maintenance of phospholipid membranes. Ganglioside lipid components (ceramide) are synthesized in the endoplasmic reticulum and glycosylated by glycosyltransferase in the Golgi network compartments. Lipid bilayer membranes function as electrical capacitors, enabling charge separation and storage of electrochemical energy in the form of ion gradients.</td>
</tr>
<tr>
<td>Ceruloplasmin (CP)</td>
<td>Copper transport, oxidation</td>
<td>Also known as ferro-oxidase I. Multicopper oxidase containing 65–90% of vertebrate serum copper; human plasma level ~200–500 mg/L. Six tightly bound copper ions at two distinct molecular sites; one appears to activate oxygen; the other appears to be involved in electron transport. CP likely major copper transport molecule in higher mammals by binding to cellular membrane receptors and releasing Cu. Aceruloplasmin individuals (humans and knockout mice) show severe difficulties with iron overload in central nervous system (CNS); deficiency may manifest as neurological damage but minimal (if any) difficulties with Cu metabolism. Wilson disease patients have low levels of CP, but normal iron metabolism.</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>Electron transport, terminal oxidase</td>
<td>Terminal enzyme in the respiratory chain, located in the inner membrane of mitochondria and bacteria. Catalyzes the reduction of O₂ to water; pumps one proton across membrane for each proton consumed in the reaction to generate electro-chemical gradients that can be used for other cellular purposes (e.g., ATP synthesis). Deficiency may lead to brain abnormalities, hypothermia, muscle weakness. Catalyses conversion of dopamine to norepinephrine. Deficiency may lead to neurological effects, possible hypothermia.</td>
</tr>
<tr>
<td>Dopamine β-hydroxylase</td>
<td>Norepinephrine synthesis</td>
<td>Catalyses the oxidation of primary amines to norepinephrine; hydrogen peroxide often produced. In fungi, may be involved in breaking down the plant cell wall prior to invasion.</td>
</tr>
<tr>
<td>Galactose oxidase</td>
<td>Carbohydrate metabolism</td>
<td>Blue respiratory protein (oxygen carrier) of decapod crustaceans such as lobster, crabs, and crayfish (contrast with iron-containing blood pigments such as hemoglobin (red), myoglobin (red), chlorocruorins (green), and hemerythrins (violet).</td>
</tr>
<tr>
<td>Haemocyanin</td>
<td>Oxygen transport</td>
<td></td>
</tr>
<tr>
<td>Laccase</td>
<td>Terminal oxidase</td>
<td>Found in plants and fungi. Catalyzes the four-electron reduction of O₂ to H₂O. Depolymerizes lignin.</td>
</tr>
<tr>
<td>Lysyl oxidase (LOX)</td>
<td>Cross-linking of collagen and elastin</td>
<td>Secreted enzyme catalyzing the deamination of peptidyl lysine residues forming inter- or intrachain covalent cross-links in elastin and collagens. Critical extracellular matrix integrity, the breakdown of which can lead to cancer invasiveness and metastasis. Also localized to the nucleus where it may function in chromatin organization. May also block the ras oncogene pathway.</td>
</tr>
<tr>
<td>Enzyme/Protein</td>
<td>Function</td>
<td>Notes</td>
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</tr>
<tr>
<td>Monoamine oxidase</td>
<td>Neurotransmitter synthesis</td>
<td>Found predominantly on the outer mitochondrial membrane and throughout CNS. Two isozymes both of which oxidize the neurotransmitters dopamine, epinephrine, tyramine, and tryptamine, although monoamine oxidase (MAO)-A selectively oxidizes serotonin; MAO-B oxidizes phenylethylamine and benzylamine. Deficiencies of MAO-A result in disturbed systemic amine metabolism, borderline mental retardation and possible cardiovascular and behavioral abnormalities. Monoamine oxidase inhibitors (MAOIs) prevent the action of MAO in breaking down dopamine. As dopamine appears to enhance mood, these drugs function as stimulants or antidepressants.</td>
</tr>
<tr>
<td>Peptidyl glucine a amidating monooxygenase (PAM)</td>
<td>Peptide processing</td>
<td>Multifunctional protein involved in the maturation of bioactive hormones (e.g., neurotransmitters and growth hormones) containing two enzymes that act sequentially to catalyze the alpha-amidation of neuroendocrine peptides. Glucose increased both the level of gastrin gene expression and maturation to carboxyamidated peptides, indicating that glucose influences the activity of the amidation enzyme complex, peptidylglycine alpha-amidating monooxygenase (PAM), in insulin cells.</td>
</tr>
<tr>
<td>Plastocyanin</td>
<td>Electron transfer in plants</td>
<td>Small (10.5-kD) protein functioning as an electron carrier between the cytochrome b6f photosystem 1 (PS 1) complexes in the photosynthetic electron-transfer chain.</td>
</tr>
</tbody>
</table>
| Polyphenol oxidase/tyrosinase | Quinone biosynthesis
Pigment (melanin) synthesis; amino acid metabolism | Polyphenol oxidase oxidizes tyrosine to dihydroxyphenylalanine which in turn is oxidized to o-quinone. Widely distributed in plants, animals, and humans. Oxidizes tyrosine to the pigment melanin in mammals and causes the cut surfaces of many fruits and vegetables to darken (browning reactions). Deficiency leads to hypopigmentation. |
| Prostaglandin reductase | Prostaglandin biosynthesis | Responsible for reduction of 15-oxoprostaglandins to 13,14-dihydro derivatives. Placental form is NAD dependent. |
| Rusticyanin | Electron transport | Found in fungi and gram-negative bacteria. Can tolerate extremes of pH. |
| Stellacyanin | Electron transport; superoxide radicals | Found in fungi and some plants including the sap of Japanese lacquer trees, cucumber peel, horseradish roots, and Arabidopsis thaliana. Appear to be elements of cell wall, and may be involved with oxidative cross-linking reactions, along with peroxidases, ascorbic acid oxidase, and laccase. |
| Superoxide dismutase (SODi (Cu-Zn SOD)) | Destruction of superoxide radicals | Found in cytoplasm of human and bovine cells; during dismutation of superoxide radical (O2·−), Cu at active site of enzyme is reduced to yield H2O2. Mutation/ altered function of SOD 1 may be involved in amyotrophic lateral sclerosis. Chicken and rat SOD is Mn based, while E. coli SOD is Fe based. Deficiency results in sensitivity to oxidative stress. |
| Uricase | Nucleic acid metabolism | Termination of purine catabolism in all mammals excluding humans, higher apes, and the Dalmatian dog; converts uric acid to allantoin. Also found in tissues of lower mammals and in some plants and microorganisms. |

Note. Adapted from IPCS (1998) and Ralph and McArdle (2001).
APPENDIX B: SUMMARY TABLES OF SELECTED ANIMAL AND HUMAN STUDIES ON COPPER TOXICITY ASSOCIATED WITH EITHER EXCESS OR DEFICIENCY

Copper is an essential trace element (ETE), and a wide range of studies have been conducted on copper deficiency. Historically, copper toxicity has rarely been observed in the general population, and thus many fewer studies have been concerned with investigating toxicity due to excess; identified cases are generally limited to individuals with genetic susceptibility to copper overload.

Studies of copper toxicity due to excess or deficiency vary widely in terms of study objectives, model used, design, test protocol, statistical analysis, and the degree of detail in terms of experimental reporting and results. Most copper toxicity studies have employed one or, at most, two doses, and thus are of limited usefulness for dose-response evaluation. Further, many of these studies have been conducted at doses that overwhelm the homeostatic system and that are unlikely to be representative of environmental concentrations.

Most human clinical studies are of relatively short duration, and thus do not provide sufficient information on the long-term health consequences of copper excess and deficiency. Copper deficiency studies typically test only one copper-deficient dose, and compare the results in this group with those from a concurrent control group of copper-adequate animals. Recently, some studies have employed two doses of graded copper deficiency, one considered to be severely deficient and the other, marginally deficient. These studies have some utility for dose-response assessment. Further, the protocol for the study of nutritional deficiencies is fairly standardized across animal experiments, and thus, it might be possible to pool (or examine together) findings from several different copper deficiency studies for evaluation of dose response, particularly if the studies come from the same laboratory. The major limitation of deficiency studies is that the same dose range for copper deficiency (e.g., 0.3–0.6 mg/kg feed) and copper adequacy (e.g., 6–10 mg/kg feed) status is used across most studies, generating a lot of hazard information but little on dose response.

A depletion–repletion protocol has also been used in human and animal studies, in which subjects are administered a diet low in copper in order to induce copper depletion; selected endpoints are measured during the copper depletion period. Subsequently, subjects are given increased dietary copper to replete diminished body stores; changes in selected endpoints associated with copper repletion are monitored. These studies provide evidence for a causal relationship between selected endpoints and a change in copper status.

Criteria for Evaluating Studies for Dose-Response Utility

The extremely large size of the copper database precludes listing all available studies on copper excess and deficiency. Therefore, only a subset of representative studies is reported. In addition to presentation of experimental details and identification of a LOAEL and/or NOAEL (if possible), the quality of studies and their utility for dose-response assessment were rated. High-quality studies were preferred; however, a number of studies of medium or low quality were also included because they were:

- The only ones available (e.g., most reproductive/developmental animal studies).
- Widely cited in reviews of copper toxicity and thus are well known.

Criteria for evaluating the quality and usefulness of animal studies for dose response included, but were not limited to:

- Whether the animal species/strain was considered to be a suitable animal model.
- Whether the study design was adequate in terms of number of dose groups and number of animals per dose group; studies were considered to be limited if there was only one dose group or if the sample sizes were small.
• The degree of completeness of data reporting (e.g., reporting of indices of maternal toxicity in reproductive/developmental toxicity studies; tabular presentation of data; toxicological significance evaluation).
• Whether statistical analysis had been conducted; studies were considered to be limited if there were no statistics presented and it was not possible to estimate significance due to insufficient information and/or small magnitude of differences between control and treated groups.
• Whether the study adequately characterized dose response or was only adequate for hazard evaluation.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Copper species/study type/exposure route/duration</th>
<th>Number of grps/dose levels</th>
<th>Effects</th>
<th>LOAEL (^c) (mg/kg/d)(^d)</th>
<th>NOAEL (^e) (mg/kg/d)</th>
<th>Comments</th>
<th>Study quality</th>
<th>Useful for dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haywood and Loughran (1985)</td>
<td>Male Wistar rats</td>
<td>CuSO(_4), subacute, dietary, 3 wk</td>
<td>Two, added to diet: 0, 270 mg/kg/d</td>
<td>Lethargy, liver necrosis and inflammation</td>
<td>270</td>
<td>N/A (^f)</td>
<td>Only one dose, small sample sizes, no statistics</td>
<td>Medium</td>
<td>Limited</td>
</tr>
<tr>
<td>Hebert et al. (1993)</td>
<td>Male and female F344 rats</td>
<td>CuSO(_4) pentahydrate, subacute, drinking water, 15 d</td>
<td>Six, added to diet: ppm: 0, 300, 1000, 3000, 10,000, 30,000; mg/kg/d: M: 0, 41, 113, 175, 140, 379; F: 39, 102, 121, 120, 279 mg/kg/d</td>
<td>Clinical signs of toxicity at 3000 ppm; effects at higher doses attributed to ↓ water intake and ↓ wt gain due to feed taste aversion; ↑ size and no. protein droplets in male renal tubules at 300 and 1000 ppm; but not at 3000 ppm, no dose response</td>
<td>121.0 CuSO(_4) 30.8 Cu</td>
<td>102.0 CuSO(_4) 6.0 Cu</td>
<td>Range-finding study. All findings at two highest doses attributed to attributed to exceedence of maximum tolerated dose (MTD) Not clear whether ↑ protein droplets in renal tubules associated with α-microglobulin accumulation, a finding specific to male rats and not relevant to human risk assessment.</td>
<td>High</td>
<td>Limited</td>
</tr>
<tr>
<td>Hebert et al. (1993)</td>
<td>Male and female B6C3F1 mice</td>
<td>CuSO(_4) pentahydrate, subacute, drinking water, 15 d</td>
<td>Six, added to diet: ppm: 0, 300, 1000, 3000, 10,000, 30,000, mg/kg/d: M: 0, 41, 95, 226, 524, 1442, F: 0, 58, 140, 245, 683, 1296 mg/kg/d</td>
<td>Early death, clinical signs of toxicity consistent with dehydration; organ wt changes attributed to dehydration resulting from ↓ body wts, due ↓ food consumption. No treatment-related lesions.</td>
<td>226 CuSO(_4) 57.5 Cu</td>
<td>95 CuSO(_4) 24.2 Cu</td>
<td>Range-finding study. Not useful for dose-response assessment.</td>
<td>High</td>
<td>Limited</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Treatment</td>
<td>Dose Schedule</td>
<td>Key Findings</td>
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<tr>
<td>Hebert et al. (1993)</td>
<td>Male and female F344 rats</td>
<td>CuSO₄ pentahydrate, subacute, dietary, 15 d</td>
<td>ppm: 0, 1000, 2000, 4000, 8000, 16,000; mg/kg/d: M: 0, 92, 180, 363, 777, 1275; F: 0, 89, 174, 367, 769, 1121</td>
<td>No mortality or morbidity; ↓ body wt at 2 highest doses attributed to taste aversion; hyperplasia and hyperkeratosis of forestomach at 4 highest doses; ↑ liver inflammation &amp; ↓ hematopoietic cells in bone marrow at 2 highest doses; ↑ protein droplets in renal tubules at 3 highest doses; 769.0 CuSO₄ 195.7 Cu (forestomach) 174.0 CuSO₄ 44.3 Cu (forestomach) 89.0 CuSO₄ 22.7 Cu (liver) 196.8 Cu (forestomach) 92.1 Cu (liver)</td>
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<tr>
<td>Hebert et al. (1993)</td>
<td>Male and female B6C3F1 mice</td>
<td>CuSO₄ pentahydrate, subacute, dietary, 15 d</td>
<td>ppm: 0, 1000, 2000, 4000, 8000, 16,000; mg/kg/d: M: 0, 168, 362, 773, 1154, 2817; F: 0, 210, 408, 849, 1563, 3068</td>
<td>No mortality or morbidity; dose-related ↓ in body wt at 2 highest doses attributed to taste aversion, forestomach hyperplasia and hyperkeratosis at 3 highest dose groups, less severe than in rats</td>
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<tr>
<td>Haywood (1980)</td>
<td>Male Wistar rats</td>
<td>CuSO₄ subchronic, dietary, 15 wk with serial sacrifices at wk 1, 2, 3, 6, 9, 15</td>
<td>ppm: 0, 2000; mg Cu/kg/d: 0, 165</td>
<td>Liver inflammation, bile duct hyperplasia at wk 6, with recovery by wk 15; renal discoloration, eosinophilic droplets in renal tubules beginning at wk 3, with recovery by wk 15</td>
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</table>

Range-finding study. ↓ body wts & changes in organ wts at higher doses may be due to feed inpalatability and not Cu toxicity. Forestomach lesions in the squamous mucosa of limited significance to humans as humans do not have a forestomach; however, they are indicative of gastrointestinal irritation. Only adverse effects were in forestomach; limited relevance as humans do not have a forestomach. Only one dose, small sample sizes, only liver and kidney examined, statistics only performed on plasma liver enzymes and ceruloplasmin levels. Reversibility of organ effects suggests that adaptation to Cu overload occurs in dosed animals.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Copper species/study type/exposure route/duration</th>
<th>Number of grps/dose levels</th>
<th>Effects</th>
<th>LOAEL&lt;sup&gt;c&lt;/sup&gt; (mg/kg/d)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NOAEL&lt;sup&gt;e&lt;/sup&gt; (mg/kg/d)</th>
<th>Comments</th>
<th>Study quality</th>
<th>Useful for dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haywood and Comerford</td>
<td>Male Wistar rats</td>
<td>CuSO&lt;sub&gt;4&lt;/sub&gt;, subchronic, dietary, 15 wk with serial sacrifices at wk 1, 2, 3, 6, 9, 15</td>
<td>Six, added to diet, ppm: 0, 2000; mg Cu/kg/d: 0, 165</td>
<td>Fluctuations in plasma and blood Cu levels at wk 1 and 9, relative to controls; serum liver enzyme ALT increased, no changes in AP, bilirubin, ceruloplasmin</td>
<td>165 N/A</td>
<td>N/A</td>
<td>Only endpoints measured were some blood and clinical chemistry parameters.</td>
<td>Medium</td>
<td>No</td>
</tr>
<tr>
<td>Haywood and Loughran</td>
<td>Male Wistar rats</td>
<td>CuSO&lt;sub&gt;4&lt;/sub&gt;, subchronic, dietary, 15 wk with serial sacrifices at wk 1, 2, 3, 4, 5, 6</td>
<td>Five, added to diet: ppm: 0, 3000, 4000, 5000, 6000; mg Cu/kg/d: 0, 247, 330, 412, 494</td>
<td>Mortality and clinical toxicity at highest dose; significantly ↓ body wt. Liver and kidney histopathology progressing from focal to diffuse necrosis during first 5-6 wk at all doses. Recovery (tissue regeneration) occurred beginning wk 5-6 through wk 15 at all doses except highest, in which liver injury developed into chronic hepatitis</td>
<td>247 N/A</td>
<td>N/A</td>
<td>Only liver and kidney examined, small sample sizes, and very high doses. Results indicate that at all doses except the highest, adaptation and recovery to Cu overload occur in treated animals.</td>
<td>High</td>
<td>Limited</td>
</tr>
<tr>
<td>Liu and Medeiros</td>
<td>Male Wistar or SHR rats</td>
<td>CuCO&lt;sub&gt;3&lt;/sub&gt;, subchronic, dietary, 15 wk</td>
<td>Two, added to diet: ppm: 0, 100; mg Cu/kg/d: 0, 8.2</td>
<td>Wistar: ↑ hemoglobin, ↓ triglycerides, ↑ blood pressure from wk 3-15, SHR: ↑ hemoglobin, ↓ liver wk., ↑ blood pressure at wk 9 and 15 Blood pressure ↑ higher in Wistar than SHR. Rats.</td>
<td>8.2 N/A</td>
<td>N/A</td>
<td>Body wt, blood pressure, hemoglobin, total cholesterol, triglycerides, liver and heart weights measured. No pathology or histopathology. Only one dose tested, adequate sample sizes, statistics performed.</td>
<td>Medium</td>
<td>Limited</td>
</tr>
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</table>
Llewellyn et al. (1985) Male Holtzman rats Cu acetate, subchronic, dietary, 21 wk

Two, added to diet: mg Cu/kg/d: 0, 45

↓ body wt (~23%); no wt changes in heart, spleen, lung, kidney; ↑ relative tests wt and ↓ femur length attributable to ↓ body wt gain associated with taste aversion; liver wt data unclear

45 N/A Radiography and measurement of long bones performed. Other endpoints were body wt and organ wts. Only one dose tested.

Hebert et al. (1993) Male and female F344 rats CuSO₄ pentahydrate, subchronic, dietary, 92 d

Six, added to diet: ppm: 0, 500, 1000, 2000, 4000, 8000; mg Cu/kg/d: M: 0, 8.1, 16.3, 32.8, 65.9, 140.2; F: 0, 8.7, 17.3, 34.4, 68, 134.4

No mortality or clinical toxicity; ↓ body wts at 2 highest doses; ↓ water intake; hematology and clinical chemistry changes consistent with hepatic injury and/or dehydration; forestomach lesions and ↑ in size and number of protein droplets in renal tubules of both sexes at 3 highest doses, ↑ in liver inflammation at 2 highest doses

34 Cu (liver, kidney) 17 Cu (liver, kidney) Authors equivocal about whether ↑ in protein droplets in renal tubules was associated with α₂-microglobulin effect, possibly due to Cu accumulation. Accumulation of Cu in liver & kidney accompanied by accumulation of Zn.

Mice less sensitive than rats to Cu toxicity.

Hebert et al. (1993) Male and female B6C3F1 mice CuSO₄ pentahydrate, subchronic, dietary, 92 days

Six, added to diet: ppm: 0, 2000, 4000, 8000, 16,000; mg Cu/kg/d: M: 0, 44, 97.2, 187.3, 397.8, 814.7; F: 0, 52.2, 125.7, 266.7, 536, 1058

No mortality or clinical toxicity; dose-related ↓ in body wt gain; no changes in food consumption but ↓ in water intake; hematology and clinical chemistry changes consistent with hepatic injury and/or dehydration; forestomach lesions in 3 highest dose groups; no other pathology

187 Cu (males, forestomach) 97 Cu (males, forestomach) Forestomach lesions of limited relevance to humans. No liver or kidney pathology/histopathology. Accumulation of Cu in liver only at highest dose; no accumulation of Zn, Ca, Mg. Mice less sensitive than rats to Cu toxicity.

High Limited (Continued)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Copper species/study type/exposure route/duration</th>
<th>Number of grps/dose levels</th>
<th>Effects</th>
<th>LOAEL&lt;sup&gt;c&lt;/sup&gt; (mg/kg/d)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>NOAEL&lt;sup&gt;e&lt;/sup&gt; (mg/kg/d)</th>
<th>Comments</th>
<th>Study quality</th>
<th>Useful for dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shanaman (1972)</td>
<td>Beagle dogs</td>
<td>Cu gluconate, subchronic, dietary, 6–12 mo</td>
<td>Four, added to diet: mg Cu/kg/d: 0, 0.42, 2.1, 8.4</td>
<td>No significant effects on mortality, body wt gain, organ weights, urinalysis, or hematology. At 6 and 12 mo dose-dependent increase in Cu in kidney, liver, and spleen, which reversed to some extent following cessation of treatment.</td>
<td>N/A</td>
<td>8.4</td>
<td>The NOAEL was the highest dose tested. Data taken from secondary source; original study unavailable; therefore, limited in utility for dose response.</td>
<td>Low</td>
<td>Limited</td>
</tr>
<tr>
<td>Fuentealba et al. (2000)</td>
<td>Rats (strain not given), 3 groups: Pregnant F Young M/F Adult M/F</td>
<td>CuSO&lt;sub&gt;4&lt;/sub&gt;, dietary, Gestation Birth to 12 wk 18 wk of age</td>
<td>Two, added to diet: ppm: 0, 1500 mg/kg/d: M: 0, 88 (average); F: 0, 118 (average)</td>
<td>Increased liver serum enzymes (AP, ALT, GGT, SDH), most pronounced in young rats; no changes in bilirubin. Liver histopathology (necrotic foci and single cell necrosis), also more marked in young relative to adult rats. No increase in hepatic metallothionein. Hepatic Cu accumulation greater in young rats relative to adults.</td>
<td>88</td>
<td>N/A</td>
<td>Results suggest age-related differences in susceptibility to liver injury. However, dose/kg was higher in young rats, because body weights were lower. No sex-related differences observed. Only one dose tested.</td>
<td>Medium</td>
<td>Limited</td>
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<tr>
<td>Authors</td>
<td>Species</td>
<td>Treatment</td>
<td>Dose Description</td>
<td>Effect Description</td>
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<tr>
<td>Fuentealba et al. (1989)</td>
<td>Male rats (strain not given)</td>
<td>CuSO₄, subchronic, dietary, 16 wk with serial analyses of liver changes at wk 1, 2, 3, 4, 5, 6, 8, 12, 16</td>
<td>Two, added to diet: ppm: 0, 1500; mg/kg/d: 0, 124</td>
<td>Increase in hepatic Cu beginning at wk 1 and reaching maximum conc. at wk 5–6, primarily in periportal and mid-zone areas. Liver histopathology beginning at wk 1, becoming most severe at wk 4–6, then regressing at wk 8–12. At wk 16, mild histopathology remained (enlarged hepatocytes, hyaline bodies, bile duct hyperplasia).</td>
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<tr>
<td>Aburto et al. (2001)</td>
<td>Male F344</td>
<td>CuSO₄, subchronic, dietary, 3 mo</td>
<td>Six, added to diet: ppm: 0, 750, 1000, 1250, 1500, 2000; mg Cu/kg/d: 0, 62, 82, 103, 124, 165</td>
<td>Dose-related decrease in body wt, statistically significant in two highest dose groups; nonsignificant dose response decrease in food consumption; dose-response increase in liver injury as measured by necro-inflammatory foci. No changes in MDA (indicative of lipid peroxidation).</td>
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<tr>
<td>Reference</td>
<td>Species</td>
<td>Copper species/study type/exposure route/duration</td>
<td>Number of grps/dose levels</td>
<td>Effects</td>
<td>LOAEL(^c) (mg/kg/d)(^d)</td>
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<tr>
<td>Cristofori et al. (1992)</td>
<td>Female rats (strain not given)</td>
<td>Cu species not given, subchronic, dietary, 8 wk, with interim sacrifice at 30 d</td>
<td>Two, added to diet: ppm: 0, 2000; mg Cu/kg/d: 0, 20</td>
<td>No changes in serum enzymes ALT, AST, AP; no changes in creatinine and urea levels. No pathology or histopathology in liver, kidney, brain. Cu accumulation in liver, kidney, paw tissue, but not in plasma, blood cells, or brain.</td>
<td>N/A</td>
<td>20</td>
<td>No liver toxicity observed. Only one dose tested.</td>
<td>Medium</td>
<td>Limited</td>
</tr>
<tr>
<td>Rana and Kumar (1980)</td>
<td>Male rats (strain not given)</td>
<td>CuSO(_4), subacute, gavage, 20 d</td>
<td>Two, added to diet: ppm: 0, 1000; mg Cu/kg/d: 0, 40</td>
<td>Decreased hemoglobin, hematocrit, red blood cell count, no change in white blood cell count; liver (centrilobular necrosis/fibrosis) and kidney (tubular necrosis) histopathology.</td>
<td>40</td>
<td>N/A</td>
<td>Only one dose tested. Cu administered by gavage (bolus dose).</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Study</td>
<td>Adverse Effects</td>
<td>Reference</td>
<td>Treatment Details</td>
<td>Notes</td>
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<tr>
<td>Keen et al. (1982)</td>
<td>Mice at the higher doses did not carry pregnancies to term; mice treated with the highest dose only on GD 7–12 and given doses of −33 mg/kg/d before and after this period during gestation had resorption frequency of 50%. Surviving fetuses were not visibly malformed and had Cu concentrations only slightly higher than fetuses from dams fed −33 mg/kg/d Cu throughout pregnancy.</td>
<td>Secondary source.</td>
<td>Three: added to diet: mg/kg/d: −33, −67, −267</td>
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<tr>
<td>Kasama and Tanaka (1988)</td>
<td>CuSO₄₂⁻ developmental Dams: dietary GD to PND 13 (lactation) Neonates: ip injections on PND 7 and 10</td>
<td>Low</td>
<td>Dams: Two, added to diet: mg Cu/kg/d: 0, 1.3–1.6 Neonates: Two, mg/kg injection: 0, 5</td>
<td>N/A</td>
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<td></td>
<td>Effect level of Cu dietary administration could not be determined because neonates were intraperitoneally dosed during lactation; this latter route is not relevant to humans.</td>
<td>N/A</td>
<td>Effect level of Cu dietary administration could not be determined because neonates were intraperitoneally dosed during lactation; this latter route is not relevant to humans.</td>
<td>Low</td>
<td>No</td>
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<tr>
<td>Reference</td>
<td>Species</td>
<td>Copper species/study type/exposure route/duration</td>
<td>Number of grps/dose levels</td>
<td>Effects</td>
<td>LOAEL&lt;sup&gt;c&lt;/sup&gt; (mg/kg/d)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NOAEL&lt;sup&gt;c&lt;/sup&gt; (mg/kg/d)</td>
<td>Comments</td>
<td>Study quality</td>
<td>Useful for dose response</td>
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<tr>
<td>Lecyk (1980)</td>
<td>Male and female C57BL and DBA mice</td>
<td>CuSO₄&lt;sub&gt;4&lt;/sub&gt;, developmental, dietary during premating, mating, and gestation until GD 19 sacrifice.</td>
<td>Seven, added to diet: mg/kg/d: 0, 27, 53, 80, 106, 150, 213</td>
<td>Malformations (hydrocephalus, encephaloceles, skeletal) in two highest dose groups; ↓ in mean litter size, no. of live fetuses, mean fetal body wt in four highest dose groups.</td>
<td>80</td>
<td>53</td>
<td>Study poorly reported; no data presented on maternal toxicity (e.g., body wt, body wt gain, other indices).</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Auerlich et al. (1982)</td>
<td>Male and female mink (standard dark)</td>
<td>CuSO₄&lt;sub&gt;4&lt;/sub&gt;, developmental, dietary, 9 mo premating and 3 mo postmating</td>
<td>Five, added to diet: ppm: 0, 25, 50, 100, 200; mg Cu/kg/d: 0, 3, 6, 12, 24.</td>
<td>No overt adult toxicity; no kit malformations reported; kit wt. ↓ at 4 wk, but not at birth, in second highest dose group; kit mortality ↑ in two highest dose groups from birth thru postnatal wk 4 (38% and 32% respectively compared with 12% in controls).</td>
<td>6</td>
<td>12</td>
<td>No statistical significance reported. No information given on maternal toxicity (body wt, body wt gain, other indices).</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Llewellyn et al. (1985)</td>
<td>Male Holtzman rats</td>
<td>Cu acetate, subchronic, dietary, 21 wk</td>
<td>Two, added to diet: mg/kg/d: 0, 45</td>
<td>↓ body wt, ↑ relative tests wt</td>
<td>45</td>
<td>N/A</td>
<td>Only one dose tested. ↑ Relative tests wt. attributed to significantly ↓ body wt; not considered to be toxicologically significant</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Study</td>
<td>Species</td>
<td>Treatment Details</td>
<td>Outcomes</td>
<td>Study Quality</td>
<td>Study validity</td>
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<tr>
<td>Haddad et al. (1991)</td>
<td>Female Wistar rats</td>
<td>Cu acetate, developmental, drinking water, 7 wk prior to mating through GD 11.5, GD 22.5, or birth, when fetuses were examined</td>
<td>No signs of toxicity in treated females; no fetal malformations; ↓ growth in 11.5-d-old fetuses; delayed ossification in 21.5-d-old fetuses.</td>
<td>Poor study description and reporting. Not possible to determine effects level because administered dose in treated group was graded—increasing concentrations up to 65 mg Cu/kg/day given during treatment period.</td>
<td>Low</td>
<td>No</td>
<td></td>
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<tr>
<td>Harrison et al. (1954)</td>
<td>Male and female Sprague-Dawley rats</td>
<td>Cu gluconate, chronic, dietary, 40–44 wk</td>
<td>Hypertrophy of uterus, ovary, seminal vesicles reported in paper, but tabular data showed ↓ wts of uterus and ovary, and no wt change in seminal vesicles; no histopathology observed in any of these tissues</td>
<td>Study inadequately described and analyzed; reproductive data inconsistent in text versus tables.</td>
<td>Low</td>
<td>No</td>
<td></td>
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<tr>
<td>Hebert et al. (1993)</td>
<td>Male and female F344 rats</td>
<td>CuSO₄, specialized reproductive, dietary, 92 d; vaginal cytology and sperm parameters measured</td>
<td>M: No changes in vaginal cytology, estrus cycle length, estrus cycle staging; F: No changes in sperm count, morphology, motility, or density</td>
<td>NOAEL was the highest dose tested</td>
<td>High</td>
<td>Yes</td>
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</table>

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<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Copper species/study type/exposure route/duration</th>
<th>Number of groups/dose levels</th>
<th>Effects</th>
<th>LOAEL&lt;sup&gt;c&lt;/sup&gt; (mg/kg/d)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>NOAEL&lt;sup&gt;e&lt;/sup&gt; (mg/kg/d)</th>
<th>Comments</th>
<th>Study quality</th>
<th>Useful for dose response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferm and Hanlon</td>
<td>Golden hamsters</td>
<td>Cu species not given, developmental, intravenous injection on GD 8, sacrifice on d 4–5 postinjection</td>
<td>Two, 0.25 mg Cu/ml, 0.80 mg Cu/ml</td>
<td>↑ embryonic resorptions; ↑ malformations</td>
<td>N/A</td>
<td>N/A</td>
<td>Data in tables inconsistent with text; study incompletely reported</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Ackerman et al.</td>
<td>Male impala in Kruger National Park, South Africa</td>
<td>Cu species not given, specialized, reproductive, analysis of impala sperm</td>
<td>Three: (1) captive animals from pristine area (control); (2) captive animals from pristine area with 5000 mg Cu/kg added to diet; (3) free-ranging animals near copper mine</td>
<td>↑ Sperm abnormalities in all Cu-treated animals, correlated positively with ↑ liver Cu concentrations</td>
<td>N/A</td>
<td>N/A</td>
<td>Semiecological study, not sufficiently quantified for dose-response assessment</td>
<td>Medium</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup>Due to the large number of studies in the copper toxicity database, only a subset of studies relevant to hazard and dose-response characterization are presented. ↑ Increased; ↓ decreased; wt, weights; rel, relative; SHR, spontaneously hypertensive rats; M, males; F, females; GD, gestation day; PND, postnatal day; ip, intraperitoneally.

<sup>b</sup>grps, groups.

<sup>c</sup>mg/kg, milligrams per kilogram body weight.

<sup>d</sup>LOAEL = low-observable-effects level.

<sup>e</sup>NOAEL = no-observable-effects level.

<sup>f</sup>N/A = not available.
**TABLE B-2.** Selected Studies of Excess Copper Toxicity in Humans: Case Reports, Clinical and Epidemiologic Investigations

<table>
<thead>
<tr>
<th>Observations</th>
<th>Effects Levels</th>
<th>Study Quality</th>
<th>Comments</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Case Report: Nausea, vomiting, diarrhea, weakness, abdominal cramps and headache reported by 10/15 nurses who ingested alcoholic cocktail(s) at a party that were prepared and stored in cocktail shaker that leached copper. Reconstruction of the episode by simulation suggested that copper ingestion varied between 5.2 and 32 mg, based on number of glasses consumed/case. Potentially confounding variables included alcohol consumption and fasted state.</td>
<td>5.2–32 mg/L</td>
<td>Low</td>
<td>Doses and dose-response reconstructed retrospectively in simulation of incident report. Study used previously in risk assessment for establishment of safe drinking water levels, with 5.2 mg/L considered to be an equivocal LOAEL.</td>
<td>Wyllie (1957)</td>
</tr>
<tr>
<td>Case Report: Three children (1–2.5 yr old) in preschool presented with extensive weight loss and recurring diarrhea. Drinking water contained 0.22–1 mg Cu/L. Symptoms disappeared when water source changed. Water consumption not measured.</td>
<td>N/A</td>
<td>Low</td>
<td>Incomplete reporting; other factors potentially associated with symptoms not considered. No control for confounding (e.g., bacterial, viral causes of diarrhea from food or drinking water), pre-existing gastrointestinal illness, other factors (e.g., stress). Not useful for dose-response assessment.</td>
<td>Stenhammar (1979)</td>
</tr>
<tr>
<td>Case Report: Association between copper content in first-draw drinking water (0.35–6.5 mg/L) and diarrhea in children attending new kindergartens; symptoms resolved when children were home for several days and reappeared when they returned to the kindergarten. Water consumption not measured.</td>
<td>N/A</td>
<td>Low</td>
<td>No potential control for confounding factors (e.g., bacterial, viral causes of diarrhea from food or drinking water, pre-existing gastrointestinal illness, other factors (e.g., stress). Not useful for dose-response assessment.</td>
<td>Berg and Lundh (1981)</td>
</tr>
<tr>
<td>Case Report, Repeated Dose: A 26-yr-old man developed liver failure after consuming a nutritional supplement providing 30 mg Cu/day for 2 yr, and increasing intake up to 60 mg Cu/day for an additional year. Subject presented with jaundice, abdominal swelling, “maltlike”; hepatosplenomegaly, but normal serum Cu, ceruloplasmin, bilirubin, and Cu-containing enzymes. Urinary Cu highly elevated (1,300 mg/24 h; normal &lt;0.076 mg/24 h). Subject developed acute renal and liver failure, recovered following emergency liver transplant; Cu liver concentrations = 3230 μg/g dry wt versus 20–50 μg/g (normal).</td>
<td>LOAEL = 30–60 mg/kg/day</td>
<td>Low</td>
<td>Good report of case study but not useful for dose response. Histology of removed liver resembled that of Wilson disease and Indian childhood cirrhosis. No identified genetic susceptibility in patient, parents and siblings.</td>
<td>O'Donohue et al. (1993, 1999)</td>
</tr>
<tr>
<td>Epidemiologic Study: In a Vermont house at the end of a copper main, 3 of 4 family members (father, two children) had repeated acute gastrointestinal upset episodes (nausea, vomiting, abdominal pain) during a 1.5-yr period. Symptoms appeared in morning, within 5–20 min of drinking breakfast juice made with tap water. Median level of Cu in tap water = 3.3 mg/L; single maximum level = 7.8 mg/L. No symptoms seen in two other families, of similar age/sex distribution on same street, exposed to lower Cu levels (median values = 1.58 and 0.02 mg/L, respectively). Copper levels in hair were significantly elevated in the symptomatic family. Symptoms ceased with change of water source.</td>
<td>Median 3.1 mg/L, max 7.8 mg/L</td>
<td>Medium</td>
<td>Some data on dose response, and levels of copper in drinking water that can induce acute gastrointestinal effects in some individuals. Highest copper levels in first-draw water.</td>
<td>Spitalny et al. (1984)</td>
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<tr>
<th>Observations</th>
<th>Effects Levels</th>
<th>Study Quality</th>
<th>Comments</th>
<th>Reference</th>
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<tr>
<td>Clinical Study, Repeated-Dose: Three men and four women ingested 10 mg Cu/d in the form of capsules containing copper gluconate. Seven other subjects received placebo capsules. Samples (blood, urine, and hair) were collected at study commencement and following 6 and 12 wk of treatment, analysed for Cu levels. Some hematology and clinical chemistry performed, no significant treatment-related changes observed during 12-wk period in (1) Cu levels (hair, serum or urine), (2) hematocrit, mean corpuscular volume; serum cholesterol, triglycerides; serum enzymes, AP, GGT, LDH, or SGOT. No differences between treated and control groups in incidences of nausea, diarrhoea and heartburn.</td>
<td>&gt;10 mg/d</td>
<td>Medium</td>
<td>Form of copper administered (capsule) in this study differs from that in drinking-water studies. Small number of subjects tested. Study reporting deficiencies: brief report with few details on methodology and results not presented in detail/tables. Therefore, limited utility for dose response. However, adequate for hazard characterization. Study authors conclude repeated oral intake of 10 mg Cu/d not associated with adverse health effects in adults.</td>
<td>Pratt et al. (1985)</td>
</tr>
<tr>
<td>Case Report: Cluster Analysis: Residents of a new housing development with well drinking water complained of gastrointestinal symptoms (nausea, diarrhea, vomiting, and abdominal cramps); some also reported “unusual irritability” and recurrent headaches. Blue stains on bathroom and laundry items were noted. Based on questionnaire results, symptoms were reported in 57% of resident population (27 adults and 15 children), were more prevalent among those &lt;18 yr old (RR = 1.80; 95% CI = 1.10–2.94) than adults. Water samples reported to contain 0.29–1.2 mg Cu/L; water sampling methodology not specified. Early morning, first-draw Cu levels were 0.16–0.65 mg/L; gastrointestinal symptoms were highly correlated with consumption of first-draw water (RR = 5.24; 95% CI = 1.85–14.91). Sample numbers not specified. Other possible water contaminants not sampled for. Samples not taken from tap water in homes of individuals exhibiting symptoms. Symptoms appeared to subside with reduced consumption of first-draw water, and an “apparent but unspecified” improvement in water quality over time. Gastrointestinal symptoms were highly negatively correlated with a threshold occupancy date (RR = 2.00; 95% CI = 1.24–3.23; p = .009); the longer the resident time in the development, the fewer the symptoms.</td>
<td>N/A</td>
<td>Low</td>
<td>Exposure data inadequate. Water samples not specific to individuals showing symptoms. Therefore, no information on individual exposures available. Sampling methodology and number of samples taken not specified. Confounding variables not controlled for. Data inadequate to establish effect levels. Finding of decrease in no. of individuals reporting symptoms with increasing residence time in new dwelling suggests either adaptive response and/or recall bias among longer term residents, and/or some other systematic factor associated with new building construction but not measured. No utility for dose-response assessment.</td>
<td>Knobeloch et al. (1994)</td>
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<tr>
<td>Study Type</td>
<td>Concentration Range</td>
<td>Exposure Level</td>
<td>Study Details</td>
<td>Authors/Year</td>
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<td>Epidemiologic, repeated dose: Data from the Massachusetts Department of Health were reviewed to identify any cases of death due to liver disease among children from three towns where the concentration of copper in drinking water ranged from 8.5 to 8.8 mg/L. Among 64,124 child-years of exposure and 135 deaths identified, none were due to liver disease.</td>
<td>&gt;8.5–8.8 mg/L</td>
<td>Low</td>
<td>Authors concluded that reported cases of infancy/childhood cirrhosis in Europe not solely due to elevated copper in drinking water but also associated with genetic metabolic disorder. Ecological study with limited predictive ability. Health data obtained from death certificates.</td>
<td>Scheinberg and Sternlieb (1994)</td>
</tr>
<tr>
<td>Epidemiologic Study, repeated dose: In Nebraska residents, gastrointestinal symptoms associated with drinking tap water containing &gt;3.0 mg Cu/L (n = 157) compared with symptoms from tap water Cu exposure of ≤1.3 mg/L (n = 176), and 2.0–2.9 mg/L (n = 176). No elevated incidence of gastrointestinal effects in high-dose cohort relative to mid- and low-dose cohorts. In nested case-control study, no significant differences were found in the Cu concentration of first-draw water of subjects with (n = 25) or without (n = 27) gastrointestinal illness, as reported in telephone or personal interviews. Pregnant women and those with preexisting diseases unrelated to Cu excluded from study.</td>
<td>&gt;3.0 mg/L</td>
<td>Medium</td>
<td>Limited exposure data. Considerable variability in Cu content of drinking water. Criteria for gastrointestinal illness were not specified by the authors. Data collected retrospectively with a lag time of 14 d; possibility of recall bias.</td>
<td>Buchanan et al. (1999)</td>
</tr>
<tr>
<td>Epidemiologic Study: A survey of 2100 state and local public health departments, departments of agriculture and water utilities. Association between clinical toxicity and incidence of failure in water backflow prevention in soft drink machines examined in these facilities. Seventy incidents affecting 156 people were attributed to copper toxicity. Reported range of symptoms included nausea, vomiting, diarrhea, chills, and dizziness. Copper concentration data was available for 24 incidents and ranged from 3.5 to 19.6 mg Cu/L; however, case-specific copper exposure and fluid consumption were not known.</td>
<td>N/A</td>
<td>Low</td>
<td>Information on individual exposures to Cu and on other possible confounding metals/chemicals.</td>
<td>Low et al. (1996)</td>
</tr>
<tr>
<td>Epidemiologic Study: Exposure of German infants to increased copper concentrations in drinking water assessed by water sampling from 2944 households with infants. Mean copper concentrations in composite samples of random and stagnant water = 0.44 and 0.56 mg/L, respectively. Pediatric examination of infants (n = 541) in households (28%) with 0.8 mg Cu/L, or greater in water and defined minimum infant ingestion of tap water showed no evidence of liver disease. In subset of infants with blood samples taken, no liver function effects, as measured by serum liver enzymes, total bilirubin were observed; no changes in serum Cu or ceruloplasmin were noted. No dose-response relationship between ingested Cu and serum parameters.</td>
<td>No effects; NOAEL = 0.8 mg Cu/L</td>
<td>Medium</td>
<td>Large sample size (n = 541). Individual exposure information assessed. Infants examined individually by pediatrician and blood samples taken. No confirmed indication of liver effects in infants whose food was prepared with tap water with &quot;elevated&quot; copper concentrations. Authors concluded that no evidence of hazard due to copper pipes connected to public water supplies.</td>
<td>Zietz et al., (2003a)</td>
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<thead>
<tr>
<th>Observations</th>
<th>Effects Levels</th>
<th>Study Quality</th>
<th>Comments</th>
<th>Reference</th>
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<tr>
<td>Epidemiologic Study: Study design similar to Zietz et al. (2003a) above, except conducted in different location (Lower Saxony) and with smaller population of infants aged up to 12 mo. Mean Cu levels in stagnant water samples ((n = 1619)) and random daytime samples ((n = 1660)), were 0.18 and 0.11 mg/L, respectively; maximum concentrations were 6.4 and 3.0 mg/L, respectively; 153 households had Cu levels (\geq 0.5) mg/L in either or both sample type (mean = 0.55 and 0.59 mg/L, respectively.) Fourteen infants from households with (\geq 0.8) mg/L and who ingested (&gt;200) ml water/day were given pediatric examination; 11 had blood samples taken. No evidence of liver dysfunction was detected; no changes in serum liver enzymes; no gastrointestinal effects (nausea, vomiting).</td>
<td>NOAEL = 0.8 mg Cu/L</td>
<td>High</td>
<td>Individual exposure information used (household-specific and daily ingestion rate). Infants examined individually by pediatrician and blood samples taken. No gastrointestinal or liver effects observed. Authors concluded that there was no association between Cu exposure in drinking-water pipes and health hazards measured.</td>
<td>Zeitz et al. (2003b)</td>
</tr>
<tr>
<td>Epidemiologic Study: Acute gastrointestinal illnesses (diarrhea and vomiting) studied in 430 children aged 9–21 mo exposed to copper in drinking water. Samples ((n = 4703)) taken in homes of children; mean daily intake of copper and maximum copper concentration in samples of consumed water used as exposure measures. Cumulative incidence of acute diarrhea and vomiting assessed during 12-wk follow-up period, excluding those caused by viral and bacterial infections. Mean Cu ingestion level = 0.612 mg/L (10th and 90th percentiles = 0.04 and 1.57 mg/L, respectively). No significant associations between daily Cu intake or maximal Cu concentration in drinking water and risk of diarrhea or vomiting.</td>
<td>No effects NOAEL = 0.612 mg Cu/L (mean)</td>
<td>High</td>
<td>Large sample size ((n = 430)); approximately 10 samples of tap water per household taken. Individual exposure information assessed, using daily intake of copper or maximal Cu concentration in drinking water. Authors conclude that an association between Cu ingestion in drinking water and risk of acute gastrointestinal illness in young children is unlikely.</td>
<td>Petterson et al. (2003)</td>
</tr>
<tr>
<td>Epidemiological Study: Adverse gastrointestinal effects (nausea, colic, vomiting, diarrhea) reported in 29 subjects from geographically diverse areas in Germany; 13 subjects (45%) aged &lt;2 yr.; 7 aged 2–18 yr (24%), and 9 adults (31%); 5 subjects (17%) reported as having “greening” hair. All subjects lived in dwellings with copper plumbing. Following appearance of symptoms, single water samples from hot and cold taps was collected from 23 subjects’ households (79%) for each of the following: (1) first-draw 100 ml, (2) 2nd liter; and (3) 5th liter. Method of analysis was not described. Cu levels in drinking water, reported as single value or range for each household, were approximately 0.1–12 mg Cu/L for infants, 0.1–3.5 mg Cu/L for children, and 0.1–15 mg Cu/L for adults. Significant variation in exposure levels were observed both at different times of day and from day to day.</td>
<td>Varies</td>
<td>Low</td>
<td>Exposure characterization inadequate. Retrospective study. Variability in copper levels over time, lack of individual water consumption and exposure data, and no consideration of potential confounding variables (e.g., preexisting gastrointestinal disease, exposure to disease-causing pathogens or other chemical contaminants in drinking water) limit the findings in this study.</td>
<td>Eife (1999)</td>
</tr>
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</table>
Case Report: Gastrointestinal illness among 43 people at a hotel was associated with elevated copper levels in drinking water. All of the "many" water samples contained ≤4.7 mg Cu/L, except one with 156 mg/L. Water consumption was not measured.

Clinical study of copper taste threshold: Taste threshold for copper sulfate and chloride in tap water, deionized water, and mineral water healthy adult volunteers determined to be 2.5–3.5 mg/L.

Clinical Study: 60 healthy adult women randomly received added Cu (as copper sulfate) in their drinking water at 0, 1, 3, and 5 mg Cu/L for 2-wk periods, separated by a 1-wk period on standard tap water. Subjects recorded their water consumption and gastrointestinal symptoms daily. Nausea, vomiting and abdominal pain, but not diarrhea, were significantly related to Cu concentrations, with combined incidence rates of 5, 2, 17, and 15% at 0, 1, 3, and 5 mg/L added copper, respectively. Daily water consumption averaged 1.64 L. Serum Cu, ceruloplasmin and liver enzymes were not different before and after the exposure period. Symptoms disappeared when copper ingestion was discontinued.

Clinical Study: Chilean women (n = 47–61/group). Each subject drank water containing 0, 2, 4, 6, 8, 10, or 12 mg/L (0, 0.4, 0.8, 1.2, 1.6, 2, or 2.4 mg Cu, respectively). Weekly dosing regime used randomized Latin square design. Nausea most frequently reporting with threshold dose of 4 mg/L (10% of subjects). At doses of 8–12 mg/L, nausea incidence appeared to plateau (19–21% of subjects). Nausea with vomiting occurred less frequently, highest incidence at 12 mg/L (12% of subjects). Administration of copper in orange-flavored drink reduced the cumulative frequency of reported nausea.

Clinical Study: 180 healthy adult volunteers (60 each in Ireland, Chile, United States) Each subject drank 200 mL of water containing either 0, 2, 4, 6, or 8 mg/L (0, 0.4, 0.8, 1.2, 1.6 mg Cu, respectively). Each subject received each of the doses in a randomized order once a week for 5 wk. Symptoms noted up to 60 min after ingestion; 24-h follow up conducted to monitor for additional symptoms. Nausea reported in 8, 7, 11, 25, and 44 subjects at 0, 2, 4, 6, and 8 mg/L, respectively, generally within 15 min of drinking. No significant sex or regional differences. Frequency of nausea and some abdominal pain increased with increasing dose.

<table>
<thead>
<tr>
<th>Study</th>
<th>NOAEL (mg Cu/L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kramer et al. (1996)</td>
<td>N/A</td>
<td>Low</td>
</tr>
<tr>
<td>Zacarias et al. (2001)</td>
<td>2.5–3.5 mg/L</td>
<td>High</td>
</tr>
<tr>
<td>Pizarro et al. (1999b)</td>
<td>NOAEL = 3–5 mg/L</td>
<td>Medium</td>
</tr>
<tr>
<td>Olivares et al. (2001)</td>
<td>NOAEL = 4 mg/L</td>
<td>High</td>
</tr>
<tr>
<td>Araya et al. (2001)</td>
<td>NOAEL = 4 mg/L</td>
<td>High</td>
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</tbody>
</table>

(Continued)
TABLE B-2. (Continued)

<table>
<thead>
<tr>
<th>Observations</th>
<th>Effects Levels</th>
<th>Study Quality</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Study:</strong> Double-blind, $3 \times 3$ (volume $\times$ dose) factorial study; adult females (total pooled $n = 269$), approximately 70 each in Ireland, Chile, China, United States. Each subject drank 100, 150, or 200 ml water containing either 0, 1.6, 0.4, 0.8, or 1.2 mg Cu. Nausea was the most prevalent symptom, generally reported within first 15 min following ingestion. Increasing dose increased incidence of nausea. Increasing volume of water decreased Cu-induced nausea.</td>
<td>NOAEL = 4 mg Cu/L = 0.8 mg Cu</td>
<td>High</td>
<td>Well-designed study designed to replicate and confirm previous study by Araya et al. (2003). Useful for dose-response assessment of acute toxicity.</td>
<td>Araya et al. (2003a)</td>
</tr>
<tr>
<td><strong>Clinical Study, Repeated-Dosing:</strong> 2-mo, randomized, controlled, double-blind study with 1365 healthy adult volunteers in Chile. Subjects prepared water to be consumed all day at home, using tap water and a Cu stock solution with &lt;0.01 (control), 2, 4, or 6 mg Cu/L as cupric sulfate. Gastrointestinal symptoms higher in 6-mg Cu group than control group. In a subset of 195 subjects, no changes were observed in indicators of Cu status (serum and erythrocyte Cu, peripheral mononuclear cell Cu, serum ceruloplasmin, nonceruloplasmin-bound Cu fraction, superoxide dismutase activity, hemoglobin, and liver enzyme activities). Liver function tests were normal.</td>
<td>NOAEL = 4 mg/L = 0.8 mg Cu</td>
<td>High</td>
<td>Well-designed repeated-dose study. As compared with bolus dosing in acute toxicity experiments, subjects drank copper-containing water or fluids prepared with this water throughout the day. Useful for dose-response assessment of short-term repeated-dose toxicity.</td>
<td>Araya et al. (2003b)</td>
</tr>
<tr>
<td><strong>Clinical Study:</strong> 128 healthy infants were randomized into one of two groups: drinking water with &lt;0.1 mg Cu/L ($n = 48$; 27 formula fed, 21 breast fed) or with 2 mg Cu/L ($n = 80$; 56 formula fed, 24 breast fed) from 3 to 12 mo of age. No differences seen in growth, morbidity, liver function, serum Cu, or ceruloplasmin (except at 9 mo) related to Cu intake (some differences related to formula versus breast-feeding were seen). No evidence of toxicity associated with water containing 2 mg Cu/L.</td>
<td>NOAEL &gt; 2 mg/L = &gt;0.4 mg Cu</td>
<td>High</td>
<td>Fewer cases of diarrhea among breast-fed vs. formula-fed infants, no adverse effects associated with copper level in water</td>
<td>Olivares et al. (1998)</td>
</tr>
<tr>
<td>Reference</td>
<td>Species</td>
<td>Study type/ exposure duration</td>
<td>Number of groups/ dose levels (mg Cu/kg diet)</td>
<td>Effects</td>
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<tr>
<td><strong>Repeated-dose studies</strong></td>
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<tr>
<td>Lynch and Klevey (1994)</td>
<td>Male and female Swiss-Webster mice</td>
<td>Subacute, 43–49 d, beginning at 6 wk of age</td>
<td>Two: CuD: 0.3 CuA: 8.4</td>
<td>CuD: Both sexes had anemia, ↓ plasma ceruloplasmin, and erythrocyte SOD,↑ and enlarged hearts. Females exhibited ↑ mortality, ↓ body wt, ↑ atrial thrombosis Cardiac edema and renal and liver Cu depletion more severe in females than males.</td>
</tr>
<tr>
<td>Rayssiguier et al. (1993)</td>
<td>Male Wistar rats</td>
<td>Subacute, 6 wk, beginning at weaning</td>
<td>Two: CuD: 0.6 CuA: 7.5</td>
<td>Hypercholesterolemia, associated with ↑ plasma apolipoprotein B, alterations in lipid composition of triglycerides, ↑ in measures of lipoperoxidation.</td>
</tr>
<tr>
<td>Gomi and Matsuo (1995)</td>
<td>Female F344 rats</td>
<td>Subchronic, 10 wk, 6-mo-old (young) and 24-mo-old (old) rats.</td>
<td>Two: CuD: 0.4 CuA: 5.7</td>
<td>In both age groups, ↓ Cu contents of cerebrum, livers, lungs, and serum, no change in Cu content of muscle or hearts; ↓ plasma ceruloplasmin activity. In young, but not old, rats, ↓ SOD activity in cerebrum, lung, liver.</td>
</tr>
<tr>
<td>Schuschke et al. (1995)</td>
<td>Male Sprague-Dawley rats</td>
<td>Subacute, 1, 3 or 5 wks starting at weaning</td>
<td>Three: CuD: 1.5 Marginally deficient (CuM) CuM: 2.0 CuA: 6.0</td>
<td>↓ Liver Cu in CuD and CuM groups after 3 wk; ↑ bleeding time after micropuncture in CuD at all time periods, in CuM at 5 wk. No differences in platelet thrombus formation. Pos. correlation between liver Cu conc. and bleeding time.</td>
</tr>
<tr>
<td>Reference</td>
<td>Species</td>
<td>Study type/exposure duration</td>
<td>Number of groups/dose levels (mg Cu/kg diet)</td>
<td>Effects</td>
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<tr>
<td>Rock et al. (1995)</td>
<td>Male Wistar rats</td>
<td>Subacute, 6 wk, beginning at weaning</td>
<td>Two: CuD: 0.6 CuA: 7.5</td>
<td>↓ Red blood cell survival, ↑ fluidity of erythrocyte membrane ↓ Cholesterol: phospholipid ratios, ↑ lipid peroxidation, ↑ red blood cell hemolysis ↓ Cholesterol: phospholipid ratios, ↑ lipid peroxidation, ↑ red blood cell hemolysis</td>
</tr>
<tr>
<td>Bode et al. (1992)</td>
<td>Male Sprague-Dawley rat</td>
<td>Subacute, 4 wk, beginning at weaning</td>
<td>Two: CuD: 0.4 CuA: 4.2</td>
<td>↓ Cu in serum, skeletal muscle, liver, kidney, ↓ ceruloplasmin; enlarged hearts, anemia, hypercholesterolemia; ↓ in mitochondrial respiration rates in heart and liver but not kidney.</td>
</tr>
<tr>
<td>Lear and Prohaska (1997)</td>
<td>Male Holtzman rats</td>
<td>Subacute, 5–6 wk</td>
<td>Two: CuD: 0.36 CuA: 0.36 + 20 mg/L drinking water</td>
<td>↓ Plasma ceruloplasmin activity and hemoglobin levels; ↑ heart wts and cardiac hypertrophy; no change in body wt; ↓ specific activities of all cuproenzymes in cardiac tissue except ventricular PAM$^{c}$. ↓ Norepinephrine and higher dopamine concentrations in cardiac tissue.</td>
</tr>
<tr>
<td>Nelson et al. (1992)</td>
<td>Male Sprague-Dawley rat</td>
<td>Subacute, 6 wk</td>
<td>Three: CuD: 0.8 CuM$^{d}$: 1.7 CuA: 6.7</td>
<td>Dose-dependent ↓ in body wt and aortic SOD activity in CuD and CuM groups relative to controls. Also dose-dependent ↓ in aortic prostacyclin synthesis, and ↑ in aortic lipid peroxidation.</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Species</td>
<td>Study Type</td>
<td>Duration</td>
<td>CuD</td>
</tr>
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<tr>
<td>Allen (1996)</td>
<td>Male Sprague-Dawley rat</td>
<td>Subchronic, 20 wk, beginning at weaning</td>
<td>Two: CuD: 0.45 CuM: 4.3 CuA: 5.75</td>
<td>CuD rats had ↓ body wts starting wk 6, ↓ growth efficiency and relative food intake, with recovery toward control values during second half of experimental period. At end of study, organ Cu content and serum ceruloplasmin significantly ↓ in CuD rats. CuM diet, in conjunction with high-fat diet, produced abnormalities in cardiac ultrastructure and EKG alterations compared to other groups. Not observed with low-fat diet.</td>
</tr>
<tr>
<td>Fields and Lewis (1997)</td>
<td>Sprague-Dawley rat</td>
<td>Subacute, 4 wk beginning at weaning</td>
<td>CuD: 0.6 CuM: 4.3 CuA: 6.0</td>
<td>Significant ↓ in glandular mass of pancreas with associated ↓ lipase and amylase activity and ↑ insulin levels; males more severely affected than females; plasma insulin ↑ in females, but not in males.</td>
</tr>
<tr>
<td>Mao et al. (1999)</td>
<td>Sprague-Dawley rat</td>
<td>Subchronic, 12 wk beginning at weaning</td>
<td>CuM: 4.3 CuA: 10 CuM and CuA groups given either (1) high-fat or (2) low-fat diets</td>
<td>CuM diet, in conjunction with high-fat diet, produced abnormalities in cardiac ultrastructure and EKG alterations compared to other groups. Not observed with low-fat diet.</td>
</tr>
<tr>
<td>Hamilton et al. (2000)</td>
<td>C57BL/6 mouse</td>
<td>Subchronic, 14 wk beginning at weaning</td>
<td>Three: CuD: 0.2 CuM: 0.6 CuA: 6.0</td>
<td>↑ Aortic lesion areas and ↑ serum cholesterol were observed in both deficient groups; dose-related ↓ in liver Cu, serum ceruloplasmin oxidase, SOD, COX with ↓ in Cu dietary intake.</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Study type/exposure duration</th>
<th>Number of groups/dose levels (mg Cu/kg diet)</th>
<th>Effects</th>
<th>LOAEL (mg Cu/kg diet)</th>
<th>NOAEL (mg Cu/kg diet)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mao et al. (1998)</td>
<td>Sprague-Dawley rat</td>
<td>Subacute, 5 wk, beginning at weaning</td>
<td>Two: CuD: 1.0 CuA: 6.7</td>
<td>Electron microscopy used for cardiac image analysis. CuD rats had larger myocytes, ↑ area of mitochondria, and ↑ ratio of mitochondria: myofibril and mitochondria: myocyte.</td>
<td>1.0</td>
<td>6.7</td>
<td>The study authors concluded that increase in the absolute mitochondria area was a major contributing factor to cardiac hypertrophy induced by Cu deficiency. Pathology resembled human forms of genetic mitochondrial cardiomyopathies, suggesting that the Cu-deficient rat may be a useful model for humans. Only one dose tested. Borderline marginal-severe deficiency. Limited utility for dose response.</td>
</tr>
<tr>
<td>Prohaska and Brokate (2001)</td>
<td>Sprague-Dawley rats</td>
<td>Developmental, pregnant dams from GD7 thru lactation, weanlings from PND 22–30</td>
<td>Two: CuD: 0.44 CuA: 0.44 + 20 mg/L drinking water</td>
<td>Weanlings: ↓ body wt; anemia; ↓ adrenal wt; alterations in adrenal and brain enzymes, DBM, and tyrosine monooxygenase; changes in brain proteins</td>
<td>0.44 + 20 mg/L drinking water</td>
<td>0.44 + 20 mg/L drinking water</td>
<td>Study authors concluded that alterations in brain enzymes and proteins are associated with Cu deficiency-induced physiological stress. Only one dose tested. Severe copper deficiency. Limited utility for dose response.</td>
</tr>
<tr>
<td>Prohaska and Hoffman (1996)</td>
<td>Sprague-Dawley rats</td>
<td>Developmental, pregnant dams from GD 7 to PND 21, weanlings from PND 22 to 30, followed by repletion for 1, 3, 5 mo</td>
<td>Two: CuD: 0.44 CuA: 0.44 + 20 mg/L drinking water</td>
<td>Weanlings: ↓ in regional brain Cu conc.; ↓ in liver Cu and plasma Cu; diminished auditory startle response after 1, 3, 5, mo of repletion. No effect on foot splay (tactile startle response). Repletion did not alter outcome. Postweanling Rats deprived of Cu for 5 wk following weaning exhibited normal auditory startle response.</td>
<td>0.44 diet</td>
<td>0.44 + 20 mg/L drinking water</td>
<td>Study authors concluded that neurochemical and behavioral abnormalities persist in rats after perinatal Cu deficiency. Timing of Cu deficiency important during brain development. Only one dose tested. Severe deficiency. Limited utility for dose response.</td>
</tr>
</tbody>
</table>
Prohaska and Wells (1975)  
Sprague-Dawley rats  
Developmental, pregnant dams from GD 7 to PND 21. Weanlings from PND 22 to 30, followed by repletion; tested at ages 2, 4, 6 mo

Two: CuD: 0.44 CuA: 0.44 + 20 mg/mL drinking water

Weanlings: ↑ relative brain and heart wt; no changes in body wt, or hemoglobin; ↓ brain Cu, ↓ liver Cu and ↓ ceruloplasmin diamin oxidase. Repletion restored liver Cu and ceruloplasmin oxidase activity but brain Cu remained ↓.

0.44 diet + 20 mg/L drinking water

Only one dose tested. Limited utility for dose response.

Dutt and Mills (1960)  
Rats  
Reproductive, treatment during premating, mating, and gestation.

One: CuD: 0.4 CuD: No live litters.

CuD: No live litters. 0.4 N/A  
Copper deficiency caused spontaneous resorptions; reproduction was completely inhibited. Severe deficiency.

Hopkins and Failla (1995)  
Sprague-Dawley rats  
Developmental, pregnant dams from GD 11 thru lactation; weanlings from weaning until 6 mo of age

Two: CuM: 2.8 CuA: 6.7

Offspring: No changes in body wt gain, heart, concentrations of Cu or cuproenzymes in serum and most tissues. Brain Cu at 6 mo irreversibly decreased in CuM group. Impaired T-lymphocyte and neutrophil function in male, but not female, rats.

2.8 6.7  
Marginal deficiency. One dose tested. Some utility for dose response.

Hunt and Idso (1995)  
Sprague-Dawley rats  
Developmental, pregnant dams: GD 1 through lactation; pups evaluated on PND 23

Three: CuD: 1.4 CuM: 1.8 CuA: 4.3

CuD and CuM pups had smaller cell nuclei in dentate gyrus of brain; CuD pups also had smaller cells in hippocampus; effects in dentate gyrus of CuD pups more severe than in CuM pups.

1.8 4.3  
Study authors concluded that copper plays a role in the morphological maturation of these brain regions. Hippocampus is involved in higher brain functions, including learning and memory. Two doses tested, evidence of dose response for some measures. Some utility for dose response.
### TABLE B-3. (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Study type/exposure duration</th>
<th>Number of groups/dose levels (mg Cu/kg diet)</th>
<th>Effects</th>
<th>LOAEL (mg Cu/kg diet)</th>
<th>NOAEL (mg Cu/kg diet)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prohaska et al.</td>
<td>Sprague-Dawley rats</td>
<td>Developmental, pregnant dams: GD 7 through lactation; weanlings from PND 22 to 30 followed by repletion until PND 60.</td>
<td>Two: CuD: 0.43 CuA: 0.43 + 20 mg/L drinking water</td>
<td>Characteristic signs of Cu deficiency, including 90% ↓ in liver Cu; ↓ activities of cuproenzymes SOD, COX, PAM in heart and midbrain; ↑ in DBM in midbrain. Following repletion, PAM and COX activity still ↑ in heart, and DBM still ↑ in midbrain.</td>
<td>0.43</td>
<td>0.43 + 20 mg/L drinking water</td>
<td>Study authors suggested that neuropeptide maturation is compromised by Cu deficiency. Only one dose tested. Severe deficiency. Limited utility for dose response.</td>
</tr>
<tr>
<td>Wildman et al.</td>
<td>Sprague-Dawley</td>
<td>Developmental, pregnant dams: GD 11 thru lactation; pups from PND 22 to 5.5 mo of age</td>
<td>Three, CuM: 2.8 CuA: 6.7 CuM/CuD: 2.8 reduced to 1.3 from 4 to 5.5 mo of age</td>
<td>No differences in conventional measures of Cu status, including growth, rel heart wt, Cu tissue concentrations, ceruloplasmin activity, and tissue SOD; ↑ in number and volume of lipid droplets, and ↑ pathological abnormalities in cardiac tissue in CuM. In CuM/D, ↓ serum and liver Cu, but no effects in heart Cu content or cardiac ultrastructure (as compared with CuM).</td>
<td>2.8</td>
<td>6.7</td>
<td>The study authors concluded that abnormalities in cardiac ultrastructure occur in developing rats ingesting a marginally deficient diet, despite minimal changes in conventional markers of Cu status. One dose tested during repro/dev period. Marginal deficiency. Some utility for dose response.</td>
</tr>
<tr>
<td>Carlton and Kelly</td>
<td>Female Holtzman rats</td>
<td>Developmental, dams from weaning through mating, gestation, lactation; offspring treated from weaning through PND 30–50</td>
<td>Two: CuD: 1.0 CuA: 9.0</td>
<td>Within 6 wk of age, neurologic dysfunction observed, including hyper irritability after noise stimulation, catatonic postures, convulsions. Lesions noted in cerebrum, corpus striatum, and thalamic region; neural tissue was spongy, edematous, necrotic; swelling, hemorrhage &amp; liquefactive necrosis noted in most severely affected animals.</td>
<td>1</td>
<td>9</td>
<td>Study authors concluded that these lesions are consistent with changes induced by severe tissue hypoxia. Only one dose tested. Borderline marginal–severe deficiency. Limited utility for dose response.</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Species</td>
<td>Stage of Study</td>
<td>CuD Dose</td>
<td>CuA Dose</td>
<td>CuD pups showed significant↓ in serum Cu (10-fold less) and in serum ceruloplasmin oxidase activity (100-fold less); ↓ SOD in peripheral leukocytes and bone marrow cells; ↓ in markers characterizing neutrophil (granulocyte) function.</td>
<td>Status</td>
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<tr>
<td>Percival (1998)</td>
<td>Mouse (strain not specified)</td>
<td>Developmental, dams, from PND 1 thru weaning; pups from weaning to 4 wk of age</td>
<td>Two: CuD: 0.6 CuA: 6.0</td>
<td>0.6</td>
<td>Study authors suggest that Cu deficiency compromises immune function of neutrophil populations. Neutrophils are predicted to be an effective and valuable tool for assessing Cu status in human populations. Only one dose tested. Severe copper deficiency. Limited utility for dose response.</td>
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<tr>
<td>Prohaska and Bailey (1995)</td>
<td>Sprague-Dawley rats</td>
<td>Developmental, pregnant dams from GD 7 thru lactation, pups from weaning (PND 22) to PND 30.</td>
<td>Two: CuD: 0.4 CuA: 0.4 + 20 mg Cu/L drinking water</td>
<td>0.4</td>
<td>The authors concluded that perinatal Cu deficiency affects the distribution of both copper and catechol amines in rat brain. Only one dose tested. Severe deficiency. Limited utility for dose response.</td>
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<tr>
<td>Karimbakas et al. (1998)</td>
<td>ICR mouse</td>
<td>Developmental, dams from parturition thru weaning; pups fed same diet as dams for additional 2–3 wk.</td>
<td>Two: CuD: 1.05 CuA: 6.0</td>
<td>1.05</td>
<td>The study authors suggested that Cu deficiency arrests the maturation of granulocytes in mice. Only one dose tested. Borderline marginal–severe deficiency. Limited utility for dose response.</td>
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<tr>
<td>Reference</td>
<td>Species</td>
<td>Study type/exposure duration</td>
<td>Number of groups/dose levels (mg Cu/kg diet)</td>
<td>Effects</td>
<td>LOAEL (mg Cu/kg diet)</td>
<td>NOAEL (mg Cu/kg diet)</td>
<td>Comments</td>
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<tr>
<td>Fascetti et al. (2000)</td>
<td>Female domestic cats</td>
<td>Reproductive depletion for 4 mos, followed by repletion during pre mating and mating</td>
<td>Two, depletion: CuD: 0.84 CuA: 10.8 Three, repletion: 4.0, 5.8, 10.8</td>
<td>Severe deficiency induced prior to mating. Negative, linear relationship between dietary Cu and mean number of days for conception to occur during mating period with proven male breeders.</td>
<td>N/A</td>
<td>N/A</td>
<td>Study shows that an inverse relationship between dietary copper levels and time interval for females to conceive during mating period. Dietary Cu deficiency decreased reproductive efficiency in cats. No differences in resorptions, no. kittens/litter, birth defects, kitten mortality or birth wts. Were observed among groups. Insufficient data for dose-response assessment; cats not suitable animal models for humans. Authors suggest that current standard diet of 5 mg Cu/kg feed not optimal for cat reproduction.</td>
</tr>
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</table>

*LOAELs and NOAELs for selected systemic and reproductive/developmental toxicity studies given in mg Cu/kg diet or mg Cu/kg/diet + added mg Cu/L drinking water, as reported in each cited paper. Conversion to mg/kg/d was not performed. However, approximate estimates of daily intake using this dose metric can be calculated using species- and age- (or life stage) specific default assumptions for body weights and food consumption rates (U.S. EPA, 1988).

*CuD = copper-deficient.
*CuA = copper-adequate.
*CuM = copper marginally deficient.
*SOD = superoxide dismutase.
*PAM = peptidylglycine α-monooxygenase.
*COX = cytochrome c oxidase.
*GD = gestation day.
*PND = postnatal day.
*DBM = dopamine β-monooxygenase.
### TABLE B-4. Selected Dietary Studies of Clinical Copper Deficiency in Humans

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Study summary</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>24 healthy adult males aged 21–57 yr</td>
<td>Clinical Study, Depletion–Repletion: Subjects received diets low in copper (1.03 mg/d) and containing either 20% of the calories as fructose or cornstarch. During treatment, 4 subjects developed heart-related abnormalities (1 myocardial infarction, 2 severe tachycardia and 1 type II second-degree heart block), and were removed from the study. There were no changes in serum Cu or ceruloplasmin, but fructose ingestion significantly reduced erythrocyte superoxide dismutase (SOD). When 3 mg Cu/d was added to subjects' diets for 3 wk (repletion), SOD levels were significantly increased in those previously fed fructose, but not starch. These results suggest that the type of ingested dietary carbohydrate can differentially affect indices of human Cu status. The study authors concluded that copper deficiency could play a role in the etiology of coronary heart disease.</td>
<td>Reiser et al. (1985)</td>
</tr>
<tr>
<td>24 healthy adult males aged 21–57 yr</td>
<td>Clinical Study, Depletion–Repletion: Subjects given an experimental diet inadequate in copper (0.36 mg/d) for 11 wk showed a significant increase in LDL cholesterol and significant decrease in HDL cholesterol when compared to either their pretest self-selected diets (0.57 mg Cu/d) or a repletion diet (1.41 mg Cu/d).</td>
<td>Reiser et al. (1987)</td>
</tr>
<tr>
<td>8 healthy adult males aged 21–36 yr</td>
<td>Clinical Study, Depletion–Repletion: The subjects were fed diets low in copper (0.89 ± 0.10 mg/d), for 105 to 120 d. Serum cholesterol was significantly elevated by the end of the 15-wk depletion. Of four subjects tested for glucose clearance, two had impaired clearance function. One individual in negative copper balance exhibited significant reductions in plasma Cu, erythrocyte SOD, and immunoreactive ceruloplasmin. Another two men with slightly negative Cu balance showed a trend toward lower plasma Cu and SOD. The study authors concluded that intakes below 0.9 mg Cu/d induce clinical signs of copper deficiency in healthy adults.</td>
<td>Milne et al. (1990)</td>
</tr>
<tr>
<td>11 healthy adult males aged 20–59 yr</td>
<td>Clinical Study, Depletion–Repletion: The subjects ingested diets with medium (1.6 mg/d), low (0.7 mg/d), and high (6.0 mg/d) Cu intakes in depletion–repletion design, for periods of 8 consecutive weeks, with a minimum of 4 weeks washout between periods. On the last day of each dietary period fasting blood and first-void urine samples were collected. Biochemical indices of bone turnover were assessed. Urinary creatinine, serum Cu and ceruloplasmin, and serum osteocalcin (biomarker of bone formation) were unaffected by dietary Cu intake. Significantly elevated urinary pyridinoline (Pyr) and deoxypyridinoline (Dpyr) (biomarkers of bone resorptions) were observed, averaging 30% and 25% increase, respectively, in subjects when tested following depletion period. The rate of urinary Pyr cross-link excretion was reversed during the subsequent 8-wk repletion period. Study authors concluded that increased bone resorption may contribute to the demineralization of bone in individuals who are marginally deficient in copper.</td>
<td>Baker et al. (1999)</td>
</tr>
<tr>
<td>11 healthy adult males aged 21–32 yr</td>
<td>Clinical study, Depletion–Repletion: Measures of immune response were examined in 11 subjects who were fed 0.66, 0.38, and 2.49 mg Cu/d for 24, 42, and 24 d, respectively. Under conditions of lower dietary intake, there was a significant decrease in the proliferation of peripheral blood mononuclear cells when cultured with the following mitogenic compounds: phytohemagglutinin, concanavalin A, and pokeweed. An increase in the percentage of circulating B lymphocyte cells was also observed. Repletion prevented further decreases in these measures but did not restore them to prestudy levels.</td>
<td>Kelley et al. (1995)</td>
</tr>
<tr>
<td>21 postmenopausal women</td>
<td>Clinical Study, Depletion–Repletion: The effects of deficient and moderately excessive intakes of zinc (Zn) on copper metabolism and in humans fed low- and high-copper diets were assessed. The women were fed 2 mg Cu and 9 mg Zn for 10 days, then divided into two groups: one given 1 mg Cu/d and the other, 3 mg Cu/d. Following equilibration, both groups were fed a basal diet providing 3 mg Zn/day for 90 days, followed by another 10-day equilibration period before dietary Zn was increased to 53 mg/d for 90 d. The results showed that women were in positive copper balance only when diet provided 3 mg Cu and 53 mg Zn per day. Serum cholesterol was higher in subjects fed 1 mg Cu/d than those given 3 mg Cu/d. Three women fed marginal copper (1 mg/d) exhibited an increase in ventricular premature dischargers, resulting in obligatory supplementation with copper before the end of the study. None of the women receiving 3 mg Cu/d had significant changes in electrocardiogram activity. Interaction between Zn and Cu occurred; inadequate zinc was more effective in inducing changes associated with decreased copper status than a moderately high Zn intake. The authors concluded that an intake of 1 mg/d may be inadequate for postmenopausal women.</td>
<td>Milne et al. (2001)</td>
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<td>12 healthy adult males aged 21 to 32 yr</td>
<td>Clinical Study, Depletion–Repletion: Subjects were given dietary copper at intakes of 0.38, 0.66, and 2.49 mg/d for 24, 42, and 24 d, respectively. Skin biopsies were taken at the start of the study (baseline) and at the end of each dietary copper period. Lysyl oxidase from skin was extracted and measured enzymatically. There was a 24% decrease in lysyl oxidase activity when dietary copper was reduced from 0.66 to 0.38 mg/d. During repletion, the activity of lysyl oxidase significantly increased, returning to baseline. The authors concluded that the activity of this copper enzyme, essential for cross-linking, normal maturation, and maintenance of collagen, was decreased when dietary Cu intake was deficient. They also suggested that cardiac abnormalities associated with copper deficiency may result in part from defective collagen structure associated with decreased lysyl oxidase activity.</td>
<td>Werman et al. (1997)</td>
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<tr>
<th>Subjects</th>
<th>Study summary</th>
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<td>6 handicapped patients aged 4-24 yr fed an enteral diet containing 15 μg/100 kcal for 1-5 yr</td>
<td>Case Studies: Symptoms of copper deficiency in patients included bone abnormalities, neutropenia, macrocytic and normochromic anemia; serum copper concentrations ranged from 0.9 to 7.2:1000/L and ceruloplasmin concentrations from 30 to 125 mg/d. Symptoms resolved with copper supplementation.</td>
<td>Higuchi et al. (1988)</td>
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<td>11 copper-deficient infants (plasma copper &lt; 70 μg/L and ceruloplasmin &lt;200 mg/L) and 10 control infants</td>
<td>Prospective Case-Control Study: The growth of copper-deficient infants was evaluated 1 mo before and 1 mo after copper supplementation with 0.80 mg/kg body weight. Following supplementation, weight/length and weight/age indices increased significantly and daily energy intake was significantly higher in the copper-deficient group compared with the control group. Daily weight gain also increased significantly in the copper-deficient group following supplementation.</td>
<td>Castillo-Duran and Uauy (1988)</td>
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<td>7 and 10-mo-old infants</td>
<td>Case Study: An infant who received total parenteral nutrition (TPN) from birth to 7 mo presented with soft tissue calcifications and osteoporosis. Plasma copper and ceruloplasmin levels were severely reduced. The infant did not survive; autopsy revealed a marked reduction in liver copper content. Another infant (preterm) required TPN for the first 4 mo of life because of bowel resection at age of 10 d. At age of 10 mo, the infant presented with symptoms of severe copper deficiency, including neutropenia, osteoporosis, anemia, and abnormalities of the metaphyses and subperiosteal new bone formation. These symptoms resolved following supplementation with 1 mg Cu/L in infant formula.</td>
<td>Heller et al. (1978)</td>
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<td>7-mo-old infant</td>
<td>Case Study: A preterm low-birth-weight (2050 g) infant, fed exclusively with powdered milk formula, presented with diarrhea, severe anemia, neutropenia, and other signs of hypocupremia. Bone evaluation via radiography showed evidence of osteoporosis, including a fracture of the right fibula and flaring and cupping of the metaphyses of the long bones. These abnormalities resolved following copper supplementation.</td>
<td>Tanaka et al. (1980)</td>
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<td>Two 6-mo-old infants</td>
<td>Case Study: One infant was fed exclusively with cow's milk following birth and presented with microcytic anemia and neutropenia; serum Cu and ceruloplasmin concentrations were very low. Another infant, who was fed a diet consisting mainly of cow's milk, presented with macrocytic anemia; concentrations of serum Cu and ceruloplasmin were markedly decreased. Radiologic evaluation demonstrated bone abnormalities, including increased density of the preparatory calcification areas and spur formation at the proximal parts of the femurs. In both cases, symptoms resolved after chicken, meat, and vegetables were added to the diets.</td>
<td>Levy et al. (1985)</td>
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<td>3-mo-old infant</td>
<td>Case Study: A very-low-birth-weight preterm infant (1140 g) was given an infant formula low in copper and developed symptoms of severe copper deficiency including apnea, neutropenia, anemia, metaphyseal flaring, and cupping. Plasma Cu and ceruloplasmin levels were very low. These symptoms resolved with copper supplementation.</td>
<td>Al-Rashid and Spangler (1971)</td>
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<td>6-mo-old infant</td>
<td>Case Study: A very-low-birth-weight preterm infant (1140 g) who was fed exclusively with cow's milk developed symptoms of copper deficiency, including sideroblastic anemia, neutropenia, osteoporosis with blurring and cupping of the metaphyses, skin depigmentation, enlarged and distended scalp blood vessels, and hypotonia. Ceruloplasmin levels were very low. The infant was treated with 3 mg Cu/d for several months, and the symptoms disappeared.</td>
<td>Ashkenazi et al. (1973)</td>
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<td>30-yr-old woman</td>
<td>Case Study: A woman receiving parenteral nutrition unsupplemented with copper developed anemia, severe neutropenia, and other symptoms of hypocupremia. Ceruloplasmin levels were very low. The parenteral solution was supplemented with copper so that she received 4 mg Cu/d. Following supplementation, her reticulocyte count, neutrophil count, and hemoglobin levels were significantly increased.</td>
<td>Zidar et al. (1977)</td>
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