Risk Assessment — Zinc

General information

Chemistry

Zinc is an abundant group IIB post-transition metallic element. It occurs in nature as the sulphide (ZnS), in blende and sphalerite, as the silicate ($ZnSiO_4$), in calamine, willemite and zinc spar, and as the oxide (ZnO) in zincite. Within this risk assessment document the word zinc refers to ionic zinc except where specific zinc compounds are mentioned.

Natural occurrence

Zinc is present in the earth's crust and in seawater. Zinc is found in all plant and animal tissues, particularly inside the nuclei.

Occurrence in food, food supplements and medicines

Meat and cereal products are rich in zinc. Zinc salts are also widely available in food supplements. In the UK, zinc supplements provide intakes up to 50 mg/day.

Zinc is present in a number of licensed medicines both alone and with other ingredients, and is used for the treatment and prevention of deficiency. The maximum daily dose of products available from a pharmacist is 150 mg, and of products available on the General Sales List is 5 mg.

Other sources of exposure

Intake of zinc from drinking water is normally very low, but may be increased by dissolution of zinc from pipes and contaminated wells. The UK current regulatory limit in water (based on taste and appearance) is 5 mg/L. Many cases of food poisoning incidents have resulted from the storage of food or drink in galvanised containers.

Recommended amounts

The UK RNI ranges set by COMA are 5.5-9.5 mg/day for males and 4.0-7.0 mg/day for females (COMA, 1991). The US Recommended Daily Allowance (RDA) is 15 mg/day for males and 12 mg/day for females.

Analysis of tissue levels and zinc status

Reliable, validated measures of zinc status are not available. Plasma, blood, hair and urinary zinc can be measured, but these may be affected by conditions unrelated to zinc deficiency.

Brief overview of non-nutritional beneficial effects.

Zinc sulphate is used to treat Wilson's disease. Studies have also reported its use to enhance wound healing and slow macular degeneration. Zinc has also been claimed to ameliorate a variety of conditions including the common cold.

Function

Zinc is an essential constituent of more than two hundred metalloenzymes. Zinc plays a key role in the synthesis and stabilisation of genetic material and is necessary for cell division and the synthesis and degradation of carbohydrates, lipids and proteins.

Deficiency

Zinc deficiency results in effects including poor prenatal development, growth retardation, mental retardation, impaired nerve conduction and nerve damage, reproductive failure, dermatitis, hair loss, diarrhoea, loss of appetite, loss of taste and smell, anaemia, susceptibility to infections, delayed wound healing and macular degeneration.

Interactions

Zinc and copper are mutually antagonistic, each interfering with the gastrointestinal uptake of the other thus potentially leading to imbalance. Similarly, zinc and iron compete for absorption. Excess zinc may also decrease magnesium and calcium uptake, and conversely, high levels of calcium in the diet can decrease zinc absorption. Zinc salts reduce the bioavailability of fluoroquinoline antibiotics.

Absorption and bioavailability

Absorption of zinc salts from food is approximately 20-40%, being higher from fish and meat but lower from cereals, where phytate content impairs absorption. The absorption of zinc salts depends on their solubility. Zinc is absorbed both by passive diffusion and an unknown membrane carrier process, which requires energy.

Distribution and metabolism

The mechanism for transfer across the gut wall is by both passive diffusion and an unknown membrane associated carrier-mediated process, which requires energy and occurs throughout the small intestine. Following internalisation in the intestinal cell, zinc associates with metallothionein. Once in plasma, zinc binds to a number of proteins including albumin, transferrin, α-2-macroglobulin and ceruloplasmin (ferroxidase). Little is known about uptake, but zinc is found in all cells, particularly inside the nuclei, of all tissue types. Some tissues are particularly rich in zinc. These include the choroid of the eye, adrenal gland, skin, certain areas of the brain, pancreas and the prostate gland.

Excretion

Since zinc is not stored, the balance between absorption and excretion (homeostasis) is essential to the maintenance of a broad spectrum of zinc-dependent functions. Zinc excretion is largely via the faeces, which contain unabsorbed zinc as well as biliary and pancreatic secretions.

Toxicity

Human data

The symptoms of acute zinc salt toxicity include abdominal pain, nausea and vomiting. Other reported effects include lethargy, anaemia and dizziness. Prolonged use of high doses of zinc can result in secondary deficiency of copper. Symptoms of this include hypocupraemia, impaired iron mobilisation, anaemia, leukopenia, neutropenia, decreased superoxide dismutase (SOD) (particularly erythrocyte SOD (ESOD)), decreased ceruloplasmin, decreased cytochrome c oxidase, increased plasma cholesterol, increased LDL:HDL cholesterol, decreased glucose clearance, decreased methionine and leucine enkephalins, abnormal cardiac function and impairment of pancreatic enzymes, amylase and lipase. It has also been suggested that excess zinc is atherogenic.

Supplementation trials

A variety of supplementation studies are available. Many of these investigate the effects of zinc supplementation on various medical conditions or physiological systems. However, some work has also been undertaken suggesting that high levels of zinc can adversely affect copper and iron status.

Animal data

Very high doses of zinc can cause minor neural degeneration, acinar cell necrosis and metaplasia in the pancreas, decreased haematocrit and decreased white blood cell count. Very high doses have also been shown to cause reproductive toxicity in rats. Lower doses have resulted in reduced ceruloplasmin activity and decreased haemoglobin levels.

Carcinogenicity and genotoxicity

Zinc has been found to give positive results in some *in vitro* and *in vivo* genotoxicity tests. No data have been identified on the carcinogenicity of zinc.

Vulnerable groups

A small study suggests that zinc supplementation increases the levels of glycosylated haemoglobin in diabetics.

Genetic variations

Sufferers from haemochromatosis may absorb larger amounts of zinc indicating possible increased risk of zinc-induced copper deficiency.

Mechanisms of toxicity

No data have been identified on the mechanisms of toxicity.

Dose response characterisation

In humans, the acute toxic effects of zinc occur at doses of approximately 200 mg or more. ESOD activity, thought to be the most sensitive measure of copper status, was shown to decrease following supplementation with 50 mg/day or more for 12 days. However, other studies have reported no effect at doses of 50 mg/day. Supplementation with 50 mg/day for up to 10 weeks has further resulted in reductions in haematocrit and serum ferritin. Doses greater than 100 mg/day have resulted in altered ratios of HDL:LDL cholesterol. Copper deficiency and sideroblastic anaemia, associated with chronic zinc ingestion, were reported in one individual who had taken non-prescribed zinc supplements of 26.6 – 40 mg/day for at least 2 years.

In animal studies, acute effects occur at doses greater than 480 mg/kg bw/day. Reproductive toxicity in rats has been observed at doses above 200 mg/kg bw/day.

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).

Secondary copper deficiency

General

Sandstead, 1995

This paper discusses the inhibitory effect of zinc on copper absorption and the risk of secondary copper deficiency increasing with high zinc:copper molar ratios. The signs of secondary copper deficiency include decreased ESOD, increased LDL cholesterol, decreased HDL cholesterol, decreased glucose clearance, decreased methionine and leucine enkephalins and abnormal cardiac function. ESOD activity is thought to be particularly sensitive to decreased copper status. The author suggested a preliminary reference dose of 9 mg/day for 60 kg adults for over the counter (OTC) zinc, assuming high bioavailability and uncertain copper intakes.

The Age-Related Eye Disease Study Research Group, 2002

The effects of supplementation for 5 years with 80 mg/day zinc (as zinc oxide) plus either antioxidants (500 mg/day vitamin C, 268 mg/day vitamin E, 15 mg/day ß-carotene) or 2 mg/day copper (as copper oxide) on serum levels of zinc, copper, lipids and haematocrit were reported in this paper. No significant differences in serum haematocrit, copper or lipids were observed at the end of the 5-year period compared to placebo controls. However, only 20% of participants were followed up for the full 5 years and no group received zinc alone. It is therefore difficult to draw conclusions from this study.

HDL, LDL and cholesterol

Hooper et al., 1980

Twelve healthy male volunteers (23-25 years) were given 160 mg/day zinc (as zinc sulphate, taken with meals) for 5 weeks. Eight control subjects received placebo treatment. After 5 weeks treatment, a 25% reduction ($p<0.001$) in HDL-cholesterol levels was measured. No effect on total cholesterol, LDL-cholesterol, or triglyceride was found. No decline in these parameters was apparent in the controls. HDL levels continued to decline for a further two weeks after cessation of treatment. Eleven weeks after the cessation of treatment, the HDL values had returned to near baseline. Plasma copper was not decreased throughout. The amounts of zinc and copper provided by food and beverages were not stated. This was a small scale, presumably single-blinded, study.

Pachotikarn et al., 1985

Twenty three male volunteers were given 50 mg zinc/day (as gluconate) for 6 weeks. Subjects were asked to avoid foods high in copper, phytate and fibre during the study. A statistically non-significant increase (approximately 16%) in HDL cholesterol and a decrease in total cholesterol with a significant decrease in diastolic blood pressure were measured. Dietary zinc and copper intakes were in the range 11.9-12.4 and 0.93-1.01 mg/day during the study. The zinc:copper intake ratio was estimated to be 60:1. Haematocrit, haemoglobin and plasma copper levels were not significantly altered from baseline. No control group was included in the study, so it is not possible to assess the potential effect of dietary changes on the parameters measured.

Black et al., 1988

Groups of 9-13 male volunteers (19-29 years) were given supplements of 0 (placebo), 50 or 75 mg zinc/day (as gluconate, consumed after breakfast with water) for 12 weeks. HDL cholesterol was decreased by approximately 14% at weeks 6 and 12 of treatment in the higher dose group and at week 12 in the lower dose group. However, at weeks 8 and 10, levels of HDL cholesterol in both treated groups were higher than the controls. It was noted that week 10 was a holiday in which the volunteers left the campus. Serum zinc levels increased in the high but not the low dose group. Serum copper levels were not altered. Mean dietary copper levels were 2.1, 1.8 and 1.7 mg/day for the placebo, 50 mg and 75 mg zinc supplement groups, respectively. Mean dietary zinc intakes were 12.6, 14.1 and 9.8 mg/day. This was a double-blind study. Details of diet were collected to establish copper and zinc intake from food. The number of participants was small and the protocol demanding since a number of dietary restrictions were requested (high phytate and high copper foods). This was monitored by the use of bi-weekly 3-day dietary records. However, limited compliance with the dietary restrictions may explain some of the variations. In the high dose group, total and saturated fat and protein intakes were lower than in the other groups.

Samman and Roberts, 1988

As part of a double blind crossover study, 20 female (mean age 27 years) and 21 male volunteers (mean age 28 years) were given 150 mg (3 x 50mg) zinc/day (as sulphate) for 6 weeks. Plasma zinc increased significantly on treatment. There were no changes in total plasma cholesterol levels in either sex. However, in females, LDL cholesterol was decreased by 9% while ESOD and caeruloplasmin activities were reduced. Plasma copper and haematocrit remained unchanged. Gastrointestinal and other effects were reported by some of the subjects leading to 6 females dropping out of the study (see also Samman and Roberts, 1987 below). Differences between the sexes were attributed to females receiving a

higher dose on a mg/kg basis (mean bodyweights were 73.9 and 61.3 for males and females respectively). Copper intake was not stated.

ESOD and other enzyme activities

Taper et al., 1980

Groups of 5 or 6 adult women of child bearing age, were fed dietary regimes containing 2 mg/day copper and 8, 16, or 24 mg/days zinc for 18 days. Plasma levels of zinc and copper were increased and decreased, respectively. However, zinc had no effect on copper retention at any dose and the negative copper balance observed in each treatment group was unrelated to zinc dose. There was no functional assessment of copper status in this study.

Fischer et al., 1984

Groups of 13 healthy males, were given a placebo or a supplement of 50 mg zinc/day (as gluconate taken as two doses) for 6 weeks. The volunteers were divided so that baseline caeruloplasmin and ESOD activity were the same within the two groups. In the treatment group plasma zinc levels were elevated. ESOD activity decreased following 4 weeks of treatment being reduced by a maximum of 20% after 6 weeks. Plasma copper and caeruloplasmin activity were unaffected by zinc treatment. The amounts of zinc and copper provided by food and beverages were not stated. The time taken for ESOD to decline was thought to be related to erythrocyte turnover. This was a reasonable double blind study, though the numbers are small and there is no information on dietary copper or zinc intake.

Festa, 1985

Nine healthy male volunteers were given a basal egg-white diet that provided recommended levels of all essential nutrients, with the exceptions of zinc and protein where the diet provided 1.8 mg zinc and 16.4 g nitrogen daily. Copper intake was 2.63 mg/day. Zinc carbonate was added to give a total zinc intake of 20.7 mg/day (week 1), 18.5 mg/day (weeks 3, 5, 6, 8, 9), 1.8 mg/d (week 2) 1.8 or 8.0 mg/day (week 4) and 4, 6 or 8 mg/day (week 7). It was reported that a total zinc intake of 18.5 mg/day for 2 weeks following on from a week at a lower intake resulted in reduced apparent retention and increased excretion of copper. Mean plasma copper concentrations remained within the normal range throughout the study, but mean faecal copper excretion was elevated over copper intake in week 6. However, the biological significance of this result is uncertain. Negative copper balance was not repeated in week 9 and there was no measurement of any functional index of copper status.

Yadrick et al., 1989

Nine female volunteers (25-40 years) were given supplements of 50 mg/day zinc (as twice daily gluconate capsules) for 10 weeks. ESOD activity, haematocrit and serum ferritin were significantly reduced (by 50%, 5% and 30% respectively) compared with pre-treatment levels. Effects on ferritin and haematocrit but not ESOD were ameliorated when equal (mg for mg) iron supplementation was given to a second group of nine women (at the start of study, the volunteers were divided so that baseline ferritin and ESOD levels were the same). The amount of zinc and copper provided by food and beverages during the study was not stated. This was a reasonable double blind study, though the numbers were small and there is no information on dietary copper or zinc intake.

Abdallah and Samman, 1993

Six healthy female volunteers (age range 18-36 years), were given zinc capsules (50 mg/day as zinc sulphate, taken with breakfast) for 12 days. A significant, 20%, decrease in ESOD activity was measured. Dietary zinc was estimated as 9-12 mg/day. Copper intake was not stated. Small increases in erythrocyte and plasma zinc were observed but were not significant. Stomach cramps and a 'burning sensation' after swallowing were reported by some volunteers. The study involved small numbers and there was no control group. The results are reasonably consistent with those of Yadrick (see above).

Cunningham et al., 1994

Following supplementation with 50 mg/day zinc (as gluconate) for 28 days, 45% and 20% increases in HbA_{1c} were reported in insulin dependent diabetes mellitus (IDDM) patients (n=14, 18-37 years) and non-IDDM individuals (n=15, 23-38 years), respectively, suggesting glycosylation becomes altered in a milieu of zinc excess. In this study, plasma and erythrocyte copper did not differ significantly from baseline levels. Dietary zinc was estimated to be 11.2 and 8.7 mg/day in the IDDM and non-IDDM individuals respectively, reflecting the sex distribution in the groups. Copper intake was not stated. Small numbers were involved in this study and there were no placebo control subjects.

Davis et al., 2000

A study was conducted on a metabolic ward, 25 healthy post-menopausal women were fed diets containing low (1 mg/day) or high (10 mg/day) copper for 180 days. For one 90 day period, the women were given a supplement of 50 mg zinc/day and for the other 90 day period, a placebo. The basal diet contained 3 mg zinc/day. Zinc supplementation significantly increased extracellular superoxide dismutase activity but did not affect erythrocyte superoxide dismutase. This effect was more apparent when the subjects received the low copper diet. The study is unusual in that it was conducted on a metabolic ward, where a high degree of control over the zinc and copper intakes of the subjects was possible.

Other adverse effects

Samman and Roberts, 1987

This paper describes details of the side effects that occurred in the study of Samman and Roberts (1988), described above. Symptoms were reported by 84% of the female and 18% of the male volunteers. These included headaches, abdominal cramps, nausea, loss of appetite and vomiting. The symptoms were particularly apparent when small meals or no food was taken with the supplements (contrary to instructions). It was considered that, although a sex difference could not be ruled out, the higher number of symptoms in the female volunteers could be due to their lower body weight. Six subjects dropped out of the study, 5 due to the abdominal cramps and one due to persistent headaches. All were women receiving a dose of 0.86 mg/kg.

Hale et al., 1989

Health questionnaires, electrocardiograms, and laboratory and medication use data from a health screening program for older people were compared for 69 participants taking zinc supplements (20 to 150 mg/day for an average duration of 8 years) with 1,832 subjects who were not taking supplements. No association was found between zinc supplementation and increased risk of developing cardiovascular disease. The zinc treated group had lower mean creatinine and total protein levels. After controlling for age and sex the group had lower serum uric acid and higher mean cell haemoglobin levels. The red blood cell count was decreased in women taking zinc. No significant differences were found in triglyceride or cholesterol levels but HDL cholesterol was not measured. Limited details and no actual values for the parameters were provided on this study. No data on dietary intake of zinc or copper were provided.

Exposure assessment

Food	Mean: 9.8 mg/day 97.5 percentile:17 mg/day (from 1986/87 NDNS)
Water	up to 10 mg/day (assuming 2 L/day consumption at maximum UK concentration of 5 mg/L)
Supplements	up to 50 mg/day (Annex 4)
Estimated maximum intake:	17 + 10 + 50 = 77 mg/day

No potential high intake groups were identified.

Risk assessment

Consumption of zinc supplements by human volunteers has been reported to cause gastrointestinal effects, including cramping and nausea. This was particularly apparent when the supplements were taken with little or no food.

Excess levels of dietary zinc interfere with the gastrointestinal absorption of copper, potentially leading to secondary copper deficiency. Signs of this include decreased ESOD (erythrocyte superoxide dismutase) activity, increased LDL cholesterol and decreased HDL cholesterol, decreased glucose clearance and abnormal cardiac function. One of the most sensitive markers of this appears to be ESOD activity. Supplemental doses of 50 mg cause significant decreases in the activity of this enzyme.

Iron and zinc in the diet each affect the gastrointestinal absorption of the other. Few data are available, but high levels of iron are known to interfere with zinc uptake, and more limited data suggest that the reverse interaction also occurs. This has not been considered in detail.

Two vulnerable groups have been identified; diabetics, since the study by Cunningham *et al.* (1994) indicates that HbA_{1c} is increased in conditions of zinc excess, and sufferers of haemochromatosis who have enhanced gastrointestinal absorption of iron, cobalt, lead and possibly zinc, potentially predisposing them to accumulation of zinc. However, there are insufficient data to establish the level of zinc intake at which adverse effects could occur in these groups.

Data are also available from animal studies that indicate high zinc levels can have a negative effect on copper balance. These have not been considered in detail in the risk assessment, as considerable data from human studies are available.

ESTABLISHMENT OF SAFE UPPER LEVEL

Key studies: Yadrick *et al.* (1989), Fischer *et al.* (1984), Black *et al.* (1988), Cunningham *et al.* (1994), Davis *et al*. (2000).

LOAEL:	50 mg/day
Uncertainty factor:	2 (LOAEL to NOAEL extrapolation)
Safe Upper Level: for daily consumption over a lifetime.	50/2 = 25 mg zinc/day for supplemental zinc

Zinc affects iron and copper uptake at supplemental doses of 50 mg/day and above. However, this is not apparent in all studies. The key endpoint is the reduction of copper absorption by zinc. Where the contribution of dietary zinc was also assessed, the lowest level at which effects were apparent (reduced activity of the copper-dependent enzyme ESOD being the most sensitive) was approximately 58-62 mg/day (50 mg supplements plus approximately 8-12 mg/day from food). There is no evidence of adverse effects from dietary zinc intake. In addition, since the contribution of zinc from the diet in many of the studies is uncertain, and may have been altered under study conditions, it is appropriate to set a safe upper level for supplemental zinc but not total intake. Taking the LOAEL as 50 mg/day and by applying an uncertainty factor of 2 to account for LOAEL to NOAEL extrapolation, a Safe Upper Level of 50/2 = 25 mg/day for supplemental zinc is derived (equivalent to 0.42 mg/kg bw/day in a 60 kg adult). An uncertainty factor of 2 has been used rather than 3 since the effect concerned is a small and inconsistent change in a biochemical parameter. No uncertainty factor is needed for inter-individual variability because this safe upper level is supported by a large number of human studies. Assuming a maximum intake of 17 mg/day from food, a total intake of 42 mg/day (equivalent to 0.7 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects.

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