

## General Information

### Chemistry

Copper has two valency states, cuprous (copper I) and cupric (copper II). It occurs in nature mainly in the form of its oxide,  $\text{Cu}_2\text{O}$  and sometimes as the chloride,  $\text{CuCl}_2$  which, in the presence of humidity and oxygen, is oxidised to the basic copper (II) chloride,  $\text{Cu}(\text{OH})\text{Cl}$ . The most important copper compounds in the aquatic environment are cupric chloride, cuprous nitrate and cupric sulphate. Within this risk assessment the word copper refers to ionic copper, except when specific copper compounds are mentioned.

### Natural occurrence.

Copper is found in a wide variety of mineral salts and organic compounds as well as in the metallic form.

### Occurrence in food, food supplements and medicines

Food is the major source of copper intake, with particularly high concentrations being found in nuts (8 mg/kg), shellfish and offal (40 mg/kg).

Copper is present in mineral and multivitamin plus mineral dietary supplements at doses up to 2 mg/day. Licensed medicines may be obtained under the supervision of a pharmacist as Pharmacy (P) medicines, with a maximum recommended dose of 4 mg, or from various retail outlets as General Sales List (GSL) products, with a maximum recommended daily dose of 1 mg.

### Other sources of exposure

Other sources of copper exposure include emissions from mines, smelters and foundries. Environmental copper can also arise from the burning of coal for power generation and from municipal waste incinerators. However, the contribution of airborne copper to total daily intake is negligible. Copper is also used as an anti-fouling agent for boats. Buffered copper sulphate can be used as a pesticide in organic farming.

High concentrations of copper can be dissolved from copper plumbing, depending on the hardness, pH and quality of the water. Dissolved copper levels are higher in acidic waters, especially where pipe runs are long and water can stagnate. Thus, drinking water can make an important contribution to copper intake in some circumstances (the UK standard is 3 mg/L, based on aesthetic considerations). However, levels in UK water are generally less than 1 mg/L.

### Recommended amounts

COMA has set a RNI of 1.2 mg/day for copper (COMA, 1991). The US safe designation for adults is 1.5-3.0 mg/day.

### Analysis of tissue levels and copper status

Present analytical methods lack sensitivity, particularly for marginal copper deficiency. The use of erythrocyte copper-zinc superoxide dismutase activity as a measure of copper status is under investigation. Tissue copper measurements considered to be of use are serum copper, caeruloplasmin, urinary copper and hair copper all of which are depressed in frankly copper-deficient subjects. Plasma copper levels may be raised in pregnancy, infection and inflammation.

### Brief overview of non-nutritional beneficial effects

Copper supplements are generally only used to correct copper deficiency. Benefits have been claimed for copper-containing supplements as anti-oxidants for general use, and specifically for arthritis and osteoporosis. There is speculation that copper might have a role in preventing age-related decline in tissue function, and possibly in ensuring healthy foetal brain development.

### Function

Copper is an essential micronutrient normally subject to effective homeostatic control. It is involved in the function of several enzymes, including cytochrome c oxidase, amino acid oxidase, superoxide dismutase and monoamine oxidase. Copper is thought to be required for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism, myocardial contractility and brain development.

### Deficiency

Copper deficiency may result from an inherited defect, such as Menke's syndrome, or may be an acquired condition. Common clinical features include anaemia, neutropenia and bone abnormalities. Less frequent signs and symptoms include hypopigmentation of the hair, hypotonia, impaired growth, increased susceptibility to infection, abnormalities in metabolism of glucose and cholesterol and cardiovascular changes.

### Interactions

Various dietary components affect the absorption of copper. For example, absorption is higher from a diet high in animal protein compared with plant protein. Milk proteins have varying effects on copper status, with whey protein having a negative effect on copper absorption. High dietary ascorbic acid has been suggested to reduce intestinal absorption of copper, but it also increases hepatic uptake and biliary excretion of copper. Studies in rats have shown that dietary fructose exacerbates the effects of copper deficiency, by elevating faecal and urinary excretion of copper, but this has not been demonstrated in humans. Other animal studies suggest that when copper status is low, copper absorption is affected by high concentrations of iron, and to a greater extent when high concentration ascorbic acid is also present.

Zinc and copper may interact, with high concentrations of one element inhibiting the absorption of the other.

### Absorption and bioavailability

Copper is mainly absorbed in the duodenum bound to specific proteins as Cu(II). Both passive diffusion and carrier mediated transfer occur. A small amount of copper is absorbed from the stomach. Absorption of copper is decreased when copper ingestion is high. Cellular mechanisms such as induction of metallothionein, which sequesters copper, have also been suggested to be part of this homeostatic process. Infants are apparently unable to absorb copper to the same extent as adults, and are often found to be in negative copper balance.

### Distribution and metabolism

Copper distribution occurs in two phases, via specific carrier proteins, first to the liver and then to other body tissues. On leaving intestinal cells, copper is initially bound predominantly to albumin and transcuprein and transported mainly to the liver hepatocytes, where it is incorporated into caeruloplasmin (the major form for transport to other tissues), excreted into the bile or incorporated into superoxide dismutase (SOD). Absorbed copper is incorporated into 3 main fractions in the liver cytosol; a high molecular weight pool, a 30,000 kDa pool (which appears to be SOD), and a 10,000 kDa pool (mainly composed of metallothionein). Copper binds to a range of unidentified components of both high and low molecular weight, but none of these has yet been related to the copper complexes identified in the liver.

### Excretion

Homeostasis of copper is maintained largely by the rate of excretion. The major route of copper excretion is via the bile, which is directly correlated with absorbed dose.

## Toxicity

### Human data

Acute copper toxicity is rare, but can occur as a result of food or beverage contamination. However, the emetic properties and unpleasant taste of copper salts prevent their frequent accidental or deliberate ingestion. Signs of acute copper toxicity include salivation, epigastric pain, nausea, vomiting and diarrhoea. Individual susceptibility varies, but vomiting has been associated with consumption of beverages contaminated with copper ranging from 25 to 840 mg/L. Intakes of 25 – 75 mg copper have been quoted as emetic doses but lower intakes have resulted in the same symptoms when taken on an empty stomach. Ingestion of  $\geq 100$  g copper sulphate may produce intravascular haemolysis, acute hepatic failure, acute tubular renal failure, shock, coma or death.

Few data are available on chronic copper toxicity in humans.

Indian childhood cirrhosis (ICC) is a fatal disorder associated with accumulation of massive levels of copper in the liver. Although ICC has been attributed to boiling and storing milk in copper and brass vessels, thus elevating copper content, there also appears to be an element of genetic predisposition in many cases of ICC. Isolated cases of idiopathic copper toxicosis (ICT), identical in nature to ICC, have also been reported in non-Indian communities in the US and Europe.

Wilson's disease is an autosomal recessive inherited disorder of copper metabolism, which is normally manifest in late childhood. There is a failure of normal copper excretion into the bile and of incorporation into caeruloplasmin. As a result, copper accumulates and causes toxicity, primarily in the liver and brain. Clinical manifestations may include liver disease, and neurological and psychiatric disturbances.

Increased copper levels in adults with untreated Wilson's disease, or in children who have recovered from ICC, have been associated with a possible increased incidence of hepatoma. High copper levels have also been cited as a possible risk factor for heart disease.

In the normal population, there is no evidence that elevated copper intake is associated with cancer incidence.

#### *Supplementation studies*

Few adverse effects have been reported in human supplementation studies where copper has been given in food or as a tablet. For example, in a study in which 10 mg/day was given for 12 weeks no changes in serum enzyme activities was apparent. However in other studies enzyme activities increased in subjects with lower than median copper status. In studies of copper given in solution, increased incidences of vomiting, diarrhoea and other gastrointestinal symptoms have been reported.

#### **Animal data**

Toxicity is highly species dependent. For example, while pigs and rats are relatively tolerant, sheep develop copper toxicosis at low dietary intakes of copper.

Repeat dose studies in rats and mice have demonstrated lesions in the forestomach (attributable to irritation). Liver and kidney toxicity, anaemia, and changes in systolic blood pressure and enzyme activity have also been reported in rats.

Hepatic failure has been demonstrated in rabbits receiving 10 mg/kg copper sulphate by gavage for 33-440 days.

Limited effects on reproduction in rats have also have been reported. These include changes to the reproductive organs and growth retardation in the offspring. At higher doses, developmental abnormalities have been observed.

#### **Carcinogenicity and genotoxicity**

The Long-Evans Cinnamon (LEC) rat is a rodent model of Wilson's disease, characterised by caeruloplasmin deficiency, hepatic copper accumulation, and hepatocellular injury ultimately resulting in hepatocellular carcinoma.

Copper compounds were negative in the majority of bacterial mutation assays reported.

Chromosomal aberrations have been produced in isolated rat hepatocytes incubated with copper sulphate.

### **Mechanisms of toxicity**

The liver mitochondrion appears to be an important target in copper hepatotoxicity, and oxidative damage may be involved in its pathogenesis.

### **Dose response characterisation**

In humans, mild gastrointestinal effects have been reported following consumption of water containing 3 mg copper/L, probably due to the irritant effect of copper in solution. Metabolic and supplementation studies suggest that dietary doses of 7-10 mg copper/day do not result in adverse effects.

In laboratory animals, forestomach lesions were seen in rats given feed containing 2000 mg/kg or greater copper sulphate for 13 weeks (equivalent to 32 mg copper/kg bw/day in male rats and 46 mg copper/kg bw/day in female rats). In mice the lesion occurred in animals given 4000 mg/kg bw/day (equivalent to 184 mg copper/kg bw/day in male mice and 262 mg copper/kg bw/day in female mice). In the same study, liver toxicity in rats was observed at doses of 65-67 mg/kg bw/day copper (and in one male receiving 32 mg/kg bw/day) and kidney toxicity and anaemia was observed in rats at doses of 32-34 mg copper/kg bw/day. Other effects such as changes in blood pressure and enzyme activities have been reported at lower doses. Effects on the reproductive organs of rats have been observed at doses  $\geq$  45 mg copper/kg bw/day. Adverse effects on foetal development in rats and mice occur at doses of 65-80 mg copper/kg bw/day, with foetal abnormalities in mice being observed at 159 mg copper/kg bw/day.

### **Vulnerable groups**

Haemodialysis patients and subjects with chronic liver disease are potentially sensitive to copper excess. See also 'Genetic variations' below.

Children may be at increased risk of copper toxicity due to a combination of efficient uptake and immature biliary excretion. However, copper is accumulated in the third trimester of pregnancy without apparent adverse effect, suggesting that neonates may be resistant to high levels of hepatic copper.

### **Genetic variations**

Subjects with Wilson's Disease and, possibly, ICC are sensitive to excess copper intake.

## Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD).

*Pratt et al., 1985*

As part of a double-blind study of back pain management, 14 adult volunteers received a supplement of 10 mg copper/day as copper gluconate for 12 weeks or a placebo. There was no increase in the levels of copper, zinc or magnesium in serum, urine or hair. Haematocrit, triglyceride, SGOT, GGT, LDH and cholesterol levels were not significantly affected by treatment. The authors concluded that the results supported the view that excess copper was excreted and homeostasis maintained in non-Wilson's disease subjects. Adverse effects of nausea, heart burn and diarrhoea occurred in copper and placebo takers, but the numbers stated are too few for reliable comparison.

*Turnland et al., 1989*

Eleven young male volunteers were confined to a metabolic ward for 90 days and given a sequence of adequate (1.68 mg/day), low (0.785 mg/day) and high (7.53 mg/day) copper diets for 24, 42 and 24 days respectively. Absorption of a stable copper isotope  $^{65}\text{Cu}$  (as  $^{65}\text{CuO}$ ) from the high copper diet, was lower (12%) than that absorbed (36.3%) from the adequate copper diet, and from the low copper diet (55.6% for the first 35 days of treatment), and declined linearly with time. Thus in the last 6 days of the study, copper balance was negative in the high dose treatment group suggesting endogenous excretion of copper retained earlier in the study. The authors concluded that where copper intake was high, reduced fractional absorption was insufficient to prevent the absorption of some excess copper, but that the excess was then eliminated by increased endogenous losses. No adverse effects were noted, but because the experiment was designed to investigate copper balance, markers of copper toxicity were not considered.

*Olivares et al., 1998*

Healthy infants living in Santiago were randomly assigned to two groups receiving drinking water containing  $<0.1$  mg/L ( $n = 48$ ) or 2 mg/L ( $n = 80$ ) copper (as copper sulphate) from 3-12 months of age. The mothers of the infants also consumed the same water. Within the groups, the infants were divided into 2 subgroups: those who were formula-fed and those who were breast-fed. Formula-fed infants in the low and high copper intake groups received 0.8-1.2 and 2.3-2.5 mg copper/day, respectively. Breast-fed infants in the low and high copper intake groups received 0.1-1.6 and 0.1-2.0 mg copper/day, respectively. Values for copper intake included copper intake from food, but not from breast milk. At 6, 9 and 12 months serum concentrations of copper, caeruloplasmin, transaminases and  $\gamma$ -glutamyl transferases were measured, as an indication of copper status. No adverse effects were observed, but small differences were apparent in biochemical estimates of copper status. There were no significant differences in copper status between the 4 groups of infants during the study. However, there were differences between the breast-fed and formula-fed infants in serum copper and erythrocyte metallothionein levels and in the serum caeruloplasmin activity of individuals with high and low copper intake levels at points during the study. A greater incidence of diarrhoea in formula-fed infants would seem likely to reflect their enhanced risk of enteric infection. Overall, the authors concluded that consumption of drinking water containing copper at the WHO guideline level of 2 mg/L did not result in adverse effects.

*Pizarro et al., 1999*

Sixty healthy adult female volunteers living in urban Santiago were randomised into four groups (n =15) who received drinking water containing 0, 1, 3 or 5 mg copper/L for 2 weeks followed by a further week of standard tap water. The copper was added in the form of copper sulphate. A double-blind latin square design was used so that each volunteer experienced all of the copper concentrations and acted as their own control. Untreated tap water contained 0.02 mg copper/L. Average dietary intake of copper by the study population was estimated to be 1.7 mg/day. Water consumption and gastrointestinal symptoms were recorded daily. Average water consumption was 1.64 L/day regardless of copper concentration. Twenty-one subjects reported gastrointestinal disturbances during the study (nine subjects presented with diarrhoea (some with abdominal pain and vomiting) and twelve subjects presented with abdominal pain, nausea or vomiting. One third of the 60 subjects had mild diarrhoea during the study but this was not associated with copper concentration. A copper concentration of 3 mg/L or above was associated with an increased incidence of nausea, vomiting or abdominal pain (5, 2, 17 and 15% in the 0, 1, 3 and 5 mg/L groups respectively). Final serum copper, caeruloplasmin and liver enzyme levels were measured in all the volunteers but did not differ from baseline. The numbers involved were small and the incidence of diarrhoea in the whole group appears quite high; the reasons for the latter were discussed. The authors suggested that the subjects could have been more sensitive to symptoms since they were specifically asked to record them.

### Animal studies

*Hébert et al., 1993*

Rats and mice were given up to 10,000 mg copper/kg (estimated intake up to 35 mg copper/kg bw/day in male rats, 30 mg copper/kg bw/day in female rats, 131 mg copper/kg bw/day in male mice and 171 mg copper/kg bw/day in female mice) copper sulphate pentahydrate in drinking water for 2 weeks. Concentrations of 300-1000 mg copper/kg sulphate (up to 28 mg copper/kg bw/day in rats and up to 35 mg copper/kg bw/day in mice) did not have any overt adverse effects on F344 rats or B6C3F1 mice, whereas concentrations of 3000–10,000 mg copper sulphate/kg (equivalent to 30-44 mg copper/kg bw/day in rats and 56-171 mg copper/kg bw/day in mice) were rapidly lethal. Copper exposure was lower in the top dose rats due to markedly reduced drinking water consumption. Body weights were depressed at > 3000 mg/kg copper sulphate (30 and 44 mg copper/kg bw/day in female and male rats and 56 and 61 mg copper/kg bw/day in male and female mice) in both species. Clinical signs observed at these dose levels included ruffled fur, emaciation, abnormal posturing, hypoactivity, dyspnea and tremors. The only histological lesions observed in rats (males only) were increased numbers of protein droplets in the epithelium of the proximal convoluted tubules. In mice in the highest dose group, most tissues showed cellular depletion, due to decreased water consumption. No effects were produced at 1000 mg copper sulphate/kg (equivalent to 26 mg copper/kg bw in female rats and 24 and 35 mg copper/kg bw in male and female mice) in female rats and mice, but protein droplets were reported in male rats at all doses.

Rats and mice were given up to 16,000 mg copper sulphate pentahydrate/kg in feed for 2 weeks. Concentrations of 4000-16000 mg copper sulphate/kg (equivalent to 91 mg copper/kg bw/day and above in male rats, 159 mg copper/kg bw/day and above in female rats, 193 mg copper/kg bw/day and above in male mice and 212 mg copper/kg bw/day and above in female mice) caused significant reductions in body weight gain in both species. Body weights were reduced in rats given 8000 mg/kg or more copper sulphate (equivalent to 192 mg copper/kg bw/day in females and 194 mg copper/kg bw/day in males) and in mice in the top dose group (704 mg copper/kg bw/day for male mice and 767 mg

copper/kg bw/day for female mice). Hyperplasia and hyperkeratosis on the limiting ridge of the forestomach were present in both species (this was more severe in mice) and was attributed to irritation. A dose-related increase in liver damage (inflammation and changes in clinical chemistry) was apparent in the rats, as was damage to the renal tubules. Depletion of iron stores occurred in rats but not in mice, and histologic changes indicated microcytic anaemia also in rats. The NOAEL for the 2 week feed studies was 1000 mg copper sulphate/kg in rats (equivalent to 23 mg copper/kg bw/day) in males and females respectively based on liver, kidney and forestomach effects. In mice, effects on the liver and kidney were not observed but the NOAEL based on the forestomach was 2000 mg/kg copper sulphate (equivalent to 91 and 102 mg copper/kg bw) in males and females respectively.

A 13 week feeding study in F344 rats and B6C3F1 mice was also carried out. The rats and mice received diet containing 500-8000 mg/kg and 1000-16,000 mg/kg copper sulphate pentahydrate respectively. Body weights were significantly reduced in male rats at 4000 and 8000 mg/kg copper sulphate (equivalent to 65 mg copper/kg bw/day and 138 mg copper/kg bw/day) and in females at 8000 mg/kg (equivalent to 132 mg copper/kg bw/day). A dose related decrease in body weights was apparent in mice at doses of 4000 mg/kg copper sulphate or more (equivalent to 184 mg copper/kg bw/day and above in males and 262 mg copper/kg bw/day and above in females). Hyperplasia and hyperkeratosis on the limiting ridge of the forestomach were present in both species, though rats were more severely affected at the same dose levels. This was attributed to the irritant effects of the copper sulphate. Damage to the renal tubules, and a dose-related increase in liver damage (inflammation and changes in clinical chemistry) was apparent in the rats. Depletion of iron stores occurred in rats but not in mice, and changes in haematologic parameters at 13 weeks indicated microcytic anaemia in rats confirming the findings of the two week feed study. The NOAEL for the 13 week studies was 1000 mg/kg copper sulphate in rats (equivalent to 16 and 17 mg/kg bw/day copper in males and females respectively) based on liver, kidney and forestomach effects. In mice, effects on the liver and kidney were not observed but the NOAEL based on reduced body weights was 1000 mg/kg (43 mg copper/kg bw/day) in male mice and 2000 mg/kg (124 mg copper/kg bw/day) in females. Forestomach effects were reported in the mice at 4000 mg/kg or more copper sulphate.

## Exposure assessment

Total exposure/intake:

Food	1.4 mg/day (mean from 1986/87 NDNS) 3.0 mg/day (97.5 <sup>th</sup> percentile from 1986/87 NDNS)
Supplements	up to 2 mg/day (Annex 4; OTC, 2001)
Drinking Water	up to 6 mg/day (assuming 2 L/day consumption and the maximum permitted water copper concentration of 3 mg/L.)
Estimated maximum daily intake:	$3.0 + 2 + 6 = 11 \text{ mg}$

No potential high intake groups were identified.

## Risk assessment

Acute copper toxicity in humans is rare due to the emetic properties and unpleasant taste of the compounds. There are relatively few data on lower level or chronic oral copper exposure in man. Copper is kept under tight homeostatic control to prevent the accumulation of excess amounts. Where dietary copper is high, absorption is reduced and, in particular, biliary excretion increased. Other mechanisms, which sequester excess copper within the cell, may also occur. Copper toxicity occurs when such defences are overwhelmed. Thus, in man, liver toxicity has only been seen in genetically determined conditions such as Wilson's disease and in Indian Childhood Cirrhosis where hepatic copper accumulation occurs. There is no evidence for copper carcinogenicity in the general population, although an elevated incidence of hepatoma has been suggested in untreated Wilson's disease patients or subjects recovering from ICC.

In the general human population, the key adverse effects usually associated with excess copper intake are gastrointestinal, resulting from consumption of copper in water or beverages.

There are some animal data for copper, although few from adequate chronic studies. Forestomach lesions, liver and kidney toxicity and anaemia were reported in a sub-chronic toxicity study. Reproductive effects have been reported in laboratory animals, but these findings are not consistent.

### ESTABLISHMENT OF SAFE UPPER LEVEL

Key studies:	Hebert <i>et al.</i> , (1993)
Supporting studies:	Turnland <i>et al.</i> (1989), Pratt <i>et al.</i> (1985)
NOAEL:	16 mg/kg bw/day from a 13-week study in rats.
Uncertainty Factors:	10 for inter-species variation x 10 for intra-individual variation (total 100)
Safe Upper Level for total daily consumption over a lifetime:	$16/100 = 0.16$ mg/kg bw/day (equivalent to 10 mg/day in a 60 kg adult).

Copper is kept under tight homeostatic control to prevent the accumulation of excess amounts. Copper toxicity occurs when such defences are overwhelmed.

Balance studies and supplementation studies in small numbers of human volunteers suggest that doses of 7.5 mg to 10 mg copper/day in food or supplements are not associated with adverse effects. In controlled studies on the effects of copper levels in drinking water. Pizarro *et al.* (1999) reported that water containing 3 mg copper/L was associated with gastrointestinal disturbance in adults, whereas water containing 1 mg/L was not. Olivares and colleagues reported that water containing 2 mg copper/L (resulting in intakes of up to 2.5 mg/day in formula fed infants and 2 mg/day + an undefined contribution from breast milk in breast-fed infants) were not associated with adverse effects. No differences in liver function tests related to copper intakes were apparent. It is noted that there was a

higher rate of diarrhoea in formula-fed infants. However, since breast-feeding is known to protect against diarrhoea this is not unexpected. The gastrointestinal disturbance reported by Pizarro *et al.* appears to be the result of a direct irritant effect from copper in water and is not so apparent when copper is present in a food matrix.

Whilst liver toxicity occurs in humans who accumulate large quantities of copper in the liver, this is as a result of genetically determined conditions such as Wilson's disease and Indian Childhood Cirrhosis and is not relevant to the general population. Copper balance studies indicate that decreased absorption and increased excretion occurs when excess copper is consumed so that homeostasis is maintained.

A NOAEL of 16 mg/kg bw/day was identified in a well-reported sub-chronic toxicity study in rats. Higher doses resulted in damage to the forestomach, kidney and liver. If uncertainty factors of 10 for inter-species variation and 10 for intra-individual variation (total 100) are applied to this, a Safe Upper Level of 0.16 mg/kg bw day for total intake of copper is derived. This is equivalent to 10 mg/day in a 60 kg adult. This is consistent with the data from small scale human studies which suggest that up to 10 mg/day supplemental copper may be without adverse effect. The worst-case maximum estimated daily exposure from food and water is 9 mg/day copper suggesting that there is a margin of 1 mg/day for supplementation or other additional intake.

The exposure estimate suggests that individuals in the UK could theoretically consume in excess of 6 mg/day copper from water alone if it was assumed that 2 L/day water was consumed containing copper at the statutory limit of 3 mg/L. This was also the dose associated with gastrointestinal effects in the Pizarro study. However, in practice copper levels in UK drinking water are much lower, so this level of exposure is unlikely to occur. There is no evidence that copper intakes in water in the UK present any risk to health.

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