

*Zinc*

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Metal Series No. 2

# Zinc

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Edited by  
Andrew Langley and Sam Mangas

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## Preface

The National Environmental Health Forum has been established by the Directors of Environmental Health from each State and Territory and the Commonwealth with a secretariat provided by the Commonwealth Department of Health and Family Services.

The National Environmental Health Forum is publishing a range of monographs to give expert advice and guidance on a variety of important and topical environmental health matters. This publication is the first in the metals series. A list of published monographs appears on page 8.

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1. Floods: An environmental health practitioner's emergency management guide (1999)

## Essentiality of zinc in human nutrition

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### 1. Introduction

The essentiality of zinc for higher plants (Somner and Lipman 1926) and animals (Hove et al. 1938; Todd et al. 1934) has been known for more than 60 years. Zinc deficiency was discovered, nearly 40 years ago, to be the cause of growth stunting and delayed sexual maturation among Egyptian (Prasad et al. 1963; Sandstead et al. 1967) and Iranian adolescents (Halsted et al. 1972). Since then zinc deficiency has been reported to affect children of many countries (Gibson 1994). Other groups at particular risk are women of child-bearing age and elderly (Sandstead 1995; Sandstead 1991b).

The main cause of human zinc deficiency is consumption of diets that contain little highly bioavailable zinc. Flesh foods, especially red meat, are the best dietary sources of bioavailable zinc (Sandstead et al. 1990). About 25% of zinc in red meat is retained by individuals with normal zinc status (Gallaher et al. 1988). Foods rich in phytate (Sandström and Sandberg 1992), dietary fiber (Knudsen et al. 1996) and Maillard browning products (Lykken et al. 1986) inhibit zinc retention. Secondary causes of zinc deficiency are illnesses that impair food intake, intestinal absorption and/or increase losses of zinc in intestinal contents or urine (Sandstead et al. 1976).

### 2. Zinc and growth

Zinc deficiency impairs growth by decreasing activity of enzymes that mediate synthesis of nucleic acids (Duncan and Hurley 1978; Terhune and Sandstead 1972) and proteins (Hicks and Wallwork 1987) and by decreasing Insulin-like Growth Factor (IGF) (Ninh et al. 1996) and other growth factors such as thymulin (Prasad et al. 1993). In addition energy utilisation is impaired (Greeley and Sandstead 1983). As a consequence of these phenomena zinc deficiency causes poor healing (Haeger and Lanner 1974; Sandstead et al. 1970), low immunity (Fraker et al. 1993; Prasad et al. 1993), dermatitis (Arakawa et al. 1976; Kay et al. 1976) and impaired fetal growth and maturation (Cherry et al. 1989; Goldenberg et al. 1995; Jameson et al. 1991; Meadows et al. 1981; Simmer and Thompson 1984).

### 3. Zinc and brain function

The essentiality of zinc for brain development and function was discovered through research on animals (Frederickson 1989; Golub et al. 1995; Sandstead 1985). Zinc status affects growth and maturation of neurons, synthesis of certain neurotransmitters, and the function of certain receptors (Wallwork and Sandstead 1993). In addition, zinc status affects calcium channels (Browning and O'Dell 1994; Browning and O'Dell 1995).

Severe zinc deficiency impairs thought processes (Henkin et al. 1975). Less severe deficiency has more subtle effects on neuropsychological functions that are not perceived by affected individuals.

This phenomenon was illustrated by a randomised double-blind depletion-repletion study of 11 men fed 1, 2, 3, 4 or 10 mg zinc per 2500 k calories daily for intervals of 35 days, while living in a controlled environment (Penland 1997). Intakes of 1-4 mg decreased ( $p < 0.05$ ) several neuromotor and cognitive functions, as compared to when 10 mg zinc was fed.

Consistent with these findings an 8 week pilot double-blind randomised controlled repletion trial of 30 mg zinc daily and selected micronutrients in 11 women with low serum ferritin concentrations found improved ( $p < 0.004$ ) short term visual memory (Wechsler 1981). In contrast 6 women given only micronutrients showed no significant change in function (Sandstead 1992).

### 3.1 A study of Chinese children

Based on the above my colleagues (see acknowledgments) and I measured effects of zinc repletion on neuropsychological functions of 6-9 year old urban and rural first graders from Qingdao, Shanghai and Chongqing, PRC (Penland 1997). Fourteen hundred children were studied. Treatments (20 mg zinc (Rx 1); 20 mg zinc with selected micronutrients (Rx 2); and micronutrients alone (Rx 3) were administered double-blind by teachers six days weekly. The micronutrient mixture was based on guidelines of the US National Academy of Sciences, Food and Nutrition Board (Committee 1989). Nutrients that can interfere with zinc retention were excluded. Main outcomes were change in knee height (Cronk et al. 1989) and change in neuropsychological functions. Laboratory indices included plasma and hair zinc. Data analysis was done by the Analysis of Variance; group comparisons were by two-tailed t-tests with Bonferroni adjustment for multiple comparisons. Some of the findings from the first 720 subjects follow.

Tables 1 and 2 show the change in length of the lower leg of children from Chongqing (CQ) and Qingdao (QD). The increase in knee height after Rx 2 and Rx 3 was substantially greater than after Rx 1. Technical problems interfered with the measurements in Shanghai.

**Table 1:** Change in knee height (mm), Spring 1994

City	n	p	Rx 1	Rx 2	Rx 3
CQ	123	0.0001	5.4 <sup>a</sup>	10.9 <sup>b</sup>	9.1 <sup>c</sup>
QD	120	0.0011	5.5 <sup>a</sup>	7.5 <sup>b</sup>	6.7 <sup>c</sup>

Different superscripts in the same row indicate significant differences ( $p < 0.001$ )

n = number of subjects

p = significance of over-all change

Rx 1 = 20 mg zinc

Rx 2 = 20 mg zinc with micronutrients

Rx 3 = micronutrients

**Table 2:** Change in knee height (mm), Fall 1994

City	n	p	Rx 1	Rx 2	Rx 3
CQ	116	0.0001	3.0 <sup>a</sup>	5.5 <sup>b</sup>	4.5 <sup>c</sup>
QD	124	0.0012	6.3 <sup>ac</sup>	10.1 <sup>b</sup>	5.7 <sup>c</sup>

Different superscripts in the same row indicate significant difference ( $p < 0.003$ )

Serum zinc data are shown in tables 3 and 4. Data from Qingdao from the fall of '94 were not available at this writing. Baseline mean fasting serum zinc of children from Chongqing and Qingdao were similar and slightly in excess of levels considered 'low'.

Very little increase occurred after zinc alone. Substantial increases occurred after repletion with zinc with micronutrients (Rx 2), and micronutrients alone (Rx 3). We speculate the increases were a reflection of mobilisation of zinc from bone during the growth process. Baseline mean serum zinc of children from Shanghai was 'normal', suggesting less severe zinc deprivation than at the other locations. It should be noted however that serum zinc is an insensitive indicator of zinc status (Sandstead 1991).

**Table 3: Change in serum zinc ( $\mu\text{mol/L}$ ), Spring 1994**

City	n	Baseline	CV %	p	Rx 1	Rx 2	Rx 3
CQ	110	12.15	32	0.0001	2.00 <sup>a</sup>	11.69 <sup>b</sup>	7.84 <sup>c</sup>
QD	114	12.15	32	0.0001	2.31 <sup>a</sup>	11.07 <sup>b</sup>	8.46 <sup>c</sup>
SH	111	15.75	16	0.0001	-0.45 <sup>a</sup>	2.32 <sup>b</sup>	2.25 <sup>bc</sup>

Different superscripts indicate difference ( $p < 0.001$ )

CV % = coefficient of variation

**Table 4: Change in serum zinc (Tmol/L), Fall 1994**

City	n	Baseline	CV%	p	Rx 1	Rx 2	Rx 3
CQ	101	12.15	32	0.0001	1.82 <sup>a</sup>	11.08 <sup>b</sup>	9.65 <sup>bc</sup>
SH	115	14.28	14	0.0088	0.11 <sup>a</sup>	1.77 <sup>b</sup>	1.46 <sup>bc</sup>

Different superscripts in the same row indicate significant difference ( $p < 0.02$ )

Table 5 shows that hair zinc concentration decreased after repletion. Data from the fall were unavailable at the time of this writing. The decrease in hair zinc after treatment was similar to that which occurred in a severely zinc deficient man after zinc repletion (Pekarek et al. 1979).

**Table 5: Change in hair zinc ( $\mu\text{g/g}$ ), Spring 1994**

City	n	Baseline	CV%	p	Rx 1	Rx 2	Rx 3
CQ	113	128	25	0.3	-33.1	-32.1	-21.6
QD	119	109	30	0.2	-26	-37	-26
SH	119	107	26	0.02	-50 <sup>a</sup>	-51 <sup>ab</sup>	-32 <sup>c</sup>

Different superscripts in the same row indicate significant difference ( $p < 0.05$ )

Cognitive and neuromotor findings from the spring are shown in Table 6. Zinc repletion alone (Rx 1) or with micronutrients (Rx 2) improved ( $p < 0.05$ ) continuous performance, matching of complex shapes, visual memory of complex shapes, concept formation (measured by oddity tasks), and neuromotor functions (tapping and tracking).

**Table 6: Change in neuropsychological tasks, Spring 1994 (all locations,  $n = 372$ )**

Task <sup>2</sup>	Rx 1	Rx 2	Rx 3
Continuous Performance	5.30 $\pm$ 2.19 <sup>3a</sup>	2.56 $\pm$ 2.29 <sup>ab</sup>	-3.32 $\pm$ 2.17 <sup>b</sup>
Matching Designs	3.72 $\pm$ 0.98 <sup>a</sup>	1.89 $\pm$ 0.99 <sup>ab</sup>	0.23 $\pm$ 0.97 <sup>b</sup>
Memory of Designs	-4.17 $\pm$ 2.43 <sup>ab</sup>	-8.33 $\pm$ 2.33 <sup>a</sup>	0.05 $\pm$ 2.23 <sup>b</sup>
Concepts by Oddity	-6.10 $\pm$ 2.44 <sup>a</sup>	-6.76 $\pm$ 2.31 <sup>a</sup>	1.97 $\pm$ 2.21 <sup>b</sup>
Tapping	11.6 $\pm$ 1.6 <sup>a</sup>	12.3 $\pm$ 1.6 <sup>a</sup>	0.0 $\pm$ 1.6 <sup>b</sup>
Tracking	26.1 $\pm$ 2.6 <sup>a</sup>	34.0 $\pm$ 2.5 <sup>b</sup>	21.0 $\pm$ 2.5 <sup>a</sup>

<sup>1</sup> Standard difference scores ((week 10 - baseline) / week 10 + baseline);

<sup>2</sup> Continuous performance & Matching designs (percent correct), Memory of designs (reaction time), Concepts by oddity (trials to learning criterion), Tapping (number of taps), Tracking (percent time on target);

<sup>3</sup> Mean  $\pm$  SEM;

<sup>ab</sup> Means with different superscripts differ significantly ( $p < 0.05$ )

#### 4. Significance of human zinc deficiency

Zinc deficiency appears common in populations with low dietary access to flesh foods (Gibson 1994). Individuals with low iron status from dietary lack are likely to be low in zinc. Consistent with this fact were findings from a study of young US women (Yokoi et al. 1994). Regression analysis of food frequency data found red meat one of five predictors of serum ferritin concentration ( $n = 38$ ,  $R^2 = 0.53$ ,  $p = 0.0001$ ) and one of four predictors of zinc status, as indicated by the plasma zinc disappearance constant ( $k$ ) ( $n = 19$ ,  $R^2 = 0.63$ ,  $p = 0.005$ ). Serum ferritin concentration, an indicator of body iron stores, was related to zinc status, indexed by the 30-60 minute disappearance of injected  $^{67}\text{Zn}$  from plasma and the zinc turnover rate. Serum ferritin concentration was lower when plasma zinc was  $< 70 \mu\text{g/dL}$  ( $p < 0.03$ ) in 18 subjects in whom the disappearance of injected  $^{67}\text{Zn}$  from plasma was measured, and plasma zinc disappearance and plasma zinc turnover were increased when serum ferritin was less than  $20 \mu\text{g/L}$  ( $p < 0.05$  and  $0.01$ ). When plasma zinc concentration was  $< 70 \mu\text{g/dL}$  the disappearance of injected  $^{67}\text{Zn}$  was increased ( $p < 0.05$ ). Regression analysis found that serum ferritin concentrations and the disappearance of injected  $^{67}\text{Zn}$  were inversely and non-linearly related ( $n = 18$ ,  $R^2 = 0.777$ ,  $p < 0.0003$ ). The non-linearity was probably caused by an increased intestinal absorption of zinc as iron status decreased (Pollack et al. 1965).

Iron deficiency affects most of the children and women of child bearing age in less developed countries. It is highly likely these individuals are also zinc deficient. Major adverse effects of zinc deficiency on populations include growth stunting (Prentice 1993), which that affects at least 30 % of children (Keller 1988); increased morbidity from infections (Ninh et al. 1996); decreased fetal growth (Goldenberg et al. 1995); teratology (Sever and Emanuel 1973); and impaired neuropsychological functions among children and adults. Thus zinc deficiency compromises entire populations where the food supply is inadequate. The impact on the gross national product of countries where the problem is common must be staggering.

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## Zinc: Nutritional aspects

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### 1. Zinc: Nutrition issues

The essentiality of zinc for plant and animal life has been recognised for more than half a century. During this time much has been learnt about the role of zinc in human nutrition but knowledge in some areas is still limited.

Several important aspects of zinc nutriture in relation to humans (Table1) will be discussed in this review.

**Table 1:** *Nutritional aspects of zinc in humans*

<b>Topic</b>	<b>Principal issue</b>
Zinc in the body	tissue distribution and mobility
Zinc in foods	distribution and socioeconomic relevance
Zinc absorption	site, mechanisms and homeostatic control
Zinc bioavailability	factors affecting fractional absorption
Zinc RDI*	impact of absorption efficiency from food
Biochemistry of zinc	role in enzymes, membranes, cell signalling, neurobiology
Zinc deficiency	symptoms and severity
Plasma zinc	response to dietary depletion
Zinc related teratology	rapid onset and relationship to feeding cycle
Zinc toxicity	acute versus chronic

\* RDI - Recommended Dietary Intake

### 2. Zinc in the body

The adult human body contains in total about 2g of zinc which is distributed principally in the large body organs (Table 2), although the absolute concentration of zinc is higher in certain tissues where the metal appears to be required to serve particular biochemical functions.

**Table 2:** *Distribution of zinc in body tissues (Dreosti 1992, WHO/FAO/IAEA 1996)*

Tissue	Zinc concentration ( $\mu\text{g/g}$ wet wt)	% of body zinc
Muscle	50	57
Bone	100	30
Skin	30	5
Liver	60	5
Brain	10	1
Prostate	200	<1
Retina	200	<1
Plasma	1	<1

Of special note is the fact that although many tissues contain significant levels of zinc, this zinc is not readily mobilised and does not appear to represent a meaningful reserve of available zinc during periods of dietary zinc deficiency.

### 3. Zinc in foods

Because of its wide involvement in many aspects of cellular function zinc is found in most foods, especially in animal and fish products, whole grain cereals, legumes and nuts (Table 3). Generally, however, zinc occurs at lower levels in plant foods and is considerably less available.

**Table 3:** *Zinc in foods (Dreosti 1992, WHO/FAO/IAEA 1996)*

Foodstuff	Zinc concentration ( $\mu\text{g/g}$ wet wt)
Oysters, shellfish	100-1000
Red meat	20-50
Cheese (Cheddar)	40
Whole grains	30-40
Legumes, pulses, nuts	20-35
Fish, poultry	10-20
Maize, rice	10-15
Wheat	10
Roots, tubers	5
Fruit	1

Calculations based on nutrient density in relation to total energy intake have indicated that, in general, foods with a zinc content below  $15\mu\text{g/g}$  (wet wt) will supply less than would be required to meet the overall adult USA RDA of  $15\text{mg/day}$  (Mertz 1980). On this basis zinc-rich foods tend to fall into the expensive category, while the staple foods of the majority of people in many parts of the world must be rated as poor suppliers of zinc.

This problem is well illustrated in Table 4 where the overall intake of zinc in the diet of vegetarians and Third World Countries is often well below  $15\mu\text{g/g}$ , a situation exacerbated by the poor availability of zinc from many of these predominantly vegetable-based diets.

**Table 4: Dietary zinc intakes around the world (Dreosti 1992, Hambidge et al 1986, Sandström 1989)**

<b>Topic</b>	<b>Zinc intake (mg/day)</b>
Australia	13
Belgium	15
Brazil	7.3
China	12.0
Holland	14.0
UK	9.0
USA	8.5-13.0
Tokelau islands	4.5
Vegetarians	6.4-9.2

A further issue affecting even those communities with access to diets replete in readily available zinc is the possible occurrence of a “conditioned” zinc deficiency which occurs despite an apparently adequate dietary zinc intake because of other life-style factors which either reduce zinc absorption from food (eg. complexing agents, competing cations, diarrhoea) or increase zinc loss in other ways (eg. diuretic agents, gastro-intestinal tract disturbances, trauma) (Dreosti 1992).

#### 4. Zinc absorption

Zinc is absorbed mainly from the small intestine although precisely at what point in this organ maximum absorption occurs is not yet clear (Dreosti 1990, WHO/FAO/IAEA 1996).

Most evidence suggests that zinc is absorbed into the intestinal mucosa by at least two mechanisms, one of which is passive and the other carrier-mediated. (Lönnerdal 1989, WHO/FAO/IAEA 1996). In part, uptake is saturable and in part non-saturable, however active, energy-dependent absorption does not seem to occur.

Homeostatic control of zinc uptake has been demonstrated repeatedly to operate in animals and appears also to take place in humans (Table 5). Control has been determined to occur principally through rapid secretion of zinc into the gastrointestinal tract followed by a slower adjustment to the rate of absorption. Urinary zinc secretion is of minor significance. Overall, homeostatic control in humans can increase zinc retention on a zinc-deficient diet to be 2-3 fold above levels operating with adequate zinc status.

**Table 5: Homeostatic control of zinc absorption\***

<b>Parameter (mg/day)</b>	<b>Dietary Zinc (mg/day)</b>			
	<b>0.3</b>	<b>5-7</b>	<b>15-17</b>	<b>30</b>
Urinary zinc	0.2	0.5-0.6	0.5-0.6	0.7
Faecal zinc	0.5	5.0-7.0	11.0-15.0	30.0
Zinc absorbed	n/a	3.3	4.5	6.5
% of intake retained	n/a	45-70	32	21

\* Approximate values derived from data obtained from King and Turnlund (1989) and WHO/FAO/IAEA(1996).

#### 5. Zinc bioavailability

The fractional absorption of zinc from the diet generally ranges between 5% and 65%, depending on the type of food with which the zinc is associated and on the pre-existing zinc status. Low existing zinc status and low levels of zinc in the diet tend to increase the efficiency with which zinc is absorbed, as also do sulphur-containing amino acids (Table 6). On the other hand some factors such

as phytate which is destroyed by fermentation/germination serve to bind zinc and make it less bioavailable, others may compete with zinc absorptive mechanisms as occurs with iron.

**Table 6: Factors affecting zinc bioavailability**

Promoters	Inhibitors
Low zinc status	Phytate (when ratio >15:1)
Low dietary zinc	Fibre
Amino acids	Competing cations
Organic acids	Iron (>25mg)
Animal protein	Calcium
Fermentation/germination	Undigested protein

## 6. Zinc RDI

Most studies suggest that the adult zinc requirement is of the order of 5.5mg/day (Dreosti 1990). Ten countries have RDIs for zinc with an average of 13.2mg/day (Dreosti 1990). Because of the many factors affecting zinc bioavailability, especially the form of zinc in the diet and the prevailing zinc status, no single RDI for zinc is applicable for all countries. In consequence RDIs are often set assuming a bioavailability appropriate for a particular country, or indicating a range of RDIs intended to cover poorly available zinc from largely vegetable-based diets, to diets rich in animal protein from which the zinc is more readily absorbed (Table 7). The impact of pre-existing zinc status on the zinc RDI has not yet been fully factored into estimating most RDIs.

**Table 7: Zinc RDIs (Dreosti 1990, WHO/FAO/IAEA 1996)**

Group	Australia*	USA**	WHO/FAO/IAEA <sup>+</sup>
Children	4.5-12	10-15	4-24
Women	12	15	6-20
Men	12	15	8-26
Pregnancy	+4	+3	+2-6
Lactation	+6	+7	+2-5

\* Based on an absorption efficiency of 30%

\*\* Based on an absorption efficiency of 40%

<sup>+</sup> Based on absorption efficiencies ranging from low (15%) to high (55%)

## 7. Biochemistry of zinc

Zinc is involved in the structure and function of more than 200 enzymes which collectively are represented in all major biochemical categories. For this reason adequate zinc is critical for normal cell function and normal metabolism, and a deficiency of zinc is manifest in the impairment of many key physiological activities.

The biochemistry of zinc is well reviewed by Williams (1989) and serves to provide a scientific basis for many of the symptoms and pathophysiological manifestations of zinc deficiency.

In particular, predictable and profound changes can rapidly be induced in plasma zinc levels by subjecting rats to feeding/fasting cycles with a zinc-deficient diet (Record 1991). In effect, plasma zinc levels remain at normal levels when animals consume a restricted intake of zinc-deficient food which probably reflects a catabolic state, but when followed by a period of unrestricted feeding plasma zinc levels fall precipitately by around 50% in 12 hours as the animals enter a period of anabolism.

## 8. Zinc deficiency

Clinically, zinc deficiency is reflected as a continuum of symptoms of increasing severity which arise to a large extent because of the critical role of zinc in cell division and the profound impact impairment of this process will have on growth, immunocompetence, skin integrity and hair growth and, in more severe cases, on fetal development (Table 8).

**Table 8: Symptoms of zinc deficiency**

Mild	Moderate	Severe
Impaired growth velocity	Growth retardation	Neuropsychiatric complications
Impaired immunocompetence	Male hypogonadism	Skin disorders, dermatitis and keratosis
Taste dysfunction	Dysphagia	Acromotrichia and alopecia
		Impaired wound healing
		Teratology

## 9. Plasma zinc

Plasma zinc levels respond very rapidly to a low dietary zinc intake largely because of the limited capacity for the metal to be readily mobilised from other body zinc deposits. For this reason plasma zinc levels in animals and in humans have been observed to fall by up to 50% within 24 hours of receiving an inadequate dietary zinc intake. Recognising that one third of plasma zinc occurs as a macroglobulin fraction which plays no part in transporting zinc, the amount of available zinc for cell growth in a zinc-deprived animal rapidly becomes a limiting factor (Record 1991, Dreosti 1992).

## 10. Zinc-related teratology

Zinc deficiency-related teratology has been demonstrated repeatedly in animals and has been speculated to occur to some extent in humans (Record 1991, McMichael et al 1994). In animals, the nature and severity of the teratogenesis can be greatly influenced by timing the feeding cycle to coincide with a particular phase of embryonic organogenesis.

## 11. Zinc toxicity

In general, because of the emetic nature of excess dietary zinc, acute zinc toxicity is often avoided by regurgitation of the ingested zinc, although cases have been reported which involve severe damage to the kidneys and pancreas (Table 9). Of possibly greater significance however are the effects of chronic mild zinc excess resulting in reduced copper absorption and copper deficiency-related anaemia.

**Table 9: Symptoms of zinc toxicity**

Dietary intake (mg/day)	Symptoms
>1000	Vomiting, fever, damage to kidneys and pancreas
200-500	Gastric disturbance, nausea, dizziness
>100	Reduced copper absorption

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## Risk assessment for essential trace elements: A Homeostatic Model

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### 1. Introduction

Most, if not all, countries developing public health programmes in chemical safety do so in two phases. The first phase, an evaluation of all available data by scientists, is generally referred to as the risk assessment process. This process attempts to quantify the dose-response relationships in humans in as quantitative a manner as possible. In the second phase public health officials (risk managers) consider the advice from the scientists as well as socio-economic, political and other concerns. This risk management phase is the sole responsibility of Member States or regional political bodies and will not be considered further in this paper.

For chemicals without known biological functions, the formal risk assessment process, first described by the US National Research Council (NRC 1983), is widely considered as an appropriate framework within which scientific experts can provide advice and develop, where possible, exposure guidance values (health-based guidance values) for total intake or for exposures from air, water, food, etc. It is a well developed methodology (NRC 1983; NRC 1994; IPCS 1994) and has been used to develop health-based guidance values nationally (Barnes and Dourson, 1988) and internationally (IPCS 1987; IPCS 1994; WHO 1996).

A major assumption made in using this paradigm for assessing risk is that zero exposure to chemicals is not harmful. That is, a conservative application of scientific principles is most protective and therefore preferred. This is obviously not appropriate when evaluating human health, or

environmental risks, from exposure to essential trace elements (ETE) such as zinc, copper, selenium, etc. There are known risks from insufficient intakes (deficiency) as well as adverse effects from exposures in excess of the recommended daily levels (toxicity). Therefore, any tolerable intake (TI) recommended must prevent the onset of toxicity but also be sufficient to provide an intake at which the risk of deficiency for the general population is minimised. This infers the need for a range of values rather than a single number.

Often, the rigid application of the risk assessment process has resulted in values lower than the recommended daily intakes for large segments of the normal healthy population (Abernathy et al., 1993; Sandstead, 1993). Obviously, it is not good public health policy to recommend exposure levels for protection from toxicity which are lower than the well recognised needs of humans.

One purpose of this paper will be to present some ideas on how the scientific principles of toxicology and nutrition can be applied together for the evaluation of non-carcinogenic ETEs. The ideas presented have been based on the work of several groups (academic, governmental and private non-governmental organisations) already aware of this problem (Beaton, 1988; Abernathy et al., 1993; Nordberg and Skerfving, 1993; and Mertz et al., 1994).

The toxicity and nutrition needs of ETEs are both important international public health problems which the International Programme on Chemical Safety (IPCS) wishes to address together. In providing scientific advice to Member States on health effects from chemicals it is imperative that nutritional needs and toxicities be balanced when evaluating ETEs.

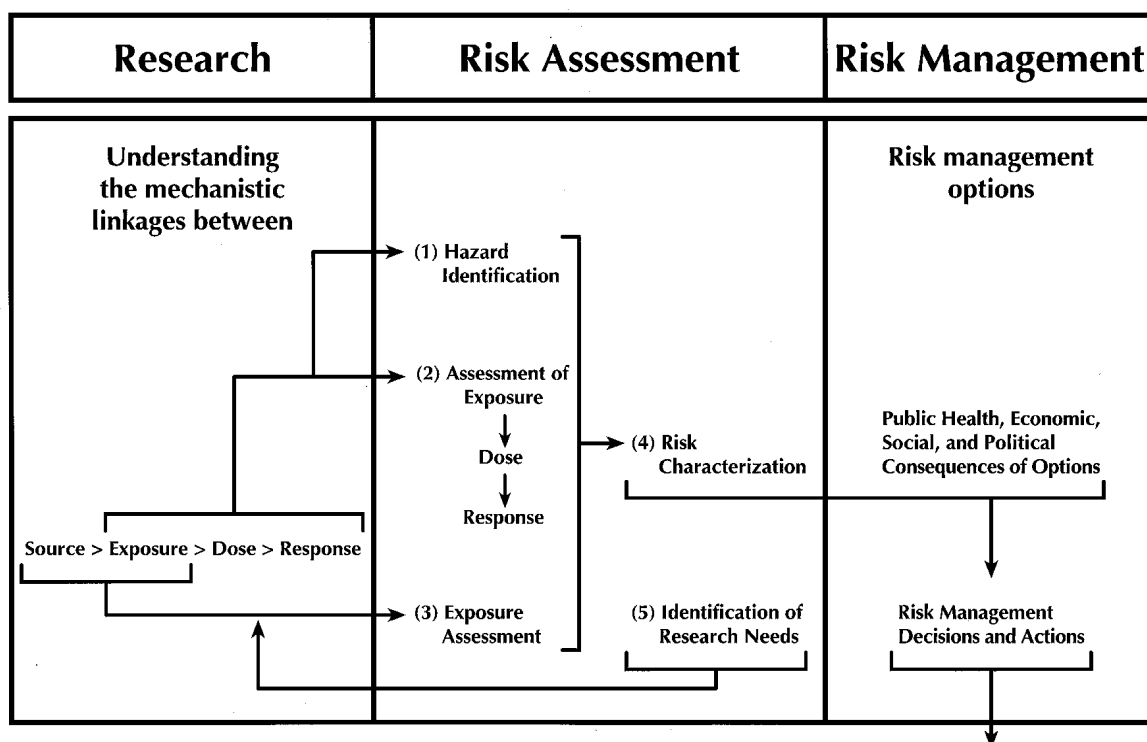
If such a methodology can be developed, and agreed upon internationally, IPCS will have succeeded in its objective to improve and harmonise internationally the methodology used to assess risks from chemical exposures including ETEs. Such an internationally agreed upon methodology would also assist IPCS in its development of Environmental Health Criteria monographs, another major objective of the Programme.

Since this workshop is focused on zinc, this ETE will be used as an example in the development of the homeostatic model. However, the principles and methods described are applicable to any other non-carcinogenic ETE.

## **2. Risk assessment within IPCS**

### *2.1 Concepts and definitions*

The four step process for risk assessment (hazard identification, dose-response relationship, exposure assessment and risk characterisation) first proposed in 1983 (NRC 1983) is still considered by IPCS as an appropriate framework for the evaluation of health data related to the effects of chemical exposures (see Figure 1). Figure 1 is an adaptation of the original scheme developed in 1983 (NRC 1994). It emphasises more clearly the iterative nature of the process, particularly as it relates to the identification of research needs at each step in order to decrease the uncertainty of evaluations given to the risk manager. For ETEs this will be an extremely important step in developing more certain evaluations. Much of the uncertainty in risk assessments, and conflicts between the nutritional and toxicological aspects of ETE, result from an inadequate data base on toxicity and mechanism(s) of action as well as a lack of understanding of the biology involved in the homeostatic processes within all mammals.



*Figure 1: NAS/NRC risk assessment/management paradigm (NRC 1994).*

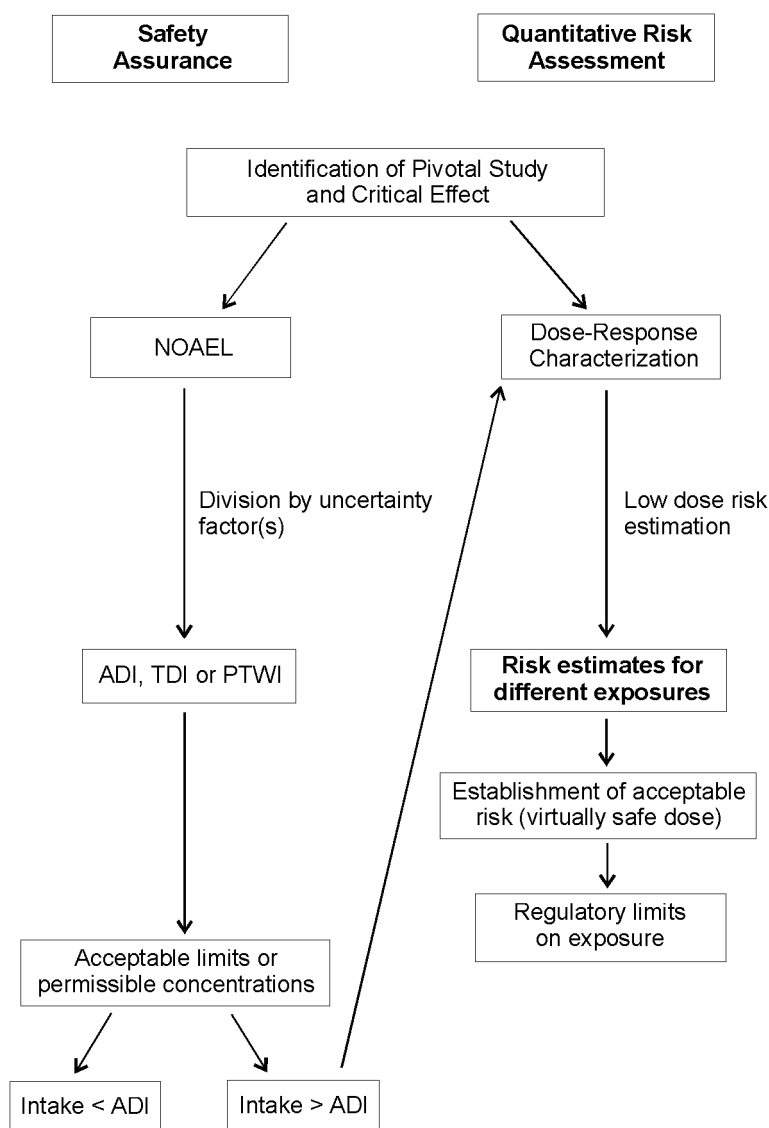
Many terms and definitions have been proposed for the health-based guidance values developed from the dose-response relationships. These include: ADI - acceptable daily intake (WHO 1987); TI - tolerable intake (daily, TDI or weekly, TWI) (IPCS 1994); PTW(D)I - provisional tolerable weekly (daily) intake (WHO 1987). These are all defined as estimates of exposure (intake) of a substance that are considered without appreciable risk to most of the population over a lifetime of exposure. There is an understanding that such values may not be fully protective of sensitive subgroups.

Although the term "tolerable" is used to indicate a "tolerated" level, not necessarily acceptable (IPCS 1994), the US Environmental Protection Agency prefers to utilize the term RfD - daily reference dose (Barnes and Dourson, 1988). The RfD is an estimate of a daily human exposure which is considered to be without appreciable risk to the human population (including sensitive subgroups) for a lifetime exposure with uncertainty spanning an order of magnitude. The protection provided to sensitive subgroups by the RfD is a major difference between the definitions used by other groups worldwide (IPCS 1994; IPCS 1987) and is a very different concept from that used to develop recommended daily allowances (RDAs) where daily allowances provide adequate nutrition for about 97.5 percent of the population (NRC 1989; Mertz 1994).

## *2.2 Methodology to develop health-based guidance values (HBGV) for non-essential chemicals*

The methodology used to develop HBGVs from the dose-response curve through the use of uncertainty factors (UF) has been well described (IPCS 1987; IPCS 1994; Dourson 1994; Kroes et al., 1993). However, a few points need to be highlighted here.

For non-cancer causing chemicals (including ETEs), the process to develop HBGVs is actually a safety assessment process rather than a probabilistic risk assessment. A comparison between these two methodologies is given in Figure 2.



**Figure 2:** Comparison of the safety assessment and probability models for assessment risks from chemical exposures.

All available data are reviewed and evaluated, a critical effect identified and the critical study (or studies) evaluated for the dose-response relationship(s) reported. In choosing the critical study in animals such factors as: lowest level of exposure causing effects; relevance of animal model to humans; duration of exposure; and overall quality of study are utilised. Where human data of acceptable quality are available it is preferred that these data be utilised in order to avoid the uncertainty of interspecies extrapolations. From the critical study a no-observable-effect-level (NOAEL) or lowest-observable adverse-effect-level (LOAEL) is identified and used to develop an HBGV.

Most often there is much scientific uncertainty with the data used to identify the NOAEL or LOAEL, for example, its relevance to humans (interspecies extrapolation) and the variation in susceptibility of individual humans (intraspecies variability). Other factors leading to uncertainty include adequacy of the pivotal study, severity of effect in animals and time of exposure used to generate the data. The use of UFs or Modifying Factors (MFs) actually reduces the dose-rate to humans compared to that in animals to account for these uncertainties within the data base.

Traditionally, an UF of 10 for intraspecies variability and one of 10 for interspecies differences in susceptibility have been used ( $UF_1 \times UF_2$ ) for a total UF of 100 (IPCS 1987). Other factors ranging from 1-10 have been suggested as MFs to account for other uncertainties arising from concerns over

the adequacy of the data (exposure duration if less than chronic, minimal number animals, etc.), severity of end-point (e.g. reproductive or general systemic effects), and use of a LOAEL when a NOAEL cannot be determined. It has been proposed (Renwick 1993; IPCS 1994) to utilize available pharmacokinetics data to modify the traditional UFs and MFs, however, such data are usually lacking and the traditional UF approach is still used. The TI is then determined by dividing the NOAEL with the product of all UFs considered appropriate ( $TI = NOAEL \text{ (or LOAEL)} \div (UF_1 \times UF_2 \times MF)$ ). Where human data are used to develop a TI, UFs of 10 or less are normally considered protective. Where good dose-response results have been reported UFs between 1 and 3 have been used. As a general rule UFs or MFs resulting in values greater than 5000 should lead one to question whether the overall quality of the data base allows one to derive a TI with any degree of certainty.

### 3. Development of recommended dietary allowances

In this paper we are discussing the assessment of risks from trace elements which have met the generally agreed upon criteria for essentiality in mammals (Frieden 1984). The methodology used to assess essentiality will not be discussed here.

It is also beyond the scope of this paper to discuss in detail the methodology used to develop recommended dietary allowances (RDAs) or the estimated safe and adequate daily dietary intake (ESADDI). They are described fully by the US National Research Council Committee Food and Nutrition Board (NRC 1989; Mertz 1994) and the World Health Organisation (WHO 1996).

However, the definitions of these values and a few of the principles underlying their derivation need to be understood in order to evaluate the risks from ETEs fully through the collaboration of nutritional scientists and toxicologists.

The RDA is defined as: "levels of intake of an essential nutrient that, on the basis of scientific knowledge, are judged to be adequate to meet the known nutrient needs of practically all health persons" (NRC 1989). Where scientific data are insufficient to develop an RDA but sufficient to estimate a safe and adequate dietary intake an ESADDI is calculated. Similar to a provisional tolerable intake value an ESADDI provides interim guidance until an RDA can be supported.

In summary, RDAs are determined by: (i) estimating the average physiological requirement for a healthy representative sample of a variety of age and sex groups in the human population; (ii) assuming a gaussian distribution and a coefficient of variation (usually 15%) within the population. The RDA is actually the average requirement + 2 standard deviations (SD) and actually represents the 97.5 percentile of the nutrient requirement of the healthy population. The data used in calculating an RDA come usually from human studies, although an ESADDI for selenium (50-200 µg/day) was derived by extrapolation from animal studies (Mertz 1994).

#### 3.1 Principles underlying derivation of recommended dietary allowances and tolerable intakes

A summary of some important differences in the assumptions underlying the derivation of RDAs and TIs is given in Table 1. These differences need to be reconciled if we are to have scientifically-based assessments of an ETE, fully protective for both low and high exposures. For example, data on bioavailability and nutrient and dietary interactions are usually available in the literature on nutrition but largely ignored by toxicologists more concerned with the derivation of TIs protective of even sub-groups at excess within the population.

**Table 1:** Comparison of principles underlying derivation of Recommended Dietary Allowances (RDA) and Tolerable Intakes (TIs)

Principle	RDA	TI <sup>(a)</sup>
Use of data	Bioavailability, nutrient and dietary interactions - all considered	Usually only toxicity considered
Population protected	97.5% of several age and sex groups	Most with RfD 100% protective
Clinical significance of end-point	Deficiency states can lead to severe clinical effects	Adverse effect often without known clinical significance

\* The US EPA RfD is considered similar to a tolerable intake (TI) although it is more protective because it takes into account sensitive subgroups.

For ETEs, the use of diet as the only source of exposure considered for an RDA is not too critical since food makes by far the major contribution to exposure from an ETE. In a few cases (zinc and copper) drinking-water can be a significant source but usually not greater than 5 to 10 percent.

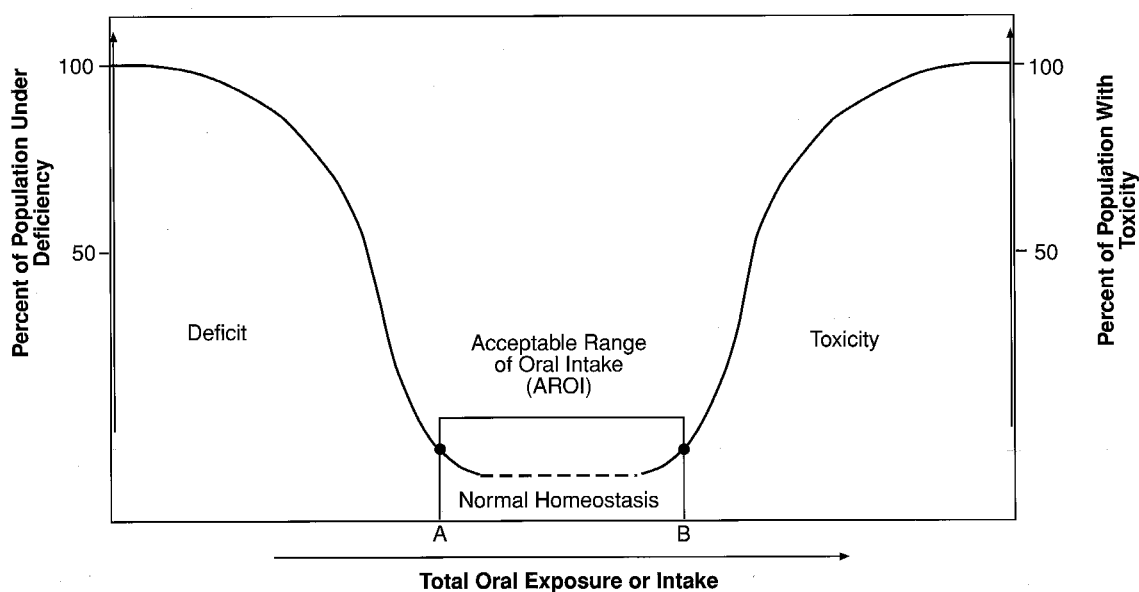
A comparison of the methodological approaches used in deriving RDAs and TIs has been made by Bowman and Risher (1994). Despite the obvious differences in the underlying principles shown in Table 1 these authors conclude that the methodological approaches are not in conflict, with apparent conflicts more reflective of inappropriate or overly rigid application of the two values. It is agreed that neither the TI nor the RDA are precise indicators of actual exposures where toxicity will appear in an individual or signs of deficiency become evident. However, the potential for conflict between levels protective from toxic effects and levels required for normal homeostasis will remain until the scientific principles become more similar and the toxicologist makes better use of all available data to decrease the reliance on default positions such as rigidly mandated UFs or MFs.

#### 4. Principles and methods for assessing human health risks from exposure to essential trace elements - An Homeostatic Model

##### 4.1 *Scientific principles*

##### 4.1.1 **The acceptable range of oral intake for an ETE**

For an ETE there are risks associated with low (deficiency) as well as high (toxic) intakes. If one plots total intakes increasing from zero versus percent of population showing signs of deficiency or toxicity one obtains a U-shaped curve shown in Figure 3. There is an Acceptable Range of Oral Intake (AROI) below which (Point A) the risk from the adverse effects of deficiency increases in the population and at the upper end of this range (Point B) the risk (probability) from adverse toxic effects increases in a dose-dependent fashion. The range of intakes between A and B in Figure 1 has been termed the AROI, however, nutritionists have termed this range as one of "optimal nutrition" or the region of normal homeostasis. For this reason I have termed this approach a homeostatic model for human health risk assessment of ETEs. A similar concept was proposed by Beaton (1988) and the region of normal homeostasis (A to B in Figure 1) was termed "a safe range of intake".



**Figure 3:** Percent of population subjected to deficiency and toxic effects according to exposure or intake of the essential trace element (ETE). As intake drops below A (lower limit of the AROI) risk for deficiency increases, reaching 100 percent at extremely low intakes. As intakes increase beyond B a progressively larger proportion of subjects will exhibit effects of toxicity.

Whatever term one wishes to use for the range of intakes A to B in Figure 3, it must be made clear that neither the lower or upper boundary of the AROI should be considered an absolute value, below or above which adverse effects in a population or individual will be initiated. In fact, RDAs and TIs are not guidance values for individuals, but for populations. Therefore, at intakes greater than B the incident rate will increase or the severity of effect noted may change with dose but all individuals will not be automatically affected at doses above Point B in Figure 3. The same concept holds for intakes lower than A with regards to signs of deficiency.

Although the term AROI is used in this paper to facilitate comparison with the RDA, which only considers dietary intake, the concept of a range of intakes within which normal homeostasis is maintained can be applied to situations where other routes of exposure are significant. One would then refer to an Acceptable Range of Total Intake (ARTI).

#### 4.1.2 Boundaries of the AROI

Some of the problems in setting the boundaries of the AROI arise from a lack of knowledge of signs or biomarkers of marginal states be they signs of deficiency or toxicity. Also, the rigid application of large UF and MF to the NOAEL without concern for bioavailability, nutrient and dietary interactions and homeostatic mechanisms can lead to Point B being given a lower value than Point A in Figure 3.

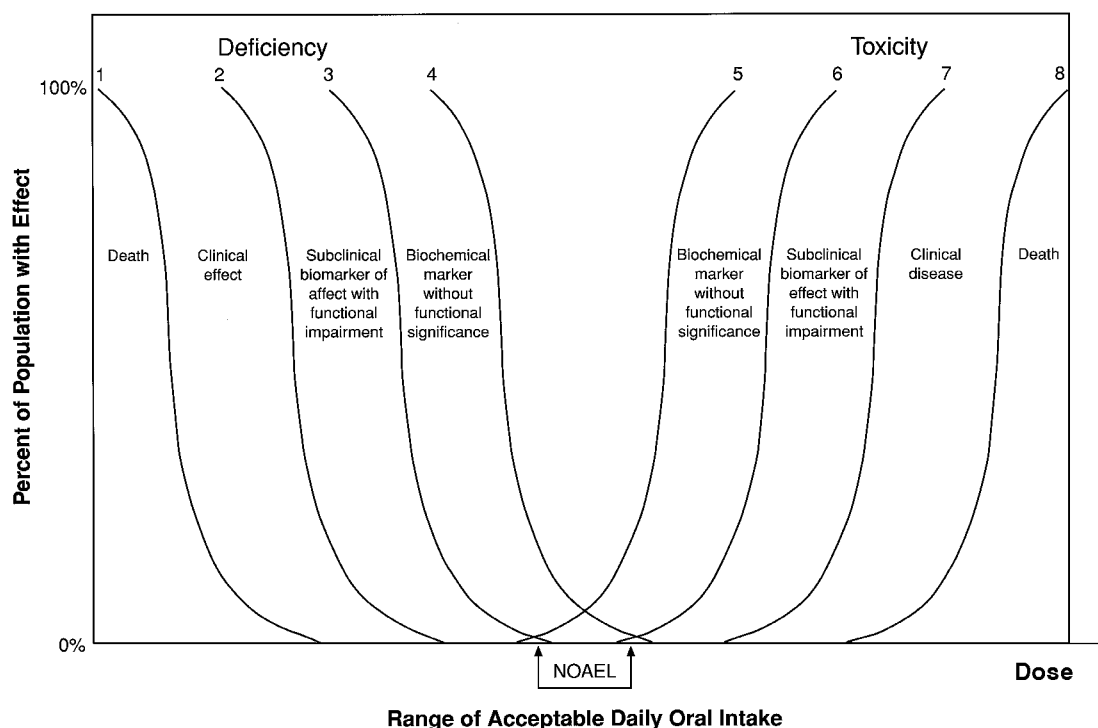
Uncertainty Factors (UF) are applied in delineating intake A and intake B. However, in estimating the protective levels of over-exposure the UFs are orders of magnitude greater than those used to determine RDAs (which are usually 30-50% over the normative requirements) (Mertz 1993). The UFs used for RDAs are typically 2-3 compared to the UFs used to derive intake Bs which may be as high as 5000. As more information on human nutritional requirements and homeostatic mechanisms is obtained even these small uncertainty factors may decrease. There would appear to be a need for better data on the toxic effects of ETEs and a willingness for toxicologists to modify the default position of rigidly applied conservative UFs and instead use modified UFs supported by the available data.

In deriving the RfD (oral) for zinc, the US EPA, using the rather rigid application of an UF of 10 to a LOAEL in a human study, suggested an RfD of 0.09 mg/kg bw/day (6 mg/kg/day for a 70 kg adult compared to the RDA of 15 mg/day). Abernathy et al. (1993) suggested the use of an UF of 3 on the

same LOAEL to account for "nutritional and energy requirements". No consideration was given to the bioavailability of the zinc supplement used in the study versus that of dietary zinc nor to possible zinc/copper interactions at the dose used. With an UF of 3 the RfD was calculated as 0.3 mg/kg bw/day (20 mg/day for a 70 kg adult). It should be noted that this RfD still does not provide the RDA for infants, pre-adolescent children or possibly lactating women (Abernathy et al., 1993). The nutritional literature on zinc requirements is fairly robust whereas the clinical significance of the adverse end-points used (lowered HDL cholesterol) is still a matter of scientific debate. Therefore, it seems to be crucial for toxicologists to attempt to validate the TIs when they are lower than the normal intake of healthy populations. This is particularly so for zinc when the effect noted may be due to a nutrient interaction resulting from high internal levels of zinc from the extremely bioavailable form of zinc, used in a small human study.

#### 4.1.3 Comparability of effects used to define deficiency and toxicity

A set of theoretical curves (see Figure 4), developed by Dr G. Nordberg, University of Umea, Sweden (personal communication, 1995), illustrates the subject of this section. Although, as stated earlier, markers of marginal deficiency states or minimal toxicity are often lacking. It is imperative that nutritionists and toxicologists work together to ensure that the boundaries of the AROI have been defined by end-points of similar clinical significance.



**Figure 4:** Theoretical dose-response curves for various effects occurring in a population at various levels of intake (doses) of an essential trace element. While lethal effects and clinical disease must always be prevented, subclinical effects indicating impairment of organ function are often identified as critical effects. The lower end of the dose-response curve for such critical effects related to deficiency (curve 3) and toxicity (curve 6) define the range of acceptable daily oral intakes. Biochemical effects without functional significance (#4 and #5) are considered without health impact and should not be taken as critical effects.

In defining the AROI for zinc we are faced with the lack of obvious markers for mild zinc deficiency. Reduced growth rate and impaired resistance to infection are often used as manifestations of mild zinc deficiency in human (WHO 1996). Similarly, markers of minimal zinc toxicity in humans are not available. Probably the most studied effect of excess zinc exposures in humans is the effect on copper utilisation with a subsequent decrease in erythrocyte copper-zinc superoxide dismutase (E-SOD), although the functional role of this enzyme in red blood cells remains unclear. Even though the

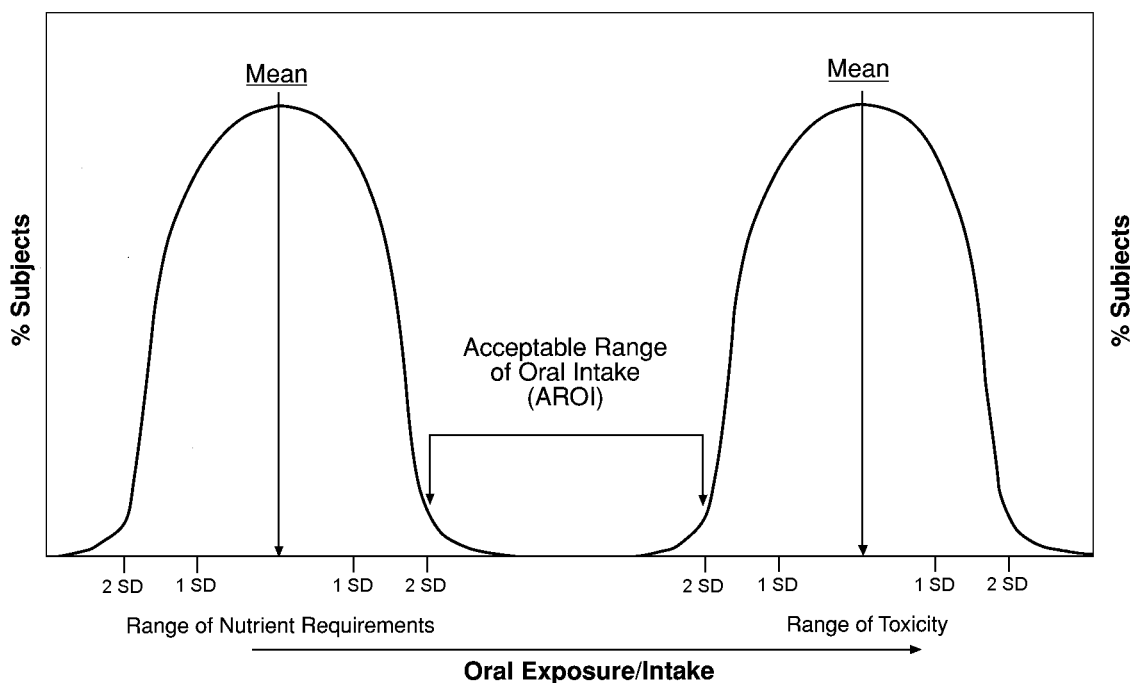
information from studies in humans regarding the boundaries for the AROI is limited, perhaps a starting point in defining the range could be the customary intakes determined in healthy populations in various regions of the world.

#### 4.1.4 A safe range of population mean intakes - an AROI

To work with nutritional scientists in developing an AROI, toxicologists must be familiar with the concept of population mean intakes and the level of protection given by this concept. The gaussian distribution shown in Figure 5 represents the averaging of a series of intake distributions in a large population homogeneous as to age, sex, and other physiological characteristics believed to affect requirements. As shown, this mean intake distribution plus 2 SD would be protective from deficiency in 97.5 percent of the population. In a recent report (WHO 1996) this concept is described fully. It is important to remember that the boundaries of the AROI are not expected to protect members of the population at special risk, they refer to the homogeneous population.

The concept of probability of adverse effects from excess exposure from the population mean intake is not usually used in toxicological risk assessment for non-essential chemicals. The normal distribution of intakes for toxicity is shown in Figure 5 to highlight the fact such a principle may need to be applied in deriving the upper (toxic) boundary of the AROI which should be protective of most members of an homogeneous population. Although a statistical definition for most is lacking, it could be derived where data are available, based on the distribution of toxic effects as intakes exceed the population mean. The toxicologist may need to consider using a type of bench-mark approach of the dose-response relationship whereby an Effective Dose<sub>50</sub> (ED<sub>50</sub>) is defined and, based on normal variability or interpolation of the dose-response curve, an ED<sub>2.5</sub> is determined (IPCS 1994).

This would be similar to the protection provided by the RDA. Where, for reasons of scientific concern or socio-economic policy more protection from toxicity is deemed necessary one could apply an UF to the ED<sub>2.5</sub> to define the upper boundary of the AROI.



**Figure 5:** Ideal model for distribution of intakes to meet nutritional requirements of a health population and prevent toxicity. The lower limit to the AROI should cover the requirements of most (97.5%) of the population while the higher limit of the AROI should protect most of the population from toxic effects.

#### 4.2 *The AROI concept in human health risk assessment - a homeostatic model*

Although the term "homeostatic model" has been chosen, it is really similar to the "safe range of intake" model proposed by Beaton (1988). Both models are supported by the scientific principles shown in Table 2. Many of these principles were discussed at a workshop in 1992 (Mertz 1993; Mertz et al. 1994).

When the principles in Table 2 are applied within the framework shown in Figure 6, it should be possible to provide guidance to all countries on the exposure levels for any ETE which would provide adequate nutrition and be without risk from toxicity. The iterative nature of this proposed scheme makes it possible to identify research needs and accommodate special groups within the population with advisories regarding exposures. It is essential that this scheme be applied by nutritional scientists and toxicologists working together to address such aspects as data quality, critical effects, dose-response, bioavailability and nutrient interactions. It is essential that all scientists involved practice sound scientific judgement during each step and make it clear whenever default positions are taken in lieu of scientific data.

**Table 2:** *Principles underlying use of the Homeostatic Model in human health risk assessment*

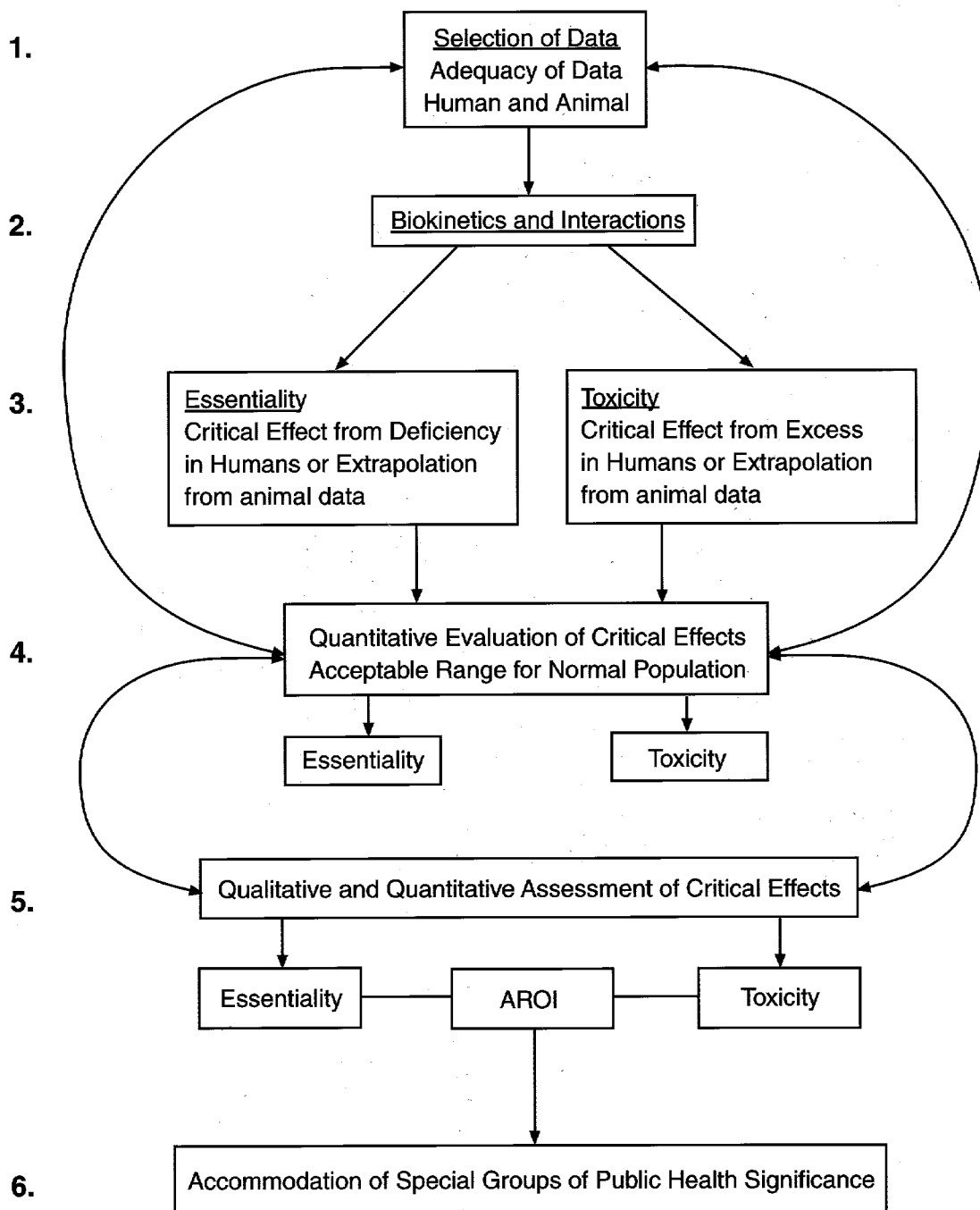
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(1)	For all ETEs there is a "zone of safe and adequate exposure" -a zone compatible with good health - an acceptable range of oral intake (AROI).
(2)	Both nutritional scientists and toxicologists must be involved in developing an AROI.
(3)	Data on toxicity and deficiency should receive equally critical evaluation.
(4)	The concept of bioavailability (biologically effective dose) should be applied and nutrient interactions considered when known.
(5)	Chemical species studied and the route and mode of application should be fully described.
(6)	Biological end-points used to define the lower (RDA) and upper (toxic) boundaries of the AROI should have similar degrees of clinical significance.
(7)	Safety margins and uncertainty factors are used to determine both boundaries of the range. They are usually higher for overexposure but need to be applied taking nutritional needs into account.

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\* Adapted from Mertz, 1993.

The scheme in Figure 6 should not be considered a novel way of assessing health risks. It really represents the thought processes used by those scientifically competent and versed in the procedures used to carry out evaluation of data on ETEs whether from studies on experimental animals or humans. The end result should be a range of recommended exposures fully protective of human health, something a single number for an ETE can never do.

**STEPS**

*Fig. 6: Application of principles for the assessment of risk from essential trace elements.*

### 5. Deriving an AROI for zinc

In concluding this paper it would seem appropriate to validate the model proposed. This validation will be done using zinc as an example of an ETE.

Earlier (section 4.1.2), a brief discussion was given of the attempts by the US EPA to develop an RfD (oral) for zinc (Abernathy et al., 1993). A more detailed look at the methodology used and a comparison with the analysis of this same database by Sandstead (1993) helps to support the call for a

more thorough analysis and evaluation of all information (both nutritional and toxicological) available when setting an RfD (TI) for an ETE such as zinc.

In this short summary it is impossible to review in detail the evaluation made by the US EPA of available studies on the effect of zinc supplementation in humans. Details can be found in Cantilli et al. (1994) and Abernathy et al. (1993). The co-critical effects examined were alteration of copper homeostasis as measured by E-SOD (Yadrick et al., 1989) and decreased serum HDL cholesterol (Black et al., 1988). It was concluded that the E-SOD inhibition was a more consistent finding in humans and results, assuming a 60 kg human, in a minimal adverse effect (LOAEL) of 1 mg/kg/day. An UF of 3 was used instead of the usual 10 applied to a LOAEL, since the effect was considered "minimally adverse". As shown in Table 3 this value is very close to the RDA for adults and is, in fact, in the middle of the range of the RDAs proposed for 10% and 20% zinc bioavailability. As reported by the authors, the RfD of 0.3 mg/kg bw/day does not meet the needs of infants and children and lactating females (Cantilli et al., 1994). It has also been reported (Sandstead 1993) that this RfD is below the RDA for adolescents at 15% bioavailability. No consideration during the development of this RfD was given to the fact that the zinc used in the human studies was the gluconate salt with a bioavailability approaching 100 per cent compared to the 10 to 30 percent bioavailability found for zinc in normal diets (WHO 1996).

If one considers the RDA as the lower limit of the AROI and uses the RfD as the upper limit, there appears to be an extremely narrow AROI for zinc, not confirmed by the much larger reported intakes of dietary zinc by apparently normal healthy populations worldwide (WHO 1996). This was acknowledged by Cantilli et al. (1994) when they proposed using an UF of 1 since the study was in humans, but applying MFs to the LOAEL based on study design and the nature of the end-point (while still showing no concern over the bioavailability of the test substance). A MF of 2 applied to the LOAEL of 60 mg/kg bw for 60 kg females results in a RfD of 0.5 mg/kg bw. For a 60 kg adult female this will result in an intake of zinc equivalent to 30 mg/kg/day, an amount easily consumed by millions of humans worldwide from a mixture of dietary zinc and zinc in multivitamin supplements without apparent deleterious effects.

The variation in TIs calculated from the same data as used by the US EPA, but taking into consideration the available data on zinc bioavailability, can be seen in Table 3. Sandstead (1993) assumed 90% absorption of zinc gluconate with zero retention of dietary zinc on the basis of known mechanisms of zinc absorption. The LOAEL for zinc in zinc supplements was calculated to be 45 mg/day and using an UF of 3 and a 60 kg body weight an RfD of 0.25 mg/kg bw/day was calculated for individuals consuming highly bioavailable (90%) zinc supplements. RfDs were derived for high (30%) and low (15%) bioavailable diets, using as a reference the internal dose (45 mg/day) calculated for the Yadrick et al. (1989) study.

**Table 3: An acceptable range of oral intake (AROI) for zinc**

Age Group	RDA (mg/day)		RfD (mg/day/60 kg)		
	Balance (USA)	FER-WHO (Availability) 20-10%	US EPA <sup>(a)</sup>	Sandstead <sup>(b)</sup> (Availability) 15-30-90%	
Infants	(0-6 months)	5	6.3 - 12.5	3/10 kg	
	(6-12 months)	5	5.5 - 11.0		
Children	(1-10 years)	10	8-16	7.5/25 kg	
Adolescent	- M	15	14-28	15/50 kg	
	- F	12	11-22		
Adults	- M	15	11-22	20	110 - 50 - 15
	- F	12	11-22	18	100 - 50 - 15
Pregnancy		15	15-30		
Lactation		19	27-55	18	

(a) Abernathy et al., (1993); (b) Sandstead (1993)

For example, it was calculated that 150 mg of dietary zinc was necessary in a high bioavailable diet to provide the 45 mg daily intake which resulted in E-SOD inhibition in humans. On this basis, a 60 kg female having an intake of 100 mg zinc/day from a highly bioavailable diet would be at the RfD (50 mg/60kg bw/day) (see Table 3).

Based on an evaluation of all available data, including bioavailability, markedly higher TIs are derived from the same data set than when only toxicological data are considered. Because of deficiencies of data related to bioavailability and nutrient interactions, this comparison cannot say which AROI is more protective of the general population. In view of the variation within the human populations for which RDAs or TIs are calculated it would seem certain that one number will not be found to be protective for both deficiency and toxicity. Risk assessments for ETEs will need to develop a range (AROI).

There is still uncertainty regarding the RDA derivations, however, it is suggested that this uncertainty is less than that surrounding any TI derived without consideration of the available data from human nutrition. The analysis by Sandstead (1993) supports the idea that nutritionists and toxicologists can work together to develop scientifically supported AROIs for ETEs, thus providing more appropriate public health advice worldwide than that provided by a single number. For zinc the upper boundary for the AROI would, for adult females, range from 11 to 100 mg zinc/day depending upon bioavailability. Given that humans consume a mixture of dietary and supplemental zinc perhaps the range of 11 to 50 mg/day would be more protective and scientifically sound. This needs to be discussed between nutritional scientists and toxicologists. It would be hoped that a critical evaluation of all available data would convince the toxicologists that the nutritionally-related data are appropriate to include in the evaluation of risk from ETEs. This would result in a less rigid and more scientific approach with less reliance on default positions by regulatory scientists.

There are certainly many research needs which must be filled before we can successfully apply the proposed homeostatic model, however, it is hoped that this proposal from IPCS will stimulate such studies and cooperation in order to protect both human health and the environment from the adverse consequences of both deficient and toxic exposure to ETEs.

### Acknowledgements

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## Environmental risk assessment for essential elements

### Case study: Zinc

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#### 1. Introduction

In risk assessment methodology, the “ Predicted Environmental Concentration ” (PEC) of a substance is compared with its "Predicted No Effect Concentration" (PNEC), the environmental level at which no adverse effect on ecosystem function is to be expected.

Environmental concentrations can be estimated from model predictions, but follow preferably from direct environmental monitoring.

The PNEC is derived from ecotoxicity data obtained on distinct organisms under laboratory conditions. In spite of the artificial nature of such data and their lack of connection with the higher levels of ecosystem organisation, this approach might be the only one feasible for assessing the multitude of man-made existing and new (organic) chemicals.

Current methods for PNEC determination include among others:

- a) the "safety factor" approach (EU TGD 1996) and
- b) the statistical extrapolation model, as used by the Dutch National Institute for human health and environment (Aldenberg & Slob 1991).

These methods were initially developed for man-made chemicals, not naturally present in the environment. However, when applied to the natural essential elements, several conceptual problems and inconsistencies with biological reality arise.

In the following, some specific features of essential elements will be highlighted and examples of the problems related to the two above-mentioned methods will be discussed shortly.

Subsequently, an alternative approach will be presented for the PNEC determination of essential metal elements, integrating at the same time their natural occurrence, their essentiality and potential ecotoxicity. Specific criteria for data relevance, following from this alternative approach, will be discussed.

The approach will be applied to zinc in a risk assessment for the European lowland river freshwater habitat. Some predictions on future trends in zinc exposure will also be made.

#### 2. Natural essential elements

Essential elements (EEs), e.g. Zn, Cu, Mn, Ni, Co, Se,... are needed by biota to fulfil specific functions in metabolism. Organisms take up the EEs from their natural environment, either directly or from food. EEs (e.g. zinc : Table 1) occur in the natural environment in natural concentration ranges. As indicated in Table 1 for the surface waters, natural zinc concentration ranges observed in different parts of the same environmental compartment can vary substantially too. Within the natural environment, some general "habitat-types" can thus be distinguished, characterised by a different natural EE concentration range (Table 1). Attention is drawn to the fact that for environmental risk assessment, the bioavailable EE fraction, not the total EE concentration, should be considered.

**Table 1:** Natural zinc levels (total zinc) in the environment

	<b>Range</b>
<b>Air</b> (rural) ( $\mu\text{g}/\text{m}^3$ )	0.01 - 0.2
<b>Soil</b> (general) (mg/kg DW)	10 - 300
<i>In igneous and sedimentary rocks</i> <sup>(1)</sup> (ppm)	
- basaltic igneous	48 - 240
- granitic igneous	5 - 140
- shales and clays	18 - 180
- sandstones	2 - 41
- black shales	34 - 1500
<i>Ore bodies</i> (%) 5 - 10 <sup>(2)</sup>	
<b>Surface water</b> ( $\mu\text{g}/\text{l}$ )	
<i>Habitat-type:</i>	
- Open ocean (surface)	0.001 - 0.06
- Coastal seas / inland	0.5 - 1
- Freshwater:	
Alluvial lowland rivers rich in nutrients and oligo-elements (e.g. European lowland)	5 - 40 <sup>(3)</sup>
Mountain rivers from old, strongly leached geological formations (e.g. Rocky Mountains)	< 10 <sup>(4)</sup>
Big lakes (e.g. great lakes US)	0.09 - 0.3 (dissolved) <sup>(5)</sup>
EE-enriched streams floating through mineralisation areas	> 200 <sup>(6)</sup>

<sup>(1)</sup> Thornton I. 1996<sup>(2)</sup> MG 1994<sup>(3)</sup> Zuurdeeg 1992<sup>(4)</sup> Kiffney & Clements 1996<sup>(5)</sup> Nriagu 1996<sup>(6)</sup> Whitton et al 1982<sup>(7)</sup> van Tilborg 1996

Since all organisms are dependent upon EEs for optimal growth and development, all species have been conditioned during the course of evolution to the EE concentration in their natural habitat and to the physico-chemical conditions of that habitat, which determine the EE's bioavailability. In nature, organisms belonging to different habitat-types will thus be conditioned to different natural EE concentration ranges. This is illustrated by measured data on 3 organisms (Fig. 1), belonging to habitat-types with different natural zinc concentration ranges (see also point III below).

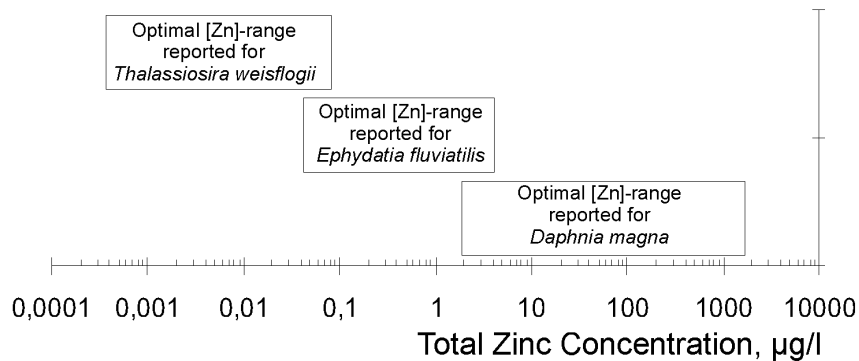


Figure 1: Deficiency-toxicity response to zinc of 3 aquatic organisms, belonging to 3 different habitat-types in terms of natural zinc concentration.

- a) *Thalassiosira weissflogii*: marine; natural concentration 0,0065 µg Zn/l (Anderson et al 1978)
- b) *Ephydatia fluviatilis*: Lake Pontchartrain (brackish); natural zinc concentration: < 1 µg/l (Francis & Harrison 1988)
- c) *Daphnia magna*: freshwater; natural zinc concentration: 5-40 µg/l (Keating et al 1989; Zuurdeeg 1992).

### 3. The optimal concentration range for essential elements (OCEE)

For each species and for each EE, an "Optimal Concentration range for Essential Elements" (OCEE) can be observed (Fig. 2). Within this OCEE, the species can satisfy its metabolic requirements for the EE, and develop and perform in an optimal way. Logically, the OCEE is linked with the natural (bioavailable) concentration range of that EE in the species' natural habitat (Fig. 2). The OCEE is further determined by the species' homeostatic capacity, that allows it to regulate actively its internal EE levels to a certain extent, and maintain them at optimal levels under varying external EE availabilities. This homeostatic regulation has, however, its limits: when the external EE concentration gets too high or too low, homeostatic capacity will not be sufficient and toxicity or deficiency can occur, resp. (Fig. 2).

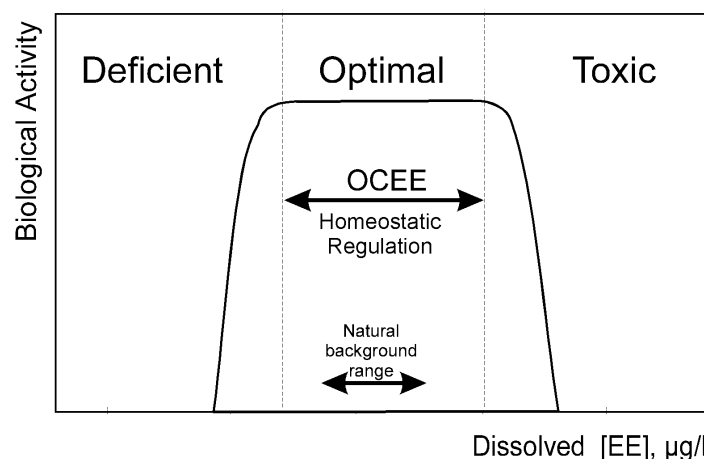


Figure 2: The Optimal Concentration range for Essential Elements (OCEE) for a species in a given habitat-type, as defined by a) the natural EE concentration range in that habitat-type, and b) by the species' homeostatic regulation capacity.

The OCEE's of all species, living in a same natural habitat, will concur to a certain extent around the natural EE concentration range, and show variation on the toxicity and deficiency boundaries, according to the species' homeostatic regulation capacity (including specific uptake mechanisms).

The OCEE can for the essential micronutrients be regarded as the concentration range, in which EE regulation allows for optimal metabolic activity. In toxicological terms, the OCEE thus represents a stress-free EE concentration range, where no deficiency- or toxicity-stress on metabolism occurs.

This has been demonstrated in plants (*Phaseolus vulgaris*), grown on increasing zinc concentration in the substrate: both a toxicological endpoint (growth rate) and metabolic stress reactions (stress enzymes) were affected when the same internal threshold level was exceeded (Van Assche et al 1988).

#### 4. Problems with PNEC determination for EEs following general methodologies

As a general approach in risk assessment, a safety factor is applied to the lowest ecotoxicity values observed in a database on a given substance. The safety factor varies with the uncertainty of the ecotoxicity value that is referred to. However, when applied to a natural EE, such an approach often leads to PNECs well below the EE's natural concentration range. At the same time, such PNECs would also be situated at concentrations that are deficient for many organisms in the ecosystem. The safety factor approach is thus not applicable to essential elements.

As an alternative to the safety factor approach, the statistical extrapolation model (Aldenberg & Slob 1991) has been used, when enough relevant data are available. This model assumes a logistic distribution for different chronic ecotoxicity data (no observed effect concentrations : NOECs) observed on a number of species, belonging to an ecosystem. Such a distribution is symmetrical i.e. the chances for occurrence for more sensitive species (below the mean value) equal those for less sensitive species. Based on such distribution, the lower 5 percentile value is determined, the "Maximal Tolerable Concentration" (MTC), at which 95% of the species is protected (accordingly, 5 % of "sensitive" organisms are at risk).

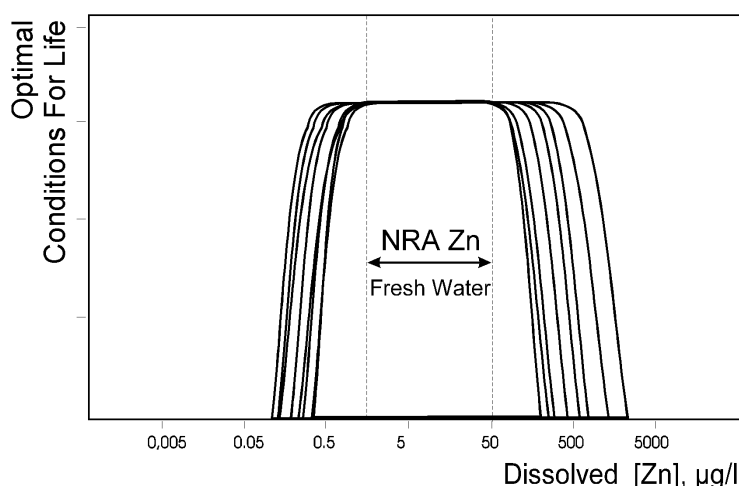
However, it has been noted that in the case of EEs, there is no logistic distribution in the low concentration range, since such concentrations are essential (Van Straalen & Verkleij 1991). This creates a problem for the application of the model for PNEC determination since the MTC is determined precisely in this low concentration range. Concentrations that are essential cannot put at the same time "sensitive" organisms at risk.

The model's use was justified on the basis of its statistical significance, but it is obvious that a statistical approach can mask more complex biological phenomena: e.g. the combination of data from species belonging to a natural habitat a) very low in zinc, b) with moderate zinc levels and c) enriched in zinc, would statistically yield a logistic distribution with one 5 percentile value, while in reality, three different data sets were combined, each reflecting the ecotoxicity response of organisms conditioned to these three different natural habitat-types. Based on statistical considerations, data from zinc effects on marine organisms and freshwater organisms have been considered together in one data set, resulting in a common 5 percentile value for both habitat-types (Janus 1993) (For a more detailed discussion of the statistical extrapolation model and its applicability for EE MTC derivation, see van Tilborg 1996).

#### 5. The OCEE - No Risk Area Concept as a basis for PNEC determination for essential elements

##### 5.1 The OCEE-No Risk Area Concept

The purpose of a PNEC determination is to set a level, at which the biodiversity structure and the function of an ecosystem are protected. Under III, it was outlined that the OCEE of a species represents a concentration range where metabolic requirements for the EE are satisfied and within which no stress occurs. Since all the OCEEs of the species belonging to a common ecosystem-type are related to the natural, bioavailable EE concentration range, they will all be centred around that natural range. The inner area of overlap of the OCEEs is defined as the "No Risk Area" (NRA) since, within its boundaries, none of the species will be subjected to toxicity or deficiency stress (Fig. 3).



**Figure 3:** The No Risk Area (NRA), determined by the inner envelope of the overlapping OCEE curves of the species, belonging to a given habitat-type (example: zinc in the freshwater). Within the NRA, none of the species is subjected to deficiency or toxicity stress.

Like the OCEE (characterizing a species' response to a given EE), the NRA (characterising an ecosystem-type response to a given EE) has two boundaries : one at the deficiency side, one at the toxicity side. The boundaries of the NRA are determined by the species with the most limited homeostatic regulation capacity. Within the NRA, none of the species suffers from EE stress and the ecosystem is unaffected. Since the NRA, like its composing OCEEs, is linked to the natural EE concentration range, it is specific for a given habitat-type, i.e. habitat-types characterised by different natural EE concentration ranges will be characterised by organisms with different OCEEs (e.g. in Fig. 1) and, consequently, by NRAs situated around these ranges.

In nature, the natural availability of EEs will generally prevent deficiency occurring. With the exception of the open ocean, deficiency can be induced only under artificial conditions, e.g. in the laboratory (EE deficient culture conditions) or in agricultural practice (as a result of e.g. liming, the EE bioavailability can significantly decrease, causing deficiency in crops).

## 5.2 PNEC derivation

For environmental risk assessment, it is the toxicity-PNEC that must be determined. The NRA's upper (toxicity) boundary is determined by the biological species with the lowest No Observed Effect Concentration (NOEC) value. This value is used for PNEC determination.

Different habitat types, characterised by different NRAs, will thus have different PNECs. Since one (lowest) NOEC value can determine the PNEC, all NOEC values and in particular this lowest value should be highly reliable and their relevance for the habitat-type which is assessed should be assured. The test showing the lowest NOEC should be reproducible and be conducted by preference under stringent quality control.

This implies the consideration of a few EE-specific criteria for data relevancy (see next point). It is also clear that a rather extensive data set should be available (e.g. > 10 reliable chronic NOEC values obtained on different species from different taxa and trophic levels), to assess the NRA.

For most EEs, such extensive and relevant data sets are available indeed. However, it is noted that data obtained by ecotoxicity testing following standard procedures (e.g. from the OECD) are scarce.

## 6. Criteria for data relevancy following the OCEE-NRA concept

Apart from the classical quality criteria that are considered when using ecotoxicity data for risk assessment, some metal-specific criteria, mainly related to the relevance of laboratory data for risk assessment should be taken into account. Indeed, organisms conditioned to natural habitat types with

different natural bioavailable EE concentration range are expected to react differently when subjected to EE test concentrations in the laboratory.

For this reason, the natural origin of test organisms used in laboratory tests should be well documented, and only data obtained on organisms originating from the habitat type for which the risk assessment is being made, should be used. This implies that risk assessments should be habitat-specific, and that data sets for organisms from different origins (e.g. marine and freshwater) should not be mixed.

Moreover, organisms in general, and unicellular green algae in particular, subjected to suboptimal (slightly deficient or slightly excessive) bioavailable EE concentrations have the capacity to adapt to this situation. As a result of this, organisms cultured in the laboratory under EE conditions deviating from their natural habitat can become adapted and will show an ecotoxicity response which is different from the one, observed on organisms taken from the field.

It should be emphasised that such phenomena can occur rather quickly and even when EE exposure is only different in a part of the organism's life cycle : e.g. fish larvae (*Jordanella floridae*) that were not exposed as embryo to zinc, showed response at lower zinc concentration than larvae from exposed embryos (Spehar 1975).

Consequently, organisms should be cultured and tested in the laboratory in (bioavailable!) EE conditions similar to their natural habitat, and this during their whole life cycle. This implies that the physico-chemical factors influencing EE bioavailability should be representative of the natural environments, or, when this is not the case, that the ecotoxicity value obtained under the deviating laboratory conditions should be adjusted to the field conditions, before being considered for PNEC determination.

Considering the above, the following criteria for data relevance should be taken into account:

- the origin of the test species should be well identified and be relevant to the natural habitat-type for which the risk assessment is made;
- the culture and test-conditions should relate to the natural habitat-type of the species

As outlined under section 4, current approaches for PNEC determination in risk assessment conflict with EE specific characteristics. The OCEE-NRA concept integrates:

- the **natural presence** of EEs in the environment, in concentration ranges dependent on the habitat-type. It should be noted that the absolute value of the natural concentration range is as such not important for PNEC determination; it is, however, necessary to know it in order to evaluate the relevancy of test organisms for risk assessment
- the **essentiality** of EEs, as well as their **potential ecotoxicity**.

The OCEE-NRA concept thus provides a scientific framework for PNEC determination of EEs in risk assessment. Different levels of uncertainty are addressed by the concept:

- intra-species variability : the homeostatic regulation defines the species OCEE.
- inter-species variability : the NRA is the inner envelope of all species OCEEs for a given habitat-type.
- acute versus chronic data : the NRA is based on OCEEs derived from chronic tests
- laboratory versus field data : the (habitat-specific) natural EE concentration range forms the basis of the OCEE-NRA concept. The data relevance is checked with the field conditions (natural EE level, origin of test organisms, physico-chemical characteristics of natural environment).

It is emphasised that, in order to take into account inter-species variability, the NRA should be based on enough data (>10 species) from different taxa and different trophic levels and certainly including autotrophic organisms.

As a general rule, PNEC values determined by whatever method should be checked with field ecotoxicity data (e.g. in situ biomonitoring studies) (see section 8).

## 7. Exposure

Laboratory ecotoxicity tests are, as a rule, performed on soluble salts of EEs, under conditions that promote the maximal availability of the ionic forms to the organisms. In nature, however, the bioavailability of EEs and their ultimate chemical fate is strongly influenced by a variety of physico-chemical factors. It is clear that in risk assessment, PNECs, largely determined on bioavailable EE levels, should be compared with bioavailable EE concentrations in the environment, too.

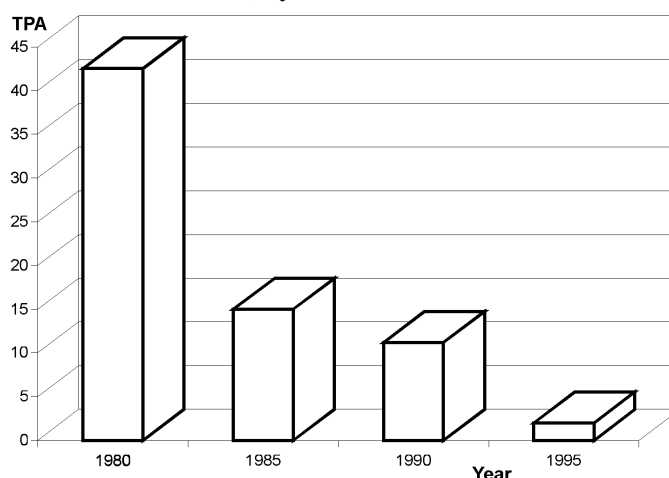
A large amount of monitoring data on EE levels in various environmental compartments is available. Unfortunately, the majority of these data is related to total EE levels, and information on bioavailable fractions is rather scarce.

Recently, more attention has been paid to this problem, and more data providing an estimation of the bioavailable fraction have come available. It is emphasised that the physico-chemical transformation of EEs in the natural environment is a complex issue and that, in any case, (recent) measured data (including estimations of the bioavailable fraction) are preferred to estimations resulting from modelling.

EEs are present in the environment due to natural biogeochemical processes and anthropogenic input. In the latter category, we distinguish between point source emissions, resulting from industrial processes, and "diffuse" emissions, resulting from the dispersive use of EEs in products. In general, point source emissions of metals to air and water have strongly decreased as a result of ever stringent emission control at industrial processes.

### 7.1 Present zinc emissions

Taking zinc as an example, zinc emissions to water from the Belgian Non-Ferrous Metals Industry strongly decreased between 1980 and 1995) by more than 90%

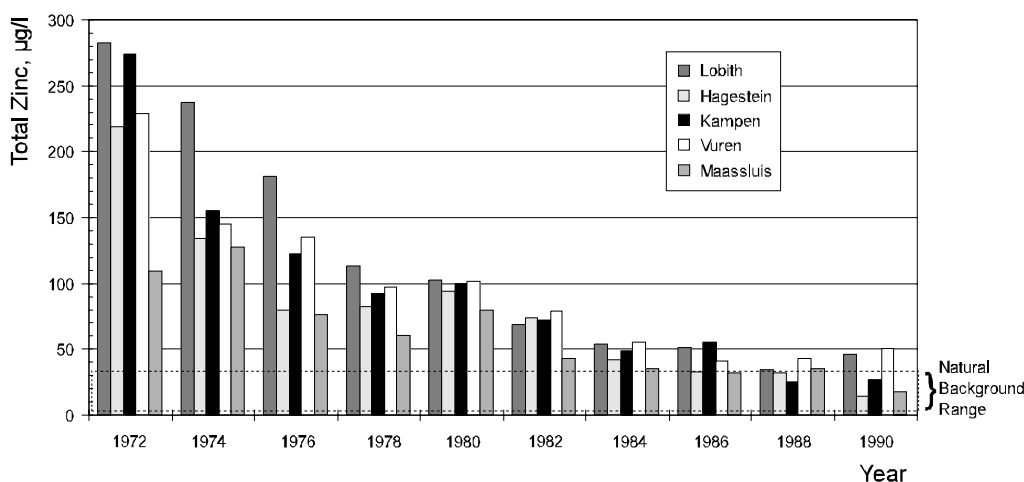


**Figure 4 :** Trend in total zinc emissions to the surface water by the Belgian non ferrous industry : 1980 - 1995 (after Royal Belgium Federation of Non-Ferrous Metals, 1995).

The zinc wash-off resulting from the corrosion of zinc structures exposed to atmospheric conditions, has been identified as an important "diffuse" source, apart from emissions from households, agriculture and traffic. As a result of marked reductions in atmospheric SO<sub>2</sub> emissions, the SO<sub>2</sub> levels in the ambient air have markedly decreased also since the 1960s. The zinc corrosion rate, predominantly related to ambient air SO<sub>2</sub> levels, has decreased accordingly (EZI 1995).

The decreases in anthropogenic zinc emissions, observed at different levels, have resulted in a strong decrease of zinc levels in the environment, e.g. in the Rhine.

(Fig. 5). The present-day levels of zinc in the Rhine (Fig. 5) show that the anthropogenic zinc input is of a same order of magnitude as the zinc load due to the natural zinc cycling.



**Figure 5 :** Trend in total zinc level in the Rhine river at 5 different sampling sites in the Netherlands: 1972-1990 (after Heymen & Vanderweyden, 1991), with an indication of the natural background range (after van Tilborg & Van Assche 1995)

## 7.2 Zinc exposure in the future

In general terms, it can be stated with certainty that, in the future, point source emissions of EEs (including zinc) will be further reduced by the general application of Best Available Technology at the process and emission control level. With respect to zinc, it is noted that the further reduction of all other SO<sub>2</sub> emissions remains a priority for international environmental policy. Within Europe, different scenarios are considered at the moment, putting forward a further SO<sub>2</sub> emission reduction between 50 % and 90 % between 1990 and 2010 (Elvingson 1996).

It is clear that the anticipated further decrease of ambient air SO<sub>2</sub> levels will result in a further decrease of the atmospheric zinc corrosion rate, too. A further general decrease in environmental zinc exposure can thus be expected in the future.

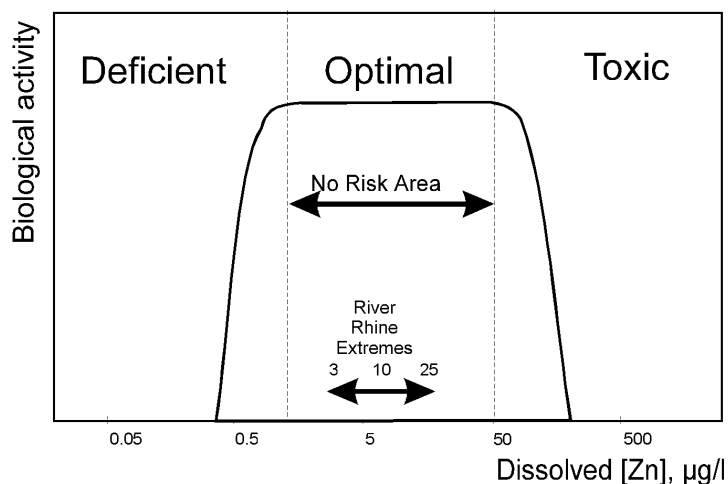
Again, the decrease of environmental EE levels is a general phenomenon that has occurred in a relatively short time frame. It is obvious that such positive trends should be taken into account in risk assessment, and that recent data should be used for exposure assessment.

It is noted also that other environmental contaminants (e.g. organic matter in surface waters) can directly influence EE bioavailability and that changes in these parameters should also be taken into account by using recent data.

## 8. Risk assessment for zinc in the European alluvial lowland river habitat

The OCEE-NRA concept outlined above applies in principle to all natural essential metallic elements. As an example, a risk assessment is made for zinc in the European alluvial lowland river habitat. The natural zinc concentrations in this habitat-type are between 5-40 µg (total) zinc/l due to natural biogeochemical processes, including seasonal variability and local variations (Zuurdeeg 1992, van Tilborg & Van Assche 1995). In terms of EE bioavailability, the habitat is further characterized by a natural hardness around 200 mg CaCO<sub>3</sub>/l.

Taking into account a) the EE-specific criteria for data relevancy outlined under IV, and b) an important physico-chemical factor for this habitat (hardness), a lowest chronic NOEC value of 50 µg Zn (dissolved)/l can be derived from a large dataset (Van Tilborg 1996). This value, observed on *Ceriodaphnia dubia* (Belanger and Cherry 1990) thus sets the PNEC for dissolved zinc in this habitat-type (Fig. 6).



**Figure 6:** Risk assessment for zinc in the European alluvial lowland river habitat. Indicated are a) the No Risk Area, bordered at the toxicity side by the PNEC, calculated under VIII, and b) the mean, minimum and maximum concentrations of dissolved zinc in the Rhine, observed over the years 1988, 1989, 1990 (the latter data are from Heymen and vd Weijden, 1991).

Preferentially, PNEC values set for a given habitat-type should be checked against data from higher organisational level e.g. ecosystem studies, and against field data e.g. observations from in situ biomonitoring studies. Unfortunately, such validation is very scarce.

For zinc, van Tilborg (1996) reviewed few relevant population studies available. The author found no observations of shifts in populations at concentrations within the OCEE-NRA described for the given habitats. There were, however, clear indications for shifts when concentrations exceeded the upper boundary of the OCEE-NRA.

In the Flanders region of Belgium, the biological quality of the freshwater is monitored by means of the Belgian Biotic Index (BBI), evaluating the presence and abundance of benthic macroinvertebrate fauna.

Measurements on an uncontaminated river (the “Kleine Nete”) show that a high quality ecosystem is present at an average zinc concentration of 60 µg Zn (total)/l (with concentrations ranging between < 20 µg/l to 140 µg/l). The high BBI of 8 observed in 1990 (VMM 1991) implies the presence of vulnerable species in normal abundance. For the same location, a BBI of the highest ecological quality level (9-10) was reported in 1992 (VMM 1993) at zinc concentrations averaging 100 µg/l (ranging between 50 and 150 µg/l). The dissolved amount of zinc was estimated to be 40-50% of total zinc.

The PNEC of 50 µg (dissolved) Zn/l, derived from the NRA, is thus confirmed by observations at the population level and in the field.

In Fig. 6, the NRA for zinc in the European lowland rivers is indicated, and compared to recent levels of dissolved zinc in the Rhine. It is clear that, even when maxima are considered, these current levels fall well within the NRA described for this habitat, and that, consequently, zinc presents no risk to the ecosystems in the Rhine.

For other European rivers, recent data on soluble zinc are lacking. Total zinc levels in freshwater roughly are 3-4 times higher than soluble zinc levels. This would result in a PNEC of 150-200 µg total zinc/l. Comparison with total zinc levels observed in European rivers (table 2) leads to the conclusion that zinc presents no risk to the European river ecosystem in general.

**Table 2: Total zinc levels in European rivers**

<i>River</i>	<i>Year</i>	<i>Zinc total (<math>\mu\text{g/l}</math>)</i>
Rhine (5 points in NL)	1990	14-50 (1)
Meuse (B-NL border)	1990	60 (2)
UK rivers (average)	1992	22 (3)
Schelde (B-NL border)	1989	50 (4)

- (1) Heymen & vd Weijden 1991  
 (2) CCRX 1991  
 (3) Harmonised monitoring scheme UK (1994)  
 (4) Goethals 1991

This general conclusion does not preclude the possibility that locally, where zinc concentrations are elevated due to uncontrolled point emissions, stress effects can occur. It is clear that in such situations proper risk management action is needed.

## 9. Conclusions

The OCEE-NRA concept provides a general framework for risk assessment of essential elements, integrating the natural occurrence of EEs, their essentiality and homeostatic regulation by organisms, and their potential ecotoxicity. It allows for the determination of a PNEC value, based on bioavailable EE concentrations.

The concept implies that separate PNECs should be determined for habitat-types, characterised by different natural EE concentration ranges. Laboratory data, used for PNEC determination, should be related to such specific habitat-types too.

Both the PNEC and the PEC should be expressed as bioavailable EE concentrations, to estimate the potential risk.

The OCEE-NRA concept is applicable to all environmental compartments. Its relevance for non-essential naturally occurring elements should be investigated.

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## Zinc in aquatic and soil environments

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Zinc is an essential trace metal; both instances of zinc excesses that result in toxicity and zinc nutrient deficiencies have been reported. For any environment there is an optimal range of zinc concentrations. Zinc concentrations in natural waters, sediments and soils can each vary by orders of magnitude. However, the availability of zinc, as well as other essential and non-essential metals, is not directly related to the total concentration of the metal in the environmental compartment. For example, some soils that are zinc deficient have higher concentrations of zinc than do other soils in which there is optimal growth of the same plant species. Clearly, to evaluate the potential ecological effects of zinc it is necessary to consider the chemical forms, or species of zinc that are present, as well as the total zinc concentration (Allen, 1993). Environmental criteria and standards must reflect the speciation to be predictive of ecosystem effects. To evaluate aquatic or terrestrial systems, it is necessary to obtain valid measurements of the concentration of zinc in environmental samples and to compare these to appropriate criteria and standards. This paper briefly reviews critical aspects of the environmental chemistry of zinc that are of importance in the evaluation of environmental quality. Because there are many similarities between the behaviour of zinc and of other trace metals in the environment, examples for other metals are introduced as appropriate.

### 1. Analysis of zinc

Zinc concentrations in natural waters are frequently in the low  $\mu\text{g/L}$  concentration range. To obtain valid data for such low concentrations it is necessary to take special precautions in sampling and analysis programs. The U.S. EPA (Prothro, 1993) has summarised the necessary components of the measurement program:

1. Use of clean techniques during each step of the measurement process to avoid contamination.
2. Use of analytical methods that have sufficiently low detection limits.
3. Avoidance of interference in the quantification step.
4. Use of blanks to assess contamination.
5. Use of sample spikes and certified reference materials to assess the effects of contamination and interference on accuracy.
6. Use of replicates to assess precision.

Although the importance of none of these items should be underestimated, the first item is of particular importance. This includes such factors as acid washing sample bottles, use of “clean hands - dirty hands” operating protocols during sampling, and use of laminar flow hoods and benches and clean rooms for laboratory measurements. It is particularly important that gloves worn must be talc-free. The analytical method should have a detection limit no greater than one-tenth that of the sample and the concentration found for the blank should not exceed one-tenth that of the blank.

The need for these precautions in sampling and analysis for environmental studies was first pointed out by Patterson and Settle (1976) with regard to the analysis of lead. Since then it has become apparent that these considerations must be applied to the sampling and analysis of all trace metals (Bruland, 1983; Nriagu et al., 1993; U.S. EPA, 1995).

Although the oceanographic community quickly realised the importance of using these clean techniques, those concerned with the analysis of freshwaters and effluents were slower to respond. This attitude changed after the publication of Windom and his colleagues (1991) that demonstrated the questionability of the data that had been collected for the U.S. Geological Survey’s NASQAN national stream quality network. They found copper concentrations about 3-fold lower, cadmium

concentrations 30-fold lower, lead concentrations 100-fold lower and zinc concentrations almost 20-fold lower by using appropriate low level sampling and analysis techniques.

Significant regulatory and economic issues arise as a consequence of results that are biased high. A summary of the results of analyses for waters of New York Harbour are presented in Table 1 (Battelle Ocean Sciences, 1991). The harbour had been routinely sampled and analysed by the New York City Department of Environmental Protection. Of the six metals studied, for 1987 only for cadmium was the annual average below the value of the Water Quality Criteria. The reported 1987 annual average zinc concentration exceeded the criteria more than three-fold. In the following year, a series of cruises was carried out by the U.S. EPA on the Research Vessel Anderson. Sampling and analysis was carried out by Battelle scientists using low level sampling and analysis techniques. The concentrations of all six elements decreased greatly, as much as 40-fold in the case of cadmium, when the 1988 results are compared to those of 1987. The average zinc concentration for the 1987 data are more than 25-fold greater than are the results that were obtained using the “clean” techniques in 1988. As a result of evaluation of the newer data, the potential ecological impacts were reassessed and an unnecessary, costly upgrade of sewage treatment plants discharging to the lower Hudson River and the New York Harbour was not instituted.

**Table 1:** Water Quality Criteria and annual average concentrations using conventional sampling and analysis methods (1987) and low level sampling and analysis methods (1988) for New York Harbour (Battelle Ocean Sciences, 1991).

<b>Metal</b>	<b>Water quality criteria 1987 (µg/L)</b>	<b>NYCDEP 1987 (µg/L)</b>	<b>Anderson 1988 (µg/L)</b>
Cadmium	9.3	4.3	0.11
Copper	2.9	13	4.6
Lead	8.6	70	3.0
Mercury	0.025	0.2	0.015
Nickel	8.3	15.6	2.6
Zinc	86	264	10

## 2. Environmental concentrations

As indicated in the preceding section, without due precaution samples can be easily contaminated and erroneously high results reported. This is particularly true for metals in natural waters. Zinc is depleted in surface sea water relative to its concentration at depth. Donat and Bruland (1995) reported that the concentration of zinc in oceanic surface water is 0.1 - 0.2 nM (0.0065 - 0.013 µg/L) while at depth it ranges from 1.6 to 8.2 nM (0.1 - 0.5 µg/L). In uncontaminated rivers the concentration of dissolved zinc ranges from 0.02 to 1.8 µg/L (Shiller and Boyle, 1985). Dissolved zinc concentrations in the Great Lakes are 0.087 - 0.277 µg/L (Nriagu et al., 1996). As is to be expected, the concentration of zinc in groundwater is more variable. Allard (1994) reviewed existing data and reported the concentration of zinc in groundwater was <0.3 to 570 µg/L. The concentration of zinc in normal soils is 10 to 900 mg/kg (Swaine, 1955) but Förstner (1994) has reported concentrations as high as 2000 mg/kg.

## 3. Chemical speciation

Frequently analyses of chemical species, rather than the total elemental concentration are required. Both bioavailability and sorption of metals are strongly dependent on the metal species that are present (Allen, 1993). Speciation of trace metals involves determination of the physical and chemical forms of the metal. This includes the evaluation of free metal ions, inorganic and organic complexes, and organometallic compounds (Table 2). Although strictly not speciation, classification by size,

including filtration is often considered speciation. Filtration is commonly used to operationally define the dissolved portion of trace metals. However, the values obtained depend on a number of factors in addition to the pore size of the membrane filter that is used (Horowitz et al., 1996). Commonly, speciation studies incorporate voltammetric methods of analysis. Batley (1989) has reviewed many of the available methods.

**Table 2:** Physical and chemical forms of zinc.

Description	Examples
Free zinc ion - hydrated	$\text{Zn}(\text{H}_2\text{O})_6^{2+}$ or $\text{Zn}^{2+}$
Inorganic ion pairs and complexes	$\text{ZnOH}^+$ , $\text{Zn}(\text{OH})_2$ , $\text{ZnHCO}_3^-$ , $\text{ZnCO}_3$
Organic complexes and chelates	Zn-SR (zinc cysteinate) Zn-OOCR (zinc glycinate) Zn-EDTA
Metals bound to high molecular weight organic matter	Zn-humates
Metals present as highly dispersed inorganic colloids	$\text{FeOOH}$ (goethite), $\text{Fe}(\text{OH})_3$ , $\text{ZnS}$
Metals adsorbed on, or occluded in inorganic colloids, manganese oxide, silica, clay, etc.)	$\equiv\text{S-O-Zn}^+$ ( $\equiv\text{S-O-H}$ is iron oxide,
Precipitates, minerals, organic detritus	

Among the most important speciation measurements are determination of free metal ion and assessment of the interaction of metals with organic matter. Samples are frequently titrated with metal, and the titration is monitored electroanalytically (Batley, 1989). Voltammetric techniques, particularly anodic and cathodic stripping voltammetry, have commonly been applied for chemical speciation of metals. Anodic stripping voltammetry is the most commonly used electrochemical technique (Bond, 1980) but cathodic stripping voltammetry has also been applied to the analysis of zinc (van den Berg, 1984). The methods provide differentiation of zinc associated with weak vis-a-vis stronger ligands and the results of these titrations are usually expressed in terms of the complexation capacity of the sample (Neubecker and Allen, 1983). Van den Berg (1985) has determined the complexation capacity of seawater for zinc has been determined by cathodic stripping voltammetry of the zinc complex of ammonium pyrrolidine dithiocarbamate (APDC).

$\text{Zn}^{2+}$ , as well as  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and other transition metals, can form inner and outer sphere complexes with a number of inorganic ligands, such as  $\text{OH}^-$ ,  $\text{HCO}_3^-$ ,  $\text{NH}_3$ , and organic ligands, such as oxalic acid and EDTA (Stumm and Morgan, 1981; Pankow, 1991). Stability constants for chemically defined ligands are available in a number of data bases. A particularly good source is the NIST Critical Stability Constants of Metal Complexes Database (U.S. Department of Commerce, 1993). Stability constants for these reactions are well-known and the distribution of species can thus be easily computed using thermodynamic principals.

The soluble metal will be present as the free aquo ion and as metal contained in inorganic and organic complexes. The formation of complexes of a divalent metal ion  $\text{M}^{2+}$  with monodentate ligands is expressed by the reaction



If the ionic strength is low, the equilibrium is

$$\beta_{j \text{ i}_a} = \frac{[j \text{ i}^{(0-a)+}]}{[j^{0+}][i^{-a}]} \quad (2)$$

where  $b_{ML_n}$  is the overall formation constant for the complex and the brackets indicate the concentrations of the enclosed species. Typically, a number of complexes are present. Then the total concentration of metal,  $C_M$ , is equal to the concentration of the free metal ion, plus the concentration of metal contained in all complexed species

$$C_j = [j^{0+}] \left( N + \sum \beta_{j \text{ i}_a} [i^{-a}] \right) \quad (3)$$

For ligands that are not monodentate, and for polynuclear reactions involving more than one metal ion, similar reactions and stability constants can be formulated.

Chemical equilibrium computer programs are useful for computing the distribution of species in samples containing defined total concentrations of metal and ligands, if appropriate stability constants are available (Nordstrom et al., 1979). Commonly used programs include MINTEQA2 (Allison et al., 1991) and MINEQL+ (Schecher and McAvoy, 1992).

The description of metal complexation with natural organic matter (NOM) is much more complicated. NOM is an unresolvable mixture of a very large number of compounds varying in their properties, including their ability to bind metal ions. Several approaches have been proposed for the modeling of metal complexation by NOM and humic substances. These include gaussian distribution models (Perdue and Lytle, 1983) and models having multiple discrete sites (Fish et al., 1986). Recently, Tipping (1994) has presented a multiple site model that is able to relatively accurately predict metal and proton binding to naturally occurring organic matter.

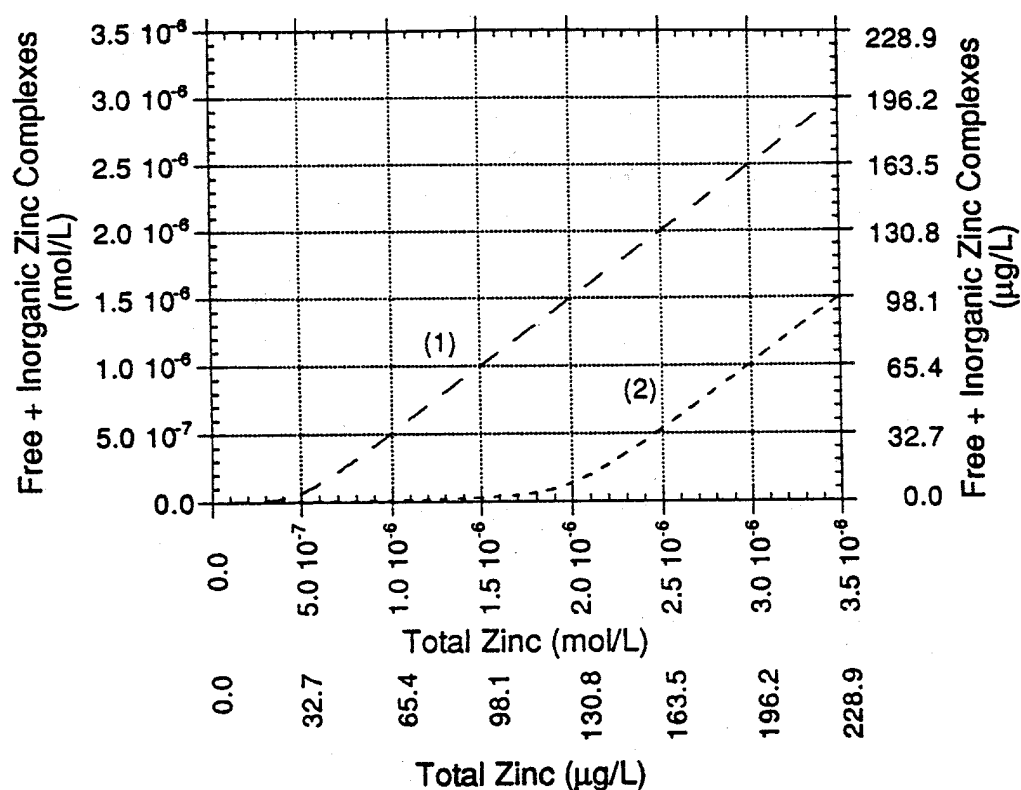
The concentration of organic matter in a natural water is not in vast excess of metal concentrations, particularly the concentrations of metals used in laboratory toxicity tests. Therefore, the speciation of metal varies as a function of the concentration of added metal. This is shown in Figure 1 for the titration of a single ligand. The titration of NOM would result in a titration with not as sharp an inflection.

#### 4. Zinc bioavailability and toxicity to aquatic organisms

The toxicity of zinc and other metals is profoundly affected by their chemical form (see reviews by Luoma, 1983; O'Donnel et al., 1985). Steemann-Nielsen and Wium-Andersen (1970) were the first to recognise that free copper ions, at the level of copper found in natural waters, are very toxic to algae. They reported that the predominant forms of copper are not free copper ions, but are organic complexes and they reported these to be "not poisonous to algae". In recently upwelled water, phytoplankton growth was limited and could be enhanced by the addition of a chelator. The understanding of the relationship between speciation and toxicity was greatly enhanced by the work of Sunda and Guillard (1976) who determined copper ion activity using a copper ion selective electrode and found that the algal growth rate was related to the free copper ion activity and not to the total copper concentration, which they had varied independently.

Because zinc is an essential element, it will be accumulated by organisms. The bioaccumulation factor will be greater if the availability of zinc is low. As a consequence of homeostasis the internal concentration of zinc will be maintained over a wide range of external zinc concentrations. Consequently, the bioaccumulation factor for an essential element such as zinc should not be used as a basis for regulation (Chapman et al., 1996). At concentrations below the homeostatic range, there

will be deficiency; at concentrations that are above the homeostatic range there will be inhibition or toxicity. Both have been demonstrated for zinc.



**Figure 1 :** Titration of a ligand with stability constant =  $2.0 \times 10^8$  for the complexation with zinc ion. Ligand concentration: (1)  $5.0 \times 10^{-7}$  mole/L (32.7 µg Zn binding capacity/L) (2)  $2.0 \times 10^{-6}$  mole/L (130.8 µg Zn binding capacity/L).

Anderson et al. (1978) showed that the growth of the coastal diatom *Thalassiosira weissflogii* depended on the concentration of zinc present in media that contained EDTA. As the EDTA concentration was increased, the total zinc concentration required to obtain the same growth rate also increased. However, for any growth rate, the zinc ion activity was the same for all concentrations of EDTA. Thus, zinc ion activity is a better predictor of bioavailability than is the total zinc concentration. Allen et al. (1980) tested the ability of a number of chelators to ameliorate the toxicity of zinc to the alga *Selenastrum capricornutum*. The conditional stability constants of the chelators at the pH of the test medium varied by more than six orders of magnitude. The results demonstrated that the growth rate was proportional to the concentration of zinc ion plus the monohydroxy zinc complex. Because the pH was constant, the growth could also have been correlated to the zinc ion activity.

Some investigators have reported that chemical species other than free, ionic metal are toxic. This has recently been critically reviewed by Campbell (1995). A mechanistic approach to understanding the influence of solution-phase complexation of the toxicity of metals was proposed by Pagenkopf (1983). He treated metal ion receptor sites on the gills of fish as chemical entities that compete with ligands in the solution for metal ions such as zinc. This allows the prediction of the effect of metal on the organism, based on equilibrium considerations such as those predicted by computer programs such as MINTEQA2 or MINEQL+. This approach also allows prediction of the effects of  $\text{Ca}^{2+}$  and  $\text{H}^+$ .

The principal receptor site for toxic metals in freshwater fish are gills where  $\text{Na}^+$  and  $\text{Ca}^{2+}$  are transported from the bulk water to the bloodstream by active, energy requiring "pumps". The channel or carrier proteins associated with these pumps occur as specific, negatively charged ligands on the gill surface. Thus, the gills of freshwater fish have the two important physiological functions of gas transport ( $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{NH}_3$ ) and active uptake of ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ). Playle and co-workers (1992, 1993a,b) and MacRae et al. (1996) have treated the specific receptor sites on the gill as competitive ligands for the binding of trace metals. They have determined conditional stability constants and site

densities for these surface complexation reactions through competitive binding experiments with ligands whose complexation constants are known. MacRae et al. (1996) showed that there is a strong relationship between the extent of saturation of the gill receptor sites and mortality. Experiments by these research groups have shown that gills indeed do compete for metals with natural DOM.

An important conclusion from the gill complexation model is that given species should not be treated as being bioavailable and other species as being not bioavailable. Rather, the presence of the gill causes chemical re-equilibration in the system. It is the degree to which the gill complexation sites are occupied by metal that determines whether toxicity will occur.

## 5. Water quality criteria and standards

The U.S. Environmental Protection Agency's Water Quality Criteria for Zinc (U.S. EPA, 1985) forms the basis for the standards for the protection of aquatic life that are promulgated by most states. The freshwater criteria for a four-day average concentration (in  $\mu\text{g/L}$ ) of zinc that is not to be exceeded more than once every three years on the average is given by the expression  $e^{(0.8473[\ln(\text{hardness})]+0.7614)}$ . The acute criteria value (in  $\mu\text{g/L}$ ), based on a one-hour average concentration should not exceed the numerical value  $e^{(0.8473[\ln(\text{hardness})]-0.7614)}$  more than once every three years on the average. For hardness of 50, 100 and 200  $\text{mg/L}$  as  $\text{CaCO}_3$  the four-day average concentrations of zinc are 59, 110 and 190  $\mu\text{g/L}$ , respectively, and the one-hour average concentrations are 65, 120, and 210  $\mu\text{g/L}$ .

A single numerical value, even when modified by water hardness, does not provide the predictability of aquatic life effects that are desired for national criteria. Therefore, provision is made for site-specific modification of the criteria. The Water Effects Ratio (WER) has been recommended to provide site specific modified criteria (Stephan et al., 1985; U.S. EPA, 1992, 1994; Prothro, 1993).

To establish a WER, toxicity tests are conducted in a site water and in a reference ("laboratory") water. Reference water tests are used as surrogates for the laboratory tests that were used to derive national criteria. The ratio of the toxicities (WER) is used as a multiplier to adjust the National Water Quality Criteria (NWQC) to account for differences in bioavailability, as measured by toxicity tests, that would be applicable to that site. For example:

$$\text{Site - Specific WQC} = \text{NWQC} \times \text{WER} = \text{NWQC} \times \frac{\text{site - water LC50}}{\text{reference - water LC50}} \quad (4)$$

Brungs et al. (1991) reported WER values for zinc as great as 2.9. WER values for copper at the same location were greater, reflecting the stronger affinity of copper, relative to zinc, for organic substances.

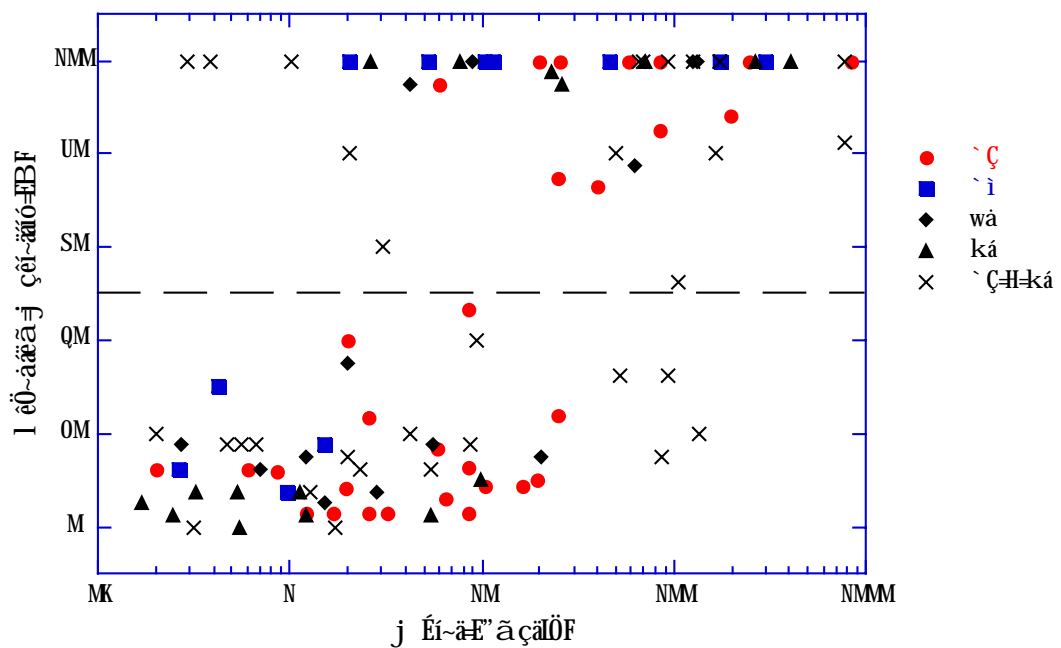
Allen and Hansen (1996) have analysed the WER procedure in terms of the change in speciation that occurs in a sample as metal is added to a sample. They indicated that the fraction of metal that is present in forms having reduced bioavailability decreases as the total concentration of metal increases. This implies that the WER for sensitive organisms will be greater for more sensitive organisms than it will be for less sensitive ones. This is in agreement with the information that has been presented by Brungs et al. (1991).

Allen and Hansen (1996) recommended that new Water Quality Criteria based on bioavailable metal be developed. Such criteria would have universal applicability and would obviate the need for such site-specific modifications as WERs.

Bergman et al (1997) recommended that the gill complexation model be used as the basis for a WQC that incorporated bioavailability by coupling biological site of action and aquatic speciation. Tipping's WHAM model be used to compute chemical speciation in a receiving water. This model, MINTEQA2 or other speciation code would need to incorporate the necessary constants for binding of metal at the gill.

## 6. Aquatic sediments

Metals and anthropogenic organic compounds are often present at elevated levels in sediments. Evaluation of sediment quality is frequently ascertained through bioassays. However, if toxicity is found, the cause must be ascertained to ensure proper disposal or treatment, to prevent further contaminant input and to allocate responsibility. This creates a difficult situation because high concentrations of metals do not necessarily lead to toxicity. Allen (1996) has discussed Sediment Quality Criteria based on total metal concentrations, such as those proposed for use in Canada (CCME, 1995; Smith et al., 1996). His analysis indicated that they did not adequately discriminate those sediments which produced biological effects from those which did not if the metal concentration was high. Figure 2 shows similar information based on the results of a number of toxicity tests in which cadmium, copper, nickel or zinc or cadmium and nickel were added to sediments. When the concentration of metal is expressed as  $\mu\text{mol/g}$  dry weight of sediment there is no relationship between the concentration of added metal and the mortality of organisms. Non-toxic sediments were found to contain as much as 3 orders of magnitude more metal than did some sediments not exhibiting toxicity.



**Figure 2 :** Toxicity of metals in sediments. Metal concentrations expressed on a dry weight normalised basis. Data courtesy of Dr. Dominic Di Toro, Manhattan College.

For example, Di Toro et al. (1990) added cadmium to samples of sediment that did not exhibit toxicity. The amount of cadmium that was required to be added before toxicity was observed differed by more than an order of magnitude for the two sediments and was greater than what is conventionally considered to represent a contaminated sediment. They found that the added cadmium displaced iron in FeS to form CdS thus rendering it non-toxic. No toxicity was found unless the amount of added cadmium exceeded the concentration of available sulfide when both are expressed on a molar basis.

Analytical methods for the measurement of acid volatile sulfide (AVS) and the concentration of simultaneously extracted metals (SEM) have been described by Allen et al. (1993). Sulfide is evolved by the addition of cold, dilute acid. It is trapped and quantified to provide the AVS value. The

dissolved concentrations of the potentially toxic metals that can displace iron in FeS, which are cadmium, copper, lead, nickel and zinc, are determined in the acid.

The SEM value is the sum of these metal concentrations on a  $\mu\text{mol/g}$  dry weight of sediment basis. The SEM/AVS ratio is useful in prediction of those sediments for which toxicity will not be exhibited. Ratios less than one have not been found to be toxic to organisms. That is, there is not toxicity if the amount of sulfide available to bind metals exceeds the concentration of metals. It should be noted that it is the absence of toxicity that is predicted.

No prediction of toxicity is provided by this procedure. This is shown in Figure 3 for the same data set shown in Figure 2. No toxicity is seen for any sediments having SEM/AVS ratios less than 1.

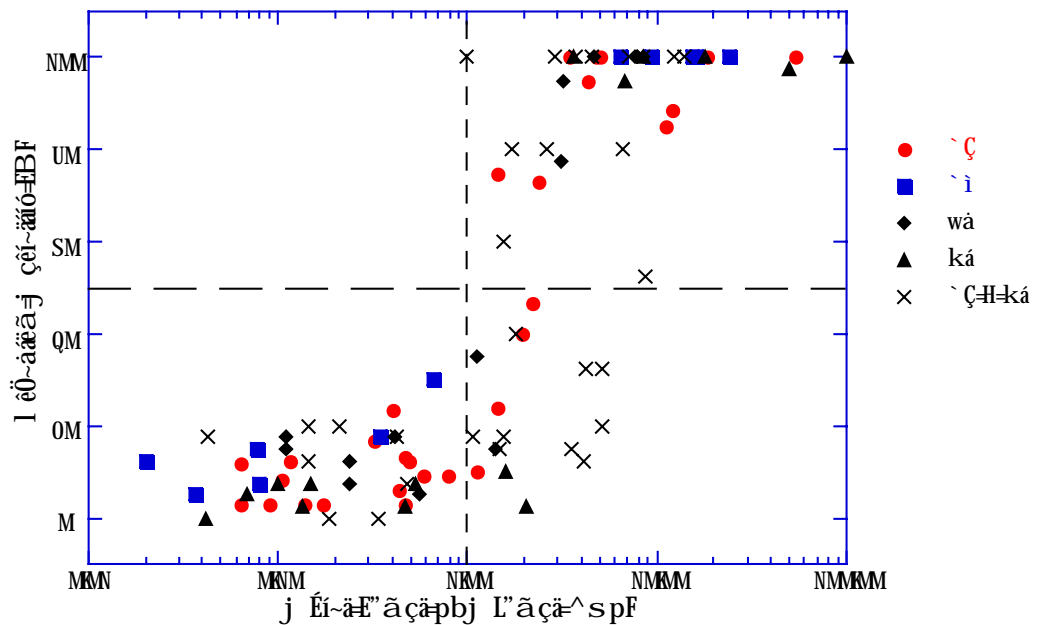
Ankley et al. (1993) found that there appeared to be copper binding in excess of the amount of sulfide present in sediment. This can be clearly seen in Figure 3 which demonstrates that, for some sediments, no toxicity was observed until the SEM/AVS ratio exceeded 5. Mahony et al. (1996) have demonstrated that this additional binding phase for cadmium, copper and lead is the organic matter contained in the sediments. Inclusion of this potential for binding of metals in addition to that of the AVS that is in excess of the SEM (i.e., SEM-AVS) should provide a good estimate of the concentration of metals that could be present in a sediment before toxicity will be observed. Because zinc also forms complexes with organic matter, it is reasonable to assume that this additional binding phase should be included in evaluation of zinc binding in sediments.

## 7. Soil quality

Of all the micronutrients required by plants, on a worldwide basis zinc is the one that is most commonly deficient. Zinc deficiency is present in 39 states in the U.S., as well as in Canada, Australia, New Zealand, Africa, Asia, South America and Europe (Adriano, 1986). However, soil zinc may also reach concentrations that are toxic to plants as a result of application of sewage sludge and industrial wastes. Figure 4 shows that the optimum yield is obtained over a relatively small range of metal concentrations in the plant. The uptake by the plant is related to the concentration of bioavailable metal in the soil.

The cation exchange capacity (CEC) has been used to develop guidelines to limit the application of metals to soils (U.S. EPA, 1979). The guidelines thus formulated, however, are not supported by long-term field experimentation (Sommers et al., 1987). In some cases, the results obtained about the effect of CEC on the bioavailability of metals to plant are in conflict. For example, Korcak and Fanning (1985) determined the uptake of zinc and cadmium, applied as metal salts, by plants grown on twelve different soils. They compared the uptake to that when the equivalent concentration of metal was added from sewage sludge. As the cation exchange capacity increased, tissue zinc and cadmium levels decreased in the metal salt treated soil. However, in the sludge-treated soil, the zinc and cadmium concentrations in the tissue increased as the cation exchange capacity increased.

Sequential extraction has frequently been used to correlate operationally defined metal species to the observed effects (Tessier et al., 1979). These procedures use increasingly strong extractants to release trace metals associated with (a) exchangeable; (b) carbonate; (c) metal oxide or reducible; (d) organic and sulfide; and (e) residual mineral phases. The procedures have been criticised (Martin et al., 1987; Kheboian and Bauer, 1987; Rendall et al., 1980; Tipping et al., 1985) as not being able to provide accurate information as to the associations of trace metals.

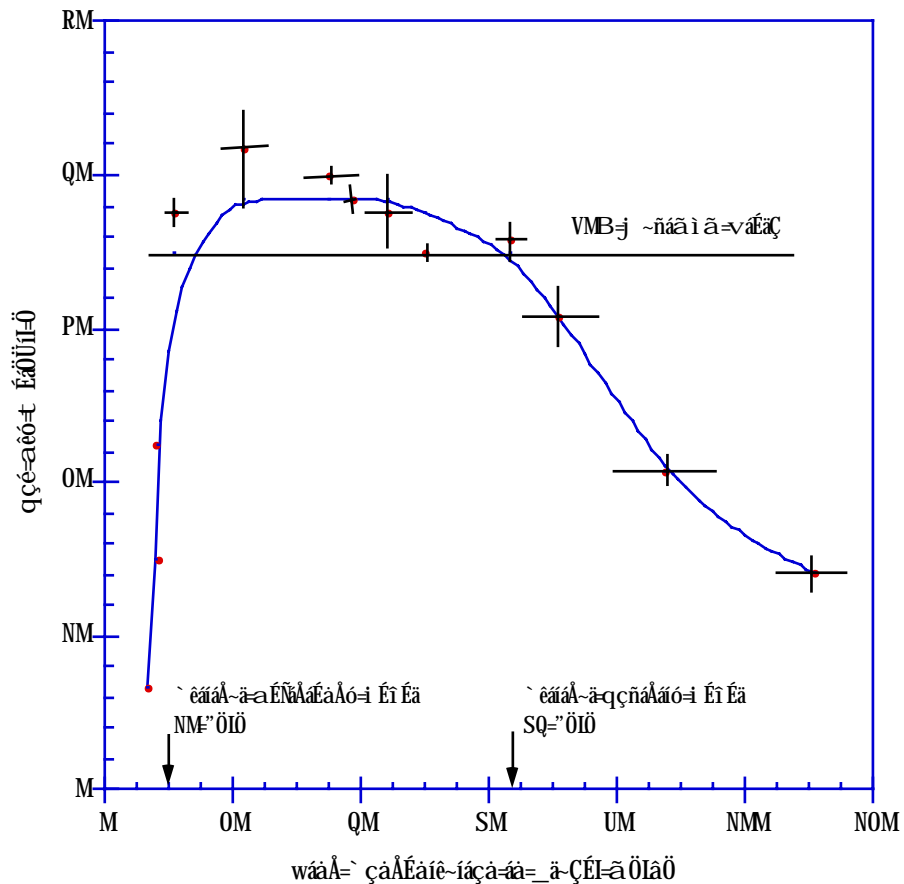


**Figure 3 :** Toxicity of metals in sediments. Metal concentrations expressed on a SEM/AVS normalised basis. Data are the same as those plotted in Figure 2. Data courtesy of Dr. Dominic Di Toro, Manhattan College.

Simpler, single extractant procedures are widely used to estimate metal availability to plants, for instance to establish the quantity of fertiliser that should be applied. Among the extractants that have been used are DTPA, EDTA, acetic acid and the mineral acids,  $\text{HNO}_3$  or  $\text{HCl}$  (Adriano, 1986). A linear relationship between the logarithm of the concentration of the metal taken up by the plant and that extracted from soil has been reported (Browne et al., 1984).

Generally, heavy metals are taken up by plants via pore water. The total metal concentration in pore water is therefore the most important factor determining the uptake. Several factors influence the pore water concentration of metals. Among these factors, pH is perhaps the most important (Anderson and Christensen, 1988; Boekhold and Van der Zee, 1992; Boekhold et al., 1991, 1993; Lee et al., 1996; Van der Zee and Van Reimsdijk, 1987). For example, Christensen (1989) determined the distribution coefficients for 63 Danish agricultural soils and found that the distribution coefficients correlated very well with soil pH ( $r = 0.72$ ). Sorption of metals from pore water is affected by pH which is an important factor in establishing both the soil solid surface charge and the soluble metal speciation.

Increases in pH weaken proton competitive binding and increase surface potential, which consequently increase both electrostatic and specific binding of metals to the solid surface. In addition, increases in pH may result in the formation of hydroxo metal species. The sorption of metals on solid surfaces has frequently been reported to significantly increase as hydroxo species became predominant (James and Healy, 1972; Elliott et al., 1986). James and Healy (1972) suggested that this resulted from the smaller solvation energy of hydroxo metal species than that of aquo species. Tewari and Lee (1975) observed that following the adsorption of  $\text{MOH}^+$ ,  $\text{M}(\text{OH})_2$  precipitates formed on the solid surface, although this would not occur in bulk solution.



**Figure 4 :** Deficiency and toxicity levels for zinc (Adapted from Ohki, 1984).

Besides pH, soil composition is also very important in determining the pore water concentration of metals. Some ligands, including hydroxide, carbonate, silicate, phosphate, and, in anoxic environments, sulfide, are important to the precipitation of metal ions. Ion exchange by 2:1 clay minerals, such as smectite and vermiculite, which have a high CEC is more important in controlling pore water composition than is ion exchange by 1:1 clay minerals, such as kaolinite and allophane, which have much smaller CEC values. In surficial soils the clay minerals are generally coated with metal oxides (Jenne, 1988) and organic matter (Hart, 1982; Davis, 1984). These soil phases constitute the most important metal-binding components.

Recent studies in our laboratory have shown that organic matter is the most important soil component controlling metal partitioning at fixed pH (Allen and Yin, 1996). The partitioning of Cd(II) to soil can be predicted to a very high degree ( $r^2 > 0.9$ ) by considering the organic matter normalised partition coefficient ( $K_{om}$ ) as a function of pH (Figure 5) (Lee et al., 1996). This study was carried out at high concentrations of metal, for which solution speciation by organic matter would have been relatively unimportant. This work suggests that soils are coated by organic matter and that other sorption phases, such as iron and manganese oxides, serve to store contaminants but are not directly involved in the partitioning of metal with pore water.

For Hg(II), which forms stronger complexes with dissolved organic matter, sorption decreased as the dissolved organic matter increased with increasing pH (Yin et al., 1996, 1997a). Increases in the initial Hg(II) concentration decreased the effect of the dissolved organic matter on sorption, and the sorption capacity of soils for Hg(II) was found to correlate with the total soil organic matter content (Yin et al., 1997b,c). Sorption of Hg(II) as a function of pH was modelled with consideration of the interactions of inorganic Hg with both solid surfaces and dissolved organic matter. The competitive binding of protons for the available sites on both solids and dissolved organic matter was considered. The final equation for total aqueous Hg concentration is (Yin, 1996):

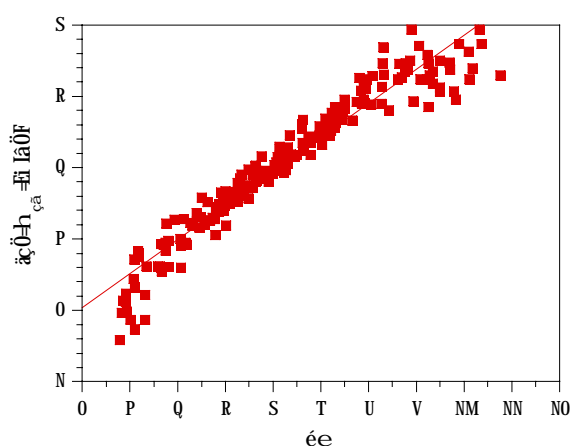
$$C_w = \frac{K_{SOH} \alpha C_{pl} + h_{ei} C_{e0} e^{+zF} + h_{e0i} C_{pl} e^{-zF}}{\alpha C_{pl} + K_{SOH} + h_{ei} C_{e0} e^{+zF} + K_{HgL} C_{e0} e^{+zF} + h_{e0i} C_{pl} e^{-zF}} \quad (5)$$

where  $C_w$  is the total aqueous Hg concentration,  $K_{SOH}$  is the solid surface protonation constant,  $K_{HgL}$  is the soluble organic ligand protonation constant,  $K_{HgL}$  is the stability constant for Hg and soluble organic ligand,  $TOC$  is the total dissolved organic C concentration at each pH value,  $C_{e0}$  is the initial Hg concentration,  $SOC$  is the soil organic C content, and  $\alpha$  is a constant correlating soil binding capacity and strength for Hg with  $SOC$ . Based on equation 5, sorption of Hg on 15 New Jersey soils at two initial concentrations,  $1 \times 10^{-7}$  M and  $1 \times 10^{-6}$  M, over a pH range of 3 to 10, was modeled by a multivariate non-linear program (Wilkinson, 1988). The predicted aqueous Hg concentrations agreed well with the measured with a regression coefficient of 0.92 (Figure 6).

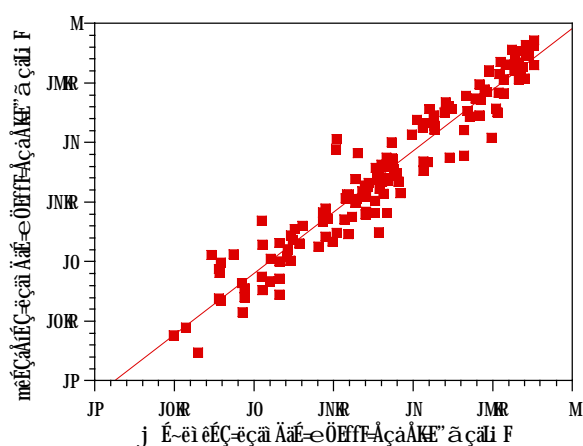
It is expected that the sorption of Zn(II) by soils would be affected by the dissolved organic matter at a low initial zinc concentration, although the extent of this effect may be small due to the weaker affinity of Zn(II) to dissolved organic matter. Because Cu(II) and Ni(II) also have strong affinity to dissolved organic matter, we expect that they would behave similarly to Hg(II).

Pore water concentrations of metals also depend on the equilibration time. The retention of metals by soil or soil components has been frequently reported to occur by a fast step followed by a slow step (Sparks, 1989). During the slow reaction process, some retained metals, which are initially available for partitioning, can penetrate into the mineral lattice (Kuo and Mikkelsen 1980; Bruemmer et al., 1988) or diffuse into intraparticle pores (Sparks et al., 1980) and become very difficult to release. Consequently, the amount of metal available for plant uptake may slowly decrease with time.

Not all soluble metal is necessarily bioavailable. Free metal ion in water has often been considered to be the toxic species that can actually be taken up. However, it is more appropriate to consider that bioavailability is the consequence of a competition for the metal among the organism, soluble ligands and sorption phases on the soil. Based on the free metal ion concentration calculated without consideration of the competitive binding of organisms, one cannot predict bioavailability. Thus, it is most appropriate to consider that the introduction of a plant or invertebrate to the soil-water system constitutes another receptor site that can compete for metal ions with the soluble ligands. The amount of metal taken up by a plant depends on its binding constant for the metal, competing ligand concentration, and pH. In the presence of ligands, the calculated free metal concentration could be very small; however, when a plant is introduced to the system, all of the metal could be taken up by the plant if the plant has much stronger affinity for the metal than other ligands. An appropriate model for the soil-pore water-plant continuum should be similar to the gill complexation model being developed for the aquatic system.



**Fig. 5 :** Log  $K_{om}$  as a function of pH for adsorption of cadmium onto fifteen New Jersey soils. Soil:water = 1g/100mL; I = 0.01 M  $\text{NaNO}_3$ ; T = 25°C. Regression coefficient  $R^2 = 0.92$ .



**Fig. 6 :** Predicted vs. measured soluble Hg(II) concentrations for adsorption of Hg(II) on 15 New Jersey soils at two initial concentrations,  $1 \times 10^{-7}$  and  $1 \times 10^{-6}$  M, over a pH range of 3 to 10. Soil:water = 1g/100mL; I = 0.01 M  $\text{NaNO}_3$ ; T = 25°C. Regression coefficient  $R^2 = 0.92$ .

## Conclusions

This review of the literature provides two important conclusions regarding the assessment of metals in the environment and the potential for metal toxicity:

1. Proper precautions must be taken in the sampling and analysis of environmental samples to ensure accurate results. Much of the existing data is of questionable quality and these results are biased high.
2. Assessment of potential impacts of zinc and other metals in water, sediment and soils cannot be judged on the basis of the total concentration of metals. Knowledge of metal speciation and of the total system chemistry is essential.

It is possible to develop Water Quality Criteria, Sediment Quality Criteria and Soil Quality Criteria that are predictive of the effects of metals on biota. Such criteria, based on sound chemical and physiological bases, would have broad applicability. Present criteria and evaluations based on observations of effects suffer from not providing the evaluator a technical basis for knowing their applicability to a new situation. Site-specific modification is a less technically acceptable process than is the development of the proposed technically sound evaluative tools.

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## Zinc and Health - Conclusions of the Health Working Group

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The Health Working Group discussion agenda was:

*Zinc pharmacokinetics*

*Zinc deficiency*

- biomarkers of deficiency
- populations at risk of deficiency

*Zinc excess*

- routes into the body
- bioavailability
- current knowledge of the homeostatic mechanisms
- toxicity of zinc
- interactions

*Further research*

## 1. Zinc pharmacokinetics

### 1.1 Bioavailability

A number of factors are known to affect the bioavailability of zinc obtained from the diet. These include the decrease in zinc availability caused by phytates, calcium (either in the diets or as a supplement), vegetarian diets, and the Maillard reaction (browning) of foodstuffs during processing.

The presence of a high level of sulphur-containing amino acids in the diet, in contrast, enhances the bioavailability of zinc.

The workshop identified the desirability of dual stable isotope studies for assessing changes in body zinc status following zinc absorption and excretion. Although costly,  $^{65}\text{Zn}$  and  $^{67}\text{Zn}$  studies could be used to measure reaction transfers from the gut into tissues and to measure clearance that would aid in the study of zinc homeostasis.

### *1.2 Current knowledge of the homeostatic mechanisms*

Homeostatic mechanisms for zinc are poorly understood. Although metallothionein is implicated in the absorption of zinc from the gut, good data are not available to clearly demonstrate its precise role in absorption. The workshop group identified this as an area in which more work is needed to improve our understanding of zinc absorption and bioavailability.

## **2. Zinc deficiency**

### *2.1 Biomarkers of zinc deficiency*

Many markers were considered of potential values, as indicators of zinc deficiency. Over 300 enzymes require zinc and all of these may have a potential to serve as biomarkers. These include erythrocyte alkaline phosphatase and erythrocyte superoxide dismutase, DNA-dependent RNA polymerase, insulin-like growth factor (IGF) alcohol clearance from the blood, serum ferritin as an indicator of dietary zinc deficiency, immune status, dark adaptation and neuromotor cognitive function. Despite extensive discussion, the workshop participants agreed that, although there are many sensitive biomarkers which may be used to indicate dietary zinc deficiency, none of these is specific to zinc. It was therefore agreed that the best option to detect deficiency is to assess a dietary intake of zinc and, if it indicates that a risk of deficiency may exist, to follow the study with the measurement of target enzymes, to indicate whether the dietary deficiency is having some effect. Subsequent controlled repletion studies would indicate the reversibility or otherwise of these effects.

### *2.2 Populations at risk of deficiency*

A number of special groups within the population were considered to be at risk of zinc deficiency for varying reasons:

<i>infant children</i>	due to greater demands from growth
<i>pregnant and lactating women</i>	due to greater demands
<i>elderly</i>	associated with lower calorie intake
<i>vegetarians</i>	lower bioavailability from food (phytate)
<i>people with liver disease</i>	increased loss in urine
<i>children with diarrhoea and intestinal malabsorption</i>	increased loss from the gut

A number of corrective strategies for the identified populations at risk could be utilised. These include: -

- Dietary manipulation, which must take into account the restrictions imposed by cost and availability of food in different cultures, and could include fermentation of food to increase bioavailability of zinc from cereals. This strategy was considered to be the most desirable as it is cheap and requires least intervention.
- Changing agricultural products, which would include plant breeding and consideration of altering fertiliser use were possible strategies which would increase the amount and bioavailability of zinc in the food.

- Supplementation, where necessary. This option would be utilised only if dietary alteration was impracticable or had failed, or in cases of overt clinical zinc deficiency. It was noted that zinc:copper ratios had to be maintained during such supplementation to avoid the consequences of zinc-mediated copper deficiency and that amounts of zinc must be within physiological requirements.

### 3. Zinc excess

#### 3.1 Potential sources of overexposure

Overexposure to zinc is known to arise from: -

- Shellfish. Zinc levels (in oysters) considerably in excess of 1000 mg/kg were found in one occurrence of acute poisoning which was rapidly manifested by gastrointestinal effects - however, other causes of poisoning including the presence of other metals in oysters were not excluded as causes;
- Drinking water from old galvanised containers which can contain up to 5 mg/L. It was considered that exposure by this route is self-limiting due to the unpleasant taste. Regular drinking water may contribute up to 0.5 mg of zinc per day.

None of the above sources was considered to contribute substantially to the exposure of the general population to zinc other than as an occasional possible source of poisoning. It was concluded that supplementation with zinc represented the greatest risk of overexposure for the population. The other potentially significant sources of exposure to selected segments of the population included exposure to zinc-containing fumes in the occupational setting and exposure to zinc chloride in smoke bombs used by the military. Other sources of exposure, such as the zinc component in insulin injections and in treatments for dermatitis were not considered to be associated with any known toxicity in man.

#### 3.2 Effects of zinc excess

The features of acute zinc over-exposure include abdominal pain, nausea and diarrhoea. In addition, exposure of animals and humans to excess zinc results in interactions with other essential micronutrients, and may lead to reduced absorption and dietary deficiency of the affected micronutrient.

The most significant interaction identified in the literature is that between zinc and copper, although interactions of zinc with iron, cadmium, calcium and other metals are documented. The interaction between zinc and copper was discussed at length at the workshop and a number of effects which may be ascribed to this interaction were identified.

##### 3.2.1 Interactions of zinc and copper

The threshold for adverse effects ascribed to copper deficiency caused by zinc excess needs to be defined. When the zinc:copper intake ratio exceeds 10, retention of copper is decreased. Changes in copper dependent metabolism have been observed at ratios above 20:1. This was demonstrated in animal studies, with some supporting data found in human studies. Therefore, the higher the copper content of the diet, the higher the level of the zinc needed to deplete the copper levels in the body. Interactions arising in situations of excess zinc with copper deficiency have been implicated in effects on haemoglobin levels (anaemia), serum lipids and other indices. A controversial association with cardiac events has been described in human studies of copper deficiency (with concurrent zinc supplementation), and this association has also been observed in animal studies.

A number of studies in animals has demonstrated that high intakes of zinc, relative to copper, induced copper deficiency and adversely affected lipid metabolism (the levels of apolipoprotein A-I and high-density-lipoprotein (HDL) cholesterol were decreased) and the myocardium. In a single human study in which 24 men were fed omnivorous diets that were deficient in copper and high in zinc (0.89 mg copper, 21.5 mg zinc), abnormal outcomes included low HDL cholesterol, low methionine and leucine

encephalin, and increased cholesterol. The study was stopped after 11 weeks because four men experienced cardiac abnormalities (one myocardial infarction, two tachycardias, and one second-degree heart block). These manifestations were similar to those that occur in copper-deficient rats (cited in Sandstead, 1995, p 622S).

The ratio of zinc:copper intake was suggested to be a factor in the induction of ill effects arising from copper deficiency in the presence of high zinc intake. One man, fed an omnivorous diet providing 0.83 mg copper and 13.7 mg zinc (Zn:Cu >16) for 105 days had decreased blood copper levels and increased total cholesterol and LDL cholesterol (Klevay et al, 1984). The experiment was stopped when an arrhythmia was detected. However, other studies were done where changes in plasma lipids were not consistent or were absent.

The Workshop participants discussed a number of supplementation trials which showed effects where high levels of oral zinc were associated with decreased levels of HDL. (Chandra, 1984; Hooper, 1980; Black 1980; Goodwin 1985) In those studies in which supplementation was discontinued and HDL levels were followed, this effect was reversed. Other studies did not show the decreased levels of HDL (Samman and Roberts 1988; Gatto and Samman 1995) or showed only transient effects (Freeland-Graves et al 1980) with oral zinc supplementation.

Following discussion of the inconsistent findings related to decreases of HDL, it was proposed that the copper status of the subjects in the trial may have influenced these findings but, in the absence of data on the copper levels, this remained speculative. Other confounding factors may have included the form of zinc supplement and diet composition (which may affect the bioavailability of the supplement).

It was pointed out that accurate measurements of the blood plasma lipids were needed to further reduce the variance of observations between different studies.

### **3.2.2 Interactions with iron**

Evidence from animal studies suggests that increased doses of zinc in the diet are often associated with decreased iron levels in the body. Human studies on this interaction were considered to be unclear though one study found a decrease in serum ferritin concentration when 50 mg zinc supplementation was given to subjects consuming omnivorous diet.

Although qualitative effects in animals may be indicative of effects in humans, it is difficult to make quantitative predictions for this effect in humans.

### **3.2.3 Interactions with cadmium**

Interactions between cadmium and zinc have been described in the literature. Most supplies of zinc salts are contaminated with cadmium (from 1:2000 to 1:5000). At low doses of zinc there is a protective effect from the renal toxicity of cadmium. Unpublished data (Nordberg, personal communication) indicate that high zinc exposures paradoxically reverse this protective effect. Simultaneous dosage with high zinc and cadmium provide a protective effect against testicular necrosis and tumours induced by very high acute doses of cadmium.

## *3.3 Biomarkers of excess zinc intake*

A range of biomarkers was considered: -

- Decreased erythrocyte superoxide dismutase (E-SOD) activity. The levels of this enzyme are unaffected by zinc deficiency and it was considered to be reasonably indicative of zinc excess.
- Decreased cytochrome C oxidase activity in platelets. While this was not a specific biomarker for zinc excess, it was considered to be the most sensitive marker for copper deficiency.

Other biomarkers of excess zinc exposure that were discussed included: -

- Plasma levels and activity of ceruloplasmin
- Plasma copper concentrations

- Tyrosinase
- Changes in plasma zinc concentrations
- Alkaline phosphatase in erythrocytes
- Changes in plasma lipids (decreased HDL)

None of these biomarkers was considered to be a specific and reliable indicator of excess exposure to zinc at an individual level, although they may be of some use in considering population exposures. It was considered important that specific and reliable indicators of zinc excess be developed for the assessment of individuals and for population surveys.

#### 4. Further research

The Workshop participants noted a number of significant data gaps which must be addressed to enable adequate hazard identification and risk assessment for both zinc excess and zinc deficiency in human populations.

Specific research needs which were identified were: -

- As dietary iron deficiency is thought to be correlated with dietary zinc deficiency, the use of serum ferritin as a potential surrogate marker of zinc deficiency needs to be evaluated.
- The use of a range of biological markers such as DNA-dependent RNA polymerase, erythrocyte alkaline phosphatase, alcohol intolerance (as a reflection of alcohol dehydrogenase levels) and immunological responses as potential surrogate markers of zinc deficiency needs to be evaluated.
- Zinc homeostasis needs better definition in respect of both its individual role and its role with other cations (copper, calcium, iron), as very little information is available. Dual isotope markers administered simultaneously by different routes appear to be a useful tool for estimation of absorption and excretion.
- More refined toxicological assessment could be achieved with an improved understanding of the relationship between the internal and the external doses of zinc, and the consequences of increases and decreases of the internal dose.
- Information on the internal concentrations of zinc in human target tissues is needed.
- In the absence of a satisfactory single measure of organ specific zinc concentrations, composite indices such as serum/plasma/leucocyte zinc concentrations, the use of  $^{65}\text{Zn}$  and  $^{67}\text{Zn}$ , and zinc challenge tests are required.
- More information on the bioavailability of zinc is required to improve exposure and risk assessment, and the management of zinc deficiency and zinc excess. The effect of factors such as high alcohol intake, diarrhoea and total parenteral nutrition on absorption of zinc requires more data.
- Given the interaction between zinc and other micronutrients, a better understanding of the optimal ratios of zinc and other micronutrients is required for situations where supplementation is being recommended.
- There are indications that zinc deficiency is associated with cognitive deficits. Prospective studies are required (especially in the elderly) to elucidate this association and the role of intervention, with possible zinc repletion studies.
- Research into the role of metallothionein and other zinc-binding proteins as biomarkers of the function of the pancreas, liver and other organs.
- Studies are needed to clarify the association between the immune function deficit and zinc status and similarly, between endocrine dysfunction and zinc status.
- Since supplementation with zinc carries the risk of inducing copper deficiency, studies to determine the appropriate zinc to copper ratios in dietary supplements are needed.

- Cardiovascular disease and/or dyslipidaemia may be a consequence of zinc-induced copper deficiency. This relationship needs to be fully examined. There is a need to define a NOEL for occupational exposure by inhalation to zinc. Studies are needed to define minimum dietary levels of zinc as defined by physiological needs.
- Further studies are needed on the relationship between dietary iron and dietary zinc.

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## Zinc and the Environment: Conclusions of the Environment Working Group

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### 1. Summary

In considering environmental exposure of zinc, the Working Group discussion principally focused on the effect of zinc in the aquatic environment, including particularly the bioavailability of zinc from total or dissolved zinc as a measure of exposure. Further discussion about the effects of zinc in soils and sediments was undertaken.

Initially, the Working Group noted that recent analytical techniques have improved oceanographic data, however caution was still required when assessing older data. It was also noted that industrial wastes were generally not measured correctly and have, in the past, been the subject of overestimation.

Discussions of zinc excess and deficiency, focussed particularly on the optimum concentration range of zinc (OCZn) as an essential element in the natural environment. The OCZn was defined as "the range of zinc concentrations below which signs of deficiencies occurred, and above which produced signs of toxicity. The group agreed that the homeostatic band width of OCZn was approximately two orders of magnitude.

While quality data exists within this optimal range, further research is required to determine the effect. The Group identified the difficulty in determining a default OCZn for a range of environments and that site specific risk assessment was required for different habitats. Considerable debate was undertaken on defining a "natural environment" or "perturbed ecosystem", and which species should be considered within those environments.

The group identified as an urgent research need, the requirement for more data on sensitive aquatic species to define the inner envelope for the optimal concentration.

In discussion on the bioavailability of zinc, it was noted that the bioavailable fraction of zinc in soil is far broader than in the aquatic environment as zinc is more readily oxidised in the soil due to the pH range and organic matter content.

It was noted that present risk assessment methodologies generally do not take essential elements into account. Current risk assessment methodologies for toxic materials which do not have essentiality utilise probabilistic and safety factor models. In the case of zinc, and other essential elements, it was considered that these models for risk assessment result in concentrations which produce deficiency as the models do not take into account upper and lower safety levels.

Further, in undertaking risk assessment it was considered that the bioavailable fraction of zinc rather than the total concentration should be considered. This would take into account the mineralisation of zinc which removes bioavailable zinc from the system.

Analytical methods for measuring the predicted bioavailability of zinc which are robust, easily performed and validated against bioassays are required, and the analytical techniques that can measure zinc speciation in ecosystems need to be improved. It was noted that models are being developed, using pH, DOC and hardness in aquatic environments to predict maximum bioavailability of zinc.

## 2. Conclusions

### 2.1 *Exposure issues*

Caution is required about older exposure data, especially environmental monitoring data.

### 2.2 *Effects assessment*

There was considerable discussion on whether there are data on enough species to define the inner envelope for optimal concentration. The group recommended this point as an urgent issue for further research.

Further research should be undertaken to understand the homeostatic capabilities of organisms with respect to their natural conditions especially species sensitive to particular habitats.

### 2.3 *Risk assessment*

Present risk assessment models in particular the Safety Factors approach and the Probabilistic (extrapolation) approach do not take deficiency levels for essential elements into account. These

models may drive the desired levels of protection to below the deficiency level and do not establish a lower as well as an upper safe limit.

For zinc the "Optimum Concentration of Essential Elements" (OCEE) concept could therefore form a valuable alternative to the presently used risk assessment models.

However several points have to be clarified in particular the limits and overlap of homeostatic capabilities of organisms, the relationship of the homeostatic mechanisms to the natural (habitat) zinc levels and the adequacy of the number of species used to define the inner envelope.

Risk assessments should be made on a "habitat type"-specific basis, in particular related to the essential elements status of that habitat.

Data quality criteria are necessary to screen the relevance of toxicity data for risk assessment, in particular site-specific (temperature, hardness, pH, organic content,...) and organism-specific (origin, culture medium) data. Only physiological adaptation mechanisms should be taken into account for the determination of the homeostatic capacities of organisms. Genetic mechanisms should not be considered.

#### *2.4 Bioavailability issues*

For risk assessment the definitions that should be used are:

Bioavailable fraction: "maximum fraction which can potentially be taken up by the organism(s)" (physico-chemically determined).

Uptake: fraction which is taken up by the organism(s) (physiologically determined)

Both bioavailability and uptake must be considered in risk assessment and management.

Total concentrations in the aquatic and soil environment are not useful to establish the bioavailability.

Regulatory criteria must be based on the bioavailable fraction of zinc. Robust analytical methods for measuring bioavailability should be (further) developed and validation against bioassays is required, in particular:

- improved analytical techniques to measure the speciation of zinc in environmental samples.
- analytical techniques to predict bioavailability in soil.

Bioconcentration factors (BCFs) are not good indicators for exposure to homeostatically controlled elements. For essential elements the BCF is a natural consequence of biological control to maintain an effective and stable internal level within the homeostatic boundaries (ref EU/OECD metals workshop Brussels Dec. -95)

#### *2.5 Aquatic environment*

Models are being developed for the aquatic ecosystem which allow the prediction of the maximum bioavailability to organisms (the models use parameters such as pH, DOC, water hardness, etc).

#### *2.6 Terrestrial / sediments environment*

Procedures are available to predict the uptake of zinc by agricultural plants especially related to zinc deficiency in crops but they do not describe the mechanisms of uptake.

The bioavailability of metals in sediments and anaerobic soils is primarily controlled by sulfide concentrations.

The soil solution/pore water concentration was found to underestimate the bioavailability of zinc for certain biological species in soil.

The behaviour and effect of zinc in organisms may depend on the exposure routes. Further research was recommended on this point.

Leach tests can give a prediction of the potential environmental mobility of zinc in soil. However they do not accurately measure the bioavailable fraction.

### *2.7 Risk management*

In order to preserve the diversity of species, ecosystems with low zinc concentrations have the same right to be protected as normal or high zinc areas.

### **Reference**

EU/OECD metals workshop Brussels (1996) “ Biodegradation/Persistence and Bioaccumulation/Biomagnification of Metals and Metal Compounds”. Technical Workshop, Canada/European Union Metals and Minerals Working Group, Brussels, Belgium, December 11-13, 1995.

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