

Copper in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

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Preface

One of the primary goals of WHO and its member states is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose ... regulations, and to make recommendations with respect to international health matters”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A “final task force” meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.

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The work of the following working group coordinators was crucial in the development of this document and others in the third edition:

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The contribution of peer reviewers is greatly appreciated. The draft text was posted on the world wide web for comments from the public. The revised text and the comments were discussed at the Final Task Force Meeting for the third edition of the GDWQ, held on 31 March to 4 April 2003, at which time the present version was finalized. The input of those who provided comments and of participants in the meeting is gratefully reflected in the final text.

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Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

AAS	atomic absorption spectrometry
ALAT	alanine aminotransferase
ARO	acceptable range of oral intake
ASAT	aspartate aminotransferase
ATPase	adenosine triphosphatase
CAS	Chemical Abstracts Service
CI	confidence interval
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency (USA)
GGT	γ -glutamyl transferase
ICP-MS	inductively coupled plasma mass spectrometry
Ig	immunoglobulin
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
RDA	recommended dietary allowance
SOD	superoxide dismutase
USA	United States of America

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1. GENERAL DESCRIPTION

1.1 Identity

Copper (CAS No. 7440-50-8) is a transition metal that is stable in its metallic state and forms monovalent (cuprous) and divalent (cupric) cations. Common copper compounds include the following:

<i>Compound</i>	<i>CAS No.</i>
Copper(II) acetate monohydrate [Cu(C ₂ H ₃ O ₂) ₂ ·H ₂ O]	6046-93-1
Copper(II) chloride [CuCl ₂]	7447-39-4
Copper(II) nitrate trihydrate [Cu(NO ₃) ₂ ·3H ₂ O]	10031-43-3
Copper(II) oxide [CuO]	1317-38-0
Copper(II) sulfate pentahydrate [CuSO ₄ ·5H ₂ O]	7758-99-8

1.2 Physicochemical properties (Lewis, 1993; Lide, 1995–1996)

<i>Compound</i>	<i>Density (g/cm³)</i>	<i>Water solubility (g/litre)</i>
Copper(II) acetate monohydrate	1.88	72
Copper(II) chloride	3.39	706
Copper(II) nitrate trihydrate	2.32	1378
Copper(II) oxide	6.32	Insoluble
Copper(II) sulfate pentahydrate	2.28	316

1.3 Organoleptic properties

Dissolved copper can sometimes impart a light blue or blue-green colour and an unpleasant metallic, bitter taste to drinking-water. The concentration at which 50% of 61 volunteers could detect the taste of copper (i.e., taste threshold) as the sulfate or chloride salt in tap or demineralized water ranged from 2.4 to 2.6 mg/litre (Zacarias et al., 2001). The taste threshold increased in the presence of other solutes (Olivares & Uauy, 1996a; Zacarias et al., 2001). Blue to green staining of porcelain sinks and plumbing fixtures occurs from copper dissolved in tap water.

1.4 Major uses

Metallic copper is malleable, ductile and a good thermal and electrical conductor. It has many commercial uses because of its versatility. Copper is used to make electrical wiring, pipes, valves, fittings, coins, cooking utensils and building materials. It is present in munitions, alloys (brass, bronze) and coatings. Copper compounds are used as or in fungicides, algicides, insecticides and wood preservatives and in electroplating, azo dye manufacture, engraving, lithography, petroleum refining and pyrotechnics. Copper compounds can be added to fertilizers and animal feeds as a nutrient to support plant and animal growth (Landner & Lindstrom, 1999; ATSDR, 2002). Copper compounds are also used as food additives (e.g., nutrient and/or colouring agent) (US FDA, 1994). Copper sulfate pentahydrate is sometimes added to

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surface water for the control of algae (NSF, 2000). Copper sulfate was once prescribed as an emetic, but this use has been discontinued owing to adverse health effects (Ellenhorn & Barceloux, 1988).

1.5 Environmental fate

The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions (US EPA, 1995). Surface oxidation of copper produces copper(I) oxide or hydroxide. In most instances, copper(I) ion is subsequently oxidized to copper(II) ion. However, copper(I) ammonium and copper(I) chloride complexes, when they form, are stable in aqueous solution.

In pure water, the copper(II) ion is the more common oxidation state (US EPA, 1995) and will form complexes with hydroxide and carbonate ions. The formation of insoluble malachite $[\text{Cu}_2(\text{OH})_2\text{CO}_3]$ is a major factor in controlling the level of free copper(II) ion in aqueous solution. Copper(II) ion is the major species in water up to pH 6; at pH 6–9.3, aqueous CuCO_3 is prevalent; and at pH 9.3–10.7, the aqueous $[\text{Cu}(\text{CO}_3)_2]^{2-}$ ion predominates (Stumm & Morgan, 1996).

Dissolved copper ions are removed from solution by sorption to clays, minerals and organic solids or by precipitation. Copper strongly adsorbs to clay materials in a pH-dependent fashion, and adsorption is increased by the presence of particulate organic materials (Barceloux, 1999; Landner & Lindstrom, 1999). Copper discharged to wastewater is concentrated in sludge during treatment. Various studies of leaching from sludge indicate that the copper is not mobile (ATSDR, 2002). Free copper ions are chelated by humic acids and polyvalent organic anions (Landner & Lindstrom, 1999).

Atmospheric copper is removed by gravitational settling, dry disposition, rain and snow.

2. ANALYTICAL METHODS

The most important analytical methods for the detection of copper in water are atomic absorption spectrometry (AAS) with flame detection, graphite furnace atomic absorption spectroscopy, inductively coupled plasma atomic emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS) and stabilized temperature platform graphite furnace atomic absorption (ISO, 1986, 1996; ASTM, 1992, 1994; US EPA, 1994). The ICP-MS technique has the lowest detection limit (0.02 $\mu\text{g}/\text{litre}$), and the AAS technique has the highest (20 $\mu\text{g}/\text{litre}$). Detection limits for the other three techniques range from 0.7 to 3 $\mu\text{g}/\text{litre}$. Measurement of dissolved copper requires sample filtration; results from unfiltered samples include dissolved and particulate copper. Simple colorimetric methods are available for measuring copper but should not be used if method sensitivity is required (IPCS, 1998). The US EPA (1991) has established 50 $\mu\text{g}/\text{litre}$ as the practical quantification limit for copper.

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Copper is present in the atmosphere from wind dispersion of particulate geological materials and particulate matter from smokestack emissions. Collectively, these sources account for only 0.4% of the copper released into the environment (Barceloux, 1999). In a nationwide study by the US EPA (1987) for the years 1977–1983, the range of copper concentrations in 23 814 air samples was 0.003–7.32 $\mu\text{g}/\text{m}^3$. Concentrations of copper determined in over 3800 samples of ambient air from 29 sites in Canada during 1984–1993 averaged 0.014 $\mu\text{g}/\text{m}^3$ (IPCS, 1998). The maximum value was 0.418 $\mu\text{g}/\text{m}^3$. Median air concentrations in the Czech Republic ranged from 0.002 to 0.123 $\mu\text{g}/\text{m}^3$, and the 90th-percentile value did not exceed 0.306 $\mu\text{g}/\text{m}^3$ (Puklova et al., 2001).

3.2 Water

Copper is found in surface water, groundwater, seawater and drinking-water, but it is primarily present in complexes or as particulate matter (ATSDR, 2002). Copper concentrations in surface waters ranged from 0.0005 to 1 mg/litre in several studies in the USA; the median value was 0.01 mg/litre (ATSDR, 2002). In the United Kingdom, the mean copper concentration in the River Stour was 0.006 mg/litre (range 0.003–0.019 mg/litre). Background levels derived from an upper catchment control site were 0.001 mg/litre. Four-fold increases in copper concentrations were apparent downstream of a sewage treatment plant (IPCS, 1998). In an unpolluted zone of the River Periyar in India, copper concentrations ranged from 0.0008 to 0.010 mg/litre (IPCS, 1998).

Copper concentrations in drinking-water vary widely as a result of variations in water characteristics, such as pH, hardness and copper availability in the distribution system. Results from a number of studies from Europe, Canada and the USA indicate that copper levels in drinking-water can range from ≤ 0.005 to >30 mg/litre, with the primary source most often being the corrosion of interior copper plumbing (US EPA, 1991; Health Canada, 1992; IPCS, 1998; US NRC, 2000). Levels of copper in running or fully flushed water tend to be low, whereas those of standing or partially flushed water samples are more variable and can be substantially higher. In a Canadian study of first-draw water samples, concentrations were >1 mg/litre in 53% of the homes in four Nova Scotia communities (ATSDR, 2002). In a study from Sweden, the 10th-percentile copper concentration in 4703 samples of unflushed water from homes in Malmo and Uppsala was 0.17 mg/litre, and the 90th-percentile value was 2.11 mg/litre (Pettersson & Rasmussen, 1999); the median concentration was 0.72 mg/litre. In Berlin, Germany, the median concentrations of two separate composite samples collected from 2944 households were 0.32 mg/litre and 0.45 mg/litre, and the maximum concentrations were 3.5 mg/litre and 4.2 mg/litre, respectively (Zeitl et al., 2003). A recent study in the Czech Republic found that only 1.5% of the samples from the distribution system had copper concentrations greater than 100 $\mu\text{g}/\text{litre}$ (Puklova et al., 2001).

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Copper concentrations in drinking-water often increase during distribution, especially in systems with an acid pH or high-carbonate waters with an alkaline pH (US EPA, 1995). In the USA, first-draw copper concentrations (after a minimum 6-h static period) must be reported to the US EPA if they exceed 1.3 mg/litre. The median values for first-draw 90th-percentile exceedances from 1991 to 1999 were slightly greater than 2 mg/litre (7307 samples). Ten per cent of the samples with exceedances had copper concentrations greater than 5 mg/litre, and 1% had concentrations greater than 10 mg/litre (US NRC, 2000). In 1990–1992, the mean copper content of freshly flushed drinking-water in German households with a central water supply was 182 µg/litre. The mean copper content of tap water from single wells was 134 µg/litre. The highest value measured in freshly flushed, centrally distributed tap water was 4.8 mg/litre, and in tap water from single wells, 2.8 mg/litre (Umweltbundesamtes, 1990–1992).

3.3 Food

Food is a principal source of copper exposure for humans. Liver and other organ meats, seafood, nuts and seeds (including whole grains) are good sources of dietary copper (IOM, 2001). Based on the results of the US Department of Agriculture 1989–1991 survey of food consumption, about 40% of dietary copper comes from yeast breads, white potatoes, tomatoes, cereals, beef and dried beans and lentils (Subar et al., 1998). Vitamin/mineral preparations for children and adults generally contain 2 mg of copper per tablet or capsule, most often as copper oxide. Infant formula contains 0.6–2 µg of copper per kcal (Olivares & Uauy, 1996b).

Data collected during the US National Health and Nutrition Examination Survey (1988–1994) and the Continuing Survey of Food Intakes by Individuals (1994–1996) indicated that the median intake of copper from the diet was 1.2–1.6 mg/day for adult males and 1.0–1.1 mg/day for adult females. The median intake for infants and young children (6 months to 3 years) was 0.6–0.7 mg/day (IOM, 2001). In Scandinavian countries, copper intakes are in the range of 1.0–2.0 mg/day for adults, 2 mg/day for lactovegetarians and 3.5 mg/day for vegans (Pettersson & Sandstrum, 1995; IPCS, 1998). The United Kingdom reported intakes of 1.2 and 1.6 mg/day for adult females and males, respectively, and 0.5 mg/day for children 1.5–4.5 years of age. Australia reported intakes of 2.2 and 1.9 mg/day for adult females and males, respectively, and 0.8 mg/day for 2-year-olds. In Germany, the dietary intake of adults was 0.95 mg/day (IPCS, 1998). Surveys in the USA indicate that about 15% of the population uses a nutritional supplement containing copper (IOM, 2001). Fortunately, clinical dietary copper deficiencies are rare, especially in developed countries (Olivares et al., 1999; IOM, 2001).

Copper is an essential nutrient. The USA and Canada recently established a recommended dietary allowance (RDA) for adults of 900 µg/day. Values for children are 340 µg/day for the first 3 years, 440 µg/day for ages 4 through 8, 700 µg/day for ages 9 through 13 and 890 µg/day for ages 14 through 18 (IOM, 2001). During

pregnancy and lactation, 1000 µg/day and 1300 µg/day are recommended, respectively. The data were not sufficient to establish RDAs for infants. However, based on the copper concentration in human milk, the IOM (2001) estimated that intakes of 200 µg/day were adequate for the first 6 months of life and 220 µg/day for the second 6 months. WHO (1996) estimated that average copper requirements are 12.5 µg/kg of body weight per day for adults and about 50 µg/kg of body weight per day for infants. The IOM (2001) recommended 10 mg/day as a tolerable upper intake level for adults from foods and supplements.

In most foods, copper is present bound to macromolecules rather than as a free ion (IOM, 2001).

3.4 Estimated total exposure and relative contribution of drinking-water

Food and water are the primary sources of copper exposure in developed countries. In general, dietary copper intakes for adults range from 1 to 3 mg/day (IPCS, 1998; IOM, 2001); use of a vitamin/mineral supplement will increase exposure by about 2 mg/day. Drinking-water contributes 0.1–1 mg/day in most situations. Thus, daily copper intakes for adults usually range from 1 to 5 mg/day. Consumption of standing or partially flushed water from a distribution system that includes copper pipes or fittings can considerably increase total daily copper exposure, especially for infants fed formula reconstituted with tap water.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

After oral exposure in mammals, absorption of copper occurs primarily in the upper gastrointestinal tract and is controlled by a complex homeostatic process that apparently involves both active and passive transport (Linder & Hazegh-Azam, 1996; Camakaris et al., 1999; Peña et al., 1999). Mucosal and serosal transport mechanisms are thought to differ, the former relying principally upon facilitated transport and the latter mediated by saturable, energy-dependent mechanisms that appear rate-limiting for typical copper intakes (Linder & Hazegh-Azam, 1996). Uptake of copper from the intestines is susceptible to competitive inhibition by other transition metals (particularly zinc or iron). The presence of dietary proteins and amino acids, complexing or precipitating anions, fructose, ascorbic acid, phytate, fulvic acid and fibre may also influence copper uptake from the gastrointestinal tract (Lönnerdal, 1996; IPCS, 1998; IOM, 2001).

Copper absorption in 11 young male adults was investigated at three dietary levels in initial (0.785, 1.68 or 7.53 mg/day) and follow-up (0.38, 0.66 or 2.49 mg/day) studies by Turnlund et al. (1989, 1998). Apparent absorption was found to vary inversely with dietary intake (ranging from 67% at 0.38 mg/day to 12% at 7.53 mg/day). However, in the 1998 study, actual absorption was more consistent (77%, 73% and 66%, respectively), with increased biliary excretion of the copper at the higher doses accounting for most of the apparent difference in absorption (Turnlund et al., 1998). Developmental age may influence copper absorption, although data from infants are

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limited (Zlotkin et al., 1995; Lönnerdal, 1996, 1997). Intestinal absorption is high in the neonatal rat, but decreases by the time of weaning. More copper is transported to the liver and less remains in the intestine with increasing postnatal age (Lönnerdal, 1996).

Within the mucosal cells, most of the copper is found in the cytosol bound to proteins, including copper chaperones (transport proteins) and metallothionein (a storage protein for copper and other metal ions). Copper transport in cells is a tightly regulated process involving a series of copper-binding proteins that protect against free radical reactions initiated by $\text{Cu}^+/\text{Cu}^{2+}$ oxidation–reduction reactions (Peña et al., 1999). Serosal transport from the mucosal cells is mediated by a *p*-type ATPase active transport system. Copper in the portal blood is bound to albumin or transcuprin; a small amount may be chelated by peptides and amino acids, especially histidine (Linder & Hazegh-Azam, 1996; Camakaris et al., 1999).

Copper uptake from the blood by the liver and distribution within the liver are not completely understood. They are presumed to involve a transport process that differs from that in the intestines (Linder & Hazegh-Azam, 1996). Within the liver, copper becomes incorporated in ceruloplasmin, superoxide dismutase (SOD) and cytochrome oxidase. Specific copper chaperones deliver copper from the plasma membrane to each of these important cuproenzymes (Peña et al., 1999). A specific *p*-type ATPase is involved with the incorporation of copper in ceruloplasmin and contributes to copper export via bile (Camakaris et al., 1999). Excess copper is bound to hepatic metallothionein (US NRC, 2000).

In post-hepatic circulation, copper is bound to ceruloplasmin, albumin, transcuprin and, to a lesser extent, certain amino acids such as histidine. Ceruloplasmin is the primary copper transport protein in systemic circulation and contains about 75% of the plasma copper (Luza & Speisky, 1996). It also has enzymatic activity as a ferroxidase and functions in the synthesis of haemoglobin. Neither ceruloplasmin nor albumin is apparently required for normal distribution of copper to non-hepatic tissues based upon normal tissue levels of copper in patients with aceruloplasminaemia (Peña et al., 1999) and in analbuminaemic rats (Linder & Hazegh-Azam, 1996).

Excluding hair and nails, the highest concentrations of copper under normal conditions are found in the liver, brain, heart and kidneys, with moderate concentrations found in the intestine, lung and spleen (Evans, 1973; Linder & Hazegh-Azam, 1996; Luza & Speisky, 1996; Barceloux, 1999). In healthy adults, the liver contains 8–10% of the body's total copper, while approximately 50% is found in muscle and bone due to their large tissue masses. In newborn infants, however, the liver contains 50–60% of the body's copper (Luza & Speisky, 1996).

Copper is required for the proper functioning of many important enzyme systems. Copper-containing enzymes include ceruloplasmin, SOD, cytochrome-c oxidase, tyrosinase, monoamine oxidase, lysyl oxidase and phenylalanine hydroxylase (Linder & Hazegh-Azam, 1996). The activity of the enzyme SOD, serum ceruloplasmin levels and serum copper concentrations can be used in the assessment of copper status (IOM,

2001). Platelet copper concentration and platelet cytochrome-c oxidase activity may be more sensitive indicators of marginal copper status than the more traditional assessment parameters listed above, but they require additional evaluation (IOM, 2001).

Copper is excreted from the body in bile, faeces, sweat, hair, menses and urine (Luza & Speisky, 1996; Cox, 1999). In humans, the major excretory pathway for absorbed copper is bile, where copper is bound to both low-molecular-weight and macromolecular species. Biliary export seems to involve glutathione-dependent and glutathione-independent processes (US NRC, 2000). Biliary copper is discharged to the intestine, where, after minimal reabsorption, it is eliminated in the faeces. In normal humans, less than 3% of the daily copper intake is excreted in the urine (Luza & Speisky, 1996). Excretion of copper in bile may be even more important than absorption in regulating total body level of copper (Turnlund et al., 1998).

There are several genetic disorders that affect copper utilization. The genetic abnormalities associated with Menkes syndrome (a deficiency disorder) and Wilson disease (a toxicity disorder) have been identified as defects in *p*-type ATPases (US NRC, 2000). There is some evidence to suggest that an autosomal recessive gene may be a predisposing factor for copper-related cases of infant or childhood cirrhosis (Müller et al., 1996; Tanner, 1999).

In Menkes syndrome and its milder variant, occipital horn syndrome, there is minimal copper absorption from the intestines, leading to a deficiency state that is independent of copper intake and often results in death during early childhood (US NRC, 2000). Children suffering from Menkes syndrome, an X chromosome-linked disorder, exhibit mental deterioration, failure to thrive, hypothermia and connective tissue abnormalities (Harris & Gitlin, 1996). The *p*-type ATPase responsible for insufficient serosal transport of copper from intestinal mucosal cells and transport across the blood-brain barrier is defective (Camakaris et al., 1999). A child with Menkes syndrome suffers from a profound copper deficiency, despite adequate dietary copper. There is no effective treatment for Menkes syndrome, although administration of copper as the dihistidine complex delays the development of symptoms (Linder & Hazeigh-Azam, 1996).

Wilson disease is an autosomal recessive disorder that leads to copper toxicity, because copper accumulates in the liver, brain and eyes. Wilson disease affects the hepatic intracellular transport of copper and its subsequent inclusion into ceruloplasmin and bile. A *p*-type ATPase is again affected, but the enzyme is different from that affected in Menkes syndrome. As is the case with Menkes syndrome, there are a number of genetic variants of this disorder (US NRC, 2000). Because copper is not incorporated into ceruloplasmin, its normal systemic distribution is impaired, and copper accumulates in the liver, brain and eyes (Harris & Gitlin, 1996). Wilson disease generally appears in late childhood and is accompanied by hepatic cirrhosis, neurological degeneration and copper deposits in the cornea of the eye (Kayser-Fleischer rings). Patients with Wilson disease are treated with chelating agents, such as penicillamine, to promote copper excretion (Yarze et al., 1992). Patients that follow

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their therapeutic regime can expect to live a normal life (Scheinberg & Sternleib, 1996). Restriction of dietary copper alone cannot influence the progression of the disease.

There is some evidence that asymptomatic carriers of a defective Wilson disease gene also have abnormal hepatic retention of dietary copper (Brewer & Yuzbasian-Gurkan, 1992). However, the data supporting this hypothesis are limited; heterozygous carriers are estimated to occur with a frequency of 1 in 100 individuals (IPCS, 1998) and thus may be a population of concern (US NRC, 2000).

Aceruloplasminaemia is an autosomal recessive disorder caused by changes in the ceruloplasmin gene that affect the ability of ceruloplasmin to bind copper (Harris & Gitlin, 1996). The symptoms of aceruloplasminaemia do not become apparent until adulthood. They include dementia, diabetes, retinal degeneration and increased tissue iron stores. Individuals with aceruloplasminaemia have apparently normal copper transport and tissue uptake despite the lack of ceruloplasmin (Peña et al., 1999).

The etiologies of Indian childhood cirrhosis, endemic Tyrolean infantile cirrhosis and idiopathic copper toxicosis are complex and may involve a combination of genetic, developmental and environmental factors (Müller et al., 1996; Pandit & Bhave, 1996; Tanner, 1999; US NRC, 2000). These disorders are characterized by liver enlargement, elevated copper deposits in liver cells, pericellular fibrosis and necrosis and are generally fatal. Poor biliary excretion of copper may play a role in the etiology of the disease.

5. EFFECTS ON LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Acute responses to copper vary with species and copper compound. Ferrets, sheep, dogs and cats are more sensitive to copper than rodents, pigs and poultry (Andrews et al., 1990; Linder & Hazegh-Azam, 1996). Soluble copper salts are more toxic than insoluble compounds.

In a classic study, Wang & Borison (1951) evaluated the acute emetic response of 107 mongrel dogs to a single dose of copper sulfate pentahydrate in aqueous solution. In this group, 20 (19%) responded to 20 mg of copper sulfate (5 mg of copper(II)) with a mean response latency of 19 ± 11 min. Ninety-one dogs (85%) responded to 40 mg of copper sulfate (10 mg of copper(II)) with a response latency of 16 ± 9 min, and all animals responded to an 80-mg dose of copper sulfate (20 mg of copper(II)) (latency 19 ± 7 min). Some of the animals were then subjected to a vagotomy, sympathectomy or vagotomy and sympathectomy. After severing of the neural pathways, the dogs were again exposed to copper sulfate. The acute dose required to elicit the emetic response increased in the vagotomized and in the vagotomized/sympathectomized dogs, as did the response latency time. The greatest effects on response threshold and latency time were seen in the vagotomized/sympathectomized dogs.

In a more recent study, copper sulfate solution was infused into the stomach and duodenum of groups of four or five ferrets with ligated pyloric sphincters (Makale & King, 1992). In one group (four ferrets), the stomach infusion preceded the duodenal infusion; in the other group (five ferrets), the duodenal infusion preceded the stomach infusion. Infusion to the stomach resulted in vomiting in seven of nine ferrets with a mean latency of 4.4 min. Infusion to the duodenum resulted in vomiting in one of nine animals. The authors concluded that the primary site of the emetic response to copper sulfate in the ferret is in the stomach. Other studies in dogs and ferrets (Andrews et al., 1990; Bhandari & Andrews, 1991; Fukui et al., 1994) confirm the importance of gastrointestinal neural pathways and receptors in copper sulfate-induced emesis. In beagle dogs, the vomiting response to 100 mg of copper sulfate per kg of body weight was reduced or eliminated by high doses of a chemical blocker of receptors for serotonin as well as severing the vagus and splanchnic nerves. Serotonin is a neuroactive compound that may activate or sensitize abdominal gastric nerves involved in the emetic response (Fukui et al., 1994).

5.2 Short-term exposure

Several short-term studies of copper toxicity have been conducted in rats and mice. Effects were largely the same in both species, but rats were slightly more sensitive than mice (Hébert et al., 1993). Accordingly, only the data from rats are reported below.

Groups of five male and five female F344/N rats were administered copper sulfate in their drinking-water for 2 weeks at estimated doses up to 36 mg of copper per kg of body weight per day (Hébert et al., 1993). A LOAEL of 10 mg of copper per kg of body weight per day was observed in male rats based on an increase in the size and number of protein droplets in the epithelial cells of the proximal convoluted tubules. No renal effects were seen in the females receiving the same dose. There was no NOAEL for males in this study; the NOAEL for females was 26 mg of copper per kg of body weight per day. However, only slightly higher doses (31 mg of copper per kg of body weight per day) were accompanied by signs of clinical toxicity.

Copper was less toxic to rats when administered in the diet than when administered in drinking-water or by gavage. This was true for 2-week and 13-week exposures. A dietary concentration of 1000 mg/kg of feed (estimated doses of 23 mg of copper per kg of body weight per day in males and females for 2 weeks and 16 mg/kg of body weight per day in males and 17 mg/kg of body weight per day in females for 13 weeks) had no adverse effects in male or female F344/N rats (Hébert et al., 1993). Dietary concentrations of 2000 mg/kg of feed (46 mg/kg of body weight per day in males and 41 mg/kg of body weight per day in females for 2 weeks and 33 mg/kg of body weight per day in males and 34 mg/kg of body weight per day in females for 13 weeks) were associated with hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the rat forestomach.

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With the 13-week exposure and the 2000 mg/kg of feed dietary concentration (33 mg/kg of body weight per day in males and 34 mg/kg of body weight per day in females), protein droplets were present in the kidneys, and liver inflammation was noted (Hébert et al., 1993). Changes in liver and kidney histopathology were dose-related; males were affected more than females. Staining of the kidney cells for $\alpha_2\mu$ globulin was negative. Dose-related decreases in haematological parameters at 4000 mg/kg of feed (66 mg/kg of body weight per day in males and 68 mg/kg of body weight per day in females) and 8000 mg/kg of feed (140 mg/kg of body weight per day in males and 134 mg/kg of body weight per day in females) were indicative of a microcytic anaemia, whereas increases in serum enzymes were indicative of liver damage. Iron stores in the spleen were depleted, especially for the highest exposure concentration.

Aflatoxin B₁ alone (0.05 mg/kg of body weight per day until weaning at 4 weeks and 0.037 mg/kg of body weight per day thereafter), copper alone (6.6 mg/kg of body weight per day or 200 mg/litre of drinking-water) or a combination of both was administered orally for 6 months to young guinea-pigs from the first or second day of life (Seffner et al., 1997). In the copper group, there were no pathomorphological changes. For the aflatoxin B₁ group, liver damage was established. In the combined group, liver injury was more frequent and more severe compared with the aflatoxin B₁ group, and biliary copper excretion was diminished compared with the copper group. Histologically, only the livers of this group exhibited degeneration, atrophy and steatosis of liver cells, inflammatory processes and more or less prominent fibrosis. These results support the possibility that a combined etiology between enhanced copper uptake and a liver-damaging agent is a plausible hypothesis for copper-associated liver disease.

5.3 Long-term exposure

Male weanling Wistar rats (four per group) were given either a standard diet containing 10–20 mg of copper per kg of feed (controls) or diets supplemented with 3000, 4000 or 5000 mg of copper per kg of feed for 15 weeks (Haywood & Loughran, 1985). The animals receiving 3000 mg of copper per kg of feed were then allowed to continue the experimental regime for the remainder of the year. Assuming that rats consume 5% of their body weight per day in food, these dietary copper concentrations would correspond to approximate doses of 0.5–1.0, 150, 200 or 250 mg of copper per kg of body weight per day. All copper-supplemented groups exhibited reductions in body weight gains relative to the control group that persisted until the end of the 15-week exposure period. For the 3000, 4000 and 5000 mg/kg of feed groups, copper concentrations in the liver peaked at 3–4 weeks, declined significantly by 6 weeks, but were still elevated at 15 weeks. Although the timing and duration varied somewhat, all supplemented groups exhibited hepatocellular necrosis during weeks 1–6, followed by a regeneration process that began after 3–5 weeks. The adaptation process noted during the latter part of the first 15 weeks of exposure continued during the 3000 mg/kg of feed group extension period. The average body weight recovered to 80% of that of the control group, and the copper concentration in the liver dropped from 1303 $\mu\text{g/g}$ at 15 weeks to 440 $\mu\text{g/g}$ at 52 weeks. However,

even at 52 weeks, hepatic copper was greater in the exposed animals than in the controls (23 µg/g).

5.4 Reproductive and developmental toxicity

There are no standard animal studies of copper toxicity and reproductive function. Sperm morphology and motility analyses, testis and epididymis weight determination and estrous cycle characterization were performed in rats as part of a subchronic dietary study (Hébert et al., 1993). No significant differences from control values were found for any of the following reproductive parameters: testis, epididymis and cauda epididymis weights, spermatid count, spermatid number per testis or per gram testis, spermatozoal motility and concentration, estrous cycle length or relative length of time spent in the various estrous stages. A NOAEL of 8000 mg per kg of diet (140 mg of copper per kg of body weight per day for male rats and 134 mg of copper per kg of body weight per day for female rats) was established for these parameters in this study.

There is some evidence from animal studies that copper can be a developmental toxicant at high doses. When mice (7–22 females per group) were fed diets supplemented with 0, 500, 1000, 1500, 2000, 3000 or 4000 mg of copper sulfate per kg of diet for 1 month (0, 65, 130, 195, 260, 390 or 520 mg/kg of body weight per day, assuming mice consume 13% of their body weight as food), fetal mortality and decreased litter size were observed in the 3000 and 4000 mg/kg of diet groups. Various skeletal and soft tissue malformations were seen in 2–9% of the surviving fetuses from the two highest dose groups (Lecyk, 1980). Data on maternal toxicity were not provided by the published report. The low concentrations of supplemental copper (500 and 1000 mg/kg of diet) had a beneficial effect on development.

For 7 weeks prior to and then during pregnancy, rats were supplied with water supplemented in step-wise fashion (details not provided) with copper acetate, up to a level of 0.185% (588 mg of copper per litre, assuming use of copper(II) acetate monohydrate; a dose of 82 mg/kg of body weight per day, assuming a 0.14 litre/kg of body weight per day water intake factor for Wistar rats) (Haddad et al., 1991). Maternal parameters were normal, except for liver and kidney effects typical of copper overload. In 11.5-day-old embryos, mean yolk sac diameter, crown–rump length and somite number were reduced significantly, with only minor retardation observed for several other developmental parameters. The number of offspring per litter and mean fetal weight were similar to controls, but most ossification centres were markedly reduced in 21.5-day-old fetuses. In newborn rats, only the numbers of cervical and caudal vertebrae and hind-limb phalanges were significantly reduced.

5.5 Mutagenicity and related end-points

Copper sulfate does not appear to induce reverse mutations in TA98 or TA100 strains of *Salmonella typhimurium* (Moriya et al., 1983). Copper(II) chloride has tested negative in strains TA98, TA102, TA1535 and TA1537 of *S. typhimurium* and *Escherichia coli* WP2 in the presence and absence of microsomal activation (Wong,

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1988; Codina et al., 1995); however, responses were positive in both the Mutatox and SOS Chromotest assays (Codina et al., 1995).

In vitro exposure of rat hepatocytes to copper sulfate caused DNA strand breakage at 1.0 mmol/litre, but not at lower concentrations (Sina et al., 1983), and unscheduled DNA repair at concentrations of 0.008–0.079 mmol/litre (Denizeau & Marion, 1989). A dose-responsive increase in DNA–protein cross-links was found in Chinese hamster ovary and non-transformed human fibroblast cell cultures when exposed to 0.5–2.0 mmol of copper sulfate per litre (Olin et al., 1996). *In vivo*, copper sulfate pentahydrate induced bone marrow chromosomal aberrations in Swiss albino mice after oral, subcutaneous and intraperitoneal exposures (Bhunya & Pati, 1987; Agarwal et al., 1990; Fahmy, 2000) and in BALB/c mice after injection (Rusov et al., 1997).

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

At high concentrations, copper may be genotoxic or enhance the genotoxicity of other agents, possibly through the generation of reactive oxygen species/free radicals or through effects on DNA-related enzyme processes. However, it should be noted that results from *in vitro* studies and *in vivo* inoculations may not be directly applicable to oral exposure circumstances, where copper is generally bound to protein or amino acid ligands.

5.6 Carcinogenicity

A number of older studies have examined the carcinogenicity of various copper compounds in laboratory animals, but all are inadequate by current methodology standards (US NRC, 2000). Nonetheless, it is evident that the limited available data provide no suggestion that copper or its salts are carcinogenic in animals having normal copper homeostasis. The US EPA (1991) classifies copper as Group D, not classifiable as to human carcinogenicity.

6. EFFECTS ON HUMANS

6.1 Acute exposure

The acute lethal dose for adults lies between 4 and 400 mg of copper(II) ion per kg of body weight, based on data from accidental ingestion and suicide cases (Chuttani et al., 1965; Jantsch et al., 1984–1985; Agarwal et al., 1993). Individuals ingesting large

doses of copper present with gastrointestinal bleeding, haematuria, intravascular haemolysis, methaemoglobinaemia, hepatocellular toxicity, acute renal failure and oliguria (Agarwal et al., 1993).

At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, diarrhoea). Records from case-study reports of gastrointestinal illness induced by copper from contaminated water or beverages plus public health department reports for 68 incidents indicate an acute onset of symptoms. Symptoms generally appear after 15–60 min of exposure; nausea and vomiting are more common than diarrhoea (Wyllie, 1957; Spitalny et al., 1984; US EPA, 1987; Knobloch et al., 1994; Low et al., 1996; Stenhammar, 1999). Among outbreaks with quantitative data, the lowest copper concentrations associated with effects were about 4 mg/litre or higher; background information on the amount of beverage consumed, sample collection and analytical methods is limited. Some studies (Knobloch et al., 1994; Stenhammar, 1999) reported lower effect levels in children.

Araya et al. (2001) conducted a double-blinded clinical study with a total of 179 subjects from three different international sites: Santiago, Chile (60); Grand Forks, North Dakota, USA (61); and Coleraine, Northern Ireland (58). Subjects fasted overnight and came to their test facility one morning per week for 5 weeks, where they drank 200 ml of copper sulfate solution in deionized-distilled water with randomly assigned concentrations of 0, 2, 4, 6 or 8 mg of copper per litre. Subjects completed a “symptoms–signs” questionnaire immediately prior to treatment, 15 min after and 24 h later by telephone; they were also directly observed in a facility lounge for 1 h after treatment. Of the gastrointestinal symptoms (nausea, abdominal pain, vomiting, diarrhoea), nausea (alone or in combination) was by far the most commonly reported symptom (by 27.3%), generally during the first 15 min after exposure; abdominal pain was the next most common. The nausea was transient, passing quickly in most cases. Vomiting in combination with nausea occurred in five instances: one each for 4 and 6 mg/litre concentrations and three for the 8 mg/litre concentration. One case of vomiting alone was experienced at the 6 mg/litre concentration but was not felt to be related to the copper exposure. Diarrhoea was reported to occur 1–24 h after treatment by five subjects (2.8%; one was a control subject) and was not associated with nausea or vomiting. The authors concluded that site, sex, age and dose order did not significantly affect response.

Using logistic regression analysis and a statistical significance level of $P \leq 0.05$, data for nausea and for total gastrointestinal symptoms defined a NOAEL of 4 mg/litre (14 incidents) and a LOAEL of 6 mg/litre (24 incidents). However, the number of individuals reporting symptoms for the 4 mg/litre concentration (14 incidents) was about twice the numbers for the control and 2 mg/litre groups (8 and 7 incidents), although not statistically significant. Odds ratios at 4 mg/litre were 3.53 ($P = 0.07$) and 1.83 ($P = 0.2$) for nausea and total gastrointestinal symptoms, respectively. Upper confidence levels on dose–response curves generated by a two-stage polynomial regression model indicated that 3% of the population would respond at 2.5–3 mg/litre, while 5% would respond at 3.5–4 mg/litre.

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A second study of very similar design was conducted in Chile by Olivares et al. (2001). Purified water containing 0, 2, 4, 6, 8 or 12 mg of copper per litre (as copper sulfate) was ingested by 61 apparently healthy volunteers. The NOAEL was determined to be 2 mg/litre and the LOAEL was 4 mg/litre based on nausea incidence. The lowest concentration at which vomiting occurred was 6 mg/litre. When copper at the same concentrations was ingested in an orange-flavoured drink, the NOAEL increased to 6 mg/litre and the LOAEL to 8 mg/litre.

In a follow-up to the Araya et al. (2001) study, 269 females at the three original locations in Chile, Ireland and the USA plus a fourth location in Shanghai, China, were tested using an adaptation of the original design. Subjects ingested 0.4, 0.8 or 1.2 mg of copper as its sulfate dissolved in bottled drinking-water using volumes of 100, 150 or 200 ml (Poirier et al., 2002). Additional samples of 0 and 1.6 mg of copper were added for the 200-ml volume. Nausea was the most frequently reported symptom. For any of the three volumes, the incidence of nausea increased with an increase in copper concentration. For a given copper mass, the nausea incidence increased as the volume decreased. There was a significant increase in nausea at a concentration of 6 mg/litre with a 200-ml sample volume, identifying 4 mg/litre as a statistical NOAEL. Using factorial analysis, the acute nausea concentration threshold was determined to be 5.3 mg/litre.

6.2 Longer-term exposure

In a prospective study that was blind for the subjects, 60 healthy adult women from Santiago, Chile, were randomized into four groups (Pizarro et al., 1999). Using a “Latin square” design, each group was sequentially exposed for 2 weeks to drinking-water containing copper sulfate at concentrations of 0, 1, 3 or 5 mg of copper per litre; the order of concentrations given differed for each group, and each 2-week exposure period was separated from the next by a week of rest. Indicators of copper homeostasis (serum copper and serum ceruloplasmin) and liver status (serum levels of hepatic enzymes) did not significantly change during the study. The incidence of gastrointestinal symptoms (one or more of diarrhoea, nausea, abdominal pain or vomiting) was significantly correlated ($P < 0.007$) to copper concentrations in drinking-water and was 5, 8, 23 and 22% at 0, 1, 3 and 5 mg/litre, respectively. The apparent response threshold was between 1 and 3 mg/litre. There was some evidence of an adaptive response for diarrhoea, but not the other symptoms.

In a second study, Pizarro et al. (2001) compared the abilities of soluble copper sulfate and insoluble copper oxide (the most common copper form in vitamin supplements) to induce gastrointestinal symptoms (one or more of nausea, abdominal pain, vomiting or diarrhoea). The double-blinded study used 45 healthy adult women (ages 18–55) from Santiago, Chile, who were randomized into three groups that differed in the order in which they were exposed to the different copper solutions (Latin square design). The study consisted of 5 exposure weeks alternating with 4 “break” weeks. Drinking-water containing 5 mg of copper per litre was provided with one of the following ratios of copper from its sulfate to copper from its oxide: 0:5, 1:4, 2:3, 3:2 and 5:0. Subjects recorded the amounts of test water consumed and any

symptoms experienced. No significant changes in indicators of copper status or liver function were observed during the study. When compared with low-copper tap water, drinking the 5 mg of copper per litre of water preparations collectively produced a 4-fold increase in the incidence of total gastrointestinal symptoms. No significant differences in symptoms were observed for the different ratios of soluble:insoluble copper. There was some evidence for an adaptive response with respect to diarrhoea, as 6 of 12 total episodes occurred during the first week, independent of copper dose.

Olivares et al. (1998) examined biomarkers of copper homeostasis and liver toxicity in a group of infants from 3 to 12 months of age. The infants all had birth weights greater than 2000 g; some were breast-fed, and others received formula preparations. Drinking-water copper concentrations for the mother and child were either <0.1 mg/litre ($n = 48$) or 2 mg/litre ($n = 80$). Blood samples were collected from the infants after an overnight fast at 6, 9 and 12 months of age and analysed for serum copper, ceruloplasmin, bilirubin, transaminases (ALAT, ASAT) and γ -glutamyl transferase (GGT), plus erythrocyte SOD and erythrocyte metallothionein. A field worker collected information on water intake and clinical signs weekly. Minor differences in biomarkers of copper nutrition were noted, but there were no consistent significant differences between groups for the biomarkers of liver toxicity. The incidence of diarrhoea was higher in formula-fed infants than in breast-fed infants.

Data on the occurrence of gastrointestinal symptoms were collected over a 2-month period in a study by Araya et al. (2003). During the study, 1365 apparently healthy adult participants used tap water containing 0.01, 2, 4 or 6 mg of copper per litre for drinking and preparation of beverages, soups and other similar products. The assignment of participants to one of the four groups was random and double-blinded. Any gastrointestinal symptoms were recorded daily by the participants in diaries. Field workers visited the homes every other day to consult with the participants and review the diary entries. At the conclusion of the experimental period, a blood sample was drawn from 60 participants (age 20–55 years) from each exposure group. Copper concentrations were measured in the serum, erythrocytes and mononuclear cells. Erythrocyte SOD activity was determined, and serum ceruloplasmin concentration was measured by nephelometry. Serum ALAT, ASAT and GGT were monitored as measures of liver function.

There was a statistically significant increase in gastrointestinal symptoms among those in the 6 mg/litre group; however, as with other similar studies, symptoms were reported by all groups, including the controls, with a dose–response pattern of 11.4%, 15.3%, 18.3% and 19.7% for the 0.01, 2, 4 and 6 mg/litre concentrations, respectively (Araya et al., 2003). There were no significant differences between groups for copper concentration in serum, erythrocytes or mononuclear cells. Similarly, there were no significant differences in ceruloplasmin, ALAT, ASAT or GGT levels or SOD activity. Forty-five of the 240 blood samples collected were not used because of indications of anaemia, infection or inflammation.

A 26-year-old male presented with symptoms of cirrhosis, liver failure and Kayser-Fleischer rings after more than 2 years of self-prescribed use of copper supplements

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(O'Donohue et al., 1993). The patient ingested 30 mg of supplemental copper per day for 2 years and 60 mg/day for a poorly defined period of up to a year. Liver damage was extensive, and a transplant was required. The diseased liver had an average copper concentration of 3230 $\mu\text{g/g}$ dry weight (normal 20–50 $\mu\text{g/g}$); tissue histopathology was similar to that seen in Indian childhood cirrhosis and Wilson disease. Based on an evaluation of the patient's family medical history and the copper excretion of his parents and sisters, he did not appear to carry the Wilson disease gene. Liver damage apparently resulted from the prolonged daily exposure to 3–6 times the recommended upper limit for dietary copper.

Buchanan et al. (1999) reported on an epidemiological investigation of the association between ingestion of drinking-water containing copper and rapid-onset gastrointestinal illness in Nebraska, USA. In August 1994, a retrospective cohort study was conducted on a total of 451 individuals from 148 households determined in 1993 to have morning first-draw copper concentrations of <1.3, 2–3 or >3 mg/litre. A standardized questionnaire was administered by telephone interview with the household adult "most knowledgeable" about family water use and health. There was no association between gastrointestinal problems during the 2 weeks prior to the interview and 1993 drinking-water copper levels.

From these participants, a nested case–control study frequency-matched 25 cases to 27 controls in three age categories (August 1994). In-person interviews were conducted, water samples were taken and a variety of potential risk factors in addition to drinking-water copper were assessed. Again, no associations were observed between gastrointestinal problems and copper concentration in drinking-water from samples collected in 1993 (tap or morning first-draw). However, water concentrations in the August 1994 samples were found to be inconsistent with those from 1993. Accordingly, a second cohort study (December 1994) was conducted on 442 individuals (145 households) from the original study. New water samples were taken and new questionnaires administered. Risks for gastrointestinal symptoms were recalculated using the December 1994 sampling results and the combined August–December case status results. Once more, no increased relative risks were apparent for the 2–3 mg/litre (0.25, 95% CI = 0.10–0.64) or >3 mg/litre (0.36, 95% CI = 0.09–1.49) households, nor was a dose–response found when households were redistributed evenly by exposure tertiles.

Dieter et al. (1999) and Eife et al. (1999) examined in detail the clinical records from children in Germany who were affected with childhood cirrhosis. For those cases that could not be ascribed to a specific medical condition, a link could be established in most cases between the affected child and formula feeding, copper plumbing and/or corrosive water, most often from private wells that do not have to meet European Union drinking-water requirements. From a total of 103 cases reported between 1982 and 1994 in Germany, Dieter et al. (1999) found that only five were possibly caused by excessive copper intakes based on elevated copper concentrations in liver. Another three cases lacked data on copper levels in liver but were plausibly linked to copper because of the use of acidic well water from homes with copper pipes in preparing formula.

Impaired liver function did not correlate with copper intake in 541 infants (from birth to 18 months of age) from 2944 households in Berlin, Germany, that had a copper concentration of ≥ 0.8 mg/litre in at least one of two composite tap water samples (Zeitz et al., 2003). Local paediatricians examined and provided records for all but 15 of the children. Blood samples from 183 infants were analysed for ASAT, ALAT, GGT, ceruloplasmin, bilirubin, immunoglobulins (IgG, IgM, IgA) and C-reactive protein. The liver was examined using palpitation and ultrasound imaging. Each of the tested children had consumed at least 200 ml of tap water per day in the 6 weeks before evaluation. Although there were some situations where the biomarkers for copper exposure and effect fell outside of the normal range, the elevated values could be accounted for by disease, infection or other non-copper-related abnormalities. Five children had abnormal ultrasound liver images, but again the abnormalities did not appear to be related to copper. Serum measures of copper status and biomarkers for liver damage were divided into quartiles based on the estimated daily and total copper intake. There were no statistically significant differences between the quartiles.

For adults without genetic disorders of copper homeostasis, long-term intake of copper in the diet at concentrations of 1–10 mg/day has no apparent adverse effects (IOM, 2001). Long-term daily intakes of copper below recommended requirements can lead to anaemia, neutropenia and bone demineralization in malnourished children (IOM, 2001). Adults are more resistant than children to the symptoms of copper deficiency. Copper intakes in the 1–10 mg/day range may have adverse effects for individuals that carry the Wilson disease gene and for infants or children genetically predisposed for one of the childhood copper cirrhosis syndromes (US NRC, 2000).

7. GUIDELINE VALUE

IPCS (1998) concluded that the upper limit of the acceptable range of oral intake (AROI) in adults is uncertain but is most likely in the range of several but not many mg/day in adults (several meaning more than 2 or 3 mg/day). This evaluation was based solely on studies of gastrointestinal effects of copper-contaminated drinking-water. The available data on toxicity in animals were not considered helpful in establishing the upper limit of the AROI due to uncertainty about an appropriate model for humans, but they help to establish a mode of action for the response.

The data on the gastrointestinal effects of copper must be used with caution, since the effects observed are influenced by temporal aspects of exposure and the concentration of ingested copper to a greater extent than the total mass or dose ingested in a 24-h period. A single glass of tap water with a concentration greater than 3 mg of copper per litre is more likely to elicit nausea than a litre of water containing the same amount (mass) of copper, but ingested episodically throughout a day.

A provisional guideline value of 2 mg/litre was established in the second edition of the Guidelines to be protective against the adverse effects of copper and to provide an adequate margin of safety in populations with normal copper homeostasis. The guideline value was provisional as a result of uncertainties in the dose–response

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relationship between copper in drinking-water and acute gastrointestinal effects in humans. Based on the studies by Araya et al. (2001, 2003), Olivares et al. (1998, 2001), Pizarro et al. (1999, 2001) and Zeitz et al. (2003), the guideline value of 2 mg/litre is no longer provisional. It should permit consumption of 2 or 3 litres of water per day, use of a nutritional supplement and copper from foods without exceeding the tolerable upper intake level of 10 mg/day (IOM, 2001) or eliciting an adverse gastrointestinal response.

There is still some uncertainty regarding the long-term effects of copper on sensitive populations, such as those with defects in the gene for Wilson disease and other metabolic disorders of copper homeostasis.

Copper tubing is used widely as a plumbing material in a variety of circumstances. In most of these, concentrations of copper will be below the guideline value. However, there are some conditions, such as highly acidic or aggressive waters, that will give rise to much higher copper concentrations, and the use of copper tubing may not be appropriate in such circumstances.

Staining of laundry and sanitary ware occurs at copper concentrations above 1 mg/litre. At levels above 2.5 mg/litre, copper imparts an undesirable bitter taste to water; at higher levels, the colour of water is also impacted.

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