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Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Copper

(expressed on 5 March 2003)

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FOREWORD

This opinion is one in the series of opinions of the Scientific Committee on Food (SCF) on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Copper is a transition metal with an atomic mass of 63.54. Three oxidation states of copper exist; cuprous (Cu^{+}), cupric (Cu^{2+}) and Cu^{0} (Uauy *et al.*, 1998). In biological systems, copper primarily exists as Cu^{2+} with minute quantities of Cu^{+} being found in solution.

2. NUTRITIONAL BACKGROUND

2.1 Function

It is well established that the trace element copper is essential for life. Copper in living organisms, including humans, forms an essential component of many enzymes (cuproenzymes) and proteins. The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen, for example cytochrome-C-oxidase and superoxide dismutase. Studies have also shown that copper is required for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism (Uauy *et al.*, 1998). Copper plays additional roles that are less well understood and may be in part non-enzymatic, such as in angiogenesis, nerve myelination and endorphin action (Linder and Hazegh-Azam, 1996).

2.2 Homeostasis

As an essential trace element, copper is third in abundance in the human body after iron and zinc and it is estimated that the adult human body contains between 50-150 mg (Turnlund, 1994). At physiological pH, there is little or no free copper in solution and “free” copper content is currently not measureable. The current best estimate of free Cu^{2+} content of human plasma is approximately $2x10^{-16}$ M (Linder, 2001). Total copper concentrations in most tissues are approximately $5x10^{-5}$ M total copper (Prohaska, 1990). Copper absorption occurs primarily in the small intestine with a small amount absorbed in the stomach. Absorption is probably by a saturable, active transport mechanism at lower levels of dietary copper; at high levels of dietary copper, passive diffusion plays a role (Turnlund, 1994). The majority of

copper is transported to the liver where it is incorporated into newly synthesised caeruloplasmin, metallothionein or cuproproteins. The major excretory route of copper is in the bile.

The percentage absorption of dietary copper is strongly influenced by the amount of copper ingested (with the percentage absorption decreasing with increasing intakes (Turnlund *et al.*, 1989; Turnlund, 1988). Turnlund *et al.* (1989) used stable-isotope methodology to study copper absorption in adults. Diets were labelled extrinsically with ^{65}Cu and copper absorption was dependent on the amount of copper in the diet. On a low copper (0.78 mg/day) diet, copper absorption was 55.6%, whereas it was 36.3% from the same diet with copper added to give total dietary intake of 1.68 mg/day and only 12.4% when copper was added to produce dietary intakes of 7.53 mg/day. A theoretical maximum absorptive capacity of 63-67% has been estimated from aggregate results of human copper absorption studies at various copper daily intakes (Wapnir, 1998). With typical diets in developed societies, however, the average copper absorption is in the 30-40% range (Wapnir, 1998).

Copper turnover is low when copper intake is low and high when intake is high. The regulation of excretion appears to be more important than the regulation of absorption in determining copper reabsorption following biliary excretion and faecal copper losses reflect dietary copper intakes. When dietary intake changes, balance over a broad range of copper intakes (0.8-5.5 mg/day) can be achieved (Turnlund, 1998). A series of studies demonstrated that a 10-fold increase in dietary copper resulted in only twice as much copper being absorbed (Turnlund, 1991). Although subjects were in positive copper balance for the first six days, when intakes were increased from 0.8 to 7.5 mg/day, average retention decreased linearly during the next 18 days until it was negative during the last six days of the trial (Turnlund *et al.*, 1989). The authors indicated that the negative balance must represent endogenous excretion of excess copper retained during the first six days of the trial. Indices of copper status, as a result of the body's regulation of copper, are resistant to change except under extreme dietary conditions. Turnlund *et al.* (1990) showed that when dietary intakes increased from 0.8 mg/day to 7.5 mg/day (for 24 days), putative indices of status, including plasma copper, erythrocyte superoxide dismutase (SOD), caeruloplasmin and urinary copper excretion were not significantly different. When dietary intake is very high, regulation is challenged. At first, balance is positive but later (approximately three weeks) it becomes negative as the excess copper retained early after a change in diet is eliminated (Turnlund *et al.*, 1989). Therefore, any intervention studies should be at least of this duration. It has been estimated that copper excretion by infants, might be as low as 50 μg Cu/kg body weight (Aggett, 1999).

2.3 Bioavailability

Dietary interactions of copper with sucrose or fructose (Schofield *et al.,* 1990; O'Dell, 1993), animal proteins, S-amino acids (Brown and Strain, 1990; Strain and Lynch, 1990; Fields *et al.*, 1993), histidine (Harvey *et al*, 1981), and ferrous iron (Yu *et al.*, 1994; Wapnir *et al.*, 1993) may inhibit copper absorption to varying degrees in animal models. Ascorbic acid supplements (1500 mg/day) (Finley and Cerklewski, 1983), molybdenum (Underwood, 1977) and other dietary factors, specifically high intakes of calcium and/or phosphorus (Snedecker *et al.*, 1982) and cadmium (Underwood, 1977), may also inhibit copper absorption in diets containing high amounts of these factors. The interaction between zinc and copper is well documented in humans (Fischer *et al.*, 1984). High levels of dietary zinc have been shown to adversely influence copper absorption and bioavailability (Turnlund, 1988). A decrease in serum/plasma copper (Festa *et al.*, 1985) and reduction in concentration of the copper

containing enzyme Cu,Zn SOD (Yadrick *et al.*, 1989) can be induced by high intakes of dietary zinc.

2.4 Dietary and other sources

The highest contents of copper in foods are in organ meats, seafood, nuts and seeds (Pennington *et al.*, 1995; Strain, 1994a). Other good sources of copper are whole bran cereals and whole grain products.

Copper piping used for water distribution can add 0.1mg/day to intakes in hard water areas but 10x this amount in acid and soft water conditions (Ralph and Arthur, 2000). The current EU standard is 2 mg/L for the maximum concentration of copper in drinking water (EU Directive 98/83).

Other sources of copper, excluding dietary intakes, include emissions from mines, smelters and foundries. Environmental copper can also arise from the burning of coal for power generation from municipal waste incinerators.

2.5 Recommended Dietary Allowances

An EU population reference intake (PRI) of 1.1 mg/day for adults was established in 1992 (SCF, 1993). In the UK, a reference nutrient intake (RNI) of 1.2 mg copper/day has been set for adults (Department of Health, 1991). Insufficient data exist, however, to set lower RNI (LRNI) and estimated average requirements (EAR) for different age groups and sexes. In the United States, new guidelines for recommended intakes have been recently published (FNB, 2001). It has been recommended that adult males and females should consume a dietary intake of 0.9 mg copper/day. Previous to this, an estimated safe and adequate dietary intake was proposed of 1.5-3 mg/day.

2.6 Typical intakes

Mean dietary copper intakes from food of adults in different European countries have been estimated with a range of 1.0-2.3 mg/day for males and 0.9-1.8 mg/day for females (Van Dokkum, 1995). The estimated dietary copper intakes in several European countries are given in Table 1. Gibson (1994) compiled several studies and found that copper intakes in adults were approximately 1-1.5 mg/day from omnivore diets. Vegetarian diets provided greater dietary intakes of copper, approximately 2.1-3.9 mg/day.

The main sources of copper in diets in Great Britain were cereals and cereal products, vegetables and potatoes. Copper is not usually used to fortify foods and copper from this source makes a negligible contribution to total copper intakes. Similarly, a very small proportion of consumers take dietary supplements containing copper, but for those few who did, median intakes from supplements were 0.1-0.5 mg/day (Church, personal communication).

2.7 Copper deficiency

Although clinically defined copper deficiency in humans is rare, it has been observed under a variety of different clinical conditions including in patients on long term total parenteral nutrition (TPN) (Dunlap *et al.,* 1974), premature infants, neonates, and previously malnourished children (Paterson and Burns, 1988; Manser *et al.*, 1980). Symptoms of severe

copper deficiency are similar to those seen in experimental animals and include anaemia, neutropaenia (Williams, 1983) and bone abnormalities (Danks, 1988), while less frequent signs are hypopigmentation (Danks, 1988), impaired growth (Castillo-Duran and Uauy, 1988), increased incidence of infections (Castillo-Duran *et al.*, 1983), alterations of phagocytic capacity of neutrophils (Heresi *et al.,* 1985) and abnormalities of glucose (Klevay *et al.*, 1986) and cholesterol metabolism (Reiser *et al.*, 1987).

Table 1. Daily intakes of copper from food in EU countries (mg/day)

Country	Type of survey	n	Method	Supplements*	Mean	97.5%
Austria[a]	Individual	2488	24h recall	Not defined	2.0	4.2
Germany[b]	Individual (M)	854	7-day dietary	-	2.2	4.0
	Individual (F)	1134	record	-	1.8	3.3
UK[c]	Individual (M)	1087		-	1.6 (1.5)	3.5
	Individual (F)	1110	7-day weighed	-	1.2 (1.1)	2.8
	Individual (M)	1087	inventory	+	1.6 (1.5)	3.5
	Individual (F)	1110		+	1.2 (1.1)	2.8
Italy[d]	Household	2734	7-day record	+	1.4	2.8
Netherlands[e]	Individual (M, F)	5958	2-day record	-	1.1	1.2
Ireland[f]	Individual (M)	662	7-day estimated	+	1.5	3.1
	Individual (F)	717	food record	+	1.2	2.7

* + data included supplements; - data excluded supplements.
[a] Elmadfa *et al.* (1998).
[b] Heseker *et al.* (1994) (VERA Study) - median values.
[c] Gregory *et al.* (1990) - values are the mean with the median in parentheses.
[d] Turrini (1996).
[e] Hulshof and Kruizinga (1999).
[f] IUNA (2001).

3. HAZARD IDENTIFICATION

3.1 Toxic effects in laboratory animals

Tolerance to high intakes of copper varies greatly from one species to another (Underwood, 1971; Osterberg, 1980). Sheep are most sensitive to copper poisoning and cases of chronic copper poisoning have been reported in lambs fed diets containing 170 mg Cu/kg dry weight (Süveges *et al*, 1971). Rats, however, have a higher tolerance to copper excess (Underwood, 1971; Osterberg, 1980). Because of species differences and the effects of zinc, iron and molybdenum in the diet, the minimum toxic copper level varies. Most rat strains are relatively tolerant of copper, but at intakes exceeding 100 mg/kg body weight, growth is impaired and extensive necrosis of hepatocytes develops (Haywood , 1980). The susceptibility to copper excess is also influenced by the chemical form. In rats, cupric chloride and cupric carbonate are more toxic than cupric nitrate, cupric acetate and cuprous oxide (JECFA, 1982). Manifestations of copper toxicity include weakness, tremors, anorexia and jaundice. As tissue copper levels increase, haemolytic crisis may ensue producing liver, kidney and brain damage.

3.2 Mechanisms of toxicity

Mechanisms of toxicity have been reviewed by Britton (1996). Evidence to date suggests that the hepatic mitochondrion is an important target in hepatic copper toxicity and that oxidant damage to the liver may be involved in the pathogenesis of copper-induced injury. In humans, chronic copper toxicity has its most pronounced effects on liver function whilst acute effects of copper toxicity are primarily observed in the gastrointestinal tract, as a local intestinal irritation effect.

3.2.1 Genotoxicity

3.2.1.1 In vitro

As reported by WHO (1998) the genotoxicity of copper compounds has not been extensively studied. Studies with copper (II) sulphate indicated that it was not mutagenic in strains TA98, TA100 and TA102 of *Salmonella typhimurium* with and without exogenous metabolic activation (Moriya *et al.*, 1983; Marzin and Phi, 1985). Furthermore copper (II) sulphate was found to be negative in the SOS Chromotest in *Escherichia coli* PQ37 (Olivier and Marzin, 1987) and in the rec-assay with *Bacillus subtilis* H17 and M45 (Matsui, 1980), both in the absence of a metabolic activation system. Conversely, copper (II) sulphate did induce a significant increase of unscheduled DNA synthesis (UDS) in cultured rat hepatocytes in a dose range between 7.9 and 78.5 µmol/L (Denizau and Marion, 1989). Copper (II) chloride showed no evidence of mutagenic activity in *S. typhimurium* strains TA98, TA102, TA1535, and TA1537 with and without metabolic activation (Wong, 1988) and was negative in the rec-assay *with B. subtilis* H17 and M45 (Nishioka, 1975; Kanematsu *et al.*, 1980). Copper (II) 8-hydroxyquinoline was weakly mutagenic in strain TA100 *of S. typhimurium* only after metabolic activation, and negative in four other strains of Salmonella and in *E. coli* WP2 hcr (Moriya *et al.*, 1983). Negative results were previously reported in strains TA98, TA100, TA1535 and TA1537, with and without metabolic activation, but the maximum concentration tested was very low (Räsänen *et al*., 1977). Copper (II) nitrate produced dose-related increases in the mutation frequency (resistance to 8-azaguanine) and in the frequency of sister chromatid exchanges in cultured V79 Chinese hamster cells. The authors reported an increase in the molecular weight of DNA, which was attributed to binding of the copper ions to the DNA (Sideris *et al.*, 1988).

3.2.1.2 In vivo

A single i.p. injection of copper (II) sulphate pentahydrate in mice induced a dose-related increase in the incidence of chromatid-type chromosome aberrations in the bone marrow 6 h after dosing between 0.28 and 1.7 mg Cu/kg body weight (Agarwal *et al.*, 1990). Only at the highest dose tested were chromosomal breaks significantly increased. No induction of micronuclei was found in mice given a single injection of copper (II) sulphate pentahydrate at 1.7, 3.4 and 5.1 mg Cu/kg body weight (Tinwell and Ashby, 1990). In a study carried out without a positive control, Bhunya and Pati (1987) reported a significant dose-related increase in the incidence of micronuclei in the bone marrow of mice after two injections at doses between 1.3 and 5 mg Cu/kg body weight per injection.

To summarise, the conflict in experimental data do not allow an adequate evaluation of the genotoxic potential of copper and copper compounds *in vivo*.

3.2.2 Carcinogenicity

Studies on the carcinogenicity of copper compounds in rats and mice have given no indication of carcinogenic potential; however, the available studies present strong limitations in the experimental protocols (small group sizes, limited extent of histopathological examination, inadequate reports) and do not allow the evaluation of the carcinogenic potential of copper compounds with any degree of certainty. These studies are summarized in IPCS (1999), Table 11.

According to the IARC evaluation (1987), copper (II) 8-hydroxyquinoline has been allocated in Group 3 "Not classifiable as to their carcinogenicity to humans", based on inadequate evidence in experimental animals.

3.2.3 Reproductive toxicity

There is some evidence to indicate an effect of exposure to copper compounds on animal reproduction. In some studies in rats, chronic oral exposure to 27-120 mg/kg bw per day of copper resulted in altered weight and/or histology of the testes, seminal vesicles, uterus or ovaries, albeit the results were not consistent. Other studies have demonstrated that exposure to copper compounds during gestation induced embryo/foetotoxic effects at doses of 12 mg of copper/kg body weight and above (IPCS, 1999).

3.3 Toxic effects in humans

Effective homeostatic controls are in place to reduce absorption and increase excretion, if excess copper is ingested. Nevertheless, there are documented cases of acute and chronic copper poisoning.

3.3.1 Acute Toxicity

Acute toxicity is infrequent in humans and is usually a consequence of contamination of food stuffs or beverages from copper containing vessels or dispensers. Acute symptoms include salivation, epigastric pain, nausea, vomiting and diarrhoea (Olivares and Uauy, 1996). Copper ions have an irritant effect on mucosal membranes and daily intakes ranging from 2 to 32mg in drinking water have been reported to cause symptoms of general gastric irritation (US EPA, 1987). Two studies (Pizarro *et al.*, 1999; Donohue, 1997) have identified the threshold for acute gastrointestinal effects from copper in water at about 4.8 mg/day (based on a level of 3 mg copper/L in the water and a mean intake of 1.6 L of water/day). A recent combined international trial determined a NOAEL and LOAEL for effects of nausea in healthy individuals who drank distilled water containing copper as the sulphate salt. An acute NOAEL and LOAEL of 4 mg and 6 mg copper/L, respectively were determined (Araya *et al.*, 2001). Preliminary unpublished data from the same research groups indicate that in a further study as volume increased, the effect of Cu-induced nausea decreased; and as copper dose increased the incidence of nausea increased. An acute NOAEL for nausea in females (more sensitive than males) was confirmed at 4 mg copper/L of bottled water (Araya *et al.*, 2003).

Fatalities from acute copper sulphate poisoning have been reported. An 11 year old female died within hours of accidentally ingesting a solution of copper sulphate (Gulliver, 1991). The postmortem blood sample was found to contain 66 μg/mL copper. In India, copper sulphate poisoning has been used as a method of suicide. Of 48 cases of copper poisoning examined, 12 were fatal and ingested doses ranged from 1 g to 100 g copper dissolved in water (Chuttani

et al., 1965). All cases were characterised by metallic taste, nausea and vomiting. Of the 12 fatal cases, seven apparently died from shock and hypotension or from subsequent renal damage with coma and uraemia. WHO have concluded that the fatal oral dose of copper salts is about 200 mg/kg body weight (WHO, 1993).

3.3.2 Chronic Toxicity

In humans, toxicity to chronic doses of copper has been less extensively studied. There are data to suggest that chronic copper exposure may cause diarrhoea in children (Stenhammar, 1979), gastrointestinal irritation from tap water (Schafer and Schumann, 1991) and acute liver failure (O'Donohue *et al.*, 1993). Scheinberg and Sternlieb (1994) retrospectively examined populations of 0-5 year olds in three towns in Massachusetts from the period 1969-1991. Copper content of the drinking water was 8 mg copper/L. Exposure covering 64,124 child-years did not reveal a single death from any form of paediatric liver disease and no gastrointestinal problems were reported.

3.3.2.2 Carcinogenicity and genotoxicity

Serum copper, caeruloplasmin and other copper binding components (such as transcuprein) are reported to be increased in cancer patients (Campbell *et al.*, 1981, Zowczak *et al*., 2001; Borella *et al*., 1997). However, elevated serum copper or caeruloplasmin in cancer does not necessarily imply increased body copper status as caeruloplasmin is an acute phase protein (DiSilvestro, 1990; Arnaud, 1994; Strain, 1994b) Copper induced DNA lesions have been shown in the liver of patients with Wilson's disease (Carmichael *et al.*, 1995). Organ dysfunction rather than cancer, however, is usually considered the cause of death from Wilson's disease in humans (Linder, 2001). Evidence linking copper toxicity to cancer is, therefore, unsubstantiated at present.

3.3.2.3 Coronary heart disease (CHD)

High copper levels have been cited as a possible risk factor for heart disease (Ferns *et al.*, 1997) and elevated serum caeruloplasmin levels have been observed in patients suffering from cardiovascular disorders (Reunanen *et al*, 1992; Manttari *et al*, 1994) However, serum copper and caeruloplasmin levels are increased as part of the acute-phase response in inflammatory conditions, such as CHD (DiSilvestro, 1990). Indeed, elevation of caeruloplasmin in CHD patients has been shown to be associated with the inflammatory process and not with pro-oxidant activity of caeruloplasmin (Klipstein-Grobusch *et al.*, 1999). Furthermore, there is no evidence of higher rates of CHD in Wilson's disease, a genetic disease associated with copper loading. There is no current evidence to link copper excess with CHD.

3.3.2.4 Neurological disease

Copper has been described as having a critical role in neurological diseases and there is speculation that copper-induced production of hydroxy radicals may contribute to the neurodegeneration in Alzheimer's disease (Multhaup *et al.*, 1996). Recent studies have also implicated copper in the pathogenesis of neuronal injury in prion-mediated encephalopathies (for review see Waggoner *et al.*, 1999); however, evidence is weak, largely speculative and there is no indication that any effects are related to copper status.

3.3.2.5 Effects of copper supplementation

A subject given supplementary copper (30 mg/day) for two years followed by 60 mg/day for an unspecified period developed acute liver failure (O'Donohue *et al.*, 1993). This level of chronic supplementation is extremely rare. Pratt *et al.* (1985) saw no evidence of liver damage or gastrointestinal effects in seven subjects given 10 mg/day supplementary copper as copper gluconate for 12 weeks. Turley *et al.* (2000) saw no effects of six week supplementation with 6 mg/day copper as copper amino acid chelates on LDL oxidizability in 24 healthy male and female subjects (FOODCUE project). Additional results from the FOODCUE project indicate no effect of copper supplementation (3 and 6 mg/day giving total copper intakes of approximately 4 and 7 mg/day respectively) on liver function and mononuclear leucocyte DNA damage as assessed by the comet assay (O'Connor *et al.*, 2003). Moreover, a protective effect of supplementation with 6 mg copper (total intake approximately 7 mg/day copper) on erythrocyte oxidizability was observed in middle-aged subjects (Rock *et al.*, 2000). Urinary pyridinoline and deoxypyridinoline (markers of bone resorption) were significantly increased after six weeks of a low copper diet (0.69 mg/day) compared with a medium copper diet (1.6 mg/day), and significantly decreased on a high copper diet (6.0 mg/day) compared with the low copper diet (Baker *et al.*, 1998).

3.3.3 Reproductive toxicity

In humans, there appears to be no evidence related to oral copper intakes and reproductive toxicity.

3.4 Sensitive subpopulations

3.4.1 Wilson's Disease

Wilson's disease is an autosomal recessive disease of copper storage caused by numerous (over 100 recognised) mutations in the ATPase gene for copper transport. Copper accumulates in the liver, the brain and the cornea of the eye. There appears to be a defect in the catabolism and excretion of caeruloplasmin copper into the bile. Presenting symptoms include hepatic, neurological, and ophthalmological involvement; low serum concentrations of copper and caeruloplasmin and an increase in urinary copper excretion (Tanner *et al.*, 1983). The worldwide incidence of Wilson's disease is 1 in 30,000 and the corresponding prevalence of the heterozygous and asymptomatic carrier of a mutated ATPase gene is 1 in 90 (Scheinberg and Sternlieb, 1996). If the disease goes untreated, copper accumulation in the liver and brain results in hepatitis, haemolytic crisis and hepatic failure may ensue.

3.4.2 Indian Childhood Cirrhosis

Indian childhood cirrhosis (ICC) is a fatal disease of infants in India associated with massive levels of copper accumulation in the liver (Portmann *et al.*, 1978, Tanner *et al.*, 1979). Clinically, ICC differs from Wilson's disease with earlier onset of hepatic abnormalities, normal or high serum concentrations and distinctive hepatic histology (Pandit and Bhave, 1996). Occurrence of ICC has been attributed to the practice of boiling and storing milk in copper and brass vessels (Bhave *et al.*, 1987). However, there also appears to be an element of genetic predisposition in many cases of ICC (Agarwal *et al.*, 1979).

3.4.3 Childhood Idiopathic Copper Toxicosis (ICT)

Idiopathic copper toxicosis has been attributed to high levels of copper (up to 6.8 mg/L) in drinking water (Müller *et al.*, 1996) and there are approximately 30 proven cases in published reports (Müller *et al.*, 1998). In a multicentre retrospective study across 16 paediatric centres in Germany for the years 1982-1994, 103 cases of histologically confirmed early childhood cirrhosis could be identified (Dieter *et al*, 1999). Excessive copper intake from copper plumbing/acid well water was the probable or sole causative environmental factor to trigger the cirrhosis in at most five of the cases. In the cases of confirmed copper associated early childhood cirrhosis, copper concentrations in private well waters were around 10 mg/L or more. Another 138 cases termed Tyrollean infantile cirrhosis have been identified in the Tyrol (western Austria) and have been associated with high dietary copper concentrations (Müller *et al.*, 1996).

4. DOSE RESPONSE ASSESSMENT

The available data clearly show that copper can cause adverse effects in humans and in domestic and laboratory animals. Liver damage is observed almost exclusively in patients with Wilson's disease and children with ICC and ICT. Acute copper toxicity in drinking water appears to have a threshold of approximately 6 mg/L (Araya *et al.*, 2001).

The occurrence of either acute or chronic copper toxicity in humans, however, is rare and tends to be confined to certain subpopulations, such as populations with high copper concentrations in drinking water, populations that utilise copper vessels (e.g. for boiling and storing milk) and those individuals who have a hereditary predisposition to a disease of copper toxicity. There appears, to date, to be little convincing evidence that excess copper is associated with the development of cancer in humans and data are inadequate to assess the reproductive developmental effects of copper excess in humans. Preliminary links between copper intakes and CHD are also inconclusive.

On the basis of the information presented in this report, a critical endpoint from which to derive an upper level (UL) is liver damage. Liver damage is selected because it is perhaps a more reliable indicator of a long-term chronic ingestion of copper. Although gastrointestinal effects of copper toxicity are better documented in humans than liver complications, gastrointestinal effects are more representative of acute copper poisoning. The aim of the UL is to identify safety of maximal copper intakes over a longer period of time.

A case study by O'Donohue *et al.* (1993) observed acute liver failure in a subject taking 30 mg copper a day for two years followed by 60 mg/day for an unspecified period. The retrospective study of Scheinberg and Sternlieb (1994) demonstrated no effect of high concentrations of copper in drinking water (8 mg/L) on the incidence of death from any form of liver disease in children (0-5 years) from three Massachusett towns over a 23 year period. No adverse effects were observed in a double blind study of 12 weeks supplementation with 10 mg copper/day as copper gluconate supplement in seven healthy men (Pratt *et al.*, 1985). A further seven healthy men took placebo for the duration of the trial. Results indicated no effect of copper supplementation on liver function. In the FOODCUE project, no adverse effects on measures of oxidative damage and liver function in 24 healthy male and female subjects given supplements of 6 mg/day copper (approximately 7 mg/day total copper intake) as copper amino acid chelates for six weeks in a cross-over design were found (Turley *et al.*, 2000; O'Connor *et al.*, 2003).

5. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

5.1 Adults

A NOAEL of 10 mg/day is based on the absence of any adverse effects on liver function (as the critical endpoint) in the study of Pratt *et al.* (1985). This was a supplementation study of seven healthy adults with 10 mg/day copper for 12 weeks. In the FOODCUE trial, where copper intakes were around 7 mg/day, no observed adverse effect of copper supplementation on liver function in 24 healthy males and females was observed (O'Connor *et al.*, 2003). In the study by Turnlund *et al.* (1991) homeostatic data indicated that a 10-fold increase in dietary copper resulted in the absorption of only twice as much copper and that indices of copper status, as a result of the body's regulation of copper, are resistant to change except under extreme dietary conditions. For example, Turnlund *et al.* (1990) showed that when dietary intakes increased from 0.8 mg/day to 7.5 mg/day (for 24 days), putative indices of status, including plasma copper, erythrocyte SOD, caeruloplasmin and urinary copper excretion were not significantly different. In the light of this evidence, the Committee decided that an UF of 2 is adequate to allow for potential variability within the normal population. An UL of 5 mg/day is derived.

5.2 Pregnancy and lactation

The upper level of 5 mg/day is not applicable during pregnancy or lactation because of inadequate data relating to this critical life stage.

5.3 Children and adolescents

Liver damage in children appears to be restricted to children with a predisposition for enhanced copper toxicity. Extrapolating adult UL values for children based on relative body weight (using reference weights) result in the values given in the table. These values are consistent with data from the studies of Scheinberg and Sternlieb (1994) and Dieter *et al.* (1999) as discussed above.

Age (years)	Tolerable Upper Intake Level (UL) for Copper (mg per day)
1-3	1
4-6	2
7-10	3
11-14	4
15-17	4

6. RISK CHARACTERISATION

The available studies show that the mean copper intakes of adults and children in EU countries are below the UL. The 97.5 percentile of total copper intakes for all age groups are close to the ULs, which, in the view of the Committee, are not a matter of concern.

The Committee notes that the additional copper intakes from drinking water may be appreciable and may need to be taken into account.

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