HEALTH RISK ASSESSMENT GUIDANCE FOR METALS

FACT SHEET

HERAG 07

ESSENTIALITY

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1. Introduction

Conventionally, previous risk assessments have focused primarily on the effects of high doses of chemicals, which ultimately may induce toxicity. However, for several metals which are essential to life, harmful effects also occur at very low levels of intake due to deficiency.

This poses a challenge to basic assumptions of risk assessment where the underlying paradigm aims at minimising exposure as far as possible. For an essential element, an unbalanced concern over high dose effects may lead to recommendations that lead to harm from deficiency.

Therefore, this fact sheet was prepared with the aim to provide guidance for a careful consideration of both nutritional essentiality and high dose toxicity in the overall risk assessment and risk characterisation procedures for essential metals and their compounds. However, at the same time, it is explicitly noted that the compilation of this fact sheet was designed to be based on a retrospective analysis of the few cases in which such assessments had been done previously in an EU risk assessment context, which in turn necessarily restricts its scope to the elements zinc and copper.

Further to this, it is the opinion of the authors and the HERAG project team that the copper voluntary EU risk assessment contains the most comprehensive discussion of how to approach a balanced risk characterisation that reflects essentiality. In addition to the practical experience from the previous metal industries' risk assessments, the methodology summarised in this fact sheet is based largely on the WHO/IPCS monograph "Principles and Methods for the Assessment of Risk from Essential Trace Elements" (WHO, 2002).

Whereas it is acknowledged that the aspect of essentiality has been recognised for several other metals (such as Iron and Selenium as examples) as well, none of these have been subject to a comprehensive (EU) risk assessment, and so therefore experience in the reflection of this aspect in the establishment of assessment factors is not available. Nevertheless, auxiliary information on iron as another important essential trace element (ETEs) is summarised briefly for comparative purposes.

Definition of essentiality

The traditional criteria for essentiality for human health are that absence or deficiency of the element from the diet produces either functional or structural abnormalities and that the abnormalities are related to, or a consequence of, specific biochemical changes that can be reversed by the presence of the essential metal (WHO, 1996). By definition, the functions fulfilled by an essential metal cannot be replaced by any other substance.

End-points frequently used in establishing the essentiality of trace elements in experimental animals are impairment of growth and development, neurological effects, inefficient reproduction, loss of tissue integrity, and defects in physiological and biochemical functions (Mertz, 1993).

Essential trace elements are subject to homeostatic control mechanisms that may include regulation of absorption and/or excretion and tissue retention. These mechanisms enable adaptation to varying nutrient intakes to ensure a safe and optimum systemic supply of ETEs for the performance of essential functions, and the efficiency of homeostatic processes may vary within populations and with levels of intake of the ETE. However, the identification of the prevalence of any such variation requires the study of large populations. Toxicity from ETEs occurs when the exposure is above or below the range which can be accommodated by homeostatic mechanisms.

Elements considered essential for human health

In their monograph (WHO, 2002) on "Principles and methods for the assessment of risk from essential trace elements", the World Health Organisation regards the following trace elements as essential for human health: Copper, Zinc, Iron, Chromium, Molybdenum, Selenium, Cobalt and Iodine.

A second group of elements is classified by WHO as "probably essential for humans": Silicon, Manganese, Nickel, Boron, Vanadium.

2. Brief overview of the assessment principles for essentiality suggested by the WHO

Introduction

The WHO concept for the assessment of essentiality (WHO, 2002) is based on defining boundaries between deficient and excess oral intakes of ETEs, finally yielding an "acceptable range of oral intake" (AROI). The AROI is designed to limit the probability of deficient and excess intakes occurring in healthy populations and is set for different age-sex groups and physiological states such as pregnancy and lactation.

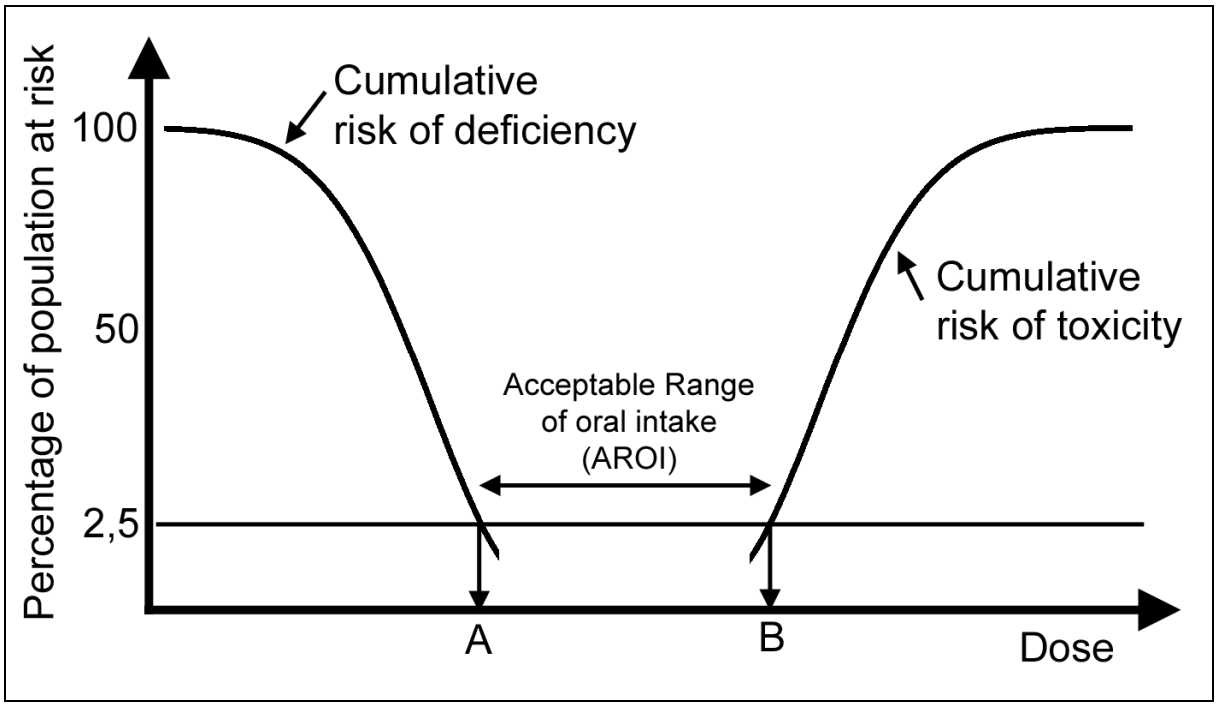

Figure 1: WHO concept on deficiency and toxicity from excess oral intake of ETEs (adapted from WHO, 2002). As ETE intakes drop below A (lower limit of AROI where 2.5% of the population under consideration will be at risk of deficiency), an increasing proportion will be at risk of deficiency. At extreme low intakes all subject will manifest deficiency. As ETE intakes exceed B (where 2.5% of the population under consideration will be at risk of toxicity), a progressively larger proportion of populations will be at risk of toxicity

Factors that influence the assessment of essentiality

Homeostatic mechanisms that involve regulation of absorption and excretion and tissue retention which enable adaptation to varying nutrient intakes must be considered in establishing an AROI. Specific portions of homeostatic control mechanisms appear to be shared among ETEs. Nutritional requirements or excess exposure impacts for one ETE can be affected by the nutritional status of another ETE. Such interactions can be highly specific and require evaluation on a case-by-case basis. Other factors such as chemical speciation, dietary composition and interactions amongst ETEs can also be critical. Chemical speciation can affect the bioavailability of ingested ETEs, and should be considered in setting recommendations for intake levels. High dietary intake levels of materials such as plant phytates can also inhibit ETE uptake and increase nutritional requirements.

Suggestions for a systematic assessment of essentiality for a given element

A detailed outline of such an assessment is presented in Appendix I, with conclusions from this given in subchapter 4, so that any further description is omitted here.

3. Selected examples of metals as essential trace elements

3.1. Brief overview of previous EU risk assessments in which essentiality was considered

To this date, essentiality has only been considered in recent EU risk assessments on copper and zinc, which are summarised in detail in this subchapter. Further below (subchapter 3.2), data made available on iron as another important essential element are also presented albeit in a less standardised format, because a detailed "essentiality assessment" in an EU regulatory context has not been conducted for this metal.

3.1.1. Copper[1]

Copper is an essential trace element for all biological organisms including humans. The essentiality of copper arises from its incorporation into a large number of proteins. Copper has the ability to cycle between stable oxidised Cu(II) and unstable reduced Cu(I) and is used by several cuproenzymes participating in fundamental redox reactions (e.g. cytochrome oxidase, Cu/Zn superoxide dismutase, dopamine-ß-hydroxylase, lysyl oxidase and ceruloplasmin). Secondly, as an essential structural component of many macromolecules, a large number of enzymes and other proteins are dependent on copper for their normal activity. The essential roles of copper are demonstrated in its physiological effects where it is critical to foetal/infant development and growth, immune function, brain development and function, bone and collagen strength, haematopoiesis, iron metabolism, cholesterol and glucose metabolism, myocardial contractility, maintenance of hair and skin, and pigment formation.

Exposure of the general population

Due to the high number of available dietary monitoring studies, the TGD default food basket was not used to assess exposure of the general population to copper. Instead, the daily dietary copper intake of the general population was estimated from a large set of market basket and duplicate diet studies. Median daily intake for adults is 1.2 mg/day. Reasonable worst case (RWC) estimates, i.e. the 10^{th}-percentile and the 90^{th}-percentile are 0.6 mg/day and 2 mg/day, respectively. These studies also allowed the derivation of age and sex-specific exposure values. The analysis revealed that elderly may have slightly lower copper intakes than younger adults.

Adverse effects due to copper deficiency

A large amount of animal and human studies are available assessing adverse health effects due to copper deficiency. Animal and human studies were ranked for quality and relevance using a set of evaluative criteria developed by Plunkett (2004). Klimisch criteria could not be fully applied as they were not designed to assess the potential adverse consequences of ETEs, for which physiological, structural and/or functional impairment due to either excessive or deficient exposures is of concern. Observed effects due to chronic deficiency were also rated for severity. Although a significant number of animal studies are available that characterise the adverse consequences of copper deficiency, the majority of these studies are limited for dose-response assessment by several factors. Human data of sufficient quality are available and were used to derive a safe threshold for deficiency (SDI). Adverse consequences of copper deficiency in humans have been observed ranging from clinical diseases to measures of cardiovascular function, bone metabolism, lipid levels, and immunological parameters indicative of impaired immune function.

Short-term studies conducted in young, healthy male volunteers indicate that a dietary intake of 1 mg Cu/day is sufficient to maintain copper balance and normal levels of some cuproenzymes and putative markers of copper status in this population. Limited information from other depletion/repletion studies suggests that peri-/post-menopausal women may benefit from intakes higher than 1 mg Cu/day, approximately 3-4 mg/day, to prevent them having a negative copper balance,

[1] Adapted and summarised from the Copper VRA (ECI, 2006). For individual references please refer to this report.

increased serum cholesterol levels, and a decrease in bone mineral density; this group is considered likely to represent a sub-population sensitive to deficiency given the mean daily intake of around 1.2 mg copper.

Adverse effects due to copper excess

A large amount of animal and human studies are available for an assessment of adverse health effects due to copper excess. Many of these studies have focused on the liver which is the target organ for copper-related toxicity. Animal and human studies were ranked for quality and relevance using the Klimisch criteria. Although a large number of human studies are available, the NOAEL was based on a 13-week animal feeding study as this was the study of highest quality. A NOAEL of 16.3 mg/kg BW was derived. The human data were used as supporting evidence for the applicability of the calculated NOAEL to humans.

Homeostatic control mechanisms for copper

Upon absorption in the gastro-intestinal tract, copper is complexed with plasma proteins and transported via the portal blood to the liver which represents the major organ in copper distribution, regulating its release. Hepatic copper is incorporated into enzymes and proteins, subsequently secreted into the blood and transported to other tissues or excreted via bile. Cellular regulation is effected by both metallothionein - though probably not as important for copper as for zinc - and a series of metallochaperones that bind copper ions and deliver copper to precise intracellular locations, thus protecting the cell from accumulation of potentially toxic, free copper ions.

There are a number of hereditary diseases which are a result of disorders in copper homeostasis. These diseases are rare and include Menke disease, a deficiency disorder, and Wilson disease, a toxicity disorder. Wilson disease, a rare autosomal recessive disorder of copper overload, occurs equally in males and females with an incidence of 1/30,000 – 1/40,000 live births. It involves impairment of hepatic copper transport, biliary excretion, and disturbances of incorporation of copper into ceruloplasmin and its systemic distribution, resulting in copper accumulation in liver, brain and eyes. Wilson's disease manifests itself in hepatic, neurological, psychological and ocular symptoms of varying severity.

The incidence of Menke disease is approximately 1/100,000 live births. The inherited defect is in the X-chromosome, resulting in most patients being male, although the disease has been reported in females. In Menke disease, copper absorption from the intestines is minimal, even under conditions of adequate dietary intake, due to impaired transport of copper from intestinal mucosal cells. Transport of copper across the blood–brain barrier is also impaired. Severe deficiency effects are induced, often resulting in death in childhood.

Variation of copper absorption with intake

A large quantity of published data is available on the absorption of copper in animals and humans following oral administration. The most reliable absorption data are from human studies in which true[2] absorption rates were determined in young men consuming a series of copper-containing diets, using stable radioisotopes of copper. Absorption rates were determined at low, normal and high intakes of copper. The studies revealed true absorption rates to be intake-dependent, decreasing with increasing daily intakes. These data reflect the homeostatic mechanisms ensuring a safe and optimal systemic supply for the performance of essential functions. For example, systemic absorption of 63-65% occurs at an intake of 1 mg/day, whereas at an intake of 8 mg/day, the systemic absorption drops to 29-32%. A graphical function was derived to allow for the determination of exposure-specific true gastro-intestinal absorption factors at varying intake levels.

[2] In contrast to *true absorption* that also reflects endogenous copper losses from biliary excretion and intestinal cells sloughing off, *apparent absorption* is merely measured as the difference between oral intake and faecal excretion, and as such does not distinguish between unabsorbed copper and endogenous copper losses.

Reflection of deficiency in human health risk assessment

Essentiality and homeostasis are addressed in the Voluntary Copper Risk Assessment (ECI, 2006) in the sections on toxicokinetics, toxicity from deficiency and excess, and in risk characterisation. Failure to maintain copper homeostasis may lead to adverse effects resulting from either deficiency or excess. The VRA report contains a detailed assessment of human health effects of copper deficiency. In order to avoid confusion with the concept of “no effect levels”, the new terms "sufficient dietary intake" (SDI) and "deficiency effects level" (DEL) were defined as follows:

> "Deficiency effect level" (DEL): For a given study, the copper dose/intake level at which potentially adverse health consequences associated with copper deficiency are first observed. For studies with only one experimental dose, the DEL is considered to be that dose if adverse health consequences are observed relative to controls (basal diet).
>
> "Sufficient dietary intake" (SDI): For a given study, the dose/intake level in a given study at which no adverse health consequences associated with copper intake are observed in any of the measured endpoints.

The argument of very efficient and highly conserved intra-species homeostatic control mechanisms was used to deviate from the MOS_{ref} approach suggested by the TGD when deriving an alternative MOS_{ref} based on a subchronic study in rats and using a human volunteer study as supplementary information as follows.

The TGD indicates an assessment factor (AF) of 10 for animal to human extrapolation (4 for toxicokinetics multiplied with 2.5 for toxicodynamics). An AF for inter-individual variability is given as 10 for the general population and 5 for workers, the latter on the basis that working populations exclude the most vulnerable individuals. A factor of 2 is given for sub-chronic (90 day) to chronic extrapolation. This gives a combined AF of 10 x 10 x 2 = 200 for the general population and 10 x 5 x 2 = 100 for workers.

Whereas no guidance on the use of AFs for ETEs is given in the TGD, WHO (2002) has reviewed the application of AFs ("uncertainty factors" in the alternative nomenclature) in relation to ETEs. They cite 10 x 10 x 2 as the conventional AF for the assessment of exogenous substances for the general population in agreement with the TGD. However, the application of this AF to ETEs, for which deficiency must also be considered, is questioned and alternative methods are explored. However, no clear guidance on the general application of AFs to ETEs is given. Consequently, the derivation of AFs to calculate MOS_{ref} values is problematical and to be considered on a case by case basis.

Application of a 10 x 10 x 2 AF directly (without correction for absorption) to the animal NOAEL (16.3 mg/kg bw) from the Hebert et al. (1993) study gives a human NOAEL of 5.7 mg Cu/day (using a default body weight of 70 kg). However, in the human volunteer study of Pratt (1985), subjects supplemented with 10 mg/day (and consuming daily dietary intakes of approximately 1-2 mg Cu/day, giving a total intake estimate of 11-12 mg/day) showed no alteration in a number of biochemical parameters. Although this study was clearly limited in its scope, these findings suggest that absorption of intakes of this magnitude is subject to efficient homeostatic control. Based on numerous studies showing that systemically absorbed dietary copper decreases with increasing dose, the estimated systemic absorption of these intakes (5.7 and 11-12 mg/day, respectively) is almost equal. An AF of 10 x 10 is therefore considered appropriate for adults.

A risk characterisation was performed for copper deficiency for the general population. Typical and reasonable worst case (10^{th}-percentiles) exposure values for the general population and specific age categories were compared with the SDI. A review of the evidence for deficiency and copper balance studies indicates that intakes below 1 mg/day may be insufficient to maintain copper status. Some evidence suggests that vulnerable individuals such as pregnant women, lactating women, adolescents, peri-menopausal women, and the elderly people may require higher intakes. The data for dietary intakes suggest a risk of marginal copper deficiency in a minority of the general population, with elderly people being the group at highest risk for copper deficiency.

3.1.2. Zinc[3]

Zinc is an essential element for humans and animals and it is required for the optimum function of over 200 enzymes. These enzymes include those required for nucleic acid, protein, and membrane metabolism, as well as cell growth and division (Vallee and Auld, 1990; South and Summers, 1990; Berg, 1990). Zinc is also a required element for the optimum activity of growth hormone and the normal exocrine and endocrine function of the pancreas (Lee et al., 1990).

Recommended daily zinc levels range from 5 mg/day for infants to 19 mg/day for women during lactation. While the Zinc RAR mentions the issue of deficiency, it does not endeavour to quantify levels of such deficiencies and its manifestations, but instead only quotes ranges of "adequate dietary intakes" from secondary sources.

Exposure of the general population

The estimated average daily dietary zinc intakes range from 5.6 to 13 mg/day in infants and children from 2 months up to 19 years and from 8.8 to 14.4 mg/day in adults aged 20-50 years. Flesh foods (i.e. meat, poultry, fish and other seafood) are rich sources of readily available zinc, while fruits and vegetables contain relatively low zinc concentrations. For omnivorous adults, more than one-third of dietary zinc can be provided by flesh foods, whereas for vegetarians, plant-based foods are the major dietary source. Mean daily intake of zinc from drinking-water is estimated to be < 0.2 mg/day (WHO, 2001).

Adverse effects due to zinc deficiency

Zinc is an ubiquitous and essential element. However, large numbers of people are believed to ingest insufficient bioavailable zinc. The effects of zinc deficiency are well documented and may be severe. They include impaired neuropsychological functions, oligospermia, growth retardation, impaired reproduction, immune disorders, dermatitis and impaired wound healing. Most of these effects are treatable with adequate amounts of zinc. The estimated absolute absorbed amount of zinc required by adults is 2.5 mg per day. This implies a dietary need at 20 % bioavailability of 12.5 mg daily. As bioavailability increases, the amount needed in the diet will decrease (WHO, 2001).

Adverse effects due to zinc excess

Toxic effects in humans are most obvious from accidental or occupational inhalation exposure to high concentrations of zinc compounds, such as from smoke bombs, or metal-fume fever, but modern occupational health and safety measures can significantly reduce potential exposure. Intentional or accidental ingestion of large amounts of zinc leads to gastrointestinal effects, such as abdominal pain, vomiting and diarrhoea. In the case of long-term intakes of large amounts of zinc at pharmacological doses (150–2000 mg/day), the effects (sideroblastic anaemia, leukopenia and hypochromic microcytic anaemia) are reversible upon discontinuation of zinc therapy and/or repletion of copper status, and are largely attributed to zinc-induced copper deficiency (WHO, 2001).

High levels of zinc may alter homeostasis for other essential elements. For example, in adults, subtle effects of zinc on copper utilisation may occur at doses of zinc near the recommended level of intake of 15 mg/day and up to about 50 mg/day. Copper requirements may be increased and copper utilisation may be impaired with changes in clinical chemistry parameters, but these effects are not consistent and depend largely upon the dietary intake of copper. Distortion of lipoprotein metabolism and concentrations associated with large doses of zinc are inferred to be a result of impaired copper utilisation. In groups with adequate copper intake, no adverse effects, with the exception of reduced copper retention, have been seen at daily zinc intakes of < 50 mg/day (Davis et al., 2000; Milne et al., 2001).

[3] Largely adapted and summarised from the EU Zinc RAR (ECB, 2004)

Homeostatic control mechanisms for zinc

Zn^{2+} absorption in the gastro-intestinal tract occurs throughout the entire small intestine, with the highest rate in the jejunum. Total absorption appears to be concentration-dependent (Lee et al., 1989). Both passive diffusion and a carrier-mediated, saturable process (Tacnet et al., 1990) are effective, the latter involving a cysteine-rich intestinal protein at low zinc concentrations. At higher zinc concentrations, a metal-binding protein (Metallothionein) is involved (Gunshin et al., 1991; Hempe and Cousins, 1992; Sturniolo et al., 1991).

There is evidence of an enteral recirculation (Nève et al., 1991), and zinc is secreted into the intestinal lumen via epithelial cells, and via bile and pancreatic secretion (Cunnane, 1988; Flanagan et al., 1983).

The gastrointestinal absorption of zinc cations can be influenced by several factors such as plant proteins (soy) and phytate (Sandstrom and Sandberg, 1992), alcohol consumption (Antonson and Vanderhoff, 1983), and in particular the presence of other trace elements in diet and overall body zinc status (Solomons, 1988).

Variation of zinc absorption with intake

Persons with adequate nutritional levels of Zn^{2+} absorb approx. 20-30% of the ingested Zn^{2+}. In a state of zinc deficiency, this may increase (Johnson et al., 1988*(r)*; Spencer et al., 1985*(r)*), while at excessive zinc intake gastrointestinal uptake can be less (Babcock et al., 1982).

The gastrointestinal absorption following single oral administration of (soluble) ^{65}Zn-chloride determined by comparison of whole body radioactivity counting and faecal excretion (determined in 6 groups of 5 healthy adult fasted volunteers) was approximately 55% of the administered doses (~1.2, 2.9 or 5.8 mg) of zinc. The absorption declined with increasing dose, leading to assumptions that zinc absorption is saturable and that in healthy persons when intake levels differ by a factor of 10, uptake levels may vary by a factor of two at the most (Payton et al., 1982). In a similar study based on total body retention, ca. 55-60% of the administered ^{65}Zn-chloride radioactivity was absorbed (Aamodt et al., 1982).

In a comparative study, the absorption of zinc from soluble zinc acetate, zinc sulphate, zinc aminoate, zinc methionine and insoluble zinc oxide was assessed in ten human volunteers dosed orally with 50 mg Zn. The bioavailability of zinc was compared on the basis of "area under the curve" plasma zinc analyses, demonstrating that the bioavailability of zinc oxide was about 60% of the bioavailability of the soluble forms (Prasad et al., 1993).

Reflection of deficiency in human health risk assessment

In the zinc RAR (rapporteur: The Netherlands), an overall oral NOAEL for repeated dose toxicity of 50 mg Zn^{2+}/day (0.83 mg/kg bw/day) was derived from a human volunteer study in women, the most sensitive population in zinc supplementation studies (Davis et al., 2000; Milne et al., 2001). Since in women clinical signs of toxicity associated with exposure excess begin to appear only at a dose three times this NOAEL, a minimal MOS of 1 was considered sufficient when comparing the human oral NOAEL with the exposure levels for workers/consumers/general population. In this, consideration was given to the fact that zinc is an essential nutrient. Thus, the minimal MOS = 1 indirectly contains a reflection of zinc as an ETE, but without quantifying deficiency levels. Toxicity from excess zinc appears to entail impaired uptake of copper and in theory could vary as a function of nutritional status for copper. However, such interactions were not explicitly considered in the risk assessment. Allowances were made for speciation (bioavailability) effects and the impact of homeostatic control mechanisms that limit gastrointestinal intake under conditions of exposure excess was recognised.

Adverse effects from deficiency were not evaluated quantitatively in detail, but nevertheless implicitly addressed by the following qualitative comparison:

The NOAEL was derived from a human volunteer study, in which a restricted amount of parameters was used. As the toxicity study with rats showed more specific adverse effects (pancreas), the results from this toxicity study are used for comparison. Starting with the NOAEL of 31.52 mg zinc monoglycerolate/kg bw/day (corresponding with 13.3 mg Zn^{2+}/kg bw/day) from a 13-week study with rats, this results in an internal NOAEL of 5.3 mg Zn^{2+}/kg bw/d or 372 mg Zn^{2+}/day for a 70-kg-worker. The calculated MOS values range from 85 - 1,240 and 35 - 98 for dermal and inhalation exposure, respectively. Comparing these values with the minimal MOS of 360, and noting that this approach will be far too conservative for the essential nutrient zinc, it is concluded that risk characterisation based on the human study is adequate to protect also against adverse effects as observed in animal studies (ECB, 2004).

3.2. Auxiliary information on the essentiality of other metals

In contrast to the subchapter above, in which the aspect of essentiality in previous EU risk assessments is summarised for copper and zinc, the data on iron is presented here in a less standardised format and far less detail, since similar assessments in an EU regulatory context have not been conducted to date. Thus, this information is merely given for auxiliary purposes to illustrate the essential character of this metal, and is not intended to be comprehensive.

3.2.1. Iron[4]

Iron is an essential element and plays a central part in many biological processes. This importance arises largely as a consequence of its ability to exist as either Fe(II) or Fe(III), making it a good candidate for being involved in reduction and oxidation reactions.

In mammals, iron is also central to oxygen transport, forming the central part of the haeme moiety of haemoglobin. The metabolism of haeme also seems to play an important part in other metabolic processes, with the haeme oxygenase enzymes underpinning important cell signalling and cell differentiation roles.

Iron is taken in with the diet primarily as Fe(III) or Fe(II). Haeme itself is largely found in meat and in other products of animal origin. It is taken into the body by a separate transporter, which has only recently been identified, but once absorbed is stored and processed in the same way as iron from other sources.

Iron in the diet is reduced to Fe(II) and transported into cells where it can be stored in ferritin, incorporated into a variety of proteins or transferred to the basal side. Unlike Cu(II), no chaperones have been identified. Iron comes out to the portal circulation and binds to transferrin.

From the gut, iron is transferred to the liver in the portal circulation and then systemically to other cells. There is no excretion of iron from within the human body. Most of the regulation takes place at the level of the gut and possibly the liver. Regulation is complex, involving several proteins such as hepcidin and HFE. Inside the cell, iron is usually found either in haeme or in iron-sulphur clusters. In ferritin, it is stored as FeOH complexes. In the mitochondria, iron is transported for incorporation into the proteins of the citric acid cycle.

There are many stages in the iron cycle where there are risks of adverse effects from either deficiency or overload (see also below). There are several inherited disorders of iron metabolism. These range from the thalassaemias, which are mostly found in southern Europe and in South-East Asia, to the anaemias such as sickle cell anaemia, common in African populations through to diseases of iron overload such as haemochromatosis, which is more common in Northern Europe. Disorders of mitochondrial iron metabolism, such as Friedrich's ataxia, are also found. Some of the disorders results from altered haemoglobin, with reductions in red cell stability, but there are also mutations which change absorption, retention and other aspects of metabolism.

[4] Summarised and adapted from McArdle (2005)

Exposure of the general population

For lack of a previous EU risk assessment, detailed standardised exposure data are not available. However, the following information is available on recommended dietary intakes for iron:

Three important types of recommendations for iron intake reference values exist, including Recommended Dietary Allowances (RDA), Adequate Intakes (AI), and Tolerable Upper Intake Levels (UL). The RDA recommends the average daily intake that is sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in each age and gender group. An AI is set when there is insufficient scientific data available to establish a RDA. AI values meet or exceed the amount needed to maintain a nutritional state of adequacy in nearly all members of a specific age and gender group. The UL, on the other hand, is the maximum daily intake unlikely to result in adverse health effects. RDAs for iron vary from 11 mg/day for m/f infants (7-12 months), to 7-18 mg/day for m/f children and adolescents (1-18 years), to 8 mg/day for male adults and 18 mg/day for female adults. There is not enough evidence available to establish a RDA for iron for infants from birth through 6 months of age. Recommended iron intake for this age group is based on an Adequate Intake (AI) that reflects the average iron intake of healthy infants fed breast milk (NIH, 2005).

Adverse effects due to iron deficiency

There are several populations who are particularly likely to suffer from deficiency. The most obvious consequence of deficiency is anaemia, but this only occurs when iron stores are very low, so that many other processes are likely to have been adversely affected before the anaemia develops. People who are growing are most susceptible. This especially includes adolescent girls and pregnant women. The former are at risk as they are growing rapidly, lose blood through menstruation and have significant hormonal changes. They often have sociological pressures that may result in a poor diet, with concomitant risk.

The National Diet and Nutrition Survey (NDNS) in the UK regularly measures food intake. They have shown that, as caloric intake falls, which it is doing at a population level, micronutrient intake also drops. This has occurred to the extent that daily intake is now below recommended levels. In men, losses are small, so there is no great problem. In pregnant women, however, the risk is much higher. A baby is born with about 1 to 1.5 g of iron. All of this comes from the mother. At best, she will save about 600 mg from the cessation of menstruation. The amount of iron absorbed by her intestine will increase, but she will still lose about 300 mg from her iron stores. If they are not sufficient, or there is not enough in her diet, then she, and her baby, will both be at risk of deficiency.

Postmenopausal women are also at risk. Although they do not menstruate, the changes in steroid hormone, and in diet, especially in older women, often mean that anaemia increases. Elderly men are also at some risk, usually from dietary insufficiency.

Finally, there are data to show that vegetarians have a higher risk of deficiency than carnivores. This is not simply due to a lower intake of haeme iron. Many vegetables contain phytates, which bind the iron and make it unavailable for absorption.

There are many and varied health consequences of iron deficiency: maternal iron deficiency during pregnancy results in hypertension and metabolic syndrome in the offspring as adults. Neurological and developmental problems have been reported in children born to anaemic mothers, though other studies have shown no relationship between maternal anaemia (within limits) and pregnancy outcome. Finally, there are data to suggest that patients with "restless legs syndrome" suffer from low iron - whether supplementation is of value is not clear.

Adverse effects due to iron excess

The traditional example of a population suffering from iron overload is that of the Bantu, who brewed beer in iron calabashes. The iron was held in many complexes and could be absorbed to a substantial extent, causing liver damage and cirrhosis. Apart from these cases, it was generally held that the mechanism of regulation of absorption, blocking iron in the intestine followed by sloughing off of the cells, was adequately protective. Recently, there are data beginning to challenge this hypothesis,

suggesting that high meat consumption and consequent (possibly) high transferrin saturation with iron was a risk factor for mortality. Generally, however, it is the populations who are given transfusion therapy for hereditary disorders who suffer most from iron overload.

More recently high levels of iron have also been associated with increased severity of diseases such as rheumatoid arthritis and heart disease. The primary link may be between folic acid and homocysteine and cardiac function, or possibly because high levels of iron act as pro-oxidants. There are only limited data explaining the link, however but as imaging methods improve and as we begin to understand more of the basic biology, a causal link or consequence will be determined.

The health consequences of overload are complex and considerable, ranging from increased risk of heart disease, inflammation and stroke to cirrhosis, kidney damage and circulatory collapse. There may be neurological symptoms and changes in muscle tone and function.

3.2.2. Other essential metals

Further comprehensive assessments of essentiality were not available at the time of the compilation of this fact sheet. In the case of chromium, the International Chromium Development Association is currently working on its risk assessment for chromium (3+). However, the outcome of this was not yet available upon finalisation of this fact sheet. Molybdenum, selenium and cobalt have until now not been subject of any such EU risk assessment, so that data comparable in detail to that given above do not exist.

4. Summary and conclusions

General considerations

To date, essentiality has only been considered in EU human health risk assessments for two cases: in the EU RAR on zinc and zinc compounds, and in the voluntary risk assessment on copper and copper compounds.

The approach adopted in the copper VRA is the most complete, and it is therefore suggested that the procedures used in that VRA form the basis for future RARs.

Conventional risk assessments (i.e., for xenobiotics) are either based on the "MOS approach" (as in the TGD), or on the derivation of "derived no effects levels" or "reference doses" by the application of assessment factors to NOAELs usually derived form animal studies.

However, this focus on the effects of high doses of chemicals tends to neglect that several metals are essential to life, and that harmful effects occur both at very low and very high levels of intake. The object of developing the aspect of essentiality for a particular metal is therefore to avoid recommendations that may place individuals into risk for adverse effects due to deficiency.

The scheme presented below summarises the suggested approach for a balanced consideration of both nutritional essentiality and high dose toxicity in the overall risk assessment and risk characterisation procedures for essential metals and their compounds.

Considerations relevant for the assessment of essentiality

In order to avoid confusion with the concept of "no effect levels" used for high dose toxicity, the new terms "sufficient dietary intake" (SDI) and "deficiency effects level" (DEL) as developed in the copper VRA are proposed to be used as boundary terms for effects at low intakes, and are defined as follows:

> "Deficiency effect level" (DEL): For a given study, the copper dose/intake level at which potentially adverse health consequences associated with copper deficiency are first observed. For studies with only one experimental dose, the DEL is considered to be that dose if adverse health consequences are observed relative to controls (basal diet).
>
> "Sufficient dietary intake" (SDI): For a given study, the dose/intake level in a given study at which no adverse health consequences associated with copper intake are observed in any of the measured endpoints.

Further, the following step-wise approach is suggested for the conduct of an "essentiality assessment" (Figure 2 overleaf). Further considerations on the individual steps are given below.

(1) Homeostatic control mechanisms are in place for Essential Trace Elements (ETEs) which may include regulation of absorption and/or excretion and tissue retention. These mechanisms enable adaptation to varying nutrient intakes, to ensure a safe and optimum systemic supply of ETEs for the performance of essential functions. The efficiency of homeostatic processes may vary within populations and with levels of intake of the ETE. However, the identification of the prevalence of any such variation requires the study of large populations. Toxicity from ETEs may be expected to occur when the exposure is above or below the range which can be accommodated by homeostatic mechanisms. For all ETEs, this homeostatic range of intakes should be identified as carefully as possible, and taken forward to risk characterisation.

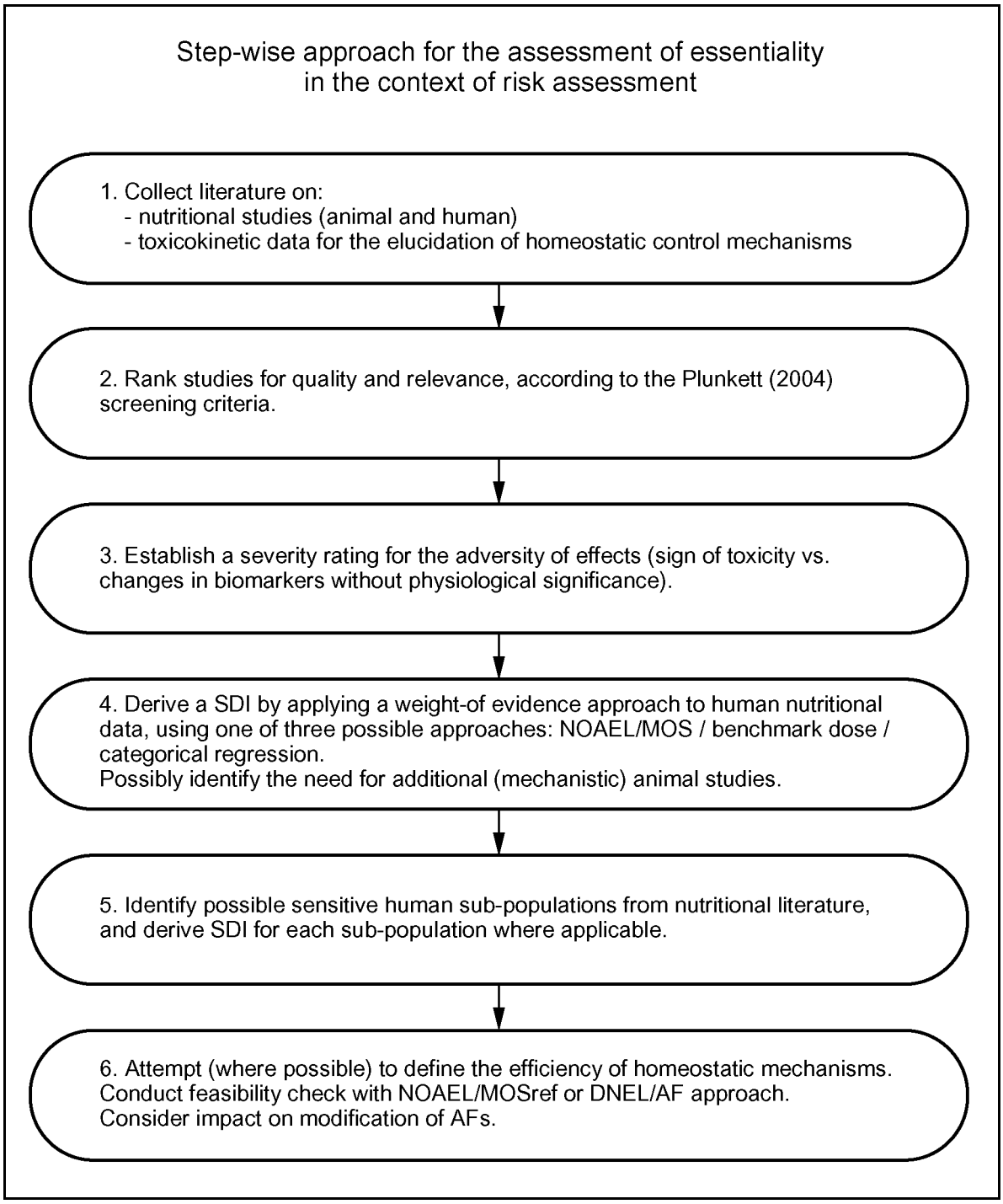

Figure 2: Suggestion for a step-wise approach to assess essentiality in the context of risk assessment

(2) For this purpose, data need to be extracted largely from nutritional studies, which have intrinsic limitations. For this, quality screening criteria (Plunkett, 2004) have been tentatively developed which have been applied to date in one example case (VRA copper). Klimisch criteria are of limited use in this context as they were not designed to assess the potential adverse consequences of ETEs, for which physiological, structural and/or functional impairment due to either excessive or deficient exposures is of concern.

(3) Any observed adverse effects due to acute and chronic deficiency should be rated for severity. A distinction between true “adverse” effects and subtle changes in biomarkers without physiological relevance should be made, where possible.

(4) Many nutritional studies only report a single or very restricted range of intakes which makes their use for risk assessment difficult. Nevertheless by applying a weight of evidence approach to these studies an approximation of the SDI may be obtained. The usability of this against the need for more animal studies should be assessed. At this stage the high quality animal studies should be identified and an animal SDI developed.

For many ETEs, human data of sufficient quality are available which can be used to derive a safe threshold for deficiency (SDI), but the range of intakes will often be limited. Many studies will report the intake at which nutritional balance (no net loss of the ETE from the body) is maintained. This balance point can be taken as the SDI. Three methods exist for determining the dose-response for this point-of-departure: (i) NOAEL approach with margin-of-safety (MOS), (ii) benchmark-dose approach, and (iii) categorical regression approach (the details, advantages and drawbacks of which are discussed in detail in the Appendix to this fact sheet). In general, the choice of method will depend strongly on the extent and quality of data.

(5) From the nutritional literature an attempt should be made to identify sensitive human sub-populations (for instance the very young, the pregnant or the elderly). If these can be identified, an estimate of the SDI for these sub-populations should also be made and should be carried forward to risk characterisation.

(6) Homeostatic mechanisms for ETEs may be both highly conserved between species and very efficient. If this can be demonstrated, this should be discussed and consideration given to the a possible deviation from the conventional EU MOS_{ref} or DNEL approaches: in particular, it is important to consider if the application of AFs to the animal NOAEL leads to an intake lower than the SDI in that species or the SDI derived from human studies.

Future considerations

In addition to the work previously conducted for copper in this context, it has been expressed within the HERAG discussions that it would be desirable to have the same concept and level of detail applied to zinc and possibly also iron as further examples.

However, it was also recognised that such work could not be made available within the timeframe for completion of this fact sheet, so that it was proposed to highlight this here as an issue for future consideration.

5. Abbreviations and References

References

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Abbreviations

AF	Assessment Factor
AI	Adequate Intake
AROI	Acceptable Range of Oral Intake
BW	Body Wight
DEL	Deficiency Effects Level
ETE	Essential Trace Element
HFE	The HFE protein is also called “hemochromatosis protein"
IPCS	International Programme on Chemical Safety
LOAEL	Lowest Observed Adverse Effect Level
MOS	Margin of Safety. Please refer also to the separate HERAG fact sheet on "Margin of Safety"

MOS_{ref}	Reference MOS, the minimum value of MOS derived using assessment factors. Please refer to the separate HERAG fact sheet on "Margin of Safety"
NOAEL	No observed adverse effect level
RA(R)	Risk Assessment (Report)
RDA	Recommended Dietary Allowance
RWC	Reasonable/realistic worst case
SDI	Sufficient Dietary Intake
TGD	Technical Guidance Document on Risk Assessment. In support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.
UL	Upper Intake Limit
VRA(R)	Voluntary Risk Assessment (Report)
WHO	World Health Organisation

Appendix I: Extended summary of assessment principles for essentiality[5]

In their monograph on "Principles and methods for the assessment of risk from essential trace elements", WHO (2002) outlines a procedure relevant for essential trace elements (ETEs) involved in human health. Based on this approach and after discussions within the HERAG project, the following can be summarised as principles for the assessment of essentiality:

Some ETEs are transitional elements on the periodic table. They are transitional because they can, to varying degrees, donate or accept one or more electrons, thereby changing their state of valency or charge. As such, the transitional ETEs can be in a reduced state (having a vacancy or incomplete complement of electrons) or oxidised (having a fuller or full complement of electrons). A wide variety of chemical reactions in the body use this feature of transitional ETEs to their advantage in helping to drive them. In this capacity, transitional ETEs act as cofactors and catalysts, influencing molecular receptor sites and stereochemical conformation of complex enzymes to expose their active sites. An element's specific ability to donate/accept an electron gives it its own unique electrochemical (or oxidation-reduction, redox) potential. For example, copper most readily accepts and donates and, accordingly, has a high redox potential. It is this feature that makes it an efficient conductor of electricity in electrical wiring and heat in heat exchangers. This feature also makes copper oxidative, so that under the right conditions it can rust, form oxides and patinas, and generate damaging oxidative free radicals in biological systems. In biological systems, transitional ETEs are largely bound to transport proteins (and chaperones) which also serve mask their redox potential and facilitate their transport to molecular targets that are very site-specific. On a molecular scale, the relationship between ETE, chaperone, and transporter greatly influences the ETE's balance (termed *homeostasis*) in the body. The body seeks to centre itself on this balancing homeostasis with respect to each ETE, and states of nutritional deficiency or excess - whether marginal, mild, severe, or something in between - are shifts away from homeostasis. Homeostasis is central to the essentiality of ETEs and an individual's nutritional status.

In humans, ETEs are acquired principally via diet and drinking water ingestion. To a lesser degree, they are acquired through dermal absorption and inhalation. Ingestion will be considered here. The dose-response principles apply equally to the other two pathways of ETE systemic absorption into the bloodstream.

Ingestion: There are many factors that determine normal concentrations of individual ETEs in the human body and appropriate amounts of daily dietary and drinking water intake to maintain those normal concentrations. Key factors include inter-individual variability (metabolic variation from person-to-person) within the human population, systemic absorption through the gut, the rate of intake (as a bolus, at intervals, or continuously), age, gender, season, nutritional status, whether in the presence of food, and chemical form of the ETE. Much research has been devoted to understanding the normal range of recommended daily dietary intake and the nature of human variation. The overall scientific weight-of-evidence in dietary and toxicological studies is used to establish (1) *dietary guidelines* of the World Health Organisation and individual countries and (2) country-specific *regulatory limits* for individual ETEs in drinking water.

In setting *dietary guidelines*, three critical concerns are considered: nutritional deficiency, essentiality, and excess. The objective is to set lower and upper limits on dietary guidelines in order to foster good nutrition over long-term life stages (*e.g.*., pregnancy, infancy, childhood, teenage years, adulthood, and old age), and to discourage deficiency and excess within these life stages, for individuals and broader populations alike.

In setting *drinking-water limits*, the concern is for excess ETE ingestion in a short, episodic event (acute ingestion via a bolus dose) and over an extended period of time up to lifetime (chronic ingestion). The applied assumption is that acute ingestion of excess ETE leads to an episode of gastrointestinal upset (gastrointestinal pains, nausea, vomiting, and diarrhoea in the protective process of voiding the excess before it is absorbed into the systemic bloodstream). It follows that chronic ingestion of excess leads to slower-developing, debilitating diseases of the body that are at the initial site of contact (the gut) and at remote, systemic sites in the body, such as the kidney, liver, brain, heart, and other vital organs and systems.

[5] Adapted from ECI (2005)

Scientific basis for setting nutritional guidelines and regulatory standards. The relationship between quantity (concentration ingested in a specified period of time) and the body's response is depicted in a dose-response curve, as presented in Figure A1. Experts who frame dietary guidelines and drinking water standards rely on the features of this curve.

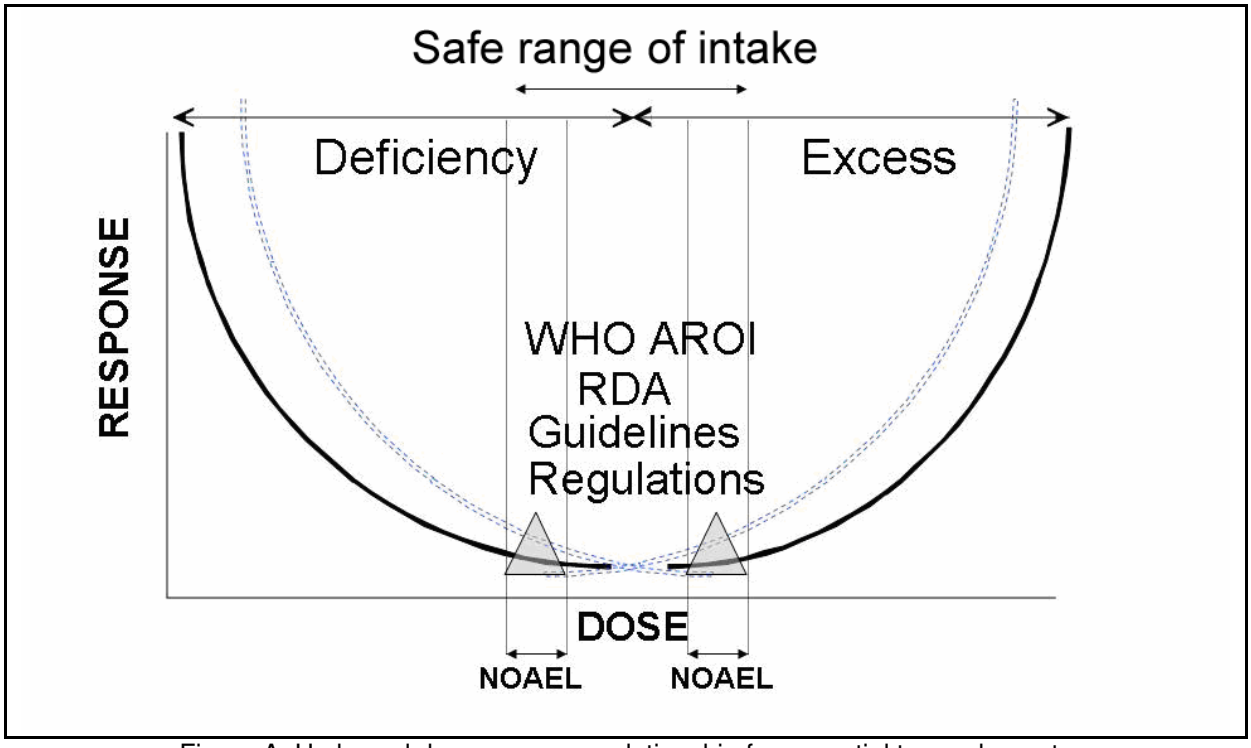

Figure A: U-shaped dose-response relationship for essential trace elements

Briefly, the dose-response curve demonstrates that from the mid-dose range the response (adverse health effect) increases as the dose either increases or decreases. Increasing the dose (excess ETE) prompts a correlated increase in response that is toxicity. Decreasing the dose (deficiency of ETE) also prompts a correlated increase in response that is adverse health associated with ETE deficiency. The term *toxicity*, by convention, is used in descriptions of excess, not deficiency. Accordingly, the right-hand side of the dose-response curve is associated with ETE excess, while the left is associated with deficiency. Together, they give the ETE dose-response curve its classic U-shape.

A critical characteristic of the curve is the no-observed-adverse-effect-level (NOAEL) - the point on the curve immediately preceding the curve's point-of-departure from the x-axis. Along the excess portion of the curve, the NOAEL represents the highest dose at which no adverse effect can be experimentally observed. Any dose higher than this point is presumed to result in some measurable toxicity.

A similar NOAEL exists for the deficiency side of the curve, as depicted in Figure A. Each ETE has its own pair of NOAELs for excess and deficiency. The NOAELs are important features when establishing upper and lower bounds on safe levels of exposure to individual ETEs. In order to avoid confusion with the toxicological concept of "no effect levels", the new terms "sufficient dietary intake" (SDI) and "deficiency effects level" (DEL) used to characterise effects from deficiency are defined as follows:

> "Deficiency effect level" (DEL): For a given study, the copper dose/intake level at which potentially adverse health consequences associated with copper deficiency are first observed. For studies with only one experimental dose, the DEL is considered to be that dose if adverse health consequences are observed relative to controls (basal diet).
>
> "Sufficient dietary intake" (SDI): For a given study, the dose/intake level in a given study at which no adverse health consequences associated with copper intake are observed in any of the measured endpoints.

Because of variation from person-to-person in the human population with regard to nutritional requirement and how ETEs are managed in states of excess or deficiency, a subset of the human population may be susceptible to (*i.e.*, unable to metabolically compensate for) small increments of ETE excess or deficiency. Unlike the normal healthy population which is able to metabolically manage slight changes in ETE nutritional status, susceptible individuals experience adverse health effects at a small incremental increase or decrease. Examples of potentially susceptible subpopulations include the foetus, infants, women in pregnancy, the elderly, those in poor nutritional status, and individuals with pre-existing genetic disorders that prevent them from acquiring, metabolizing, using, and eliminating ETEs. These individuals are represented in Figure A along the dotted lines. For them, the NOAEL for excess is presumed to be slightly lower than for the normal health population. Similarly the SDI for deficiency is presumed to be slightly higher. What is striking about this circumstance is that, for the population in its entirety with all of its inherent variation, there can never be complete satisfaction of nutritional adequacy. This is depicted in Figure A as the dotted lines crossing over, indicating that as one susceptible population is compensated (*e.g.*, for ETE excess), another is placed at risk (*e.g.*, for ETE deficiency). This makes characterisation of the NOAELs for excess and deficiency (either as discreet numbers or as ranges) critical to:

(1) Protecting as much of the human population as possible, and
(2) Understanding who may be susceptible for nutritional excess or deficiency.

This is the underlying basis of nutritional guidelines for ETEs (*e.g.*, Recommended Dietary Allowances, World Health Organisation Acceptable Rate of Intake) in foods and regulatory limits of ETEs in drinking water.

Another perspective on the interface between no-effect and adverse-effect within the human population is the overlap between (1) “safe range of intake” and excess, and (2) “safe range of intake” and deficiency, as depicted in Figure A. These two areas of overlap present inherent "zones of conflict" (as labelled in Figure B, an alternative depiction of the U-shaped curve), leading to scientific and regulatory debates about the "precise" concentrations that represent the DEL[6] (deficiency effect level) or the SDI[7] (sufficient dietary intake), and the NOAEL (no adverse effect level for toxicity). The regions (zones of conflict) of dose within which these levels are contained occur where the biological responses are low or subtle. As represented by the two triangles in Figures A and B, they represent regions of dose and response where scientific research is greatly needed to reduce uncertainty, resolve scientific debate, and produce more evidence to support more responsible scientific weight-of-evidence determinations of both levels.

One difference in the depictions of ETEs in Figures A and B is the shape of the "bottom" region of the U-shape - the most important region for ETE risk assessment. Flatter bottom (Figure B) implies that there is a distinct, broad, and fairly-well defined region of nutritional balance (homeostasis), bounded by equally distinct shifts to states of excess or deficiency. Conversely, a rounder bottom (Figure A) implies a narrower range of homeostasis, with more gradual transitions to states of excess or deficiency.

[6] "Deficiency effect level" (DEL): for a given study, the dose/intake level at which potentially adverse health consequences associated with deficiency are first observed.

[7] "Sufficient dietary intake" (SDI): for a given study, the dose/intake level in a given study at which no adverse health consequences associated with copper intake are observed in any of the measured endpoints.

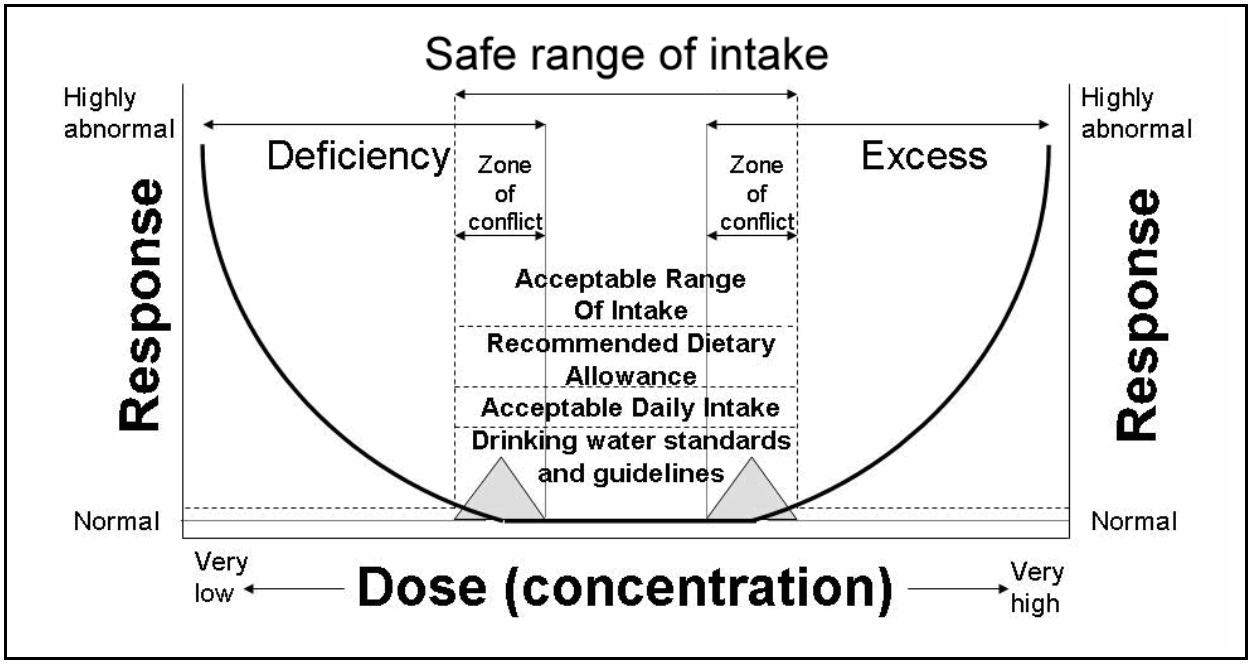

Figure B: Complexity of dose-response relationships for essential trace elements

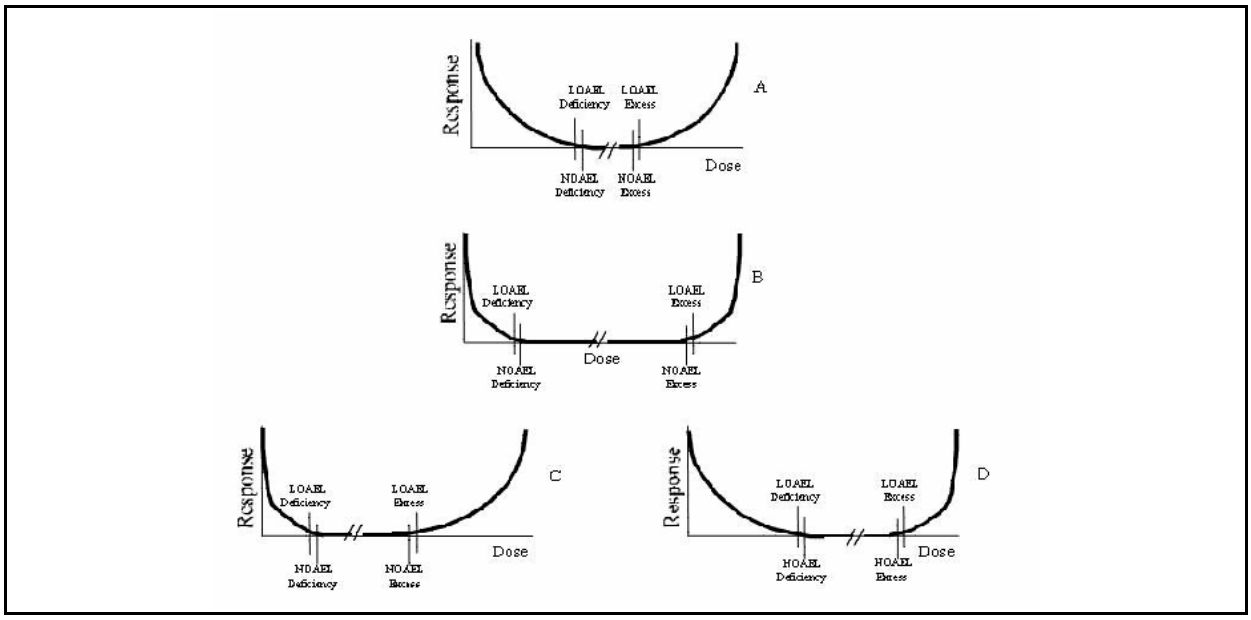

Figure C: Possible dose-response relationships for chronic effects in humans

The range of options for the shape of the U-shaped dose-response-curve is presented in Figure C, including flat, round, skewed-left, and skewed-right curves. For each ETE, the shape will greatly influence where the NOAELs for excess and deficiency lie, as indicated in the figure. For risk assessment purposes, it is important to characterise:

(1) The bottom-shape of the "U".
Which are the appropriate points of departure (NOAELs) from homeostasis?
What is the biological basis for establishing the NOAELs (usually different for excess and deficiency)?

(2) The steepness of both side-walls of the "U":
How rapidly does homeostasis degrade to excess and deficiency, and how rapidly do these conditions degrade further from mild to severe?

The presumption to this point is that the DEL/SDI and NOAEL values are the appropriate point-of-departure for dose-response assessment as it is used in risk assessment. In fact, three methods exist for determining the dose-response point-of-departure:

(1) The NOAEL approach with its applied margin-of-safety (MOS), as discussed
(2) The benchmark-dose approach, and
(3) The categorical regression approach.

The *NOAEL approach* relies on the dose at which adversity is first observed to begin as its point-of-departure. The *benchmark-dose approach* is a modelled point in the dose-response curve that represents a predetermined level of change (*e.g.*, ED_{01}, the effective dose causing a 1% change in response) when compared with controls. The *categorical regression approach* uses all available dose-response data in the literature on an ETE's health effects. In this approach, all observed health effects in all studies are "binned" within a system of discrete biological severity categories, ranging from no-effect as the least severe to death as the most severe. In this *meta*-analysis, the dose-response data captured (aggregated) within each severity category are then treated in a statistical regression model and a regression relationship is generated that represents all data. The advantages and limitation of each approach are presented in Table 1.

Table 1: Strengths and weaknesses of different dose-response methodologies

Method	Advantages	Limitations
NOAEL-MOS	Simple, traditional, familiar Universally applied	Focused on non-response health endpoint (no-observed effect) Little or no uncertainty characterisation Uses only one dose-time data point for one health effect Requires determination of a margin-of-safety which is sometimes arguable and difficult to resolve
Benchmark dose	Empirical curve-fitting Uses all dose-response data Bounds uncertainty quantitatively	Snapshot: represents one time point Uses binary severity scores Focuses on one health endpoint
Categorical regression	Applies to multiple studies with various endpoints Uses complete data sets	Relies on best professional judgement to derive severity ratings Combines different health effects Labour-intensive/costly

As an example, a preliminary categorical regression analysis of the copper dose-response literature is presented in Figure D below. Dose-response data from 92 studies with reliable data were binned into one of these four categories and a series of regression plots prepared. The most compelling argument for this approach is that it utilizes all available data, human and animal, harnessing the power of large amounts of molecular biology information on the mechanism of action and control of individual ETEs, while at the same time including the more conventional toxicological and nutritional dietary studies. The greatest limitation to this approach is that, in violation of conventional toxicological thinking, it combines data from observations of *different* health effects into *one* dose-response construct, with the justification that the data binning-severity normalisation procedure makes the ensuing statistical regression procedure legitimate.

In applying these techniques to risk assessment, it is important to recognise the dynamic tension between (1) the regulatory need to apply adequately protective margins of safety against toxicity due to excess, and (2) the potential consequence of creating a state of deficiency if the protective levels are set to low. Protection against the harm (toxicity) of excess is typically mandated by enabling legislation and regulation. Conversely, protection against the harm of deficiency is typically offered as recommended nutritional guidelines.

When considering both deficiency and excess in ETE risk assessment, the skew of the U-shape left or right can dramatically affect the outcome. Emerging evidence suggest that toxicity due to excess is induced and progresses more gradually (the body has more defence) than deficiency. In other words, once the state of deficiency is induced, it cascades into a more severe state of adversity more rapidly than does the state of excess (plot to the lower-left in Figure D). In the end, the best intention is to empower risk decision-makers with as much useful information as possible to evoke the best possible (wisest) decision and to foster consensus. Accordingly, for ETEs, the best possible strategy is to offer decision-makers a quantitative dose-response assessment that is derived using all three methods.

Other factors influencing the shape of the ETE dose-response curve and assignment of points of departure that are not discussed here, but which bear consideration, include the following: gender- and age-related sensitivities, metal-metal, metal-toxicant, metal-drug, and metal-nutrient interactions, chemical form (species) of the ETE, pre-existing nutritional status, seasonal variation in nutritional status, cultural differences in diet, and long-term sequelae of foetal and infant excess or deficiency. These should be considered qualitatively in the dose-response assessment of risk assessment.

A preliminary categorical regression analysis of the copper dose-response literature (as an example) results in the dose-response curves depicted in Figure D below. In this example, four severity category scores were used to bin data: 0 = biological effects in the homeostatic range; 1 = low level (adverse/non-adverse) effects (*e.g.*, enzyme induction); 2 = metabolic perturbation; and 3= gross toxicity (note that the crossover-point for deficiency and excess in severity category 3 (lower-right plot in Figure D) is at a lower dose than the crossover-point in category 2. As severity increases, the U-shape skews to the left).

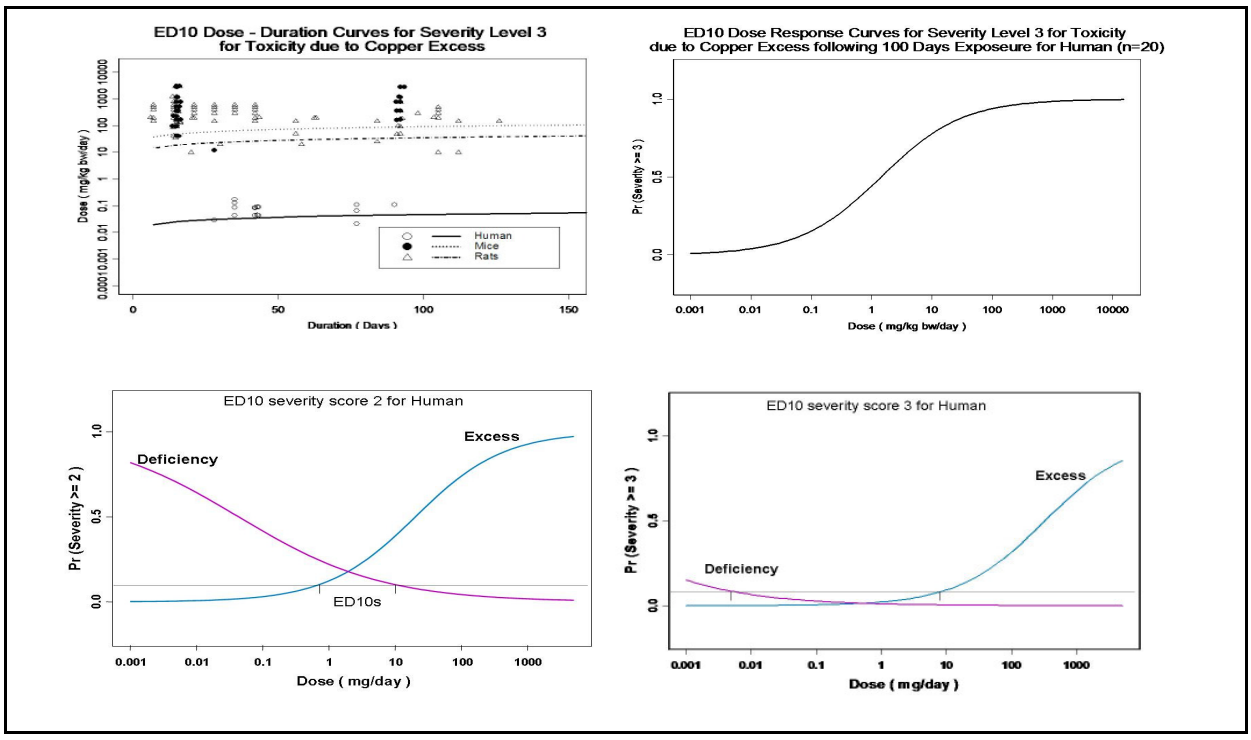

Figure D: Categorical regression for copper
(ED10 = effective dose (mg/kg bw/day) eliciting a 10% response)

Upper-left: Dose vs. duration-of-exposure plot of ED_{10} values for all dose-response data in copper excess studies of mice, rats and humans, severity category 3.
Upper-right: Curve-fitted dose vs. response plot of ED_{10} values for copper excess in humans alone exposed for 100 days (sub-chronic), severity category 3.
Lower-left: Curve-fitted dose vs. response plot of ED_{10} values for copper deficiency and excess in humans alone, severity category 2.
Lower-right: Curve-fitted dose vs. response plot of ED_{10} values for copper deficiency and excess in humans alone, severity category 3.