OPINION OF THE SCIENTIFIC COMMITTEE ON FOOD ON THE TOLERABLE UPPER INTAKE LEVEL OF ZINC

(EXPRESSED ON 5 MARCH 2002)

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

Zinc has an atomic weight of 65.37 and is classified as a group IIB post-transition metal. In biological systems, zinc exists as Zn^{2+} and is present in all tissue and fluids in the body. Total body content of zinc is between 2 and 4 g and plasma concentration is between 11 and 18 µM (approximately 0.1% of total body content). Urinary zinc excretion is between 300 to 700 µg/day. Zinc is also present in foods and supplements as salts of the divalent cation. Under European legislation the following salts of zinc: acetate, chloride, citrate, gluconate, lactate, oxide, carbonate and sulphate are included in the list of substances that can be used in the manufacture of foods for particular nutritional uses and in food supplements (the legal measure on food supplements is expected to be adopted in the immediate future). Zinc content in the most common single nutrient supplements on the market is 30 mg per capsule, range 15-50 mg and in the most common multiple nutrient supplements is 10-15 mg, range 2-20 mg.

2. NUTRITIONAL BACKGROUND

2.1. Function

Zinc is essential for growth and development, testicular maturation, neurological function, wound healing and immunocompetence. Over 300 zinc enzymes have been discovered covering all six classes of enzymes and in different species of all phyla (Christianson, 1991; Coleman, 1992; Vallee and Auld, 1990). Zinc has structural, regulatory or catalytic roles in many enzymes (Vallee and Galdes, 1984; Hambridge *et al*, 1986). Additionally, it maintains the configuration of a number of non-enzymatic proteins such as pre-secretory granules of insulin, some mammalian gene transcription proteins (Struhl, 1989) and thymulin. Well known zinc containing enzymes include superoxide dismutase, alkaline phosphatase and alcohol dehydrogenase.

2.2. Homeostasis

Absorption of zinc takes place in the small intestine and appears to be a carrier-mediated transport process which is not saturated under normal physiological conditions. At high intakes, zinc is also absorbed through a non-saturable process or passive diffusion (Sandström, 1992). Absorption of dietary zinc ranges from 15 to 60%. Mechanisms for the transport of zinc across the intestinal wall, its export into plasma and its uptake into other tissues are uncertain. Once in plasma, zinc is carried by a number of proteins that include albumin, transferrin and caeruloplasmin. Most of the absorbed zinc is excreted in the bile and eventually lost in the faeces. There appears to be no specific zinc “store” in the body.

Tissue content and activity of zinc-dependent processes are maintained over a wide range of dietary zinc intakes. When zinc intake is increased, the fractional absorption decreases and intestinal excretion increases while urinary losses remain fairly constant. Endogenous faecal zinc losses may increase several fold to maintain zinc homeostasis with high intakes (Coppen and Davies, 1987). At very low zinc intakes, absorption can increase to between 59-84% and faecal and urinary losses decrease accordingly (Baer and King, 1984; Johnson *et al*, 1993; Wada *et al*, 1985). When these primary homeostatic mechanisms are not sufficient to handle large dietary excesses of zinc, the excess zinc is lost via the hair (Jackson, 1989). The kinetics of zinc absorption and elimination follow a two-component model. The initial rapid phase has a half-life in humans of 12.5 days and the slower pool turns over with a half-life of approximately 300 days (Hambridge *et al*, 1986).

2.3. Bioavailability

Interactions with a number of dietary factors influence zinc uptake. Ligands, such as phytate, form insoluble complexes with zinc and prevent absorption. Calcium increases binding of zinc by phytate (Oberleas *et al*, 1966). Larger doses of calcium can decrease net zinc absorption (Spencer *et al*, 1984; Wood and Zheng, 1995). High iron content in the diet decreases zinc absorption. Earlier reports indicated that folic acid can also inhibit zinc retention and metabolism (Milne, 1989; Milne *et al*, 1984; Milne *et al*, 1990), but more recent evidence indicates that folic acid does not adversely affect zinc status (Kauwell *et al*, 1995). Copper and zinc compete for absorption but it appears unlikely that modestly increased intakes of copper interfere with zinc absorption. Histidine, methionine and cysteine are thought to facilitate zinc absorption (these amino acids remove zinc from the zinc-calcium-phytate complexes) (Mills, 1985).

2.4. Dietary and other sources

Good food sources of zinc include red meat, whole wheat, raisins, unrefined cereals (high content, low bioavailability) and fortified cereals. Milk, fruit and vegetables are low in zinc (Sandstead and Smith Jr, 1996).

Concentrations of zinc in tap water may be elevated as a result of dissolution of pipes and contaminated wells may lead to high exposure. Drinking water quality standards for European countries provide a zinc content not more than 5 mg/L (Anon, 1971). Exceeding this value may result in an astringent effect, opalescent appearance and a fine granular sediment. The World Health Organisation recommends that concentrations should not exceed 3 mg zinc/L (WHO, 1993).

Other sources of zinc, excluding dietary intakes, include inhalation of zinc metal or oxide fumes in industrial settings and storage of food and drink in galvanised containers.

2.5. Recommended Dietary Allowances

The European Population Reference Intake (PRI) for zinc (SCF, 1993) for adult males and females is 9.5 mg/day and 7.0 mg/day, respectively. In the UK, the Reference Nutrient Intake (RNI) for zinc is the same as the European PRI and was established in 1991 (Department of Health, 1991). Estimated Average Requirements (EAR) are 7.3 mg/day and 5.5 mg/day for males and females, respectively. In the US, new guidelines recommend daily intakes of 11 mg/day and 8 mg/day for men and women respectively (Institute of Medicine, Food and Nutrition Board, 2001).

2.6. Typical intakes

Mean intakes in Europe (excluding supplements) are 13 mg/day for males and 9 mg/day for females (Van Dokkum, 1995). The estimated mean dietary zinc intakes in several EU countries are given in Table 1. Zinc intakes from vegetarian diets have been shown to be similar to non-vegetarian diets (Hunt *et al*, 1998). However, the dietary requirement for zinc may be as much as 50% greater for vegetarians.

Table 1. Mean and 97.5 percentile zinc intake (mg/day) from food and supplements

Country	Population	n	Method	Supplements*	Mean	97.5%
Austria[a]	Individual	2488	24h recall	Not defined	11.2	21.9
Germany[b]	Individual (M) Individual (F)	854 1134	7-day dietary record	- -	12.1 9.7	20.5 16.0
UK[c]	Individual (M) Individual (F)	1087 1110	7-day weighed inventory	+ +	11.4 (10.9) 8.4 (8.2)	19.0 13.6
Italy[d]	Household	2734	7-day record	+	11	19.0
Netherlands[e]	Individual (M,F)	5958	2-day record	-	9.4	17.0
Ireland[f]	Individual (M) Individual (F)	662 717	7-day estimated food record	+ +	10.8 7.5	23.5 22.1

* + data included supplements; - data excluded supplements.
[a] Elmadfa *et al* (1998)

[b] Heseker *et al* (1994) - median values.
[c] Gregory (1990) - values are the mean with the median in parentheses
[d] Turrini (1996).
[e] Hulshof and Kruizinga (1999).
[f] IUNA (2001).

2.7. Zinc deficiency

Clinically defined zinc deficiency in humans is rare. Zinc deficiency, however, has been observed in patients on total parenteral nutrition, patients taking the chelating agent penicillamine and in acrodermatitis enteropathica, a genetic disease resulting in zinc deficiency. The main clinical manifestations of zinc deficiency are growth retardation, delay in sexual maturation, diarrhoea, increased susceptibility to infections, dermatitis, the appearance of behavioural change and alopecia. Symptoms of mild/marginal zinc deficiency include delayed wound healing, impaired resistance to infection and reduced growth rate (Walsh *et al*, 1994; WHO, 1996).

3. HAZARD IDENTIFICATION

3.1. Toxic effects in farm animals

For review see Lantzsch and Schenkel (1978). Normal zinc concentrations in major feeds and foods ranges from 20 to 80 mg/kg on a dry matter basis (Lantzsch and Schenkel, 1978). Growth rate is affected by zinc intakes, approximating 3.6 g/kg in feed on a dry matter basis, in fillies (Willoughby *et al*, 1972). In drinking water, intakes of zinc at a concentration of 8 mg/L were reported to be toxic for dairy cows (Pickup *et al*, 1954) whilst in a trial feeding dairy cows zinc in food up to 1.279 g/kg feed no toxic effects were observed (Archibald, 1944). In sheep, toxicosis has been observed at intakes of 1 g/kg feed where zinc was in the form of zinc oxide. First cases of death occurred at 3 g/kg zinc in feed (Ott *et al*, 1966). Pigs are also affected by zinc toxicity. Symptoms of non-specific arthritis, including stiffness and lameness were reported in pigs at intakes of zinc above 2 g/kg dry matter. Death occurred frequently within three weeks of treatment (Brink *et al*, 1959; Grimmett and McIntosh, 1936).

3.2. Toxic effects and mechanisms in laboratory animals and *in vitro* studies

In rats, dietary zinc intakes up to 1 g/kg body weight have been well tolerated (Kulwich *et al*, 1953; Sutton and Nelson, 1937; Whanger and Weswig, 1971), but dietary zinc intakes above 2 g/kg body weight have usually led to death (Sadasivan, 1951; Smith and Larson, 1946). Studies in laboratory animals (Sandstead, 1982; 1995) have demonstrated that elevated levels of dietary zinc can have a negative effect upon copper balance and, in part, could explain the induction of a microcytic, hypochromic anaemia in rats after ingestion of large amounts of zinc (for review, see Lantzsch and Schenkel, 1978). The mechanism whereby high zinc intakes antagonises copper status has been clarified (Cousins, 1985). High zinc intakes increase the synthesis of metallothionein in intestinal mucosal cells. Metallothionein avidly binds copper and when mucosal cells are rich in this protein, little copper is able to traverse the cells into the body. Studies in rats have shown that high levels of zinc supplementation (0.5-2 g/kg body weight) can affect iron storage and encourage depletion, interfere with iron uptake in the liver and cause anaemia as a result of higher iron turnover (Walsh *et al*, 1994). Conversely, zinc (10^{-4}M) has been shown to alleviate the toxic effects of cadmium in mice and rabbits (Chiba and KiKuchi, 1984).

Zinc is not teratogenic except when high doses (20 mg/kg body weight) are injected intraperitoneally to mice during pregnancy (Chang *et al*, 1977). Similarly, zinc does not exhibit reproductive toxicity in rats until very high concentrations of 1 g/kg body weight given during pregnancy and which caused a significant reduction in foetal growth, birth weight and still births (Cox *et al*, 1969; Heller and Burke, 1927; Schlicker and Cox, 1968). A total failure of reproduction occurred in rats on zinc intakes of 2 g/kg body weight (Sutton and Nelson, 1937). A published report (MAFF, 1998) reviews zinc toxicity in experimental animals.

3.2.1. Genotoxicity

3.2.1.1. In vitro

Zinc was negative in the majority of tests for induction of gene mutations in bacterial or mammalian cells. In particular, zinc sulphate and zinc acetate were not mutagenic in *Salmonella typhimurium* (Marzin and Vo, 1985; Gocke *et al*, 1981; Thompson *et al*, 1989); zinc 2,4-pentanedione was mutagenic in

Salmonella typhimurium, with and without S9 (Thompson *et al*, 1989). Zinc chloride was not mutagenic in the mouse lymphoma *tK* assay (Amacher and Paillet, 1980); zinc acetate was found positive, with and without S9, both in the mouse lymphoma *tK* assay and in the chromosome aberration assay in Chinese hamster ovary (CHO) cells (Thompson *et al*, 1989). Zinc chloride induced chromosomal aberrations in human lymphocytes (Deknudt and Deminatti, 1978). Zinc acetate and zinc 2,4-pentanedione were negative in the UDS assay in rat hepatocytes. Zinc chloride did not induce cell transformation in Syrian hamster (SHE) cells (Di Paolo and Casto, 1979).

3.2.1.2. In vivo

Zinc sulphate did not induce sex-linked recessive lethal mutations in *Drosophila* (Gocke *et al*, 1981), while zinc chloride induced dominant lethal mutations and sex-linked recessive lethal mutations (Carpenter and Ray, 1969). Zinc sulphate did not induce micronuclei in the mouse (Gocke *et al*, 1981); conflicting results, negative or positive at high doses, were reported for the induction of chromosomal aberrations in the mouse bone marrow by zinc chloride (Deknudt and Gerber, 1979; Vilkina *et al*, 1978; Gupta *et al*, 1991). Zinc chloride did not induce dominant lethal mutations in mice (Vilkina *et al*, 1978).

The weight of evidence from the *in vitro* and *in vivo* genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels at elevated doses, has no biologically relevant genotoxicity activity (reviewed by Walsh *et al*, 1994; WHO, 2001).

3.2.2. Carcinogenicity

No adequate experimental studies are available to evaluate the carcinogenic potential of zinc (WHO, 2001).

3.3. Toxic effects in humans

Zinc is not stored in the body and excess intakes result in reduced absorption and increased excretion. Nevertheless, there are documented cases of acute and chronic zinc poisoning.

3.3.1. Acute toxicity

Acute toxicity is infrequent in humans. Brown *et al* (1964) described several cases of food poisoning resulting from storage of food or drink in galvanised containers. Symptoms of acute zinc toxicity include nausea, vomiting, epigastric pain, abdominal cramps and diarrhoea. One study reported symptoms of lethargy and light-headedness (Murphy, 1970). This change in presenting symptoms could be a result of the type of zinc (in this case zinc sulphate) ingested (Bennett *et al,* 1997). Zinc acetate (25-50 mg, three times per day), given to Wilson's disease patients to prevent copper accumulation was reported (Henderson *et al*, 1995) to cause less dyspepsia than equivalent doses of zinc sulphate. Fosmire (1990) estimated that an emetic dose of zinc corresponds to 225-450 mg. An industrial hazard associated with inhalation of zinc oxide fumes is "metal fume fever". Subjects present with malaise, fever, headache, nausea and dryness of mouth and throat.

3.3.2. Chronic and sub-chronic toxicity

Studies of chronic and sub-chronic toxicity of zinc are well documented. Prolonged intakes of zinc supplements ranging from 50 mg/day up to 300 mg/day have been associated with a range of biochemical and physiological changes. These changes include hypocupraemia, leucopaenia, neutropaenia, sideroblastic anaemia, decreased concentrations of plasma copper and decreased activity of the copper containing enzymes, superoxide dismutase and caeruloplasmin, altered lipoprotein metabolism and impaired immune function (Sandstead, 1995). Many of these biochemical and physiological changes are similar to those observed during copper deficiency. Nevertheless, there are problems with hazard identification in that these changes are not specific to copper deficiency and the clinical relevance of some are unknown. Sensitive sub-populations may include subjects with haemochromatosis and/or insulin dependent diabetes. Zinc excess in water may decrease iron absorption (Rossander-Hulten *et al*, 1991). Hepatic zinc concentration is increased in haemochromatosis (Adams *et al*, 1991) and there is some evidence that zinc absorption may be increased (Adams *et al*, 1991; Spencer *et al*, 1988).

3.3.3. Adverse effects

3.3.3.1. Changes in copper balance

Doses of 75 mg/d of zinc have been used for some time as effective treatment for Wilson's disease. Negative copper balance can be induced in these patients with doses as low as 75 mg/day, provided that the dose is given as 3 x 25 mg of zinc (Brewer *et al*, 1993). Such doses, and those of 100 mg/day

zinc, have been shown to lack toxicity in these patients and have been used effectively to control body copper levels in individuals for 11 years or longer (Najda *et al*, 2001). Increased copper excretion and decreased copper retention (Festa *et al*, 1985; Burke *et al*, 1981) have been demonstrated also during zinc supplementation of healthy subjects. Burke *et al* (1981) observed significantly higher faecal copper excretion and significantly decreased apparent copper retention when 23.3 mg/day compared with 7.8 mg/day of zinc (fed as fortified food; copper intake, 2.33 mg/day) were fed to 5 men and 6 women, aged 56-83 years for a period of 30 days. Supplementation of diet with 18.2 mg/day zinc for 2 weeks (Festa *et al*, 1985) in young male subjects demonstrated increased faecal copper excretion and decreased apparent retention of copper. No significant change in copper balance was found when 14 adolescent girls were fed 13.4 mg/day compared with 7.4 mg/day zinc (Greger *et al*, 1978). Other studies examining alterations in copper excretion after zinc supplementation found no effect of increasing zinc intakes from 8 mg/day up to 24 mg/day for 18 days (Taper *et al*, 1980) or zinc intakes of 19.9 mg/day for 24 days (Colin *et al*, 1983). Taken together, these studies suggest that an intake of zinc of some 9 mg/day or more over recommended dietary allowances can affect balance at least in the short term. Balance studies, however, may not be indicative of homeostasis in the longer term and it may take three weeks or more before copper absorption and retention are stabilised after changes in copper intake (Turnlund *et al*, 1989). More recently, Milne *et al* (2001) found that 21 postmenopausal women fed 53 mg/day of zinc with adequate copper (3 mg/day) for 90 days maintained positive copper balance, whereas a low zinc (3 mg/day) regimen for 90 days produced a negative copper balance.

3.3.3.2. Decreased activity of copper-dependent enzymes

One of the most consistent findings of zinc supplementation studies is the decrease in erythrocyte copper-zinc superoxide dismutase (SOD) activity. Fischer et al (1984) found decreased erythrocyte SOD activity after 6 weeks of supplementation with 50 mg (2 x 25 mg)/day zinc as gluconate in 26 healthy adult men. Samman and Roberts (1988) studied the effects of 150 mg/day zinc as sulphate on healthy young women (n = 26) and men (n = 21) in a double-blind crossover trial lasting 12 weeks. Significant decreases in erythrocyte SOD activity were observed in the females only. Yadrick *et al* (1989) found significant decreases in erythrocyte SOD activity when 18 healthy adult women received 50 mg/day zinc as gluconate for 10 weeks.

Decreases in other putative indices of copper status have been observed in some studies. For example, Prasad *et al* (1993) found decreased serum copper in 44 older adults (who were zinc deficient, with high inflammatory status) given 20 mg/day zinc in an eight week crossover study. Fischer *et al* (1984) and Yadrick *et al* (1989) found no such changes. Similarly, Black *et al* (1988) who gave young men 50 mg/day or 75 mg/day for 12 weeks, found no change in serum copper concentration. Samman and Roberts (1988) found decreased activity of serum caeruloplasmin but no change in serum copper at 150 mg/day for 12 weeks. Bonham *et al* (2002b) found that zinc supplementation for 14 weeks at 40 mg/day zinc (diet and supplement) did not affect erythrocyte SOD activity and caeruloplasmin concentration or activity in 19 healthy men, whose intake was estimated at 1.2 mg/day copper, compared with placebo controls (n=19). Given the differences in the responses of these putative indices of copper status to the same or similar zinc supplementation regimens, it is possible that the observed decreased erythrocyte SOD activity is not directly related to decreased copper status.

The recent findings of Milne *et al* (2001) question the adverse physiological significance of the decreased erythrocyte SOD activity observed in a number of zinc supplementation studies.

These workers found that intake of zinc at 53 mg/day did not induce changes indicative of decreased copper status or function in 21 post-menopausal women fed low dietary copper (1 mg/day) for 90 days in a metabolic unit. Results suggested that inadequate intake of 3 mg/day zinc was a nutritional stress of copper metabolism and status. These women were in positive copper balance only when the diet provided 53 mg/day zinc and 3 mg/day copper (see section 3.2.3.1). Irrespective of dietary copper intake, high dietary (53 mg/day) zinc compared with low dietary (3 mg/day) zinc, decreased erythrocyte SOD activity but the largest fall in erythrocyte SOD activity was when the women were fed low dietary zinc and copper. Another putative index of copper status, platelet cytochrome c oxidase activity on a platelet number basis, was significantly lower during low dietary than during high dietary zinc intake. Findings with respect to another copper protein, caeruloplasmin, indicate that a moderately deficient (3 mg/day) intake of zinc is more detrimental to copper metabolism and function than a moderately high (53 mg/day) intake. When the women were fed high dietary zinc whole blood glutathione concentration and erythrocyte glutathione peroxidase activity were reduced in comparison with low dietary zinc.

In conclusion, decreased erythrocyte SOD activity, although the most consistent biochemical finding from studies measuring the influence of zinc on putative indices of copper status (Fischer *et al*, 1984;

Samman and Roberts, 1988; Yadrick *et al*, 1989; Milne *et al*, 2001), is not accompanied by adverse effects and is not considered to be a marker of decreased copper status. The physiological relevance of lowered erythrocyte SOD activity in these studies, therefore, is unclear.

3.3.3.3. Lipoprotein and cholesterol metabolism

Two studies have found that zinc supplementation at doses of 50 mg/day and 75 mg/day for 12 weeks (Black et al, 1988) and 160 mg/day for 6 weeks (Hooper *et al*, 1980) decreased high density lipoprotein (HDL) concentrations in male subjects. In contrast, Samman and Roberts (1988) observed no decrease in HDL concentrations in males with zinc supplementation doses of 150 mg/day for 12 weeks, but rather found some indication of decreased low density lipoprotein (LDL) in females. Similarly, Freeland-Graves *et al* (1982) observed no consistent change in HDL cholesterol in women after eight weeks at 100 mg/day zinc. Two more recent studies have also found no adverse changes in lipoprotein metabolism. Bonham *et al* (2002a) found no change in HDL, LDL or triglycerides in healthy men after 14 weeks of zinc intakes at 40 mg/day whereas Milne *et al* (2001) found that total and LDL cholesterol concentration decreased with 53 mg zinc supplementation for 90 days. Lower doses (20 mg/day zinc) in elderly subjects have shown no effect on lipoprotein metabolism (Boukaïba *et al*, 1993). Collectively, these data indicate no consistent adverse effects of zinc supplementation giving total intakes in the range 40-160 mg/day zinc on lipoprotein and cholesterol metabolism.

3.3.3.4. Changes in haemoglobin and blood profile

Very high intakes of zinc over long periods have resulted in anaemia and changes in red and white blood cells indicative of copper deficiency. Patients with sickle cell anaemia (Prasad *et al*, 1978) and coeliac disease (Porter *et al*, 1977) treated with 150 mg/day zinc for 23 months and 10 months respectively developed clinical signs of copper deficiency characterized by hypocupraemia, anaemia, neutropaenia and leucopaenia. These complications could be corrected by copper supplementation or cessation of zinc supplementation. Zinc supplementation at 50 mg/day zinc for 10 weeks decreased haematocrit but had no effect on haemoglobin (Yadrick *et al*, 1989) whereas doses of 150 mg/day for 12 weeks had no affect on haematocrit (Samman and Roberts, 1988). Inclusion of iron (50 mg/day) supplements ameliorated the effects on iron status in the former study. Zinc intake at 40 mg/day had no effect on full blood profile data and flow cytometric analyses of lymphocyte subsets (Bonham *et al*, 2002b). No consistent adverse effects on blood profiles, therefore, have been observed at intakes of zinc below 60 mg/day.

3.3.3.5. Reproductive effects

Dietary supplementation with zinc at 20 mg/day did not result in adverse effects of pregnancy progress or outcome in healthy pregnant women in a number of large, controlled trials (Hunt *et al*, 1984; Kynast and Saling, 1986; Mahomed *et al*, 1989). Similarly, supplementation with zinc at 30 mg/day did not result in any adverse outcomes in a double blind trial involving low income pregnant adolescents (n=268 at delivery) thought to have low zinc status (Cherry *et al*, 1989). In a smaller study, Jameson (1976) gave zinc supplements of 90 mg/day to seven pregnant women with low serum zinc concentrations and found no adverse effects. Moreover, in a follow-up study by the same author (Jameson, 1982), 133 women with low serum zinc concentrations were randomly assigned to either zinc supplementation at 45 mg/day or no supplementation and no adverse effects were reported. These data indicate that zinc supplementation at doses of 20-90 mg/day produce no adverse effects on pregnancy outcome.

3.3.3.6. Other adverse effects

Excessive intake of zinc (300 mg/day) for six weeks can impair immune responses, ie. reduction in lymphocyte stimulation response to phytohaemaglutinin as well as chemotaxis and phagocytosis of bacteria by polynuclear leucocytes (Chandra, 1984).

Although individuals with insulin-dependent diabetes mellitus have chronic hyperzincuria, they do not appear to be zinc deficient (Cunningham *et al*, 1994). Supplementing such individuals (n=7) with 50 mg/day zinc was reported to cause an elevation of HbA_{1C} in each of the seven subjects (Cunningham *et al*, 1994) but the clinical significance of this observation is unclear, given the short duration of the study and the absence of any change in blood glucose concentrations.

Zinc supplementation at 53 mg/day for 90 days can increase bone-specific alkaline phosphatase (a possible indicator of bone formation) in 25 post-menopausal women (Davis *et al*, 2000).

4. DOSE-RESPONSE ASSESSMENT

The available data clearly show that zinc can cause adverse effects in humans and in domestic and laboratory animals. In humans, the most prominent effects of acute zinc toxicity are gastrointestinal disturbances.

Chronic zinc toxicity, undoubtedly, is associated with symptoms of copper deficiency. These overt adverse effects (e.g. anaemia, neutropaenia, impaired immune responses) are evident only after feeding zinc in the form of dietary supplements in excess of 150 mg/day for long periods. It is much more difficult to identify the critical effect of zinc excess at intakes below 100-150 mg/day. Short-term balance studies would indicate adverse effects on copper retention at intakes as low as 18.2 mg/day zinc (Festa *et al*, 1985). Recent longer-term balance studies, however, indicate that positive copper balance can be maintained at 53 mg/day zinc in post-menopausal women for 90 days provided copper intakes are adequate to high (3 mg/day). High dietary zinc, however, did not exacerbate the non-positive copper balance in the women fed low (1 mg/day) dietary copper nor did the higher (3 mg/day) copper diet induce positive copper balance in the women fed low (3 mg/day) dietary zinc (Milne *et al*, 2001). The occurrence of adverse (lower HDL, higher LDL cholesterol) effects on lipoprotein metabolism is inconsistent at zinc intakes below 100 mg/day.

In conclusion, clear adverse effects on copper balance and an array of measures of copper status or lipoprotein metabolism cannot be detected at 53 mg/day zinc, when copper intakes are adequate at 3 mg/day (Davis *et al*, 2000; Milne *et al*, 2001), nor on copper status, lipoprotein metabolism, blood profile and circulating levels of peripheral blood leucocytes and lymphocyte subsets at 40 mg/day zinc (Bonham *et al*, 2002a). Collectively, these data indicate that a NOAEL for zinc is around 50 mg/day.

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

5.1. Adults

A NOAEL of 50 mg/day is based on the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) in the studies of Davis *et al* (2000), Milne *et al* (2001), Bonham *et al* (2002a, 2002b). Subjects were 25 and 21 healthy post-menopausal women in the study of Davis *et al* (2000) and Milne *et al* (2001) and 19 healthy young men in the studies of Bonham *et al* (2002a, 2002b). Duration of supplementation was for 90 days in the studies of Davis *et al* (2000) and Milne *et al* (2001) and for 14 weeks in the studies of Bonham *et al* (2002a, 2002b). Total zinc and copper intakes were tightly controlled in the metabolic studies of Davis *et al* (2000) and Milne *et al* (2001) in which the zinc intake was 53 mg/day. Total zinc intake in the studies of Bonham *et al* (2002a, 2002b) was 30 mg/day from supplements on top of 10 mg calculated from dietary intake estimates (total 40 mg/day). An UF of 2 is applied owing to the small number of subjects included in relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed. An UL of 25 mg/day is recommended.

5.2. Pregnancy and lactation

Available data indicate that pregnant women do not have increased susceptibility to zinc supplementation. Therefore the UL of 25 mg zinc per day applies also to pregnant and lactating women.

5.3. Children and adolescents

There are no data on adverse effects of zinc intakes on children and adolescents. On the other hand, there are no data to indicate that children or adolescents are more susceptible to adverse effects of zinc. Therefore, in the absence of adequate data the Committee chooses to extrapolate the UL from adults to children on a surface area (body weight$^{0.75}$) basis. The reference weights derived by the Scientific Committee on Food (SCF, 1993) are used as a basis for the calculations of surface area and UL.

Age (years)	Tolerable Upper Intake Level (UL) for Zinc (mg per day)
1-3	7
4-6	10
7-10	13
11-14	18
15-17	22

6. RISK CHARACTERISATION

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

7. REFERENCES

Adams PC, Bradley C, Frei JV (1991). Hepatic zinc in hemochromatosis. Clin Invest Med 14: 16-20.

Amacher DE and Paillet SC (1980). Induction of trifluorothimidine-resistant mutants by metal ions in L5178Y *tK* +/- cells. Mutat Res 78: 279-288.

Anonymus (1971). Einheitliche Anforderungen an die Beschaffenheit, Untersuchung und Beurteilung von Trinkwasser in Europa. Schriften Ver Wasser Boden Lufthygiene. Berlin Dahlem, 14b, 1-50.

Archibald JG (1944). Zinc in cow's milk. J Dairy Sci 27: 257-261.

Baer MJ and King JC (1984). Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am J Clin Nutr 39: 556-570.

Bennett DR, Baird CJ, Chan KM, Crookes PF, Bremner CG, Gottlieb MM, Naritoku WY (1997). Zinc toxicity following massive coin ingestion. Am J Forensic Med Pathol 18: 148-153.

Black MR, Medeiros DM, Brunett E, Welke R. (1988). Zinc supplements and serum lipids in young adult white males. Am J Clin Nutr 47: 970-975.

Bonham M, O'Connor JM, Walsh PM, McAnena LB, Downes CS, Hannigan BM, Strain JJ (2002a). Zinc supplementation has no effect on lipoprotein metabolism, hemostasis and putative indices of copper status in healthy men. Biol Trace Elem Res (in press).

Bonham M, O'Connor JM, Alexander HD, Coulter SJ, Walsh PM, McAnena LB, Downes CS, Hannigan BM, Strain JJ (2002b). Zinc supplementation has no effect on circulating levels of peripheral blood leucocytes and lymphocyte subsets in healthy adult men. Br J Nutr (in press).

Boukaïba N, Flament C, Acher A, Chappuis P, Piau A, Fusselier M, Dardenne, M, Lemonnier D (1993). A physiological amount of zinc supplementation: effects on nutritional, lipid and thymic status in an elderly population. Am J Clin Nutr 57: 566-572.

Brewer GJ, Yuzbasiyan-Gurkan V, Johnson V, Dick RD, Wang Y (1993). Treatment of Wilson's disease with zinc XII: Dose regimen requirements. Am J Med Sci 305: 199-202.

Brink MF, Becker DE, Terrill SW, Jensen AH (1959). Zinc toxicity in the weanling pig, J Anim Sci 18:836-842.

Brown MA, Thom JV, Orth GL, Cova P, Juarez J (1964). Food poisoning involving zinc contamination. Arch Environ Health 8: 657-660.

Burke DM, DeMicco FJ, Taper LJ, Ritchey SJ (1981). Copper and zinc utilisation in elderly adults. J Gerontol 36: 558-563.

Carpenter JM and Ray JH (1969). The effect of zinc chloride on the production of mutations in *Drosophila melanogaster*. Am Zool 9: 1121.

Chandra RK (1984). Excessive intake of zinc impairs immune responses. JAMA 252: 1443-1146.

Chang CH, Mann DE, Gautieri RF (1977). Teratogenicity of zinc chloride, 1,10-phenanthroline, and a zinc-1,10-phenanthroline complex in mice. J Pharm Sci 66: 1755-1758.

Cherry FF, Sandstead HH, Rojas P, Johnson LK, Baston HK and Wang XB (1989). Adolescent pregnancy: associations among body weight, zinc nutrition, and pregnancy outcome. Am J Clin Nutr 50: 945-954.

Chiba M and KiKuchi H (1984). The in vitro effects of zinc and manganese on δ-aminolevulinic acid dehydratase activity inhibited by lead or tin. Toxicol Appl Pharmacol 73: 388-394.

Christianson DW (1991). The structural biology of zinc. Adv Prot Chem 42: 281-335.

Coleman JE (1992). Zinc proteins: enzymes, storage proteins, transcription factors and replication proteins. In: Annua Review of Biochemistry (Richardson CC, Abelson JN, Meister A and Walsch ST. Editors), pp 897-946, Annual Reviews Inc., Palo Alto CA.

Colin MA, Taper LJ, Ritchey SJ (1983). Effect of dietary zinc and protein levels on the utilization of zinc and copper by adult females. J Nutr 113: 1480-1488.

Coppen DE and Davies NT (1987). Studies on the effects of dietary zinc dose on ^{65}Zn absorption *in vivo* and on the effects of Zn status on ^{65}Zn absorption and body loss in young rats. Br J Nutr 57: 35-44.

Cousins RJ (1985). Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 65: 238-309.

Cox DH, Schlicker SA, Chu RC (1969). Excess dietary zinc for the maternal rat, and zinc, iron, copper, calcium and magnesium content and enzyme activity in maternal and foetal tissues. J Nutr 98: 459-466.

Cunningham JJ, Fu A, Mearkle PL, Brown RG (1994). Hyperzincuria in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high-dose zinc supplementation. Metabolism 43: 1558-62.