# Assessment of the Trace Element Status of Individuals and Populations: The Example of Zinc and Copper[1,2]

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ABSTRACT This paper describes the proceedings of a workshop that was convened at the 11th International Symposium on Trace Elements in Man and Animals (TEMA-11) symposium to review recent advances concerning the assessment of the trace element status of individuals and populations, using zinc and copper as the primary examples to illustrate basic principles and recent advances in assessment methods. The workshop was initiated with a brief review of the importance of zinc nutriture for human health and a discussion of the likely common occurrence of zinc deficiency worldwide. This overview was followed by presentations on selected issues concerning the assessment of zinc status, with particular attention devoted to dietary assessment techniques, the use of isotopic tracers to assess zinc homeostasis and the relationship of these methods to biochemical indicators of zinc status. Because relatively little information is available on zinc toxicity, the discussion concerning the definition of excess intake of trace elements focused primarily on recent work concerning risk assessment of copper toxicity. J. Nutr. 133: 1563S–1568S, 2003.

KEY WORDS: • *zinc* • *copper* • *trace elements* • *nutritional assessment* • *toxicity*

As part of the 11th International Symposium on Trace Elements in Man and Animals (TEMA-11), a workshop was convened on the assessment of the trace element status of individuals and populations, using zinc and copper as the primary examples to illustrate basic principles and recent advances in assessment methods. The workshop, which was cosponsored by the International Zinc Nutrition Consultative Group (IZiNCG)[4], was initiated with a brief review of the importance of zinc nutriture for human health and a discussion of the likely common occurrence of zinc deficiency worldwide. This overview was followed by presentations on selected issues concerning the assessment of zinc status. Particular attention was devoted to dietary assessment techniques, the use of isotopic tracers to assess zinc homeostasis, and the relationship of these methods to biochemical indicators of zinc status. Because relatively little information is available on zinc toxicity, the discussion of excess intake of trace elements focused primarily on recent work concerning risk assessment of copper toxicity. Each of these components of the workshop is summarized briefly in the following sections.

## *Zinc and human health*

The proceedings of several recent conferences have provided detailed information on the importance of zinc for human health (1,2,3), so these relationships were reviewed only cursorily in the present workshop. Of particular interest are recent quantitative analyses demonstrating the critical role of zinc in reducing the risk and severity of common infections (4,5) and the importance of zinc for children's growth (6). Other studies suggest a prominent role for zinc for several outcomes of pregnancy (7) and for supporting adequate neurobehavioral development (8).

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[2] Workshop sponsored by the International Zinc Nutrition Consultative Group

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[4] Abbreviations used: CV, coefficient of variation; EAR, estimated average requirement; EZP, exchangeable zinc pool; FNB/IOM, Food and Nutrition Board, Institute of Medicine; FZA, fractional zinc absorption; IZiNCG, International Zinc Nutrition Consultative Group; NHANES, National Health and Nutrition Examination Survey; P:Z, phytate:zinc molar ratio; SES, socio-economic status; WHO, World Health Organization.

### *Suggestive evidence of zinc deficiency in populations*

Despite the compelling health impacts of zinc deficiency, development of appropriate intervention programs has been hampered by the lack of simple reliable low-cost biomarkers of zinc status. To address this problem, the steering committee of IZiNCG has deliberated on possible approaches to assess the risk of zinc deficiency in populations. Several types of suggestive information, such as the amount of absorbable zinc available in the national food supply and the rates of childhood stunting, have been proposed as possible methods of gaining insight into the risk of zinc deficiency in populations. These suggestive indicators have been chosen because: *1*) they are based on existing information that is readily available to country-level health planners; and *2*) they represent either an ecological risk factor (in the case of food supply data) or a health consequence strongly associated with zinc deficiency.

The use of national food-balance sheets to estimate the risk of zinc deficiency has been described in detail previously (9). Briefly, analyses were completed using the data available in the national food-balance sheets that are published annually by the Food and Agriculture Organization of the United Nations. The amounts of individual food commodities described in the respective food-balance sheets were multiplied by their zinc and phytate contents to estimate the amount of absorbable zinc in the national food supply, expressed in relation to mean daily per capita theoretical requirement of the population. Countries at relatively high, medium or low risk of inadequate amounts of absorbable zinc in the national food supply were identified, using this information.

The prevalence of growth stunting can also be used as suggestive information concerning a population's risk of zinc deficiency. In a recent meta-analysis of zinc supplementation trials in children (6), the magnitude of the growth responses attributable to zinc was significantly greater in those studies that enrolled children with preexisting stunting or underweight, defined as height-for-age or weight-for-age $<-2$ SD in relation to international reference data. By contrast, there were no significant effects of zinc supplementation in those trials that enrolled children whose initial mean anthropometric status was within normal limits. These results indicate that the national prevalence of stunting or underweight can be used as indirect evidence of the likely responsiveness of the population to supplemental zinc, and hence their likely risk of zinc deficiency. A notable advantage of this indicator is the fact that relevant anthropometric data are often collected routinely and are compiled periodically by the World Health Organization (WHO) (10).

In a forthcoming document, the IZiNCG steering committee will offer suggestions on possible approaches to assessing a population's risk of zinc deficiency. As a first step, it is recommended that existing information should be consulted on the absorbable zinc content of the national food supply and the prevalence rates of childhood stunting or underweight. It is tentatively proposed that if the national food supply is unlikely to provide sufficient absorbable zinc for at least 75% of the population and if more than 20% of children less than 5 y of age are stunted, then the country has a high risk of zinc deficiency at a level thought to be of public health concern. By contrast, if the food supply can meet the theoretical needs of at least 85% of the population and less than 10% of children are stunted, then the risk of zinc deficiency of public health consequence is relatively low. Countries with intermediate values for one or another of these two indicators would have an intermediate risk of deficiency of public health importance. Countries with a high risk of deficiency should carry out more extensive assessments of the population's zinc status and urgently consider developing intervention programs, especially in high-risk groups, such as young children from lower socioeconomic groups and pregnant women. Countries with an intermediate risk should also complete an assessment of the populations' zinc status to help decide whether further interventions are necessary. Countries with a low risk of zinc deficiency require no further initiatives at a national level, although further assessment of high-risk groups might be indicated.

Direct assessment of a population's risk of zinc deficiency relies on traditional nutritional assessment techniques, such as dietary, biochemical and functional assessment. The following sections will provide more information on selected aspects of these approaches.

### *Population assessment of zinc intake*

Assessment of the adequacy of dietary intakes of zinc can provide a measure of the risk of zinc deficiency in a population. To assess the adequacy of population zinc intakes, it is necessary to compare these intakes with appropriate dietary requirements. Dietary requirements must take the following information into account: *1*) the physiological requirements for absorbed zinc; *2*) an estimate of the proportion of dietary zinc that is absorbable, which is used to calculate the estimated average requirement (EAR) for dietary zinc intake; and *3*) an estimate of the coefficient of variation (CV) of usual intakes of zinc in the population.

Physiological requirements for zinc are defined as the amount of zinc that must be absorbed to replace all endogenous losses and to meet requirements for retained zinc. Estimates of endogenous losses of zinc in urine, integument and sweat, menstrual blood, semen and feces were recently reported in the North American publication on Dietary Reference Intakes (DRIs) (11). Based on this information, daily zinc requirements were estimated to be 3.84 mg/d for adult men and 3.30 mg/d for adult women. A factorial approach was used to estimate the physiological requirements for most other age groups, and a further increment for tissue accrual was added for children, adolescents and pregnant women. These physiological requirements represent the best available estimates, and the model used to derive them appears to be robust and applicable over a wide range of normal populations (12).

To derive dietary requirements for zinc from the physiological requirements, it is necessary to consider estimates of the proportion of dietary zinc that is absorbable. Several dietary factors affect zinc absorption as a result of physico-chemical interactions in the intestine. Phytate, a component in plants with highest concentration in seeds (cereal grains/legumes/nuts), inhibits zinc absorption, as does calcium, whereas dietary protein enhances zinc absorption. Zinc absorption is also diminished with increasing intake of zinc. The phytate:zinc molar ratio (P:Z) in the diet has been used to estimate the absorption of zinc, whereby P:Z $< 5$ is associated with relatively high absorption of zinc, P:Z 5–15 with moderate absorption and P:Z $> 15$ with low absorption (13).

Two committees charged with establishing dietary zinc requirements have provided estimates of dietary zinc absorption (11,13), and the IZiNCG is currently preparing updated guidelines suitable for international use. All three committees identified studies of dietary zinc absorption that were considered suitable for inclusion in the analysis, however, the inclusion criteria used by each committee differed notably. All committees estimated zinc absorption level by deriving a linear regression equation for absorbed zinc by total zinc intake for

each study, and determining from the equation the total zinc intake at which the physiological requirement for absorbed zinc would be met. The proportion of zinc absorbed at this level of intake was taken to be the "critical" level of absorption.

The WHO committee set three levels of zinc absorption based on the P:Z of three diet types: P:Z $<$ 5 (representing refined diets or semipurified formulas), 50% absorption; P:Z 5–15 (representing mixed diets or refined vegetarian diets), 30% absorption; P:Z $>$ 15 (unrefined diets, negligible animal protein), 15% absorption. The data used were derived from studies measuring zinc absorption from single test meals, as well as from total diets, although the availability of the latter study types were limited at the time the estimates were made.

The Food and Nutrition Board, Institute of Medicine (FNB/IOM) committee chose to use zinc absorption data only from total diet studies. The advantages of the total diet studies are that they label the meals with either radio or stable isotopes of zinc and, using fecal monitoring, are able to estimate *true* absorption for each individual by correcting for endogenous losses of intestinal zinc for each individual. On the contrary, most single meal studies have used radioisotope tracers and whole body counting methods, which apply an estimated figure as a correction factor for intestinal losses of endogenous zinc. There is also some evidence from studies of iron absorption that single-meal studies may exaggerate the enhancing and inhibiting effects of dietary factors (e.g., ascorbic acid, phytate). Even though the proportion of these factors relative to the iron content of the whole diet may be similar to that in a single test meal, they may be more widely distributed across the various meals consumed throughout the day, and therefore less concentrated in any one meal consumed. The FNB/IOM committee used 10 data points from total diet studies of zinc absorption, including four studies of semipurified diets, one study of a diet based on soy protein that was washed with ethylene diamine tetraacetic acid to remove zinc, and five studies of mixed diets. The derived absorption level was 41% for men and 48% for adult women.

The IZiNCG committee is also restricting their analysis to zinc-absorption data from total diet studies. However, to make the absorption estimate more widely applicable internationally, a larger proportion of studies of mixed diets ($n$ = 13) and vegetarian diets ($n$ = 2) were included; studies of semipurified formulas and studies where zinc salts were added to the meals were excluded. In a subsample of 12 of these studies for which dietary data were available, calcium and protein were not found to significantly affect zinc absorption, whereas phytate and zinc content had significant negative effects on the proportion of zinc absorbed. The P:Z of the latter studies ranged from 1 to 18. The estimated average critical absorption level was determined to be 28% for men and 30% for women. Many diets in developing countries have P:Z in the range of ~18–34, and thus it is desirable to have an estimate of absorption from these unrefined diet types using total diet studies. Zinc-absorption data of this type were presented during the TEMA-11 symposium (14,15), but were not available beforehand. These data may thus be used in the future to derive a preliminary estimate of zinc absorption from diets with P:Z $>$18.

Dietary zinc intake data from the 1999 Mexican National Nutrition Survey (16) were used to estimate the risk of inadequate zinc intakes by preschool children; this estimate was derived using the absorption estimate from IZiNCG and the EAR derived from FNB/IOM (adjusted for age). Because dietary intake data represented only a single day's intake from a 24-h recall for each participant ($n$ = 1016), it was not possible to estimate the CV of the distribution of usual intakes by correcting for intraindividual variation. Therefore, a CV of

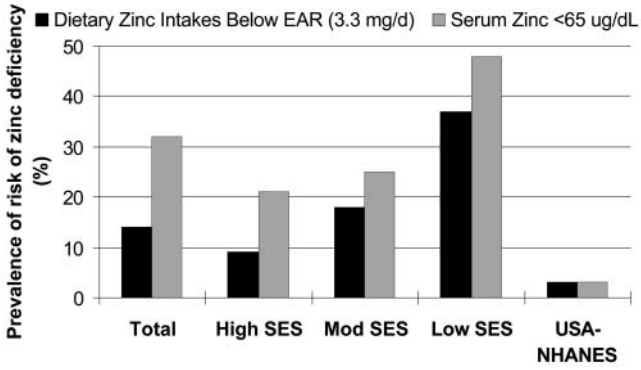



**FIGURE 1** Estimated prevalence of risk of zinc deficiency among Mexican preschool children, by socioeconomic status, and among young U.S. children, based on dietary intakes of zinc and serum zinc concentration.

usual zinc intakes of 25% was assumed, as previously derived from another population (13). The estimated proportion of the total population with intakes below the EAR (3.3 mg/d) was 14%. **Figure 1** shows the prevalence of inadequate zinc intakes increased with decreasing socioeconomic status (SES), likely related to poorer dietary quality (high SES, 9%; moderate SES, 18%; low SES, 37%). When the prevalence of low serum zinc concentrations for a subsample of this population ($n$ = 124) was also determined for each SES group, a similar distribution of risk of zinc deficiency was found (Fig. 1). Given the relatively high risk of zinc deficiency predicted by both of these estimates, and the concordance between them, it can be concluded that Mexican preschool children would likely benefit from programs to improve dietary zinc intakes, especially those of lower SES. The risk of inadequate zinc intakes (based on the EAR from FNB/IOM, 2001) among young children in the U.S. (4–6 y) derived from the National Health and Nutrition Examination Survey (NHANES) III survey (17) and the "theoretical" prevalence of low serum zinc concentrations derived from NHANES II data (18) are also shown (Fig. 1) for the purpose of comparison.

### *Kinetic markers of zinc homeostasis and their relation to other indices of zinc status*

Zinc kinetics in humans have been studied using isotopes for more than five decades. Since the early studies in the 1950s in which $^{65}$Zn disappearance from the plasma and activity in the tissues were studied, advances in analytical methods and mathematical techniques have enabled complex integration of data representing temporal and spatial components of zinc metabolism to be undertaken. The purpose of this section is threefold: *1*) to briefly review the milestones in the development of kinetic analysis of zinc metabolism during the last 50 years; *2*) to describe how kinetic parameters respond to changes in dietary zinc intake; and *3*) to discuss how these relate to biochemical markers of zinc status.

One of the first reports of the use of zinc isotopes to study zinc metabolism in humans was from Ross et al. in the 1950s (19). $^{65}$Zn was administered as an intravenous tracer to study the distribution of zinc in body tissues, including blood, liver, hip and spleen. This technique was also used by Foster et al. in 1979 (20), and the data were analyzed using the Simulated Analysis and Modeling (SAAM) software (21) to produce a multicompartment model of human zinc kinetics. This landmark model has been used as a basis for development of new models that have enabled the identification of sites of homeostatic

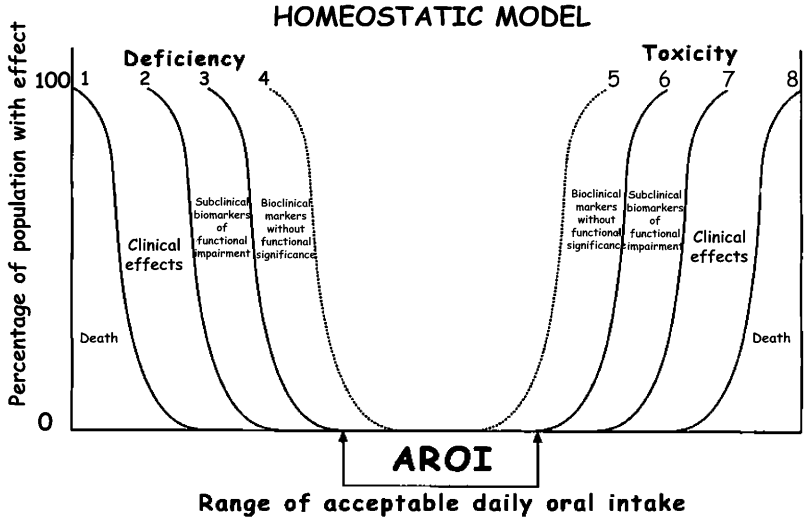
regulation of zinc (22,23). By examining the effects of oral zinc loading on kinetic parameters, five sites of homeostatic regulation were identified, namely: *1*) absorption from the gut, *2*) excretion in the urine, *3*) exchange with red blood cells, *4*) release from the muscle, and *5*) secretion into the gut.

Technological advances in mass spectrometry have enabled the increased use of stable isotopes as tracers. Stable isotopes have a number of advantages over radioisotopes, not the least being the ethical concerns regarding the use of radioisotopes of zinc in populations such as pregnant women and infants. In addition, for a given element there are often several naturally occurring stable isotopes, enabling simultaneous oral and intravenous dosing with different isotopes of the same element. Therefore, parameters associated with plasma isotope kinetics and parameters associated with uptake from and release into the gastrointestinal tract can be resolved in parallel. A major disadvantage of stable isotopes is that a significant mass must be administered to raise isotope abundance above background, which may perturb normal mineral kinetics. To compare the metabolism of stable versus radioisotopes in vivo, Wastney et al. gave a dose of $^{65}$Zn (2.02 $\mu$Ci) and $^{70}$Zn (1.99 mg) orally and compared the kinetic parameters derived from both sets of isotope data (24). They reported that the stable isotope dose resulted in a transient 3% rise in plasma-zinc concentration after 2 h, returning to baseline after 1 d. There were no significant differences in the parameter values determined from data collected over a period of 21 d.

Other disadvantages of the use of stable isotopes include the high costs of the isotopes and their analysis, and the limitations in terms of sampling sites. In contrast to radioisotopes, where data can be collected by external counting of tissues, stable isotope studies are limited to sampling of blood, urine and feces. Therefore, models derived from these data are necessarily less complex than those derived from radioisotope studies. The early models derived from zinc stable isotope data were very simple (25). More complex models became possible as analysis by inductively coupled mass spectrometry became more sensitive, thus enabling plasma isotope disappearance to be followed for a longer period of time.

Returning to the theme of this workshop, the following questions need to be addressed: *1*) Can kinetic models be useful tools in the assessment of zinc status?; and *2*) What effect does marginal or severe zinc depletion have on kinetic parameters? Two studies conducted at the USDA Western Human Nutrition Research Center (WHNRC) were designed to investigate these questions. The first examined the effect of acute severe zinc depletion (0.2 mg zinc/d) in a group of healthy young men (23), and the second examined the effect of marginal zinc intake (4.6 mg zinc/d) following a similar protocol (26). Data from the first study were analyzed using a six-compartment model, which revealed that fractional zinc absorption (FZA) increased from 0.26 to 1 and endogenous zinc excretion fell from 2.72 to 0.25 mg/d. In addition, severe zinc depletion resulted in a 36% reduction in the exchangeable zinc pool (EZP) and a 39% increase in plasma turnover. In contrast, marginal zinc depletion had no significant effect on the EZP or plasma zinc turnover, however, FZA increased. Biochemical indices of status taken during the two studies also responded to severe, but not marginal, zinc depletion. Plasma zinc concentration, alkaline phosphatase activity and urinary zinc excretion fell significantly during severe depletion, but did not change in response to marginal zinc intake.

Finally, it is useful to consider whether kinetic markers provide greater insight into zinc status than biochemical indices. One way of investigating this question is to correlate changes in the biochemical markers with changes in net loss or gain of zinc from the body during periods of zinc depletion and repletion, as measured using zinc balance studies. Regression analysis revealed that of all the parameters measured (both kinetic and biochemical), plasma zinc concentration has the strongest relationship to net loss/gain of zinc from the body during acute severe zinc depletion and repletion ($r^2 = 0.826$). This was followed by endogenous zinc excretion ($r^2 = 0.773$) and plasma zinc flux ($r^2 = 0.766$). It is well known that plasma zinc concentration as a marker of zinc status can be confounded by factors such as stress, infections, etc. Further studies are required to investigate the influence of such factors on kinetic parameters, and the stable isotope methodologies must be




Theoretical dose-response curves for various effects occurring in a population at various levels of intake (doses) of an essential trace element.

The lower end the dose response curve for such critical effects related to deficiency (curve 3) and toxicity (curve 6) defines the range of acceptable daily oral intakes.

Adapted from IPCS, 2002

**FIGURE 2** Theoretical dose-response curves for various effects occurring in a population at various levels of intake (doses) of an essential trace element (from WHO).

simplified before kinetic parameters can be used as routine clinical tools for the assessment of zinc status.

### *Risk assessment for excessive intake of trace elements*

Development of a useful conceptual model for risk assessment of essential trace elements has represented a difficult challenge for many years. The model recently published by the International Program on Chemical Safety (IPCS) recognizes the presence of an area of homeostatic regulation, in which individuals adapt to variations in exposure to an extent that varies for different elements (**Fig. 2**). However, additional empirical data are needed to help define the level of exposure at which the curves start climbing at both sides of the model, namely with insufficient or excess intake. Studies designed to generate relevant, new data should provide several points in the dose-response curve, such that the best-fit curve and confidence intervals can be calculated. This, in turn, will allow calculation of the dose of the nutrient that is necessary to elicit response in 2.5% of the population, the benchmark dose, the lowest observed adverse effect level (LOAEL), the no-adverse effect level (NOAEL) and the limits of acceptable range of intake (AROI) (**Fig. 3**).

On the excess side of the curve for copper assessment, interest has focused on the possibility that rather small amounts of copper, such as those contained in drinking water, could lead to toxic effects, i.e., that the ascending curve for acute effects would be so close to the declining curve of effects secondary to deficiency that the two curves would overlap. In recent years, studies have been completed to describe the early adverse effects of acute exposure to low copper doses, and to characterize the symptoms elicited, the shape of the response curve and the threshold concentration at which gastrointestinal symptoms (mainly nausea) increase significantly in relation to the basal prevalence (when 4 mg Cu/L of water is provided, as copper sulfate) (27,28). These results were analyzed in the recent meeting in Tokyo in which the guidelines for drinking water for human consumption were reviewed (WHO, May 23–28) and the level of 2 mg Cu/L was confirmed as safe for human consumption.

Historically, the diagnosis of trace element deficiency and excess has been based on the detection of rather gross changes in their nutritional status, such as the appearance of toxic effects. A more recent approach is to search for markers of biologically relevant changes that can be detected before toxic effects or disease conditions are established. In doing so, the difficult task is to distinguish innocuous changes that precede toxic effects from those that are associated with critical relevant functions. Olivares et al. (29) assessed infants who received milk-based formulas containing 2 mg Cu/L during the first year of life. On the basis of the volumes ingested daily, it was estimated that these children consumed 250–320 $\mu$g copper/kg BW/d (body weight/d) throughout the study period. The children grew normally and their serum copper and ceruloplasmin levels and transaminases remained within normal values for age at 6, 9 and 12 mo of age, regardless of the copper concentration administered. Although the nonceruloplasmin bound copper fraction showed a positive correlation with serum copper ($p < 0.001$), the values were all $<1.8$ $\mu$mol/L, which is substantially less than the levels of $>10$ $\mu$mol/L that have been reported in cases of Wilson's disease (30). Thus, this level of copper intake was not associated with any detectable adverse consequences.

In subsequent studies, classical markers of copper status were examined in apparently healthy adults, free of infectious/inflammatory conditions, after controlled exposure to 15–150 $\mu$g/kg BW/d for 2 mo (31). Results showed that serum copper and ceruloplasmin did not change after exposure to the higher levels of copper intake. In addition, the copper contents of peripheral blood cells were evaluated as potential markers of copper status. The hypothesis was that if copper in plasma was higher transiently because of the higher intake, these cells should modify their copper content accordingly. However, in individuals exposed to the lowest and highest copper concentrations, the erythrocyte and peripheral mononuclear cell copper contents did not differ, and intracellular red and white cell copper contents were not correlated with serum concentrations of copper or ceruloplasmin (31). This was interpreted

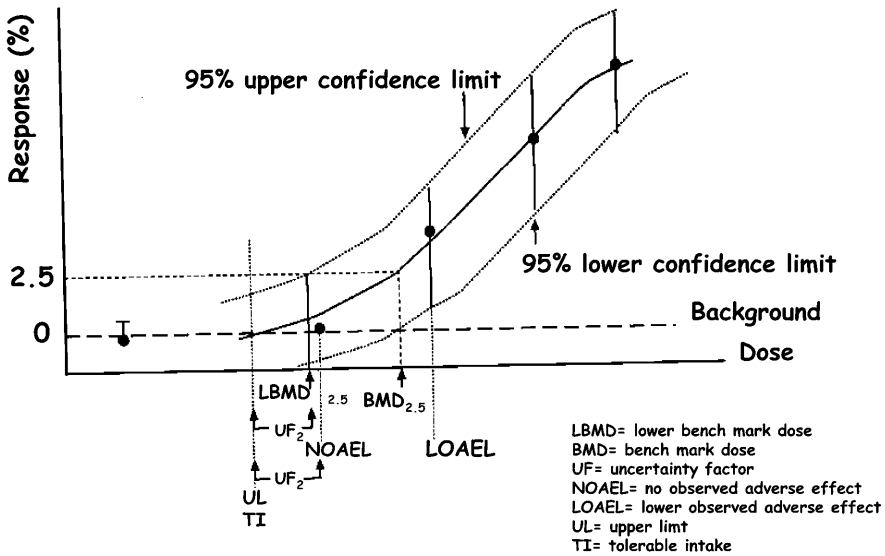



**FIGURE 3** Theoretical representation of the lower part of the dose-response curve for a minimal adverse effect in a sensitive population with the upper 95% of confidence limit of the response (from WHO).

Theoretical representation of the lower part of the dose -response curve for a minimal adverse effect in a sensitive population with the upper 95% of confidence limit of the response.

Adapted from IPCS,2002

as indicating that homeostatic regulation is efficient within the range of copper exposure tested, both in children and adults, as measured by traditional criteria of copper toxicity. Similar types of studies are needed for other trace elements.

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