DRAFT SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for zinc [1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) derived Dietary Reference Values for zinc, using a two-stage factorial approach and reference values for body weight. The first stage of estimating physiological requirements used studies that had physiologically plausible data, specifically related to intestinal excretion of endogenous faecal zinc. Adult physiological requirements were closely related to body size, and sex differences were not detectable after adjustment for body weight. Average Requirements (ARs) for dietary zinc necessary to meet physiological requirements were estimated using saturation response modelling, taking into account the inhibitory effect of dietary phytate on zinc absorption. Estimated ARs and Population Reference Intakes (PRIs) are provided for phytate intake levels of 300, 600, 900 and 1 200 mg/day, which cover the range of mean/median intakes observed in European populations. ARs range from 6.2 mg/day to 10.2 mg/day for women with a reference weight of 58.5 kg and from 7.5 to 12.7 mg/day for men with a reference weight of 68.1 kg. PRIs were derived from the zinc requirement of individuals with a body weight at the 97.5th percentile for reference weights for men and women and range from 7.5 mg/day to 12.7 mg/day for women and from 9.4 to 16.3 mg/day for men. ARs for infants from 7 months and children were estimated factorially, based on extrapolation from estimates of adult losses plus zinc needs for growth, and range from 2.4 to 11.8 mg/day. PRIs were derived by assuming a coefficient of variation of 10 % and range from 2.9 to 14.2 mg/day. For pregnancy and lactation additional zinc requirements related to fetal and maternal tissues and transfer of zinc into breast milk, respectively, were considered. Additional PRIs for pregnancy and lactation were estimated to be 1.6 mg/day and 2.9 mg/day, respectively.

KEY WORDS

zinc, Dietary Reference Value, Population Reference Intake, phytate

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[2] Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values (DRVs) for the European population, including zinc.

Zinc has a wide array of vital physiological functions. It has a catalytic role in each of the six classes of enzymes. The human transcriptome has 2 500 zinc finger proteins, which have a broad intracellular distribution and whose activities include binding of RNA molecules and involvement in protein-protein interactions. Thus, their biological roles include transcriptional and translational control/modulation and signal transduction.

The majority of dietary zinc is absorbed in the upper small intestine. The luminal contents of the duodenum and jejunum, notably phytate, can have a major impact on the percentage of zinc that is available for absorption. Absorption of zinc by the enterocyte is regulated in response to the quantity of bioavailable zinc ingested. Albumin is the major transporter of zinc in both portal and systemic circulation. Virtually no zinc circulates in a free ionised form, and the majority of total body zinc is in muscle and bone; zinc does not have an identified major storage site. The quantity of zinc secreted into and excreted from the intestinal tract depends on body zinc concentrations, and the quantities of endogenous zinc in the faeces and exogenous zinc absorbed in normal adults are related. The kidneys and integument are minor routes of loss of endogenous zinc.

Plasma/serum zinc concentration and other putative biomarkers of zinc adequacy, deficiency and excess are not useful for estimating DRVs for zinc. Zinc requirements have been estimated by the factorial approach involving two stages. The first is the estimation of physiological requirements, defined as the minimum quantity of absorbed zinc needed to match losses of endogenous zinc and to meet any additional requirements for absorbed zinc that may be necessary for growth in healthy well-nourished infants and children, and in pregnancy and lactation. The second step is the determination of the quantity of dietary zinc available for absorption that is needed to meet that physiological requirement. Fifteen studies were identified from the published literature which included data on endogenous faecal zinc and total absorbed zinc to enable estimation of physiological zinc requirements of adults. Individual's data from these studies were supplied by the authors. Data were assessed for physiological plausibility and, after careful evaluation, some data were excluded from further calculations. The final numbers of subjects contributing data to the estimate of physiological zinc requirements were 31 males and 54 females, from a total of 10 studies. Dietary phytate intakes were available for some of the included studies, either as a mean study value or as individual data. The range of dietary phytate intakes in the available data was 0-2 080 mg/day. Multiple regression analysis was used to evaluate possible relationships of physiological requirements with sex, zinc balance (difference between absorbed zinc and total losses of endogenous zinc) as well as body size. The R^2 values for the models with body weight, height, body mass index and body surface area variables were 0.46, 0.42, 0.37 and 0.47, respectively. It was decided to use further the equation relating the physiological requirement to body weight for reasons of convenience and accuracy of measurement. The equation for the physiological requirement was calculated on the basis that the physiological requirement is equivalent to total absorbed zinc when absorbed zinc minus total endogenous zinc losses equals zero at a given body weight. For deriving the dietary zinc requirement, a trivariate saturation response model of the relationship of zinc absorption to dietary zinc and phytate was established using 72 mean data sets (reflecting 650 individual measurements) reported in 18 publications. The R^2 of the fit of this model was 0.81. From this model, the Average Requirement (AR) was determined as the intercept of the total absorbed zinc needed to meet physiological requirements. Estimated ARs and Population Reference Intakes (PRIs) for zinc are provided for phytate intake levels of 300, 600, 900 and 1 200 mg/day, which cover the range of mean/median phytate intakes observed in European populations. ARs range from 6.2 mg/day to 10.2 mg/day for women with a reference body weight of 58.5 kg and from 7.5 to 12.7 mg/day for men with a reference body weight of 68.1 kg. PRIs for adults were estimated as the zinc requirement of individuals with a

body weight at the 97.5th percentile for reference body weights for men and women, respectively, and range from 7.5 mg/day to 12.7 mg/day for women and from 9.4 to 16.3 mg/day for men.

For infants from 7 months, and children, DRVs for zinc were derived using the factorial approach, taking into account endogenous zinc losses via urine, sweat and integument, faeces and, in adolescent boys and girls, also semen and menses, respectively, as well as zinc required for synthesis of new tissue for growth. Urinary and integumental losses were extrapolated based on estimates of adult losses, while endogenous faecal zinc losses were estimated by linear regression analysis of endogenous faecal zinc losses versus body weight for the subjects contributing data to the adult estimates, and for infants and young children from two studies from China and the USA. Zinc requirements for growth were taken into account based on the zinc content of tissue gained, and by estimating daily weight gains for the respective age groups. Absorption efficiency of zinc from mixed diets was assumed to be 30 %. Estimated ARs range from 2.4 mg/day in infants aged 7-11 months to 11.8 mg/day in adolescent boys. Due to the absence of reference body weights for infants and children at the 97.5th percentile, and in the absence of knowledge about the variation in requirement, PRIs for infants and children were estimated based on a coefficient (CV) of variation of 10 % and range from 2.9 to 14.2 mg/day.

The physiological requirements for pregnancy and lactation may be calculated by adding the increases in physiological requirements predicted to meet the demands for new tissue primarily by the conceptus, and the replacement of zinc secreted in breast milk. For pregnancy, an additional requirement for zinc for the four quarters of pregnancy of about 0.4 mg/day was assumed due to zinc accumulation by the fetus, placental, uterine and mammary tissue, amniotic fluid and maternal blood. The Panel decided not to use the trivariate model to estimate the dietary zinc intake required to meet the additional physiological requirement. Instead, the Panel applied a mean fractional absorption of zinc of 0.3 observed in healthy adults to the physiological requirement of 0.4 mg/day. The additional requirement for pregnant women was derived at 1.3 mg/day and the additional PRI for pregnancy was estimated based on a CV of 10 % and was 1.6 mg/day.

For lactation, taking into account breast milk zinc concentration, breast milk volume transferred and postnatal redistribution of zinc due to involution of the uterus and reduction of maternal blood volume, the additional physiological requirement averaged over six months of lactation was estimated to be 1.1 mg/day. Assuming that fractional absorption of zinc is increased 1.5-fold in lactation, and applying a fractional absorption of zinc of 0.45 to the additional physiological requirement of 1.1 mg/day, resulted in an additional dietary requirement for lactating women of 2.4 mg/day. The additional PRI for lactation, based on a CV of 10 %, was 2.9 mg/day.

Meat, legumes, eggs, fish, and grains and grain-based products constitute rich dietary zinc sources. On the basis of data from ten dietary surveys in seven EU countries, zinc intakes were assessed using food consumption data from the EFSA Comprehensive Food Consumption Database and zinc composition data from the EFSA nutrient composition database. Average zinc intakes ranged from 2.4 to 3.7 mg/day among infants (< 1 year of age), from 4.5 to 6.9 mg/day among children aged 1 to < 3 years, between 5.5 and 9.9 mg/day among children aged 3 to < 10 years, between 6.9 and 13.6 mg/day among adolescents (10 to < 18 years), and between 8.1 and 13.5 mg/day among adults. Main food groups contributing to zinc intakes were meat and meat products, grains and grain-based products, and milk and dairy products. Published data on phytate intakes in the EU are limited and indicate a wide range of dietary phytate intakes.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on nutrient and energy intakes for the European Community.[4] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then, new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context, EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002,[5] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance, EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically, advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;

[4] Scientific Committee for Food. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st series. Office for Official Publication of the European Communities, Luxembourg, 1993.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

- Protein;
- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food (SCF) published an opinion on nutrient and energy intakes for the European Community (SCF, 1993). For zinc, Population Reference Intakes (PRIs) were proposed for all population groups from 7 months onwards based on zinc requirements to replace basal losses and losses via breast milk in lactating women or an increment to supply zinc for growth in children and pregnant women, respectively. In addition, a Lowest Threshold Intake was derived for men and women (see Section 4).

2. Definition/category

2.1. Chemistry

Zinc has an atomic mass of 65.39 Da and is the 24th most abundant element in the Earth's crust. It is included in the group of transition metals although it has only one oxidation state and exists as a stable divalent cation. Considered to be of fundamental importance to the far-ranging biology of zinc is its ability for fast exchange coupled with strong binding to organic molecules, especially to thiolate and amine electron donors. Zinc does not exhibit direct redox activity, a feature which facilitates its safe transport within the body (Krezel et al., 2007). There are five naturally occurring stable isotopes of zinc, the most abundant is ^{64}Zn (48.63 % natural abundance).

2.2. Functions of zinc

2.2.1. Biochemical functions

Zinc has a wide array of vital physiological functions and is ubiquitous within every cell in the body. It is this very abundance which is thought to be the reason why it has proved so challenging to link zinc deficiency with specific phenotypic features. However, three general functional classes (catalytic, structural and regulatory) define zinc's role in biology (King and Cousins, 2014). Zinc has a structural or catalytic role or both in each of the six classes of enzymes, although unequivocal evidence of a direct link between signs of zinc deficiency and a deficiency of a specific metallo-enzyme has not yet been confirmed in humans.

The structural role of zinc is exemplified by transcription factors having zinc motifs (zinc fingers) which link with cysteine and histidine to form a tetrahedral Zn^{2+} coordination complex. The presence of zinc is necessary for the activity of these zinc fingers. The human transcriptome has 2 500 zinc finger proteins which represent 8 % of the genome and account for a significant portion of the zinc requirement (King and Cousins, 2014). Zinc fingers have a range of binding affinities, suggesting that some zinc finger-dependent transcription may be especially vulnerable to low zinc absorption. Zinc finger proteins have a broad intracellular distribution and their activities include binding of RNA molecules and involvement in protein-protein interactions. Thus, their biological roles include transcriptional and translational control/modulation and signal transduction. A combination of structural and regulatory functions are involved with the large quantities of zinc movement involved in the release of insulin, secretion of zinc-containing digestive enzymes, and acid secretion by parietal cells in the stomach (Guo et al., 2010).

Regulation of gene expression is a key biochemical role of zinc. The metal-response element (MRE)-binding transcription factor (MTF1) is thought to provide zinc-responsiveness to many genes (King and Cousins, 2014) including a master regulatory role for micro RNA genes involved in gene expression.

A second regulatory role of zinc is as a regulator of intracellular signalling, analogous to calcium but at a finer level of control, especially through regulation of kinase and phosphorylase activity (King and Cousins, 2014). Control of phosphorylation/dephosphorylation may explain effects of zinc on phosphorylated transcription factors, cell surface receptor binding of growth factors and cytokine receptors, and the major effects of zinc on virtually all aspects of the immune system.

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

There is a lack of specific health effects of zinc deficiency, apart from those observed in infants with Acrodermatitis Enteropathica (see below), and this is the consequence of its essentiality for many core biochemical processes. There is protection by homeostatic mechanisms both at a whole body and tissue level, which, during periods of rapid growth, include slowing of linear growth (i.e. bone growth). Though by no means unique to zinc deficiency, slowing of linear growth is one of the most clearly defined effects of chronic zinc deficiency. The particular vulnerability of the immune system to zinc deficiency results in part from its high rate of cell proliferation. However, the immune system also epitomises the dependence of many cellular biochemical processes on zinc. These may include atypical regulation of cytokine gene expression and signalling pathways which can disrupt the balance of cell-mediated versus humoral immunity. Failure of zinc-dependent structural factors needed for antigen presentation may enhance the risk of microbial and parasitic infections (King and Cousins, 2014) of which enteric infections have been the principal foci of interest (Black, 2003).

Acute severe zinc deficiency results from genetic defects in zinc transporters involved in the intestinal absorption of zinc and in the transfer of zinc by the mammary gland into human milk, collectively termed Acrodermatitis Enteropathica. The onset of clinical features after birth is rapid. The most superficially apparent are the skin lesions, characteristically most prominent around the body orifices and on the extremities. Diarrhoea is prominent in most but not all cases. Growth failure is progressive and these infants are susceptible to a range of immune defects and infections. Loss of appetite and of taste perception are notable and alterations in affect and mood are early phenomena of incipient zinc deficiency in children with Acrodermatitis Enteropathica when their supplemental zinc becomes inadequate. Response to treatment with zinc is profound, but without treatment there is typically a fatal outcome in Acrodermatitis Enteropathica by later infancy. Similar acute acquired zinc deficiency states have been extensively documented primarily in patients dependent on intravenous nutrition lacking zinc (Younoszai, 1983).

2.2.2.2. Excess

Chronic high zinc intakes can result in severe neurological disease attributable to copper deficiency (Hedera et al., 2009). The SCF (2002) has set a Tolerable Upper Intake Level (UL) of 25 mg/day for adults, including pregnant and lactating women, based on studies of zinc supplementation in women of up to 14 weeks. A NOAEL of 50 mg/day was based on the absence of any adverse effect on a wide range of relevant indicators of copper status in controlled metabolic studies. An Uncertainty Factor of 2 was applied. The UL for children was extrapolated from the UL for adults using body weight to the power of 0.75 and reference body weights for European children (SCF, 1993).

2.3. Physiology and metabolism

Zinc transporter gene regulation currently dominates all aspects of cellular zinc metabolism The ZnT family (SLC30a) facilitates the efflux of zinc across cell membranes and into vesicles. The ZIP transporters do the reverse. Up- or down-regulation of these genes in response to zinc intake contributes to the tight homeostatic control of zinc by the small intestine. Diet is among the factors that regulate transporter gene expression. These same families of transporters have the major role in regulating uptake, excretion and metabolism of zinc by all cells in the body. Metallothionein also has a supportive role in zinc metabolism. Polymorphisms in these genes can effect phenotypic expression.

2.3.1. Intestinal absorption

Small quantities of zinc may be absorbed throughout the entire gastrointestinal tract but the majority is absorbed in the upper small intestine. When ingested from food it will be firmly bound especially to protein thiols and nitrogen ligands. The phytate-zinc ligand is weakened at low pH (Cheryan, 1980) and the results of stable isotope studies of zinc absorption are consistent with the zinc being released from these ligands and entering a common pool in the acidic environment of the stomach and, subsequently, being bound to a variety of other organic ligands, including phytate in the alkaline medium of the distal duodenum. The form in which bioavailable zinc is presented to the apical surface of the enterocyte and the zinc transporters, especially Zip 4, has not been fully elucidated. The luminal contents of the duodenum and jejunum in particular, especially phytate, can have a major impact on the percentage of zinc available for absorption. With diets low in phytate and low in zinc, for example less than 4 mg/day, the fraction of zinc absorbed may be as high as 60 % or more. The fraction of absorbed zinc then decreases progressively with increasing dietary zinc (Hambidge et al., 2005). The uptake of zinc and its transfer to the body by the enterocyte is regulated especially in response to the quantity of bioavailable zinc ingested (Chung et al., 2008); this relation of the quantity of zinc absorbed versus that ingested is best fit with saturation response modelling (Hambidge et al., 2010).

WHO/FAO (2004) categorised diets with regard to their impact on zinc absorption being mainly influenced by the phytate–zinc molar ratio and the amount and source of dietary protein. In most European countries, the main contributors to dietary protein intake of adults are meat and meat products, followed by grains and grain-based products, and milk and dairy products. Mean protein intakes of European adults are generally above the Average Requirement (EFSA NDA Panel, 2012). Thus, for the majority of the European population consuming mixed diets, the Panel considers that the phytate content of the diet has a more profound effect on zinc availability than its protein content, and that at zinc intakes adequate to meet the requirement the absorption efficiency of zinc from the diet is moderate or high (Table 1).

Table 1: Criteria for categorising diets according to their potential absorption efficiency of zinc (adapted from WHO/FAO (2004))

Absorption efficiency	Principal dietary characteristics
High	Refined diets low in cereal fibre, low in phytic acid content, and with phytate–zinc molar ratio below five; adequate protein content principally from non-vegetable sources, such as meat and fish. At a zinc intake of 10 mg/day, a phytate-zinc molar ratio of below five is equivalent to a phytate intake of below about 500 mg/day.
Moderate	Mixed diets containing animal or fish protein. (Lacto-)ovo-vegetarian, or vegan diets not based primarily on unrefined cereal grains or high-extraction-rate flours. Phytate–zinc molar ratio of total diet within the range 5–15, or not exceeding 10 if more than 50 % of the energy intake is accounted for by unfermented, unrefined cereal grains and flours. At a zinc intake of 10 mg/day, a phytate-zinc molar ratio of 5-15 is equivalent to a phytate intake of about 500-1 500 mg/day.
Low	Diets high in unrefined, unfermented, and ungerminated cereal grain[(a)], especially when intake of animal protein is negligible. Phytate–zinc molar ratio of total diet exceeds 15; high-phytate, soya-protein products constitute the primary protein source. Diets in which, singly or collectively, approximately 50 % of the energy intake is accounted for by the following high-phytate foods: high-extraction-rate (≥ 90 %) wheat, rice, maize, grains and flours, oatmeal, and millet; sorghum, cowpeas, pigeon peas, grams, kidney beans, black-eyed beans, and groundnut flours. At a zinc intake of 10 mg/day, a phytate-zinc molar ratio exceeding 15 is equivalent to a phytate intake higher than about 1 500 mg/day.

(a): Germination of cereal grains or fermentation (e.g. leavening) of many flours can reduce antagonistic potency of phytates; if done, the diet should then be classified as having a moderate absorption efficiency of zinc.

2.3.2. Transport in blood

Albumin is the major transporter of zinc in both the portal and systemic circulation. Virtually no zinc circulates unbound. Zinc in the plasma compartment turns over 130 times per day and 80 % of circulating zinc is in the cellular components of the blood.

2.3.3. Distribution to tissues

Total body zinc in adult males is approximately 2.5 g in men and 1.5 g in women. The majority of total body zinc, i.e. about 85 %, is in muscle and bone. There are metabolic pools with both short-term and long-term turnover. The exchangeable zinc pool exchanges with plasma zinc in approximately two days and is thought to represent the most metabolically active portion of total body zinc.

Zinc uptake capacity by the human placenta is inversely related to maternal plasma zinc concentrations and increases with increasing gestational age. There are no recent data on the metabolism of zinc by the placenta and fetus at the molecular level.

2.3.4. Storage

Zinc does not have an identified major storage site. The liver provides a limited short-term store of zinc, which is readily released as needed. Twenty per cent of bone zinc, which accounts for about 30 % of total body zinc, has been reported to be released into the circulation in times of depletion at a slower rate than liver zinc. At times of increased bone turnover and tissue catabolism zinc is released adventitiously from these depots. Though muscle has the largest quantity of zinc, release of this zinc in response to zinc depletion has not been documented. Within all cells, vesicles provide sites for temporary storage.

2.3.5. Metabolism

The rapid turnover of plasma zinc reflects its exchange with all tissues and organs in the body. There is a rapidly exchanging pool of zinc that fully exchanges with zinc in plasma and accounts for about 10 % of total body zinc. This zinc is in soft tissues other than muscle and especially in the liver (Wastney et al., 1986; Miller et al., 2000).

2.3.6. Elimination

2.3.6.1. Faeces

The quantity of zinc secreted into and excreted from the intestinal tract depends on body zinc status, both short-term and long-term. The amount of endogenous zinc in the faeces and the quantity of exogenous zinc absorbed in normal adults is positively related.

2.3.6.2. Urine and sweat

The kidneys and integument are relatively minor routes of excretion of endogenous zinc. There is a weak positive relationship between absorbed zinc and urinary zinc. However, the latter declines markedly when dietary zinc is severely reduced. For subjects in normal zinc status urinary zinc losses of 0.5 mg/day for men and 0.3 mg/day for women have been calculated based on individual data from studies by Jackson et al. (1984); Turnlund et al. (1984); Lowe et al. (1997); Miller et al. (2000); King et al. (2001); Pinna et al. (2001); Sheng et al. (2009) (see Section 5.1.1). Studies of whole body surface zinc losses in men have indicated combined integumental and sweat zinc losses of 0.5 mg/day for men (Jacob et al., 1981; Milne et al., 1983; Johnson et al., 1993).

2.3.6.3. Breast milk

During lactation, the quantity of zinc transferred from the mammary gland to the exclusively (or partially) breast-fed infant decreases and this physiological decline is quite notable. Milk zinc concentrations do not appear to be associated with maternal zinc status or dietary zinc intake (Mills, 1989), and long-term ingestion of supplementary zinc (15 mg/day from two weeks *post partum* until seven months) did not affect the rate of decline of milk zinc concentration in supplemented women (Krebs et al., 1995). A comprehensive review of breast milk zinc concentrations and zinc transferred from mother to child covered 63 studies globally, including 12 from European countries (Brown et al., 2009). Zinc concentrations (mean ± SD) were: 4.11 ± 1.50 mg/L below 1 month (n = 74 observations), 1.91 ± 0.53 mg/L at 1-2 months (n = 42), 0.98 ± 0.35 mg/L at 3-5 months (n = 24), and 0.77 ± 0.22 mg/L at 6-11 months (n = 24). Taking into account breast milk volume, Brown et al. (2009) estimated a milk zinc transfer of 2.52 mg/day for the first month, 1.37 mg/day for months 1 to 2, and 0.86 mg/day for months 3 to < 6.

Additional data on breast milk zinc concentrations in mothers of term infants in Europe are given in Appendix A.

2.3.7. Interaction with other nutrients

High dose iron supplements can interfere with zinc absorption when provided simultaneously with zinc supplements. There is no interference with zinc absorption from iron added to foods.

High doses of zinc can interfere with copper absorption (see Section 2.2.2.2).

2.4. Biomarkers

A systematic review and meta-analysis of the literature examining the efficacy of potential biomarkers of zinc status was undertaken by Lowe et al. (2009). This review presented an analysis of data from more than 32 potential biomarkers, however for many there was insufficient evidence to assess their reliability.

2.4.1. Plasma zinc concentration

In apparently healths subjects, plasma and serum zinc concentration is affected by intake, both inadequate and excessive. Lowe et al. (2009) concluded that plasma zinc concentration responds to an increase in intake over short periods, but that the homeostatic mechanisms that act to maintain plasma zinc concentration within the physiologic range may prevent high plasma concentrations from being sustained over a prolonged period.

Plasma zinc concentrations are reduced with severe inherited and acquired zinc deficiency states. However, sensitivity as a biomarker is poor and, with more moderate zinc deficiency states, lacks specificity (King, 2011). Plasma zinc concentration has been recommended as a biomarker of zinc status and the population's risk of zinc deficiency by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition Consultative Group (IZiNCG) (de Benoist et al., 2007).

2.4.2. Hair zinc concentration

Low hair zinc concentrations have been associated with retarded growth (Gibson et al., 1989; Gibson et al., 1991). However, there are potential and actual confounders which may have a role in apparent age-related differences in hair zinc concentrations (Hambidge et al., 1972). Based on three randomised controlled trials (RCTs) with zinc intakes between 15 and 100 mg/day Lowe et al. (2009) concluded that hair zinc concentration increases in response to an increase in zinc intake, but that the effect of zinc depletion is inconclusive.

2.4.3. Urinary zinc concentration

Urinary zinc concentration has been found to increase in response to increases in zinc intake resulting from zinc supplementation; however, the response to zinc depletion has been reported to be inconclusive (Lowe et al., 2009).

2.4.4. Other biomarkers

The early expectation of biomarkers based on zinc transporters or metallothionein have disappointed. Candidates based on proteomic and metabolomic techniques are a current focus of research interest (Kettunen et al., 2012; Ryu et al., 2012); however, the Panel considers that they are not useful yet for deriving DRVs.

2.4.5. Conclusion on biomarkers

The Panel considers that neither plasma/serum zinc concentration nor any other putative biomarker is useful for estimating DRVs for zinc.

2.5. Effects of genotype

The most well documented and severe polymorphisms for zinc result in the clinical syndrome of Acrodermatitis Enteropathica (Zip4) and in the 'lethal mouse syndrome (ZT4)'. A similar defect in cattle (Adema Disease) is less well characterised. There are no known genotypes that would affect the estimation of DRVs for zinc. Significant results for altered putative zinc biomarkers in groups of people with differing gene variants have been documented by Lowe et al. (2013).

3. Dietary sources and intake data

3.1. Dietary sources

Meat, legumes, eggs, fish, and grains and grain-based products constitute rich dietary zinc sources.

Currently, zinc acetate, zinc bisglycinate, zinc chloride, zinc citrate, zinc gluconate, zinc lactate, zinc oxide, zinc carbonate, and zinc sulphate may be added to both foods[6] and food supplements.[7] Zinc L-ascorbate, zinc L-aspartate, zinc L-lysinate, zinc malate, zinc mono-L-methionine sulphate, zinc L-pidolate and zinc picolinate may be added to food supplements only. The zinc content of certain foods for healthy people, for example of infant and follow-on formulae, is regulated.[8]

3.2. Dietary zinc intake

Food consumption data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011a), classified according to the food classification and description system FoodEx2 (EFSA, 2011b), were used for this intake assessment. Data from ten dietary surveys in seven EU countries for which food consumption had been classified according to FoodEx2 were used after consistency checks. The countries included were Finland, Germany, Ireland, Italy, Latvia, the Netherlands and the UK. The data covered all age groups from infants to adults aged 75 years or older (see Appendix B).

Nutrient composition data for zinc were derived from the EFSA nutrient composition database which was compiled as a deliverable of a procurement project "Updated food composition database for nutrient intake" (Roe et al., 2013). Fourteen national food database compiler organisations participated in this data collation project. In case no data were available at the national level, the national data compilers were allowed to use compatible data from other countries. Food composition information of Finland, Germany, Italy, the Netherlands and the UK were used to calculate zinc intakes in these countries, assuming that the best intake estimate would be obtained when both the consumption data and the composition data are from the same country. For zinc intake estimates of Ireland and Latvia, food composition data from the UK and Germany, respectively, were used, because no specific composition data from these countries were available.

[6] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26.

[7] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[8] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1.

Average zinc intakes ranged from 2.4 to 3.7 mg/day in infants (< 1 year of age), from 4.5 to 6.9 mg/day in children aged 1 to < 3 years, between 5.5 and 9.9 mg/day in children aged 3 to < 10 years, between 6.9 and 13.6 mg/day in adolescents (10 to < 18 years), and between 8.1 and 13.5 mg/day in adults. Average daily intakes were in most cases slightly higher in males (see Appendix C) compared to females (see Appendix D), mainly due to larger quantities of food consumed per day.

Main food groups contributing to zinc intakes were meat and meat products, grains and grain-based products and milk and dairy products. Other food groups contributing to zinc intakes were composite dishes in the UK and the Netherlands and vegetables and vegetable products as well as fish and fish products in Italy (see Appendices D and E). Differences in main contributors to zinc intakes between males and females were small. When the EFSA zinc intake estimates were compared with published intake estimates from the same surveys, the EFSA average zinc intake estimates corresponded to 93-108 % of intakes reported in the literature in five countries (Finland, Germany, Italy, Netherlands and United Kingdom), except for the German VELS study where the EFSA intake assessment produced values that were 27-28 % lower compared to published figures. This underestimation may at least partly be due to differences in reporting breast milk consumption. In the data obtained by EFSA, the volume of breast milk consumed was reported only for one infant, but in the published report of the VELS study, 8.1 % of zinc intake was from mother's milk among all infants (Kersting and Clausen, 2003). The estimated Irish intakes showed to be an overestimation of about 21-23 %, likely due to differences in the practices of disaggregation of composite foods before providing the food consumption data to EFSA.

3.3. Dietary phytate intake

The range of dietary phytate intake in the few European countries for which English-language data are available varies widely (Schlemmer et al., 2009; Amirabdollahian and Ash, 2010; Prynne et al., 2010). For example, median phytate intakes reported in the UK based on the representative National Diet and Nutrition Survey ranged from 692 to 948 mg/day in men and from 538 to 807 mg/day in women of various age groups (Amirabdollahian and Ash, 2010), whereas lower intakes have been reported from studies in Scandinavian countries (Brune et al., 1989; Plaami and Kumpulainen, 1996) and in Italy (Carnovale et al., 1987) (see Appendix G).

The wide variation in phytate intakes can partially be explained by differences in dietary patterns within and between countries, for example dietary patterns dominated by plant foods are accompanied by a higher phytate intake. Besides dietary patterns, differences in food processing impacting on the phytate content of foods consumed as well as methodological problems associated with phytate intake assessment also contribute to variation between surveys. It has been estimated that adults ingest about 300 to 800 mg/day of phytate with a mixed diet and that the phytate intake increases to 700 to 1 400 mg/day for mixed diets with a high proportion of unrefined cereal grain products and legumes (Ingelmann et al., 1993; Schlemmer, 1995), whereas dietary phytate intake may be as high as 1 600 to 2 500 mg/day in adults on vegetarian diets (Bindra and Gibson, 1986; Ellis et al., 1987; Khokhar and Pushpanjali, 1994).

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The Nordic countries (Nordic Council of Ministers, 2014) estimated zinc requirements using the factorial method. For the estimate of endogenous losses and routes other than the intestine the figures of the US Institute of Medicine (IOM, 2001) were used. Endogenous intestinal losses were estimated to be 1.4 mg/day for both sexes based on the observed losses at low intakes (1-5 mg/day). Thus, it was assumed that 2.67 mg/day and 2.4 mg/day for men and women, respectively, have to be absorbed in order to replace all losses. Absorption efficiency of zinc from a mixed animal and vegetable protein diet usually consumed in the Nordic countries was assumed to be 40 %. The Average Requirement (AR) of zinc was therefore set at 6.4 mg/day and 5.7 mg/day, respectively. The inter-individual variation in requirement was set at 15 %, resulting in recommended intakes of 9 mg/day for men and 7 mg/day for women. It was noted that this recommended intake probably has a high safety margin as the ability to adapt to lower intakes appears to be substantial.

The German-speaking countries (D-A-CH, 2013) estimated obligatory daily zinc losses as 2.2 mg in men and 1.6 mg in women based on King and Turnlund (1989). To replace these losses an AR of 7.5 mg/day for men and 5.5 mg/day for women was calculated, assuming a mean zinc absorption efficiency of 30 % from mixed diets (Milne et al., 1983; Taylor et al., 1991). Adding to the AR twice a coefficient of variation (CV) of 15 % resulted in recommended intakes of 10 mg/day for men and 7 mg/day for women.

The US Institute of Medicine (IOM, 2001) applied a factorial approach to calculate the minimal quantity of absorbed zinc necessary to replace daily excretion of endogenous zinc. Losses via routes other than the intestine were regarded as unrelated to dietary zinc intakes over a wide range encompassing zinc requirements. They were calculated to be 1.27 mg/day for men and 1.0 mg/day for women, considering data on average urinary excretion, integumental losses, and losses in semen or menstrual losses, respectively. IOM determined the correlation between the losses through excretion of endogenous zinc via the intestine and the quantity of zinc absorbed based on balance studies (Jackson et al., 1984; Turnlund et al., 1984; Wada et al., 1985; Turnlund et al., 1986; Taylor et al., 1991; Hunt J et al., 1992; Lee et al., 1993) and, taking into account a constant for non-faecal endogenous losses, calculated the average total minimal quantity of absorbed zinc required to offset losses as 3.84 mg/day for men and 3.3 mg/day for women. Considering the asymptotic regression of absorbed zinc on zinc intake observed in the balance studies, Estimated Average Requirements (EARs) of 9.4 mg/day for men and 6.8 mg/day for women were determined, corresponding to average fractional zinc absorptions of 0.41 and 0.48 for men and women, respectively. IOM noted that such EARs are supported by data from zinc depletion studies considering changes in functional endpoints (Wada and King, 1986; Grider et al., 1990; Beck et al., 1997b; Beck et al., 1997a) and a study on biochemical zinc status in healthy women (Gibson et al., 2000). Recommended Dietary Allowances (RDAs) of 11 mg/day for men and 8 mg/day for women were set by adding twice a CV of 10 % to the EARs.

WHO/FAO (2004) applied a factorial approach, which involved totalling the requirements for tissue maintenance, metabolism and endogenous losses. The body's ability to adapt to different levels of zinc intake was taken into consideration by defining the normative requirement for absorbed zinc as the obligatory loss during the early phase of zinc depletion before adaptive reductions in excretion take place. The normative requirements for absorbed zinc were estimated to be 1.4 mg/day for men and 1.0 mg/day for women by adding estimations of faecal, urinary and skin losses (data derived from Milne et al. (1983); Milne et al. (1987); Taylor et al. (1991)). To translate these estimates into requirements for dietary zinc, the influence of the nature of the diet (i.e. its content of promoters and inhibitors of zinc absorption) and the efficiency of absorption of potentially available zinc were considered. Overall, three categories of diets were distinguished, characterised by high, moderate and low zinc bioavailability, and the absorption efficiency figures estimated at intakes adequate to meet the normative requirements for absorbed zinc were 50 %, 30 % and 15 %, respectively. Corresponding average individual dietary requirements were estimated to be 36, 59 and 119 µg/kg body weight per day for women and 43, 72 and 144 µg/kg body weight per day for men. Assuming an interindividual variation of zinc requirements of 25 %, the recommended nutrient intakes are 3.0, 4.9 and 9.8 mg/day for women and 4.2, 7.0 and 14.0 mg/day for men for diets of high, moderate and low zinc bioavailability, respectively.

Afssa (2001) set two levels of recommended intakes, depending on the dietary content of products of animal origin. Daily intakes of 7 mg/day for women and 9 mg/day for men were recommended if the diet contains relatively high amounts of products of animal origin (estimated intestinal zinc absorption of 30 %). Increased daily intakes of 12 mg/day for women and 14 mg/day for men were proposed if the diet contains relatively low amounts of products of animal origin (estimated intestinal absorption of 20 %).

The Netherlands Food and Nutrition Council (1992) applied a factorial approach. Total zinc losses were estimated to be 1.3-1.9 mg/day for men and 1.1-1.7 mg/day for women, considering data on average urinary excretion, integumental losses, and additional losses in semen for men. Menstrual zinc losses were considered negligible. Minimum requirements were estimated to be 5.2-7.6 mg/day for men and 4.4-6.8 mg/day for women, applying an estimated average absorption efficiency of 25 %. The Council proposed adequate ranges of intakes of 7.0-10.0 mg/day for men and 6.0 to 9.0 mg/day for women, assuming an inter-individual variation in zinc losses of 20 %.

The UK COMA (DH, 1991) assessed the zinc requirement on the basis of factorial analyses using measurements of basal losses during metabolic studies of deprivation, the turnover time of radiolabelled endogenous zinc pools, and deduction from metabolic studies of patients receiving total parenteral nutrition. Minimal zinc losses in the order of 2.2 and 1.6 mg/day in men and women, respectively, were estimated, considering data on basal faecal and urinary losses, and on losses via skin, hair, semen and menstruation, where appropriate (Hambidge et al., 1986; King and Turnlund, 1989; Taylor et al., 1991). Assuming an absorption efficiency of 30 %, these figures translate to EARs of 7.3 mg/day in men and 5.5 mg/day in women, respectively. Reference Nutrient Intakes (RNIs) of 9.5 mg/day and 7.0 mg/day were set for men and women, respectively. Based on the same considerations, the SCF also proposed PRIs of 9.5 mg/day for men and 7.0 mg/day for women (SCF, 1993).

Table 2: Overview of Dietary Reference Values for zinc for adults

	NNR (2012)	**D-A-CH (2013)**	**WHO/FAO (2004)**			**AFSSA (2001)** [(a)]		**IOM (2001)**	**SCF (1993)**	**NL (1992)** [(b)]	**DH (1991)**
			High BA (50 %)	Moderate BA (30 %)	Low BA (15 %)	IA of 20 %	IA of 30 %				
Age (years)	≥ 18	≥ 19	≥ 19	≥ 19	≥ 19	≥ 20	≥ 20	≥ 19	≥ 18	≥ 19	≥ 19
PRI											
Men (mg/day)	9	10	4.2	7.0	14.0	14	9	11	9.5	7-10	9.5
Women (mg/day)	7	7	3.0	4.9	9.8	12	7	8	7	6-9	7.0

(a): The values vary according to the bioavailability of zinc from the diet: for predominantly vegetarian diets a bioavailability of 20 % is assumed, and for balanced diets rich in animal products, including meat products, a bioavailability of 30 % is assumed.

(b): Adequate range of daily intake

BA, bioavailability; IA, intestinal absorption; NL, Netherlands Food and Nutrition Council

4.2. Infants and children

The Nordic countries (Nordic Council of Ministers, 2014) noted that data on endogenous losses of zinc at different intakes are almost completely lacking for children. It was also noted that in relation to body weight, children appear to have larger losses of zinc than adults. The need for growth was estimated to be 175 µg/kg body weight per day during the first month, decreasing to approximately 30 µg/kg body weight per day at 9-12 months (Krebs and Hambidge, 1986). For growing children the need for zinc was based on basal losses of 0.1 mg/kg body weight and a zinc content in new tissue of 30 µg/g. For adolescents, growth was assumed to result in an average zinc content in new tissue of 23 µg/g, due to an increase in fat tissue with a lower zinc content than children. The physiological needs for rapidly growing adolescents were considered to be increased by 0.3-0.4 mg/day. Applying the same principles as for adults, the recommended zinc intakes vary from 2 mg/day in the youngest age group to 12 mg/day for adolescent boys.

WHO/FAO (2004) considered evidence that the maintenance requirement in infants is influenced by the nature of the diet (Krebs and Hambidge, 1986; Krebs, 1993) and assumed endogenous losses of zinc to be 20 µg/kg body weight per day for human milk-fed infants and about 30-40 µg/kg body weight per day for infants fed formula or weaning foods. Estimated zinc increases for infant growth were set at 120-140 µg/kg body weight per day for female and male infants, respectively, for the first three months. These values decreased to 33 µg/kg body weight per day for ages 6-12 months. A bioavailability of 80 % was assumed for exclusively breast-fed infants, while a bioavailability of 15 % or 30 % was assumed for formula-fed infants depending on the type of formula. For infants up to six months of age, it was assumed that interindividual variation of zinc requirements is 12.5 % and is the same for breast-fed (derived from Vuori (1979a)) and formula-fed infants. After that age, a CV of 25 % was assumed. For other age groups an average loss of 0.57 µg/kcal of resting energy expenditure (REE) was derived by extrapolating from the adult values using the respective REEs. For ages 1-10 years, the requirements for growth were based on the assumption that new tissue contains 30 µg/g. For adolescent growth, a tissue zinc content of 23 µg/g was assumed. Taking into account that pubertal growth spurts increase physiological zinc requirements substantially, growth of adolescent males was assumed to correspond to an increase in body zinc requirement of about 0.5 mg/day.

For infants aged 0-6 months, IOM (2001) set an Adequate Intake (AI) at 2.0 mg/day that reflects the observed mean zinc intake of infants exclusively fed human milk. Human milk alone was considered an inadequate source of zinc after the first six months, and EARs for older infants and children were based on the factorial approach. Excretion of endogenous zinc was estimated by extrapolation from measured values for either adults or younger infants. Requirements for growth were derived from chemical analyses of zinc concentrations of infant and adult tissues (Widdowson and Dickerson, 1964) and average daily accretion of new tissue (Kuczmarski et al., 2000). For preadolescent children (7 months to 13 years), a conservative fractional zinc absorption of 0.3 was applied (Fairweather-Tait et al., 1995; Davidsson et al., 1996), while a fractional zinc absorption of 0.4 was used for adolescents (14 to 18 years). IOM noted that growth data from supplementation studies with zinc in children aged 7 to 12 months (Walravens et al., 1989) and 4 to 8 years (Walravens et al., 1983; Gibson et al., 1989) were consistent with the EARs derived from the factorial approach. Corresponding RDAs were set by adding twice a CV of 10 % to the EARs.

The Netherlands Food and Nutrition Council (1992) applied a factorial approach. Total zinc losses were extrapolated from adults on the basis of metabolic weight ($kg^{0.75}$). For the first half year of life the requirement for growth was estimated at 400 µg/day (Widdowson and Dickerson, 1964; Sandstead, 1973; WHO, 1973), on the basis of the increase in fat-free body mass and the zinc content per kg fat-free body mass. An estimated average absorption efficiency of 25 % was applied to derive the minimum dietary requirements. Corresponding adequate ranges of intakes were set assuming inter-individual variations of 20 % in zinc loss and 15 % in zinc requirement for growth.

The UK COMA (DH, 1991) used a factorial approach to calculate daily zinc requirements for infants and children. Growth increments were estimated on the basis of growth progressing along the 50^{th} percentile and on a lean tissue zinc content of 30 µg/g. Urine and sweat zinc losses were taken as 10 and 20 µg/kg body weight per day, respectively, and faecal losses as 77 µg/kg body weight per day. This led to a daily requirement of absorbed zinc of 1.0 mg. Taking into account an absorption efficiency of 30 % from infant formula, an EAR of 3.3 mg/day was derived. The RNI was set at 4 mg/day by adding twice a CV of 10 % to the EAR. For children over one year of age, RNIs were based on interpolated basal losses from adults and calculated increments for growth, assuming an absorption efficiency of 30 %. The SCF set the PRIs for infants and children based on the same approaches (SCF, 1993).

Table 3: Overview of Dietary Reference Values for zinc for children from four months

	NNR (2012)	D-A-CH (2013)	WHO/FAO (2004)			AFSSA (2001) [a]		IOM (2001)	SCF (1993)	NL (1992) [b]	DH (1991)
			High BA (50%)	Moderate BA (30%)	Low BA (15%)	IA of 20 %	IA of 30 %				
Age (months)	6-11	4-<12	7-12	7-12	7-12			7-12	6-11	6-12	7-12
PRI (mg/day)	5	2	0.8 [c], 2.5 [d]	4.1	8.4			3	4	3-4	5
Age (years)	1-<2	1-<4	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-4	1-3
PRI (mg/day)	5	3	2.4	4.1	8.3	8	5	3	4	3-4	5
Age (years)	2-5	4-<7	4-6	4-6	4-6	4-9	4-9	4-8	4-6	4-7	4-6
PRI (mg/day)	6	5	2.9	4.8	9.6	11	6	5	6	4-5	6.5
Age (years)	6-9	7-<10	7-9	7-9	7-9			9-13	7-10	7-10	7-10
PRI (mg/day)	7	7	3.3	5.6	11.2			8	7	4-6	7
Age (years)	10-13	10-<13	10-18	10-18	10-18	10-12	10-12	14-18	11-14	10-13	11-14
PRI											
Boys (mg/day)	11	9	5.1	8.6	17.1	14	9	11	9	5-7	9
Girls (mg/day)	8	7	4.3	7.2	14.4	13	9	9	9	5-7	9
Age (years)	14-17	13-<15				13-19	13-19		15-17	13-16	15-18
PRI											
Boys (mg/day)	12	9.5				14	11		9	7-10	9.5
Girls (mg/day)	9	7				11	9		7	7-10	7.0
Age (years)		15-<19								16-19	
PRI											
Boys (mg/day)		10								8-11	
Girls (mg/day)		7								6-9	

(a): The values vary according to the bioavailability of zinc from the diet: for predominantly vegetarian diets a bioavailability of 20 % is assumed, and for balanced diets rich in animal products, including meat products, a bioavailability of 30 % is assumed.
(b): Adequate range of daily intake
(c): Exclusively human milk-fed infants
(d): Not applicable to infants consuming human milk only
BA, bioavailability; IA, intestinal absorption; NL, Netherlands Food and Nutrition Council

4.3. Pregnancy

The Nordic countries (Nordic Council of Ministers, 2014) considered that the total need for zinc during pregnancy for the fetus, placenta and other tissues is approximately 100 mg (King, 2000), and that studies on whether or not homeostatic adjustments occur during pregnancy are inconclusive (Swanson and King, 1982; Fung et al., 1997). The recommended intakes were based on an assumed increase of the physiological requirement by 0.7 mg/day. With adjustment for absorption the additional recommended dietary intake was set to 2 mg/day.

The German-speaking countries (D-A-CH, 2013) considered that the average additional requirement of absorbed zinc during the second half of pregnancy is 0.8 mg/day and recommended an additional zinc intake of 3 mg/day from the fourth month of pregnancy onwards.

WHO/FAO (2004) considered an estimated amount of zinc retained during pregnancy of 100 mg (Lentner, 1984; Swanson and King, 1987). During the third trimester, the physiological requirement of zinc was assumed to be approximately twice as high as that of non-pregnant women.

Applying a factorial approach, IOM (2001) determined an additional requirement of 2.7 mg/day, considering the highest average daily rate of zinc accumulation by maternal and fetal tissues of 0.73 mg observed during the fourth quarter of pregnancy (Swanson and King, 1987), and an estimated average fractional absorption of zinc of 0.27 (Turnlund et al., 1991; Hunt J et al., 1992; Sian et al., 1996; Fung et al., 1997; Hunt et al., 1998; Miller et al., 1998). EARs of 10 mg/day for pregnant adolescents aged 14-18 years and of 9.5 mg/day for pregnant women were derived, and RDAs set at 12 mg/day and 11 mg/day, respectively, by adding twice a CV of 10 % to the EARs and rounding to the nearest 1 mg.

The Netherlands Food and Nutrition Council (1992) considered extra zinc requirements of 0.6, 0.9 and 1.0 mg/day during the first, second and third trimesters of pregnancy, respectively, according to WHO (1973), and set adequate ranges of intakes of 9-12 mg/day during the first trimester and of 11-15 mg/day during the second and third trimesters.

The UK COMA (DH, 1991) noted that, although there was evidence that extra zinc is required during pregnancy, studies have shown no increase in customary daily zinc intake by pregnant women and no benefit from zinc supplements (Mahomed et al., 1989). The Committee considered it probable that in healthy women metabolic adaptation ensures an adequate transfer of zinc to the fetus, and no increment was proposed for pregnant women. The SCF (1993) adopted the same approach.

Table 4: Overview of Dietary Reference Values for zinc for pregnant women

	NNR (2012)	**D-A-CH (2013)**	**WHO/FAO (2004)**			**AFSSA (2001)** [(a)]		**IOM (1998)**	**SCF (1993)**	**NL (1992)** [(b)]	**DH (1991)**
			High BA (50%)	Moderate BA (30%)	Low BA (15%)	IA of 20 %	IA of 30 %				
Age (years)								14-18			
PRI (mg/day)	9	10 [(c)]	3.4 [(d)] 4.2 [(e)] 6.0 [(f)]	5.5 [(d)] 7.0 [(e)] 10.0 [(f)]	11.0 [(d)] 14.0 [(e)] 20.0 [(f)]	16 [(g)]	11 [(g)]	12	7	9-12 [(d)] 11-15 [(e)] 11-15 [(f)]	-
Age (years)								19-50			
PRI (mg/day)								11			

(a): The values vary according to the bioavailability of zinc from the diet: for predominantly vegetarian diets a bioavailability of 20 % is assumed, and for balanced diets rich in animal products, including meat products, a bioavailability of 30 % is assumed.
(b): Adequate range of daily intake
(c): From four months
(d): First trimester
(e): Second trimester
(f): Third trimester
(g): Increases during gestation, value is for the third trimester.
BA, bioavailability; IA, intestinal absorption; NL, Netherlands Food and Nutrition Council

4.4. Lactation

The Nordic countries (Nordic Council of Ministers, 2014) considered milk zinc concentration to be 2.5 mg/L in the first month of lactation and to fall to approximately 0.7 mg/L after four months (Krebs and Hambidge, 1986). An additional requirement of 1.7 mg/day for replacement of zinc losses with human milk was assumed. Taking into account absorption efficiency, an additional dietary intake of 4 mg/day was recommended.

Assuming that fully breast-fed infants receive 1 mg zinc/day with 0.75 L of human milk, the German-speaking countries (D-A-CH, 2013) considered that the average additional requirement of absorbed zinc during lactation is 1 mg/day and recommended an additional zinc intake of 4 mg/day.

From data on maternal milk volume and milk zinc concentrations, WHO/FAO (2004) estimated the daily output of zinc in milk to be 1.4 mg/day during the first three months of lactation, 0.8 mg/day from three to six months, and 0.5 mg/day thereafter (Vuori, 1979a; Krebs and Hambidge, 1986; Casey et al., 1989). In setting the requirements for early lactation (0-3 months *post partum*), it was assumed that around 0.5 mg/day is covered by postnatal involution of the uterus and from skeletal resorption.

IOM (2001) estimated the losses of zinc in human milk to be 3 mg/L at 4 weeks to 1.2 mg/L at 24 weeks on the basis of observed average zinc concentrations in human milk (Moser-Veillon and Reynolds, 1990; Krebs et al., 1995) and an average secretion of 0.78 L milk/day. IOM also took into account that zinc is released from the *post partum* involution of the uterus and the decreased maternal blood volume (King and Turnlund, 1989), and assumed that it is available for reutilisation. Overall, the average calculated increased requirement of absorbed zinc was 1.35 mg/day. Applying a fractional zinc absorption of 0.377 during lactation (Fung et al., 1997), the additional zinc requirement was estimated to be 3.6 mg/day.

To compensate for zinc transfer into breast milk, the Netherlands Food and Nutrition Council (1992) estimated an additional requirement of 2.4 mg/day during the first month of lactation, 2.0 mg/day during the second and third months, and 1.2 mg/day thereafter (Vuori, 1979b; Ruz, 1984; Casey et al., 1985).

During lactation, the UK COMA (DH, 1991) proposed an increment of 6 mg/day during the initial four months of lactation and 2.5 mg/day thereafter, on the basis of a daily milk volume of 0.85 L and zinc losses of 2.13 mg/day and 0.94 mg/day, respectively. The SCF (1993) proposed an additional dietary intake of 5 mg/day during lactation to cover the amount of zinc transferred into the milk.

Table 5: Overview of Dietary Reference Values for zinc for lactating women

	NNR (2012)	**D-A-CH (2013)**	**WHO/FAO (2004)**			**AFSSA (2001) [(a)]**		**IOM (1998)**	**SCF (1993)**	**NL (1992) [(b)]**	**DH (1991)**
			High BA (50%)	Moderate BA (30%)	Low BA (15%)	IA of 20 %	IA of 30 %				
Age (years)								14-18			
PRI (mg/day)	11	11	5.8 [(c)] 5.3 [(d)] 4.3 [(e)]	9.5 [(c)] 8.8 [(d)] 7.2 [(e)]	19.0 [(c)] 17.5 [(d)] 14.4 [(e)]	23 [(f)]	15 [(f)]	13	12	16-20 [(c)] 13-16 [(g)]	+6 [(h)] +2.5 [(g)]
Age (years)								19-50			
PRI (mg/day)								12			

(a): The values vary according to the bioavailability of zinc from the diet: for predominantly vegetarian diets a bioavailability of 20 % is assumed, and for balanced diets rich in animal products, including meat products, a bioavailability of 30 % is assumed.
(b): Adequate range of daily intake
(c): 0-3 months
(d): 3-6 months
(e): 6-12 months
(f): Decreases during lactation, values for the first month
(g): After 3 (NL)/4 (UK) months of lactation
(h): 0-4 months
BA, bioavailability; IA, intestinal absorption; NL, Netherlands Food and Nutrition Council

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of zinc requirement of adults

The lack of sensitive specific biomarkers or clinical features of 'mild' zinc deficiency precludes the possibility of using a dose-response approach to estimating zinc requirements. Theoretically the traditional balance technique combined with urine and integumental zinc losses has the potential to provide information on zinc requirements. In practice, despite a long history of such measurements this approach has not provided satisfactory results. The small difference obtained from subtracting total faecal excretion from total ingested zinc to derive net absorption detracts from the accuracy and reliability of this approach, and it does not provide information on true zinc absorption. The advent and progressive improvement of equipment and techniques for the application of zinc stable isotopes to studies of zinc homeostasis has progressively facilitated the application of a factorial approach in the estimation of zinc requirements.

The estimation of zinc requirements by the factorial approach requires two stages. The first is the estimation of physiological requirements, defined as the minimum quantity of absorbed zinc necessary to match losses of endogenous zinc and to meet any additional requirements for absorbed zinc that may be necessary in lactation and for growth in healthy well-nourished infants and children and in pregnancy. The second step is the determination of the quantity of dietary zinc available for absorption that is necessary to meet the physiological requirement.

5.1.1. Physiological requirements

5.1.1.1. Identification of studies, data extraction, assessment of methodological quality

A total of 15 studies were identified from the published literature which included data on endogenous faecal zinc (EFZ) and total absorbed zinc (TAZ) for the estimation of physiological zinc requirements. Fourteen studies were identified by comprehensive literature searches in PubMed up to mid February 2014 using the following search string: zinc[TI] AND ((endogenous f*ecal) OR (intestinal excretion endogenous) OR (intestinal endogenous losses) OR isotope* OR compartmental OR extrinsic* OR balance) AND ((total absorbed) OR absorption OR retention OR depletion OR pool* OR metabolism), with a limit to human studies. One study was identified by hand-searching the reference list of studies retrieved by the comprehensive literature search.

Inclusion criteria were: studies of healthy adults, whole-day isotope studies of true zinc absorption, studies with information on body weight of participants, and retrieval of individual data at time of final data analyses. Second stage exclusion criteria included physiologically implausible data for EFZs and evidence of clinical disease.

After detailed review of all potential data and elimination of studies that had significant methodological limitations, the methodologies used in the studies included in the final analyses are considered to be reliable. For example, only studies that employed isotope tracer methods for determining zinc absorption were considered acceptable.

5.1.1.2. Inclusion of studies

For 15 studies, data from the individual study participants were supplied by the authors. Data quality was assessed initially by data being physiologically plausible. Initial evaluation identified two study designs (compartmental modelling and faecal isotope dilution) that included intravenous administration of a zinc stable isotope tracer and its dilution in the faeces, i.e. isotopic labelling of the endogenous zinc appearing in the faeces, and which consistently provided physiologically plausible data. The quality of a third method involving isotopic measurement of absorption coupled with gastro-intestinal balance of non-labelled zinc was judged on the physiologically plausible results (see Section 5.1.1.2 and Appendix H). Information extracted from the studies were total dietary zinc (µmol/day or mg/day), total dietary phytate (µmol/day or mg/day), total absorbed zinc (µmol/day or mg/day), faecal excretion of endogenous zinc (µmol/day or mg/day), daily urinary zinc excretion (µmol/day or mg/day), subject body weight (kg) and subject height (m) (see also Appendix I).

5.1.1.3. Inclusion of individual data

Thirteen individual data, all from studies utilising the zinc absorption-intestinal balance technique, had physiologically impossible negative values for EFZ, and accordingly were omitted from subsequent calculations. After omitting these individual data points, the remaining data from these studies (Wada et al., 1985; Hunt J et al., 1992; Hunt et al., 1995; Hunt et al., 1998) and the data from the study of Sandstrom et al. (2000) were evaluated in comparison to the data from studies which had no negative EFZ values, the core of which were data from studies using isotope tracer methods (isotope dilution, compartmental modelling) to directly measure EFZ and were, therefore, the most reliable. The EFZ data in question were found to differ from the standard data in distribution and relationships to other variables, bringing into question their accuracy (see Appendix H for details).

In view of the uncertainty about the accuracy of the EFZ results in particular studies (Wada et al., 1985; Hunt J et al., 1992; Hunt et al., 1995; Hunt et al., 1998; Sandstrom et al., 2000), the Panel decided to exclude data from these studies from subsequent consideration. Also excluded was one participant in the study of Taylor et al. (1991) who had biochemical and haematological indices of hepatitis possibly due to alcohol abuse. Exclusion involved a total of 103 data points (more details on the preliminary data analysis are given in Appendix H).

The final numbers of subjects contributing data to the estimate of physiological zinc requirements were 31 males and 54 females from a total of ten studies. These included data from all available published studies that contained the data required, including a study in China (Sian et al., 1996) which had results that fit well with those of data from studies in the USA and Europe. Dietary zinc intakes of subjects ranged from 0.8-29 mg/day (see Appendix I). Dietary phytate intakes were available for some of the studies included either as a mean study value or as individual data. The range of dietary phytate intakes in the available data was 0-2 080 mg/day. The majority of phytate intakes appeared to be in the range of 500 to 800 mg/day, and there were only three known instances of individuals having phytate intakes greater than 1 000 mg/day. While several studies used semipurified formula diets, in most studies the diets were composed of conventional foods, sometimes based on the habitual diets of the subjects. The period of time during which the subjects consumed these constant diets prior to the isotope studies varied from five days to five weeks, though seven days was the most common duration.

5.1.1.4. Estimation of endogenous zinc losses

Total endogenous zinc losses were calculated as the sum of losses via faeces, urine, combined integument and sweat (0.5 mg and 0.3 mg/day for men and women, respectively; see explanation below); semen (0.1 mg/day (Hunt CD et al., 1992; Johnson et al., 1993)); and menses (0.01 mg/day (Hess et al., 1977)), of which endogenous faecal zinc is the major component. Urinary zinc losses were reported for 57 of the 85 subjects. For the remaining 28 individuals, estimated urinary zinc losses based on sex were used. The estimated mean urinary zinc losses were 0.5 mg/day for men and 0.3 mg/day for women. These were the averages of the 53 reported values (22 men, 31 women) for subjects in normal zinc status. Integumental and sweat zinc losses were estimated from published studies. An estimate of 0.5 mg/day for men was obtained from studies of whole body surface zinc losses in men (Jacob et al., 1981; Milne et al., 1983; Johnson et al., 1993). The estimate of 0.3 mg/day for women was calculated by multiplying the value for men by the female to male ratio of sweat zinc losses observed in studies of whole body sweat zinc losses and whole body sweat rates in men and women (Cohn and Emmett, 1978; Avellini et al., 1980; Frye and Kamon, 1983; Tipton et al., 1993; DeRuisseau et al., 2002; Hazelhurst and Claassen, 2006). These studies reported female to male ratios for sweat zinc loss rates between 0.5 and 0.7 while sweat zinc concentrations were similar.

5.1.1.5. Modelling of zinc requirements

The assumptions that the regression errors were normally distributed and exhibited constant variance, and that the model was valid, were checked primarily by visual examination of plots of the residuals. Both the raw residuals and the externally studentised residuals were examined. Normality of the residuals was also tested with the D'Agostino-Pearson and Shapiro-Wilk tests. Externally studentised residuals were examined for outliers as well. The variance inflation factor was used to evaluate multicollinearity. Details of the regression diagnostics are provided in Appendix J.

A multiple regression analysis was used to evaluate possible relationships of physiological requirements with sex, the difference between absorbed zinc and total endogenous zinc losses as well as body size. A major finding from this analysis was that the physiological requirement for zinc varies significantly with body size expressed as weight, height, body mass index (BMI) or surface area. Each of the body size variables was made a covariate along with (TAZ – total endogenous zinc losses) in four separate regression models. In each case the body size variable was significant with p-values < 0.001, except for BMI which had a p-value of 0.013. The R^2 values for the models with body weight, height, BMI and body surface area variables were 0.46, 0.42, 0.37 and 0.47, respectively. A variety of other unmeasured/unmeasurable variables presumably contributed, ranging from inter-/intra-research facility variation to possible biological factors, for example the extent of up- or down-regulation of zinc transporters and other proteins involved in the absorption of zinc by the enterocyte, or variations in body zinc stores. The variable for sex was entered in each model. With the exception of BMI, none of the models demonstrated a significant sex effect, sex differences apparently being accounted for by the body size covariate. In the BMI model, sex was a significant predictor (p-value = 0.011). The equation relating the physiological requirement to body weight will be used for reasons of convenience and accuracy of measurement. The equation resulting from a least squares fit to the body weight data, and therefore relating TAZ to body weight and the difference of absorbed zinc minus total endogenous zinc losses, is:

TAZ [mg/day] = 0.642 + 0.038 x body weight [kg] + 0.716 x (TAZ – total endogenous zinc losses [mg/day]). [1]

Table 6: Details of parameter estimates for the model estimating the relationship of physiological zinc requirement with the difference between absorbed zinc and total endogenous zinc losses as well as body size corresponding to equation [1] above

Parameter	Estimate	95 % confidence limits	p-value
Intercept	0.642	-0.403, 1.687	0.23
Body weight	0.038	0.022, 0.054	<0.0001
TAZ-endogenous losses	0.716	0.512, 0.919	<0.0001

Examination of the residuals indicated that errors were normally distributed and exhibited constant variance, and that there was no deviation of the model from the data. One outlier with an externally studentised residual of 3.7 was observed. Re-examination of the source of the outlier indicated no basis for its removal; therefore, the outlier was retained. There was no evidence of collinearity of predictors.

As mentioned below, the residuals did not indicate any deviation from the linear model. Nonetheless, polynomial terms were added to the model to explore the possibility of nonlinear relationships. Only the second order polynomial of (TAZ – total endogenous losses) was significant, but this was due to the presence of the outlying point (see above). When this point was momentarily removed, no significant polynomial terms were observed. It was therefore concluded that there was no evidence of nonlinear relationships.

Because the physiological requirement is equivalent to TAZ when the difference between absorbed zinc and total endogenous zinc losses equals zero at a given body weight, the calculation of the physiological requirement derived from this model is:

Physiological zinc requirement [mg/day] = 0.642 + 0.038 x body weight [kg]. [2]

This equation is valid over a body weight range of roughly 40 to 100 kg. The size of the 95 % confidence interval (CI) for the estimation of the physiological requirements in Table 8 varies between ± 0.23 to ± 0.25.

Recent developments which have facilitated the estimation of physiological requirements include a simple model for estimating physiological requirements; the use of individual rather than mean data; recognition of the inaccuracies associated with the zinc absorption-intestinal balance technique in some studies which were omitted from final estimates; recognition of the extent of the impact of body weight on the estimation of intestinal excretion of endogenous zinc and, therefore, of physiological requirements; and recognition of the absence of a sex effect on endogenous faecal zinc losses beyond that accounted for by differences in body weight.

5.1.2. Estimation of dietary zinc intake to meet physiological requirements

Though experimental data are still limited (Hambidge et al., 2010), there are also theoretical reasons for supporting the conclusion that the relationship between TAZ and total dietary zinc (TDZ) is most appropriately fitted with saturation response modelling. Therefore, the intercept of the TAZ necessary to meet physiological requirements with the saturation response model (Morgan et al., 1975) for the population studied should give the AR for that population. Saturation response modelling is based on the assumption that zinc absorption is a carrier-mediated, saturable process, and this is used to characterise the relationship of the quantity of zinc absorbed to the quantity ingested. It is accomplished by fitting one of several appropriate models to data from isotope tracer studies of zinc absorption using nonlinear regression analysis.

5.1.2.1. Effect of dietary zinc and phytate on absorbed zinc

Though quantities vary greatly, diets containing plant foods, i.e. virtually all non-synthetic diets, contain phytate. The luminal contents of the duodenum and jejunum, especially phytate, can have a major impact on the percentage of zinc available for absorption (see Section 2.3.1). A trivariate model of TAZ as a function of dietary zinc and dietary phytate, based on saturation response modelling has been found to account for more than 80 % of the variance in TAZ (Miller et al., 2007).

5.1.2.2. Identification of studies, data extraction, assessment of methodological quality

The data used in this trivariate model of the relationship of zinc absorption to dietary zinc and phytate were 72 mean data (reflecting 650 individual measurements) reported in 18 publications. These are the data used in the development and early application of the model (Miller et al., 2007; Hambidge et al., 2010). The eligibility criteria were: studies of healthy adults, whole day isotope studies of true zinc absorption, reporting measurements of TDZ, and total dietary phytate (TDP) and TAZ. No extensive literature search was performed further to that performed by the EURRECA network on factors affecting zinc bioavailability (Lowe et al., 2013); relevant publications were identified through knowledge of the existing work of the small number of investigators in this field of research and ongoing monitoring of the new literature. All the data came from research groups having extensive experience with the application of isotope tracer methods to the study of zinc absorption. A formal assessment of the quality of the data was not performed. The data are summarised in Appendix K.

In all studies participants ate controlled diets having known quantities of zinc and phytate (in many cases dietary calcium, iron and protein were also measured) in free-living and metabolic study environments. After varying lengths of time on the study diets, zinc stable or radio isotope tracers were administered, and enrichment was measured in body tissues and/or excretions to determine absorption. TDZ, TDP and TAZ data from these studies were used to develop the saturation response zinc absorption model (Miller et al., 2007).

5.1.2.3 Modelling of the saturation response model

The assumptions that the regression errors were normally distributed and exhibited constant variance, and that the model was valid, were checked primarily by visual examination of plots of the raw and standardised residuals. Normality of the residuals was also tested with the Shapiro-Wilk test. Residuals were also examined for outliers.

The trivariate saturation response model is described by the following equation [3]:

$$TAZ = 0.5*\left(0.033*\left(1+\frac{TDP}{0.68}\right)+0.091+TDZ-\sqrt{\left(0.033*\left(1+\frac{TDP}{0.68}\right)+0.091+TDZ\right)^{2}-4*0.091*TDZ}\right)$$

where TAZ is total absorbed zinc, TDP is total dietary phytate and TDZ is total dietary zinc (all in mmol/day). Units are converted to mg/day for plots and values reported in this Opinion. The range of TDP and TDZ of the data are 0-3 730 mg/day and 4-21 mg/day, respectively. The R^2 of the fit was 0.81. TAZ predicted by this model for the range of dietary zinc intakes and selected dietary phytate levels is shown in Figure 1.

Table 7: Details of parameter estimates in the model on the relationship of zinc absorption to dietary zinc and phytate corresponding to equation [3] above

Parameter	**Estimate**	**95 % confidence limits**	**p-value**
A_{max} [(a)]	0.091	0.079, 0.108	< 0.0001
K_P [(b)]	0.678	0.290, 1.230	0.0029
K_T [(c)]	0.033	0.014, 0.062	0.0038

(a): A_{max}, maximum possible absorbed zinc
(b): K_P, zinc-phytate binding equilibrium dissociation constant, rounded to 0.68 in equation [3]
(c): K_T, zinc-transporter binding equilibrium dissociation constant

Examination of the residuals indicated that errors were normally distributed and exhibited constant variance, and that there was no deviation of the model from the data. No outliers were detected. Details of the regression diagnostics are provided in Appendix J.

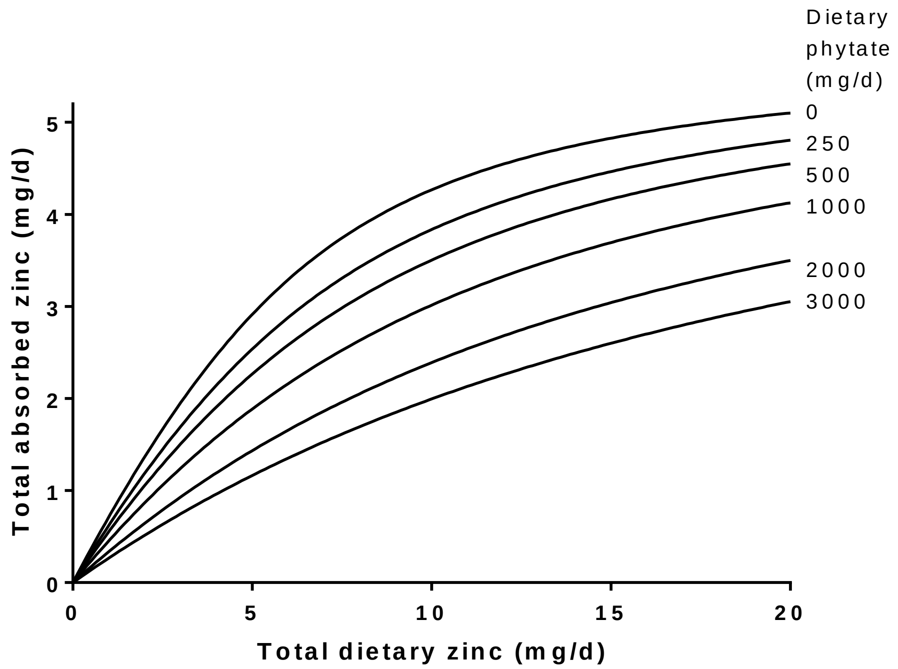

Figure 1: Saturation response model predictions of total absorbed zinc (TAZ) for selected levels of dietary phytate. Portions of the curves between total dietary zinc (TDZ) values of 0 and 4 mg/day are extrapolated as there were no zinc intake data within that range. A three-dimensional plot giving a complete range of TAZ as a function of TDZ and dietary phytate is given in Appendix L.

The dietary zinc intakes required to meet the AR associated with different body weights (as predicted by model [1]) can be derived from the intersection of the respective physiological zinc requirements (identified on the axes of the absorbed zinc) with the model curve [2] back-predicting the dietary zinc intake conditional to an expected level of phytate intake.

This is illustrated in Figure 2 derived from the established model of phytate effect (Miller et al., 2007; Hambidge et al., 2010). The curves show the relationships of absorbed zinc to dietary zinc for dietary phytate levels of 0 and 900 mg/day as predicted by the saturation response model. The horizontal dashed lines indicate the physiological requirements for males and females based on measured body weights of the subjects in the study (i.e. 59.1 kg for females and 72.7 kg for males, see Appendix I). Average dietary zinc requirements of these subjects are the corresponding dietary zinc intakes for the intersections of physiological zinc requirement values with the model curves. In Table 8, dietary zinc requirements depending on level of phytate intake are shown for the subjects who contributed data to establish the physiological requirement model.

Table 8: Average dietary zinc requirements depending on phytate intake and body weight

	Body weight (kg)	**Physiological requirement (mg Zn/day)**	**AR (mg Zn/day) for 300 mg/day of dietary phytate**	**AR (mg Zn/day) for 600 mg/day of dietary phytate**	**AR (mg Zn/day) for 900 mg/day of dietary phytate**	**AR (mg Zn/day) for 1 200 mg/day of dietary phytate**
Measured	72.7 [(a)]	3.4	8.2	10.2	12.1	14.0
	59.1 [(b)]	2.9	6.3	7.7	9.1	10.4

(a): Mean of the body weight data for men used to establish the physiological requirement (equations [1] and [2]) as described above (see Appendix I).
(b): Mean of the body weight data for women used to establish the physiological requirement (equations [1] and [2]) as described above (see Appendix I).

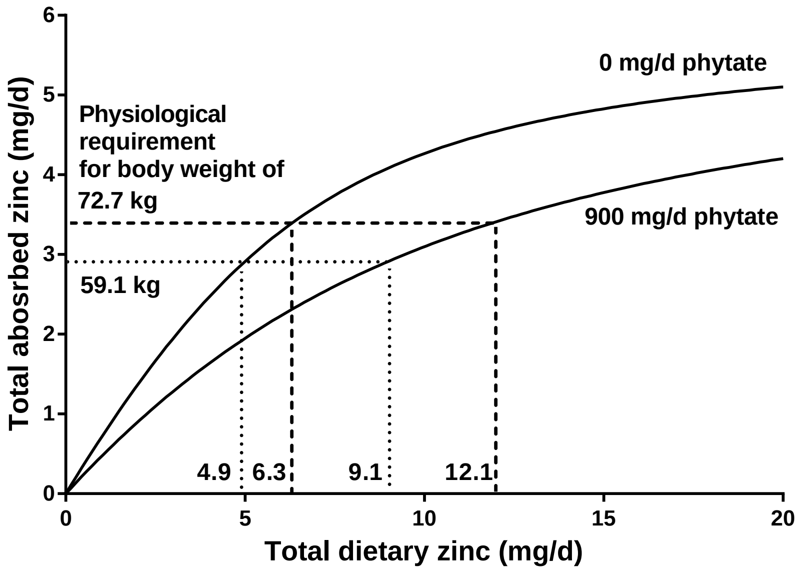

Figure 2: Relationships of absorbed zinc to dietary zinc for dietary phytate levels of 0 and 900 mg/day as predicted by the saturation response model.

No data are available for subjects older than 52 years. Though muscle mass decreases with ageing, the turnover of zinc in muscle is slow. Without the relevant experimental data, the Panel considers that the basis for setting DRVs for older adults should be the same as for younger adults.

5.2. Indicators of zinc requirements of children

No specific indicators of zinc requirements are available for older infants and children. Linear growth is affected by zinc deficiency but is far from being a specific indicator.

5.3. Indicators of zinc requirements in pregnancy and lactation

Though a variety of clinical features have been linked to zinc deficiency in pregnancy, these features are non-specific and have not been adequately substantiated.

The additional need for zinc during pregnancy can be calculated from the weight of tissues gained during gestation and the concentration of zinc in those tissues. Widdowson and Dickerson (1964) measured the concentration of zinc in 24 human fetuses and full-term infants ranging in weight from 0.75 to about 4 400 g. Using a mean measured zinc concentration of 18.4 µg/g fat-free tissue and measured fetal growth rates, Shaw (1979) calculated the rate of zinc accumulation by a human fetus growing along the 10th, 50th or 90th percentiles. The zinc accumulation rate for a fetus growing along the 50th percentile increased progressively from 0.21 mg/day at the 24th week of gestation to 0.67 mg/day at the 36th week. In addition to the fetus, placental, uterine and mammary tissue, amniotic fluid and maternal blood are also gained during gestation. Hytten (1980) calculated the total weight of the pregnancy tissues at term. Based on the total weight of those tissues and their zinc concentrations, the total zinc requirement for pregnancy has been calculated to be about 100 mg (Swanson and King, 1987). Approximately 60 % of the gain in zinc is associated with the fetus. The daily requirement for zinc in pregnancy above that of non-pregnant women can be calculated from the rate of tissue gain and the tissue zinc concentrations. The daily rates of zinc accumulation for the four quarters of pregnancy have been estimated at 0.08, 0.24, 0.53 and 0.73 mg (Swanson and King, 1987). Taking into account the cessation of zinc losses with menstruation (equivalent to about 0.01 mg/day in menstruating women), Swanson and King (1987) estimated an additional physiological requirement for zinc in the second half of pregnancy of about 0.6 mg/day. Averaging the daily rates of zinc accumulation for the four quarters of pregnancy (Swanson and King, 1987) results in an additional physiological requirement of about 0.4 mg/day for the whole pregnancy.

In lactation, additional zinc may be needed to replace zinc secreted in breast milk. Losses of zinc in breast milk have been estimated taking into account milk zinc concentrations and the amount of milk transferred, and are 2.52 mg/day for the first month, 1.37 mg/day for months 1 to 2 and 0.86 mg/day for months 3 to <6 (Brown et al., 2009) (see Section 2.3.6.3). Estimations for the additional zinc requirement in lactation also need to take into account redistribution of tissue zinc during postnatal readaptation to the non-pregnant state. *Post partum* involution of the uterus and decreased maternal blood volume have been estimated to release about 30 mg of zinc which has been accumulated during pregnancy (King and Turnlund, 1989). The Panel assumed that this endogenous zinc is available for reutilisation and decreases the additional amount of zinc required during the first month of lactation by 1 mg/day. This would reduce the additional physiological requirement to about 1.5 mg/day for the first month of lactation. It has also been postulated that bone resorption in early lactation contributes endogenous zinc for secretion in breast milk (Moser-Veillon, 1995; WHO, 1996), though the amount of zinc released from maternal bone during lactation has not been quantified (Donangelo and King, 2012).

In pregnancy and notably in early lactation, up-regulation of zinc absorption has been reported (Fung et al., 1997; Harvey et al., 2007; Donangelo and King, 2012). For example, in two longitudinal studies of zinc homeostasis during pregnancy and lactation, FAZ increased 1.3-fold ($p > 0.05$) from pre-conception to late pregnancy in 13 US women with a zinc intake of about 12 mg/day (Fung et al., 1997) and 1.5-fold ($p < 0.05$) from early (10-12 weeks) to late (34-36 weeks) pregnancy in 10 Brazilian women ingesting about 9 mg/day (Donangelo et al., 2005). FAZ increased 1.7-fold ($p = 0.023$) from pre-conception to lactation in the US women (Fung et al., 1997) and 1.4-fold ($p < 0.05$) from early pregnancy to lactation in the Brazilian women (Donangelo et al., 2005). There is some evidence to indicate that this up-regulation of zinc absorption may be sufficient to match increased requirements (Hambidge KM et al., unpublished).

5.4. Zinc intake and long-term health consequences

Mild to moderate dietary zinc depletion is a cause of several non-specific features including growth retardation, depressed immune function with susceptibility to infections, delayed wound healing, loss of appetite and loss of cognitive function. Severe restriction of dietary zinc is a cause of other clinical features including skin rashes. However, clinical features are non-specific and cannot be used for estimating DRVs. A systematic literature search and review for studies addressing zinc intake and health relationships was done by EURRECA; many studies were retrieved addressing the relationship of zinc intake with outcomes such as cognitive and immune function, depression, anorexia, diabetes mellitus, ischaemic heart disease and cancer in adults. The authors concluded that studies were heterogeneous in their methodological approaches and outcomes assessed (Lowe et al., 2013). The Panel concludes that the available evidence on zinc intakes and health outcomes cannot be used for setting DRVs for zinc.

6. Data on which to base Dietary Reference Values

The data required to derive ARs and PRIs in different population groups are the zinc intakes that are needed to replace endogenous losses, plus the quantities needed for growth and lactation, where appropriate. The factorial approach for deriving DRVs for zinc is used for all age groups.

6.1. Adults

As dietary zinc requirement depends on body weight and dietary phytate intake (Sections 5.1.1 and 5.1.2), the Panel considers it appropriate to estimate ARs and PRIs for the range of mean/median dietary phytate intakes observed in Europe. Thus, estimated ARs and PRIs are provided for phytate intake levels of 300, 600, 900 and 1 200 mg/day, which cover the range of mean/median phytate intakes observed in European populations (see Section 3.3 and Appendix G) and thus reflect the variety of European dietary patterns. Where population data on phytate intakes are available, ARs and PRIs could subsequently be adjusted using well-validated statistical models, an example of which has been used in this Opinion.

Table 9 contains estimates on ARs and PRIs for zinc based on reference body weights for a BMI of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013). PRIs for adults were estimated as the zinc requirement of individuals with a body weight at the 97.5th percentile for reference body weights for men and women, respectively, as body weight is a strong determinant of the requirement for zinc and as this approach is considered to have less uncertainty than the mathematical application of a CV of between 10 and 20 %.

Table 9: Estimations of Average Requirement (AR) and Population Reference Intake (PRI) for zinc according to phytate intake and body weight

Level of phytate intake (mg/day)	Body weight (kg)	AR	PRI [(a)]
300	58.5 [(b)]	6.2	7.5
	68.1 [(c)]	7.5	9.4
600	58.5 [(b)]	7.6	9.3
	68.1 [(c)]	9.3	11.7
900	58.5 [(b)]	8.9	11.0
	68.1 [(c)]	11.0	14.0
1 200	58.5 [(b)]	10.2	12.7
	68.1 [(c)]	12.7	16.3

(a): Dietary zinc intake of subjects with a body weight at the 97.5th percentile of the reference body weights (i.e. 79.4 kg for men and 68.1 kg for women)

(b): Median body weight of 18- to 79-year-old women based on measured body heights of 19 969 women in 13 EU Member States and assuming a BMI of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013). At this body weight, the physiological zinc requirement is 2.9 mg/day.

(c): Median body weight of 18- to 79-year-old men based on measured body heights of 16 500 men in 13 EU Member States and assuming a BMI of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013). At this body weight, the physiological zinc requirement is 3.2 mg/day.

6.2. Infants and Children

Estimation of DRVs for zinc uses a factorial approach taking into account endogenous zinc losses via urine, sweat and integument, faeces and, in adolescent boys and girls, also semen and menses, respectively, as well as zinc required for synthesis of new tissue for growth.

6.2.1. Methodology

6.2.1.1. Urinary and integumental zinc losses

After early infancy, urinary excretion rates for children on a body weight basis seem to differ very little from adult values (Krebs and Hambidge, 1986). Thus, for infants aged 7 to 11 months and children from 1 year of age, data for urinary losses were extrapolated from adult values (see Section 2.3.6.2) using isometric scaling, i.e. linear with body weight. For this, reference body weights based on the WHO Multicentre Growth Reference Study Group (2006) were used for infants and young children up to 2 years of age and based on van Buuren et al. (2012) for older children. For the age classes shown in Table 10, median body weights at midpoint ages were chosen, i.e. at age 9 months, 2, 5, 8.5, 12.5, and 16 years.

Integumental zinc losses were estimated from adult values (see Section 2.3.6.2) using allometric scaling, i.e. body weight to the power of 0.67 as a proxy for body surface area.

6.2.1.2. Endogenous faecal zinc losses

In infants aged two to four months the average intestinal excretion of endogenous zinc in exclusively breast-fed infants was approximately 50 µg/kg body weight per day (Krebs et al., 1996). For infants receiving complementary foods in addition to infant formula or human milk, endogenous faecal losses of 40 µg/kg body weight per day were assumed by WHO (1996); WHO/FAO (2004). This figure was thus used to calculate daily EFZ by multiplication with infants' reference body weight.

Linear regression analysis of EFZ versus body weight (kg) for the subjects contributing data to the adult estimates (see Section 5.1.1), for 43 young children aged 19 to 25 months from China (Sheng et al., 2006), and from a study in 45 infants aged 9 to 10 months in the US (Krebs N. et al., unpublished) gives the following equation [4]:

EFZ [mg Zn/day] = 0.0318 * body weight [kg] + 0.362, with $R^2 = 0.75$. [4]

This equation was used to estimate EFZ for children from 1 year of age.

6.2.1.3. Zinc losses in menses and semen

Zinc losses in menses and semen of 0.01 and 0.1 mg/day, respectively, have been assumed (see Section 5.1.1.4). The mean age of menarche in the EU has been reported at 12.7 years (van Buuren et al., 2012); thus, menstrual zinc losses have been taken into account for the 11 to 14-year-age group of girls, whereas zinc losses in semen have been assumed for boys from 15 years onwards.

6.2.1.4. Zinc requirement for growth

For the estimation of zinc requirement for tissue gain, a figure of 30 µg/g of new tissue has previously been used for infants and children, whereas for adolescents, a zinc content of 23 µg/g wet weight has been assumed due to an increase in fat tissue with a lower zinc content than that in younger children (WHO, 1996). Analyses of whole fetuses of various gestational ages have shown a constant zinc content of about 20 µg/g of fat-free tissue (Widdowson and Spray, 1951), and this value has also been used in factorial estimates (IOM, 2001). The Panel considers that in the absence of direct and precise data on body composition of infants and children at various postnatal ages, a figure of 20 µg zinc/g tissue gained appears to be a reasonable estimate. This value was multiplied with daily weight gains of the respective age groups. Daily weight gains of infants in the second half year of life were assumed to be 11.5 g/day, based on observed weight increments of infants in the Euro-Growth Study, where median weight gain of boys and girls was 13 g/day from month 6 to 9 and 10 g/day from month 9 to 12 (van't Hof et al., 2000). For children, daily weight gains were calculated by subtracting the median weight at the lower boundary of the age group according to van Buuren et al. (2012) from that at the higher boundary of the age group and dividing by the number of days in that age interval, assuming that one year equals 365 days.

6.2.1.5. Fractional absorption of zinc

The IOM (2001) used a figure of 0.30 for fractional absorption of zinc for older infants and children based on literature available at that time. The Panel considers that subsequent data (Manary et al., 2000; Griffin et al., 2004; Lopez de Romana et al., 2005; Mazariegos et al., 2006; Sheng et al., 2006; Griffin et al., 2007) provide no reason to modify this figure. As this figure is based on mixed diets likely to contain variable quantities of phytate, no adjustment for phytate intakes has been made.

6.2.2. Infants aged 7 to 11 months

Using a mean of 8.6 kg for weight-for-age of boys and girls aged 9 months at the 50^{th} percentile (WHO Multicentre Growth Reference Study Group, 2006), endogenous faecal zinc losses were estimated as 0.343 mg/day (see Section 6.2.1.2) and urine losses as 0.054 mg/day extrapolated from adult values (see Section 6.2.1.1). No data are available on integumental zinc losses in infants which have, therefore, been extrapolated from integumental losses in adults as described in Section 6.2.1.1, giving a figure of 0.105 mg/day. Estimated total endogenous zinc losses are 0.502 mg/day.

Based on an average weight gain of 11.5 g/day for infants in the second half year of life, the estimated zinc requirement for growth is 0.230 mg/day.

Therefore, the estimated physiological zinc requirement for infants aged 7 to 11 months is 0.732 mg/day.

Assuming a fractional absorption of zinc of 0.3 (see Section 6.2.1.4), the AR for infants aged 7 to 11 months is 2.4 mg/day. Due to the absence of reference body weights for infants at the 97.5th percentile, and with no knowledge about the variation in requirement, the PRI for infants was estimated based on a CV of 10 %, and is 2.9 mg/day.

6.2.3. Children

Components that were considered for the factorial approach for the various age groups are listed in Table 10. The number of digits used for the calculations has been retained in the table, with the exception of the AR for which an erroneous impression of accuracy would be given.

Table 10: Estimates used in the factorial approach to derive the AR for zinc for children

Age	Reference weight (kg)	Zinc losses (mg/day) [(a)] via					Estimated daily weight gain (g/day) [(b)]	Zinc need for growth (mg/day)	Physiological requirement (mg/day) [(c)]	AR (mg/day) [(d)]
		Faeces	Urine	Sweat	Semen	Menses				
1-3 years	11.9 [(e)]	0.738	0.075	0.130	-	-	6.57	0.131	1.074	3.6
4-6 years	19.0 [(f)]	0.965	0.120	0.178	-	-	6.35	0.127	1.390	4.6
7-10 years	28.7 [(g)]	1.275	0.181	0.236	-	-	8.82	0.176	1.869	6.2
11-14 years (M)	44.0 [(h)]	1.762	0.278	0.314	-	-	14.1	0.282	2.635	8.8
11-14 years (F)	45.1 [(i)]	1.797	0.285	0.319	-	0.01	12.6	0.252	2.663	8.9
15-17 years (M)	64.1 [(j)]	2.401	0.405	0.403	0.1	-	11.7	0.235	3.544	11.8
15-17 years (F)	56.4 [(k)]	2.157	0.357	0.370	-	0.01	3.78	0.076	2.969	9.9

M, males; F, females
(a): see Sections 6.2.1.1 and 6.2.1.2.
(b): see Section 6.2.1.3.
(c): Sum of losses and need for growth
(d): Estimated from the physiological requirement and assuming an absorption efficiency of 30 % from a mixed diet (see Section 6.2.4); values were rounded to the nearest 0.1
(e): Mean of body weight-for-age at 50^{th} percentile of boys and girls aged 24 months (WHO Multicentre Growth Reference Study Group, 2006)
(f): Mean of body weight at 50^{th} percentile of boys and girls aged 5 years (van Buuren et al., 2012)
(g): Mean of body weight at 50^{th} percentile of boys and girls aged 8.5 years (van Buuren et al., 2012)
(h): Mean of body weight at 50^{th} percentile of boys aged 12.5 years (van Buuren et al., 2012)
(i): Mean of body weight at 50^{th} percentile of girls aged 12.5 years (van Buuren et al., 2012)
(j): Body weight at 50^{th} percentile of boys aged 16 years (van Buuren et al., 2012)
(k): Body weight at 50^{th} percentile of girls aged 16 years (van Buuren et al., 2012)

Due to the absence of reference body weights for infants and children at the 97.5^{th} percentile, and with no knowledge about the variation in requirement, PRIs for infants and children were estimated based on a CV of 10 %.

Table 11: Summary of Average Requirements and Population Reference Values for zinc for infants and children

Age	AR (mg/day)	PRI (mg/day)
7-11 months	2.4	2.9
1-3 years	3.6	4.3
4-6 years	4.6	5.5
7-10 years	6.2	7.4
11-14 years	8.9	10.7
15-17 years (M)	11.8	14.2
15-17 years (F)	9.9	11.9

M, males; F, females

6.3. Pregnancy and lactation

Despite some evidence of up-regulation of zinc absorption during pregnancy and notably during early lactation (see Section 5.3) and evidence from one unpublished study that this may be sufficient to meet increased requirements, the Panel considers that data are insufficient to modify estimated additional physiological requirements. The most reliable indicators of zinc requirements at present are the addition of the estimated daily increment for pregnancy and the quantity of zinc secreted in milk over the first six months of lactation, adjusted for reabsorption of zinc due to redistribution of tissue zinc during postnatal readaptation to the non-pregnant state.

The additional requirements for pregnancy and lactation may be calculated by estimating the additional physiological requirement for synthesis of new tissue, primarily the conceptus, and for replacement of zinc secreted in breast milk (see Section 5.3).

For pregnancy, an additional physiological requirement of about 0.4 mg/day may be calculated for the whole pregnancy (see Section 5.3). This combined estimate likely overestimates the requirement in the first half and underestimates the requirement in the second half of pregnancy. It is unknown whether the trivariate model used to estimate dietary zinc requirements of non-pregnant non-lactating women is also suitable in pregnancy and lactation, and up-regulation of zinc absorption is likely to modify the inhibitory effect of phytate on zinc absorption. Thus, the Panel decided not to use the trivariate model to estimate the dietary zinc intake required to meet the additional physiological requirement. Instead, the Panel applied a mean FAZ of 0.30 observed in healthy adults (see Appendix I) to the physiological requirement of 0.4 mg/day and estimated the additional average dietary zinc requirement in pregnancy as 1.3 mg/day. In the absence of knowledge about the variation in requirement, the additional PRI for pregnancy was estimated based on a CV of 10 %, and was 1.6 mg/day.

For lactation, the Panel assumed that the mean increases in physiological requirement are 1.5 mg/day for the first month, 1.37 mg/day for months 1 to 2, and 0.86 mg/day for months 3 to 6 (see Section 5.3). Averaging this over six months of lactation results in an additional physiological requirement of 1.1 mg/day. Assuming that FAZ is increased 1.5-fold in lactation (see Section 5.3), and applying a FAZ of 0.45 to the additional physiological requirement of 1.1 mg/day, this results in an additional average dietary zinc requirement in lactation of 2.4 mg/day. In the absence of knowledge about the variation in requirement, the additional PRI for lactation was estimated based on a CV of 10 %, and was 2.9 mg/day.

CONCLUSIONS

The Panel concludes that ARs and PRIs for zinc can be derived for adults based on a two-stage factorial approach. The first stage comprised estimation of physiological requirements, defined as the minimum quantity of absorbed zinc necessary to match losses of endogenous zinc, and their relationship to body weight. In the second stage the quantity of dietary zinc available for absorption that is necessary to meet the physiological requirement was determined, taking into account the inhibitory effect of phytate on zinc absorption. ARs for adults were estimated as the zinc requirement at the 50th percentile of reference body weights for men and women in the EU and for phytate intakes of 300, 600, 900 and 1 200 mg/day, and PRIs for adults were estimated as the zinc requirement of individuals with a body weight at the 97.5th percentile for reference body weights for men and women, and for the same range of phytate intakes. For infants and children, ARs were estimated based on factorial calculation of losses and estimation of the need for growth. For pregnant and lactating women, the increase in physiological requirement was estimated based on the demand for new tissue primarily by the conceptus, and on the provision of zinc secreted in breast milk, respectively. ARs were derived taking into account fractional absorption of zinc. In the absence of knowledge about the variation in requirement, PRIs for infants and children and PRIs to cover the additional requirement of pregnant and lactating women were estimated based on a CV of 10 %.

Table 12: Summary of Population Reference Intakes for zinc

	Level of phytate intake (mg/day)	**Population Reference Intake (mg/day)**
Age		
7-11 months		2.9
1-3 years		4.3
4-6 years		5.5
7-10 years		7.4
11-14 years		9.4
15-17 years (M)		12.5
15-17 years (F)		10.4
≥ 18 years (M)	300	9.4
	600	11.7
	900	14.0
	1 200	16.3
≥ 18 years (F)	300	7.5
	600	9.3
	900	11.0
	1 200	12.7
Pregnancy		+ 1.6
Lactation		+ 2.9

M, males; F, females

RECOMMENDATIONS FOR RESEARCH

The Panel suggests that studies of zinc homeostasis in European populations be undertaken using state of the art techniques, and targeting the more vulnerable populations such as young children, adolescents, pregnant and lactating women, and the elderly.

The Panel recommends that additional reliable data on phytate intake in the EU be collected.

The Panel recommends that studies be undertaken to identify suitable biomarkers of zinc status. The Panel also recommends that methods to derive zinc requirements be further refined.

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APPENDICES

APPENDIX A – CONCENTRATIONS OF ZINC IN BREAST MILK FROM MOTHERS OF (PRESUMABLY) TERM INFANTS IN EUROPE

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L)			Analytical method	Comments
					mean ± SD	median	range		
Bates and Tsuchiya (1990)	57	UK	Not reported	2 months *post partum*	1.34 [(a)]			AAS	Infants assumed to be term infants on the basis of the study design and setting
				3 months *post partum*	2.06 [(a)]				
				4 months *post partum*	0.87 [(a)]				
				5 months *post partum*	0.73 [(a)]				
				6 months *post partum*	0.73 [(a)]				
Bjorklund et al. (2012)	60	Sweden	Not reported	14-21 days *post partum*	3.47 ± 0.98	3.52	1.24-5.71	ICP-MS	
Chierici et al. (1999)	11	Italy	Non-supplemented women with a dietary zinc intake of about 12 mg/day as estimated by 3-day dietary record	3 days *post partum*	8.16 ± 2.96			Inorganic mass spectrometry	
				30 days *post partum*	3.99 ± 1.01				
				90 days *post partum*	2.87 ± 1.23				
			Supplemented women receiving 20 mg/day of supplemental zinc	3 days *post partum*	5.89 ± 2.65				
				30 days *post partum*	3.36 ± 1.40				
				90 days *post partum*	2.63 ± 1.35				

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L) mean ± SD	median	range	Analytical method	Comments
Domellof et al. (2004)	86	Sweden	Not reported	9 months *post partum*	0.46 ± 0.26			AAS	Dietary intake of the mothers assessed with a 5-day food diary
Elmastas et al. (2005)	32(32)	Turkey	Not reported	2 months *post partum*	1.20 ± 0.01			FAAS with microwave digestion	
Kantola and Vartiainen (2001)	175(175)	Finland	Not reported	4 weeks *post partum*	3.00 ± 1.00			FAAS with microwave digestion	2 analyses (in 1987 and in 1993-1995)
	81(81)				1.40 ± 0.70				
Leotsinidis et al. (2005)	180(180)	Greece	Not reported	3 days *post partum*	4.91 ± 1.73	5.01	1.32-9.12	FAAS	
	95(95)			17 days *post partum*	2.99 ± 0.92	2.97	0.86-6.55		
Matos et al. (2009)	31(155)	Portugal	Not reported	7 days *post partum*	4.13 ± 1.22 [(a)]	4.04 [(a)]	2.12-6.98 [(a)]	ICP-MS	
				4 weeks *post partum*	2.22 ± 0.61 [(a)]	2.10 [(a)]	1.26-3.77 [(a)]		
				8 weeks *post partum*	1.53 ± 0.64 [(a)]	1.46 [(a)]	0.33-3.05 [(a)]		
				12 weeks *post partum*	1.11 ± 0.56 [(a)]	1.10 [(a)]	0.22-2.27 [(a)]		
				16 weeks *post partum*	1.04 ± 0.47 [(a)]	1.00 [(a)]	0.15-2.29 [(a)]		
Ortega et al. (1997)	25	Spain	Women with zinc intakes < 50% RI [(b)] from diet and supplements assessed during the 3rd trimester of pregnancy: 8.3 ± 1.0	13-14 days *post partum*	3.03 ± 0.47			Not reported	Maternal intake assessed with 5-day dietary record
				40 days *post partum*	1.86 ± 0.40				

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L) mean ± SD	median	range	Analytical method	Comments
			Women with zinc intakes > 50% RI [(b)] from diet and supplements assessed during the 3rd trimester of pregnancy: 12.3 ± 1.9	13-14 days *post partum*	3.31 ± 0.60				
				40 days *post partum*	2.15 ± 0.52				
Perrone et al. (1993)	(46)	Italy	Not reported	1 week *post partum*		36.4 ± 2.8 [(c)]		Not reported	
	(15)			2 weeks *post partum*		24.2 ± 1.6 [(c)]			
	(19)			3 weeks *post partum*		28.6 ± 6.8 [(c)]			
	(59)			> 3 weeks *post partum*		21.7 ± 1.4 [(c)]			
Piotrowska-Dept et al. (2006)	27	Poland	10.7 ± 3.3 (5.7-18.2)	0-30 days *post partum*	3.42 ± 1.62	3.29	0.53 – 7.28	AAS	Dietary zinc intakes of the mothers assessed by a 24-hour record
	18			31-90 days *post partum*	1.50 ± 0.87	1.37	0.12 – 3.58		
	8			> 90 days *post partum*	0.86 ± 0.57	0.64	0.28 – 1.51		
Rodriguez Rodriguez et al. (2000)	11(56)	Spain	Not reported	2 weeks to 5 months *post partum*	2.10 ± 1.10		0.14 – 3.99	AAS	
Salmenpera et al. (1994)	75	Finland	Non-supplemented women	4–5 days *post partum*		4.75	3.27–6.9		
	77			2 months *post partum*		1.41	1.1–2.19		

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L)			Analytical method	Comments
					mean ± SD	median	range		
	67			4 months *post partum*		0.9	0.58–1.38		
	56			6 months *post partum*		0.67	0.4–1.13		
	31			7.5 months *post partum*		0.61	0.39–0.97		
	14			9 months *post partum*		0.60	0.38–0.95		
	8			10 months *post partum*		0.61	0.42–0.87		
	6			11 months *post partum*		0.43	0.33–0.57		
	5			12 months *post partum*		0.43	0.33–0.56		
	62		Supplemented women (20 mg/day)	4–5 days *post partum*		4.94	3.50-6.98		
	58			2 months *post partum*		1.52	1.10-2.11		
	48			4 months *post partum*		0.95	0.65-1.39		
	38			6 months *post partum*		0.67	0.43-1.03		
	26			7.5 months *post partum*		0.63	0.43-0.92		
	16			9 months *post partum*		0.60	0.41-0.88		

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L) mean ± SD	median	range	Analytical method	Comments
	4			10 months *post partum*		0.41	0.36-0.48		
	5			11 months *post partum*		0.51	0.44-0.60		
	2			12 months *post partum*		0.46	0.26-0.79		
	22		Supplemented women (40 mg/day)	4–5 days *post partum*		5.18	3.33-8.04		
	24			2 months *post partum*		1.38	0.70-2.73		
	15			4 months *post partum*		1.08	0.61-1.94		
	13			6 months *post partum*		0.88	0.50-1.53		
	5			7.5 months *post partum*		0.90	0.62-1.30		
	4			9 months *post partum*		0.94	0.71-1.24		
Sievers et al. (1992)	10	Germany	Not reported	17 days *post partum*		3.6		AAS	
				35 days *post partum*		2.6			
				56 days *post partum*		1.7			
				85 days *post partum*		1.3			

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L) mean ± SD	median	range	Analytical method	Comments
				117 days *post partum*		1.2			
Silvestre et al. (2000a)	Not reported (10)	Spain	Not reported	Colostrum (number of days not reported)	8.60 ± 1.82			FAAS with microwave digestion	
				Transitional milk (number of days not reported)	3.45 ± 0.58				
				30 days *post partum*	1.97 ± 0.25				
				60 days *post partum*	1.24 ± 0.33				
				90 days *post partum*	0.89 ± 0.27				
Silvestre et al. (2000b)	62 (136)	Spain	Not reported	2 days *post partum*	7.73 ± 0.86			FAAS with microwave digestion	
				15 days *post partum*	3.15 ± 0.86				
Silvestre et al. (2001)	22 (110)	Spain	Not reported	Colostrum (number of days not reported)	7.99 ± 3.23			FAAS	
				Transitional milk (number of days not reported)	3.31 ± 1.06				
				30 days *post partum*	2.41 ± 0.90				
				60 days *post partum*	1.40 ± 0.65				
				90 days *post partum*	1.05 ± 0.71				

Reference	n (number of samples)	Country	Maternal dietary intake (mg/day) Mean ± SD	Stage of lactation	Zinc concentration (mg/L) mean ± SD	median	range	Analytical method	Comments
Stawarz et al. (2007)	5(210)	Poland	Not reported	12 weeks	17.94 ± 7.10 (c)		4.42-38.61	Volumetric method	
Ustundag et al. (2005)	20	Turkey	Not reported	Colostrum (0-7 days *post partum*)	3.08 ± 0.30			AAS	
				7-14 days *post partum*	2.72 ± 0.20				
				21 days *post partum*	2.65 ± 0.20				
				60 days *post partum*	2.81 ± 0.18				
Vuori et al. (1980)	15(15)	Finland	6-8 weeks *post partum*: 13.7 ± 2.7	6-8 weeks *post partum*	1.89 ± 0.74			FAAS	Two 7-day food records, infants assumed to be term infants on the basis of the study design and setting
	15(15)		17-22 weeks *post partum*: 12.8 ± 2.8	17-22 weeks *post partum*	0.72 ± 0.44				
Wasowicz et al. (2001)	43	Poland	Not reported	0-4 days *post partum*	8.2 ± 2.8			ICP-AES	
	46			5-9 days *post partum*	3.7 ± 1.8				
	41			10-30 days *post partum*	1.4 ± 0.7				
Yalcin et al. (2009)	47	Turkey	Not reported	2 weeks *post partum*	4.78 ± 1.83	4.5		AAS	

Studies were identified by a comprehensive literature search for publications from the year 2000 onwards, earlier publications were identified from Brown et al. (2009). The following articles based on one or two case reports are not presented in this table: Sievers and Schaub (2004), Kharfi et al. (2005), Chowanadisai et al. (2006), Coelho et al. (2006), Mandato et al. (2009); Milacic et al. (2012), Leverkus et al. (2006); Gass et al. (2010); Bieri et al. (2013); Miletta et al. (2013). FAAS, flame atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; AES, atomic emission spectroscopy

(a): After conversion from mg/g into mg/L using a conversion factor of 1.03 kg of breast milk per litre, as reported in Brown et al. (2009)

(b): RI, recommended intakes (value not reported), (c): mg/kg dry weight of breast milk

APPENDIX B – DIETARY SURVEYS IN THE COMPREHENSIVE DATABASE UPDATE DATASET INCLUDED IN THE NUTRIENT INTAKE CALCULATION AND NUMBER OF SUBJECTS IN THE DIFFERENT AGE CLASSES

Country	Dietary survey	Year	Method	Days	Age (years)	Number of subjects (b)						
						< 1 y	1 to < 3 y	3 to < 10 y	10 to < 18 y	18 to < 65 y	65 to < 75 y	≥ 75y
Finland/1	DIPP	2000-2010	Dietary record	3	<1-6	499	500	750				
Finland/2	NWSSP	2007-2008	48-hour dietary recall (a)	2x2 (a)	13- 15				306			
Finland/3	FINDIET2012	2012	48-hour dietary recall (a)	2 (a)	25-74					1295	413	
Germany/1	EsKiMo	2006	Dietary record	3	6-11			835	393			
Germany/2	VELS	2001-2002	Dietary record	6	<1- 4	158	347	299				
Ireland	NANS	2008-2010	Dietary record	4	18- 90					1274	149	77
Italy	INRAN-SCAI 2005-06	2005-2006	Dietary record	3	<1-98	16 (b)	36 (b)	193	247	2313	290	228
Latvia	FC_PREGNANTWOMEN 2011	2011	24-hour dietary recall	2	15-45				12 (b)	991 (c)		
Netherlands	VCPBasis_AVL	2007-2009	24-hour dietary recall	2	7-69			447	1142	2057	173	
UK	NDNS - Rolling Programme (1-3 years)	2008-2011	Dietary record	4	1-94		185	651	666	1266	166	139

y, years

(a): A 48-hour dietary recall comprises two consecutive days.

(b): 5^{th} or 95^{th} percentile intakes calculated over a number of subjects lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011a) and therefore for these dietary surveys/age classes the 5^{th}, 95^{th} percentile estimates will not be presented in the intake results.

(c): One subject was excluded from the dataset due to only one 24-hour dietary recall day being available, i.e. the final n = 990.

APPENDIX C – ZINC INTAKES AMONG MALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY (MG/DAY)

Survey	Age class (year)	Country	n	Mean	Intake P5	Intake P50	Intake P95
DIPP	< 1	Finland	247	3.7	0.3	3.5	9.0
VELS	< 1	Germany	84	3.0	0.8	3.0	5.5
INRAN_SCAI_2005_06	< 1	Italy	9	2.5	(a)	3.0	(a)
DIPP	1 to < 3	Finland	245	6.9	2.6	6.6	12.1
VELS	1 to < 3	Germany	173	4.9	2.7	4.8	7.3
INRAN_SCAI_2005_06	1 to < 3	Italy	20	6.6	(a)	6.8	(a)
NDNS-RollingProgra	1 to < 3	UK	107	5.8	3.3	5.6	8.9
DIPP	3 to < 10	Finland	381	9.1	5.6	8.8	13.2
EsKiMo	3 to < 10	Germany	426	9.1	5.8	8.9	13.5
VELS	3 to < 10	Germany	147	5.9	4.0	5.7	8.5
INRAN_SCAI_2005_06	3 to < 10	Italy	94	9.9	5.3	10.1	14.7
VCPBasis_AVL2007_2	3 to < 10	Netherlands	231	8.3	4.8	7.9	13.7
NDNS-RollingProgra	3 to < 10	UK	326	6.7	3.8	6.5	10.3
NWSSP07_08	10 to < 18	Finland	136	12.7	7.9	12.4	18.2
EsKiMo	10 to < 18	Germany	197	9.8	6.3	9.3	14.8
INRAN_SCAI_2005_06	10 to < 18	Italy	108	13.1	7.4	12.3	19.6
VCPBasis_AVL2007_2	10 to < 18	Netherlands	566	10.7	5.7	10.1	17.6
NDNS-RollingProgra	10 to < 18	UK	340	8.8	4.7	8.4	13.7
FINDIET2012	18 to < 65	Finland	585	12.8	6.7	12.4	20.8
NANS_2012	18 to < 65	Ireland	634	13.2	7.2	12.7	20.5
INRAN_SCAI_2005_06	18 to < 65	Italy	1068	12.2	6.7	11.8	19.0
VCPBasis_AVL2007_2	18 to < 65	Netherlands	1023	12.8	6.8	12.1	21.2
NDNS-RollingProgra	18 to < 65	UK	560	10.2	5.2	9.8	16.2
FINDIET2012	65 to < 75	Finland	210	11.0	5.9	10.7	16.6
NANS_2012	65 to < 75	Ireland	72	12.0	5.9	12.3	17.6
INRAN_SCAI_2005_06	65 to < 75	Italy	133	12.2	6.8	12.0	17.9
VCPBasis_AVL2007_2	65 to < 75	Netherlands	91	12.3	6.1	11.1	19.1
NDNS-RollingProgra	65 to < 75	UK	75	10.3	3.5	10.2	16.7
NANS_2012	≥ 75	Ireland	34	10.6	(a)	10.7	(a)
INRAN_SCAI_2005_06	≥ 75	Italy	69	11.4	7.5	11.0	16.0
NDNS-RollingProgra	≥ 75	UK	56	8.7	(a)	8.4	(a)

(a): 5^{th} or 95^{th} percentile intakes calculated over a number of subjects lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011a) and therefore for these dietary surveys/age classes the 5^{th} and 95^{th} percentile estimates will not be presented in the intake results.

APPENDIX D – ZINC INTAKES AMONG FEMALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY (MG/DAY)

Survey	Age class (year)	Country	n	Mean	Intake P5	Intake P50	Intake P95
DIPP	< 1	Finland	252	3.7	0.3	3.3	9.1
VELS	< 1	Germany	74	2.4	0.8	2.6	3.7
INRAN_SCAI_2005_06	< 1	Italy	7	3.1	(a)	2.6	(a)
DIPP	1 to < 3	Finland	255	6.9	2.3	6.5	12.3
VELS	1 to < 3	Germany	173	4.5	2.9	4.5	6.4
INRAN_SCAI_2005_06	1 to < 3	Italy	16	5.7	(a)	5.2	(a)
NDNS-RollingProgra	1 to < 3	UK	78	5.3	3.1	5.2	7.8
DIPP	3 to < 10	Finland	369	8.2	5.4	8.0	11.4
EsKiMo	3 to < 10	Germany	409	8.3	5.4	7.9	12.2
VELS	3 to < 10	Germany	149	5.5	3.5	5.2	7.6
INRAN_SCAI_2005_06	3 to < 10	Italy	99	9.5	4.9	9.0	14.2
VCPBasis_AVL2007_2	3 to < 10	Netherlands	216	8.0	4.1	7.6	13.4
NDNS-RollingProgra	3 to < 10	UK	325	6.3	3.6	6.1	9.6
NWSSP07_08	10 to < 18	Finland	170	10.0	5.9	9.8	15.4
EsKiMo	10 to < 18	Germany	196	9.1	5.6	8.9	12.9
INRAN_SCAI_2005_06	10 to < 18	Italy	139	10.6	5.8	10.3	16.2
FC_PREGNANTWOMEN_2	10 to < 18	Latvia	12	13.6	(a)	13.0	(a)
VCPBasis_AVL2007_2	10 to < 18	Netherlands	576	8.8	4.9	8.5	13.3
NDNS-RollingProgra	10 to < 18	UK	326	6.9	3.4	6.7	11.1
FINDIET2012	18 to < 65	Finland	710	9.8	5.4	9.4	15.3
NANS_2012	18 to < 65	Ireland	640	9.4	5.1	9.2	14.5
INRAN_SCAI_2005_06	18 to < 65	Italy	1245	10.1	5.6	9.7	15.5
FC_PREGNANTWOMEN_2	18 to < 65	Latvia	990	13.5	7.2	12.7	22.2
VCPBasis_AVL2007_2	18 to < 65	Netherlands	1034	9.9	5.3	9.3	16.4
NDNS-RollingProgra	18 to < 65	UK	706	8.1	4.2	8.0	12.8
FINDIET2012	65 to < 75	Finland	203	8.7	4.4	8.5	13.4
NANS_2012	65 to < 75	Ireland	77	10.1	5.5	9.7	15.1
INRAN_SCAI_2005_06	65 to < 75	Italy	157	9.7	4.5	9.7	15.2
VCPBasis_AVL2007_2	65 to < 75	Netherlands	82	8.9	4.6	8.9	14.2
NDNS-RollingProgra	65 to < 75	UK	91	8.2	5.2	8.1	12.0
NANS_2012	≥ 75	Ireland	43	9.7	(a)	9.5	(a)
INRAN_SCAI_2005_06	≥ 75	Italy	159	9.2	5.3	9.1	13.2
NDNS-RollingProgra	≥ 75	UK	83	8.3	5.2	8.0	12.5

(a): 5th or 95th percentile intakes calculated over a number of subjects lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011a) and therefore for these dietary surveys/age classes the 5th and 95th percentile estimates will not be presented in the intake results.
(b) Pregnant women only

Appendix E – Minimum and maximum % contribution of different FoodEx2 level1 food groups to zinc intakes among males

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives,flavours, baking and processing aids	<0.1 - 0.2	<0.1 - 0.6	0 - 0.7	<0.1 - 1.1	<0.1 - 0.2	0	0
Alcoholic beverages	<0.1	<0.1	<0.1	<0.1 - 0.1	0.6 - 1.5	0.7 - 2	0.2 - 2.1
Animal and vegetable fats and oils	<0.1 - 0.3	0.1 - 0.4	0.1 - 0.4	0.1 - 0.3	0.1 - 0.2	0.1 - 0.3	0.1 - 0.3
Coffee, cocoa, tea and infusions	<0.1 - 0.5	<0.1 - 1.1	0.3 - 2.5	0.6 - 2.2	0.7 - 4.2	0.7 - 6.6	0.6 - 6.6
Composite dishes	0.1 - 2.8	0.5 - 13	0.1 - 16.2	0.3 - 22.8	0.3 - 18.2	0.4 - 10	0.2 - 12.3
Eggs and egg products	0.1 - 0.8	0.4 - 3.5	0.5 - 4.2	0.5 - 3.3	0.6 - 2.4	0.9 - 2.4	1.1 - 2.3
Fish, seafood, amphibians, reptiles and invertebrates	0.2 - 0.9	0.5 - 6.8	0.3 - 6.8	0.6 - 6	0.9 - 6.3	1.8 - 7.1	2.4 - 5
Food products for young population	2.7 - 28.4	0.2 - 10.1	<0.1 - 0.6	<0.1	<0.1	-	-
Fruit and fruit products	0.6 - 4.4	1.6 - 2.9	1.1 - 2.1	0.7 - 1.1	0.8 - 1.4	1.1 - 2	1.4 - 1.9
Fruit and vegetable juices and nectars	0.1 - 1.8	0.4 - 3.3	0.4 - 3.2	0.3 - 2.1	0.1 - 1.4	0.1 - 1.4	0.1 - 0.7
Grains and grain-based products	2.3 - 29.6	16.3 - 29	18.1 - 33	18.8 - 33.1	17.1 - 26.7	16.6 - 29.5	20.3 - 32.3
Legumes, nuts, oilseeds and spices	0.2 - 1.2	1 - 2.5	0.9 - 3.1	0.8 - 2.4	1.1 - 3	0.8 - 2.5	0.6 - 1.8
Meat and meat products	1.8 - 13.4	11.7 - 25.5	15.6 - 34.7	23.2 - 40.2	26.5 - 39	24.4 - 38.6	24.5 - 33.6
Milk and dairy products	32.4 - 67.6	31.3 - 39.7	19.3 - 36.4	13.1 - 28.4	11.6 - 23.2	10.8 - 22.1	12.2 - 18.5
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0 - 0.2	0 - 1	<0.1 - 0.6	<0.1 - 0.7	<0.1 - 0.6	0 - 0.1
Seasoning, sauces and condiments	<0.1 - 0.2	0.1 - 0.7	0.1 - 0.6	0.1 - 0.6	0.2 - 0.7	0.2 - 0.5	0.2 - 0.6
Starchy roots or tubers and products thereof, sugar plants	0.6 - 3.7	1.8 - 3.6	1.9 - 4.7	1.8 - 5.6	2.1 - 4.7	2.9 - 4.8	3.2 - 5.3
Sugar, confectionery and water-based sweet desserts	<0.1 - 0.5	0.1 - 2.3	0.4 - 4.1	0.4 - 4	0.2 - 1	0.2 - 0.6	0.1 - 0.6
Vegetables and vegetable products	2.5 - 7.4	2 - 6.2	2.2 - 6.6	2.1 - 6.3	2.5 - 8.2	2.7 - 9	2.9 - 9.1
Water and water-based beverages	0.4 - 1.5	0.3 - 0.6	0.5 - 1.5	0.6 - 2	0.5 - 1.4	0.4 - 0.5	0.3 - 0.7

Appendix F – Minimum and maximum % contribution of different FoodEx2 level1 food groups to zinc intakes among females

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives,flavours, baking and processing aids	<0.1 - 0.1	0 - 0.4	0 - 0.7	<0.1 - 1.1	<0.1 - 0.2	0	0
Alcoholic beverages	<0.1	<0.1	<0.1	<0.1 - 0.1	<0.1 - 0.7	0.1 - 0.9	0.2 - 0.8
Animal and vegetable fats and oils	<0.1 - 0.4	0.1 - 0.4	0.1 - 0.4	0.1 - 0.3	0.1 - 0.2	0.1 - 0.3	0.1 - 0.3
Coffee, cocoa, tea and infusions	<0.1 - 0.4	<0.1 - 1	0.3 - 3	0.4 - 4.8	0.9 - 6.2	0.8 - 7.6	0.9 - 7.1
Composite dishes	<0.1 - 0.9	0.2 - 11.9	0.1 - 16.4	0.5 - 23.5	0.4 - 14.9	0.3 - 13.2	0.4 - 12.2
Eggs and egg products	<0.1 - 0.6	0.4 - 4	0.7 - 4.8	0.5 - 3.3	0.9 - 2.2	1.4 - 2.2	1.2 - 2.6
Fish, seafood, amphibians, reptiles and invertebrates	0.1 - 0.9	0.5 - 8.7	0.3 - 5.2	0.3 - 7.3	0.8 - 7	1.2 - 6.7	1.5 - 4.5
Food products for young population	5.2 - 24.5	0.3 - 8.5	0 - 0.2	<0.1 - 0.1	<0.1	-	<0.1
Fruit and fruit products	1.4 - 4.8	1.1 - 2.7	1.2 - 2.4	1 - 1.9	1.3 - 2.2	2.1 - 2.8	1.9 - 2.2
Fruit and vegetable juices and nectars	<0.1 - 1.9	0.3 - 3	0.4 - 3	0.4 - 2	0.2 - 1.3	0.2 - 1.2	0.3 - 1.1
Grains and grain-based products	9.8 - 28.4	16.3 - 29.7	19.1 - 33.5	19.1 - 32.9	17.8 - 37.6	18.2 - 30.8	20.4 - 31.2
Legumes, nuts, oilseeds and spices	0.3 - 2.5	0.9 - 2.5	1 - 2.5	1 - 2.4	1.3 - 3.3	1.5 - 3.5	1.1 - 2.2
Meat and meat products	9.6 - 13.1	12.8 - 22	16.1 - 35.2	20.7 - 36.6	23.6 - 34.3	22 - 32.4	20.2 - 31.7
Milk and dairy products	31.7 - 48.5	29.5 - 44.4	19.8 - 37.3	13 - 28.1	12.7 - 26.2	13.4 - 24	13.4 - 20.9
Products for non-standard diets, food imitates and food supplements or fortifying agents	0	0 - 0.4	0 - 1.3	<0.1 - 1	<0.1 - 1.4	0 - 1.6	0 - 0.9
Seasoning, sauces and condiments	<0.1 - 0.1	0.1 - 0.5	0.1 - 0.6	0.1 - 0.9	0.2 - 0.9	0.2 - 0.6	0.2 - 0.6
Starchy roots or tubers and products thereof, sugar plants	2.2 - 4.5	1.8 - 3.6	2.1 - 4.9	2.4 - 5.4	1.9 - 4.6	2.2 - 4.4	2.9 - 3.8
Sugar, confectionery and water-based sweet desserts	0.2 - 0.7	0.2 - 2.2	0.6 - 4.1	0.5 - 4.1	0.3 - 2.5	0.2 - 0.7	0.2 - 0.9
Vegetables and vegetable products	4.8 - 9.3	2 - 5.5	2.3 - 6.8	2 - 6.3	2.6 - 9.4	3.9 - 10.6	3.3 - 9.4
Water and water-based beverages	0.3 - 0.9	0.4 - 0.6	0.4 - 1.6	0.2 - 1.8	0.2 - 1.9	0.5 - 1.1	0.5 - 0.6

APPENDIX G – PHYTATE/PHYTIC ACID INTAKES IN VARIOUS EUROPEAN COUNTRIES

Study	Country	(n) Sex	Age range (years)	Phytic acid/phytate intake (mg/day) Mean ± SD or (range)	Phytic acid/phytate intake (mg/day) Median (IQR)	Phytate-zinc molar ratio Median (IQR)	Comments/methods of assessment
Adults							
Amirabdollahian and Ash (2010)	UK	(108) male	19-24	817	762 (565-940)	8.21 (6.82-10.30)	Phytate intakes were assessed based on food consumption data obtained in the National Diet and Nutrition Survey and the content of phytate in food according to published and unpublished data (the phytate content of food in the UK being unavailable). The authors acknowledged that those data may be inaccurate due to the use of non-peer-reviewed food composition data, due to the unavailability of data on the phytate content for many foods, due to the accuracy of the method to measure phytate.
		(219) male	25-34	1010	904 (659-1132)	9.11 (7.31-11.47)	
		(253) male	35-49	993	903 (670-1262)	8.80 (6.58-11.65)	
		(253) male	50-64	1094	948 (679-1314)	9.27 (7.24-12.23)	
		(371) male	65-74	891	733 (509-1112)	8.70 (6.26-11.50)	
		(200) male	75-84	938	692 (453-1145)	8.78 (6.32-12.58)	
		(62) male	> 85	1059	779 (496-1419)	8.97 (7.13-17.94)	
		(104) female	19-24	650	645 (438-790)	9.28 (7.00-12.11)	
		(210) female	25-34	756	714 (486-910)	10.50 (8.20-13.23)	
		(318) female	35-49	868	792 (568-1071)	10.27 (8.00-14.03)	
		(259) female	50-64	928	807 (599-1138)	10.51 (8.26-13.82)	
		(434) female	65-74	693	630 (426-849)	8.93 (6.43-11.26)	
		(638) female	75-84	674	549 (392-777)	8.50 (6.25-10.91)	
		(251) female	> 85	712	538 (416-772)	8.40 (6.90-11.62)	
Prynne et al. (2010)	UK	(562) male	36	662 (626, 698) [(a)]		5.7 (5.5, 6.0) [(a)]	Dietary survey following the same individuals over several years (follow-up dietary survey). Phytate intakes were assessed based on food consumption data obtained by 5-day dietary records and the phytate content of foods. The original (British) nutrient composition database was updated with phytate data for US foods for principal sources of phytate.
			43	666 (634, 698)		5.9 (5.6, 6.1)	
			53	715 (684, 747)		6.8 (6.5, 7.0)	
		(691) female	36	566 (536, 597)		6.3 (6.0, 6.6)	
			43	562 (537, 587)		6.3 (6.1, 6.5)	
			53	647 (622, 671)		7.5 (7.3, 7.8)	
Heath et al. (2005)	UK	(49) male	> 40	MBIAT: 1 436 ± 755	1328 (918-1876)	11.64 (8.24-15.07)	Phytate intakes were assessed by meal-based intake assessment tool (MBIAT)/weighed diet record (WDR) and food composition data based on published articles.
				WDR: 1 366 ± 559	1374 (855-1707)	11.03 (8.62-14.64)	
Brune et al. (1989)	Sweden	(6) male+female	24-70	369 (230-532) [(b)]			Individuals following a “typical unrestricted Swedish diet”
		(4/9) male/female	35-76	1146 (500-2927) [(b)]			

Study	Country	(n) Sex	Age range (years)	Phytic acid/phytate intake (mg/day) Mean ± SD or (range)	Median (IQR)	Phytate-zinc molar ratio Median (IQR)	Comments/methods of assessment
							Individuals following a vegetarian (omitting meat, fish, eggs) or vegan diet (omitting meat, fish, eggs and milk) Phytate intakes were assessed based on food consumption data obtained by 4-day dietary record and the phytate content of foods determined with the method described in Harland and Oberleas (1986)
Plaami and Kumpulainen (1996)	Finland	nr	nr	370			Phytic acid intake was assessed from intake of cereal products only (consumption data plus the content of phytate in cereals)
Carnovale et al. (1987)	Italy	nr	nr	ISTAT diet: 219 High-plant food diets: 796 (112-1 367) [(c)]		1.54 5.92 (0.90-11.83) [(c)]	Phytic acid content of 12 diets collected during seven days in a rural area of southern Italy; diets were characterised by a high content of plant foods. One diet representative of national meal pattern trends ("ISTAT") was also included. Phytic acid determined in whole diets according to a modification of the colorimetric method of Harland and Oberleas (1977)
Torelm and Bruce (1982)	Sweden	nr	nr	181			Calculated phytic acid intake assessed on the basis of selected foods and their content of phytic acid
Children							
Amirabdollahian and Ash (2010)	UK	(298) male	1.5-2.5	601	465 (353-733)	11.50 (8.08-17.37)	Phytate intakes were assessed based on food consumption data obtained in the National Diet and Nutrition Survey and the content of phytate in food according to published and unpublished data (the phytate content of food in the UK being unavailable).
		(300) male	2.5-3.5	636	515 (408-718)	12.41 (9.47-16.92)	
		(250) male	3.5-4.5	605	526 (406-725)	11.84 (9.03-15.83)	
		(184) male	4-6	640	576 (435-770)	10.90 (8.99-13.08)	
		(256) male	7-10	733	627 (519-831)	10.61 (9.08-13.50)	
		(237) male	11-14	792	714 (540-929)	10.39 (8.15-13.10)	
		(179) male	15-18	855	780 (616-1010)	9.34 (7.26-11.79)	
		(278) female	1.5-2.5	615	463 (332-695)	11.90 (8.10-17.18)	
		(306) female	2.5-3.5	577	483 (337-688)	11.90 (8.94-15.78)	
		(243) female	3.5-4.5	566	497 (379-680)	11.58 (9.10-16.27)	

Study	Country	(n) Sex	Age range (years)	Phytic acid/phytate intake (mg/day) Mean ± SD or (range)	Median (IQR)	Phytate-zinc molar ratio Median (IQR)	Comments/methods of assessment
		(172) female	4-6	564	494 (369-657)	10.54 (8.39-13.50)	
		(225) female	7-10	644	566 (461-740)	10.02 (8.42-12.90)	
		(238) female	11-14	657	594 (480-789)	10.60 (8.25-12.76)	
		(210) female	15-18	674	574 (459-829)	10.19 (7.90-13.91)	

nr, not reported

(a): mean and 95 % confidence intervals

(b): as reported in Schlemmer et al. (2009), values in the paper by Brune et al. (1989) are for "phytate-phosphorus"

(c): Mean (range), mean calculated from individual values given in the paper.

APPENDIX H – EVALUATING DATA WHEN EFZ WAS ESTIMATED USING THE ZINC ABSORPTION – INTESTINAL BALANCE METHOD

In the data used to estimate the physiological requirement the most critical measurements are those of endogenous faecal zinc (EFZ) and total absorbed zinc (TAZ). The techniques used to determine EFZ fall into two categories, those that measure EFZ directly with the use of isotope tracers administered intravenously and sampled in the faeces (Kirchgessner and Weigand isotope dilution, compartmental modelling), and techniques that rely on tracer measurements of zinc absorption along with measurements of elemental zinc intestinal balance to determine EFZ. The latter techniques have shortcomings and are less reliable than the direct measurement methods.

During compilation and inspection of the individual data it was observed that several of the studies using the absorption-intestinal balance technique had one or more negative EFZ values. As this is physiologically impossible, these anomalies were attributed to limitations of the intestinal balance technique and the data were removed prior to further analysis. The presence of the negative values prompted concern that the accuracy of the remaining data from these studies were also compromised. To address this concern the EFZ data were evaluated by comparison to those acquired with the more reliable, and most likely more accurate, direct measurement methods.

The EFZ and TAZ data from studies using the zinc absorption-intestinal balance method but containing no negative EFZ values (Turnlund et al., 1984; Taylor et al., 1991; Knudsen et al., 1996) are referred to as the “Balance A” data and the data from studies having negative EFZ values (Wada et al., 1985; Hunt J et al., 1992; Hunt et al., 1995; Hunt et al., 1998) are called the “Balance B” data in the following discussion. They are compared to the data from the “Direct” EFZ measurement method (Jackson et al., 1984; Sian et al., 1996; Lowe et al., 1997; Miller et al., 2000; King et al., 2001; Pinna et al., 2001; Sheng et al., 2009).

The Balance A EFZ and TAZ mean values were not different from the Direct means (two-sided t-test p-values of 0.60 and 0.95). While the Balance B TAZ means were not different from the Direct means ($p = 0.13$), the EFZ means were ($p = 0.019$). Furthermore, the distribution of the combined Direct and Balance A data (Figure 6) was found to be different from the distribution of the Balance B data as assessed with the nonparametric Anderson-Darling test ($p = 0.009$).

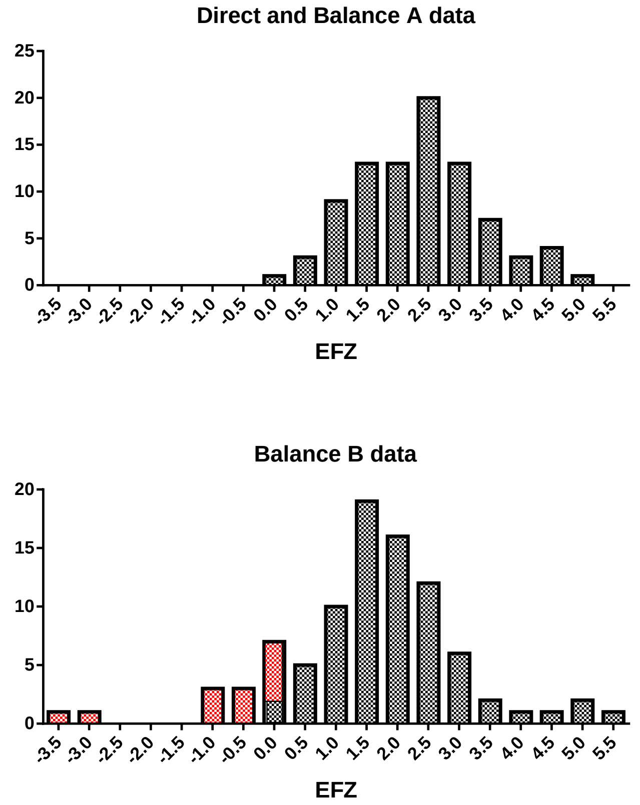


Figure 3: Frequency distributions of EFZ data. The red bars show the negative EFZ values which were removed prior to the analyses described here.

More importantly, the relationships of EFZ to TAZ and body weight were different for the Balance B data. Figure 5 shows that the Direct and Balance A data exhibited the expected positive relationship of EFZ and TAZ and had similar slopes and intercepts. In contrast, there was not a corresponding relationship in the Balance B data.

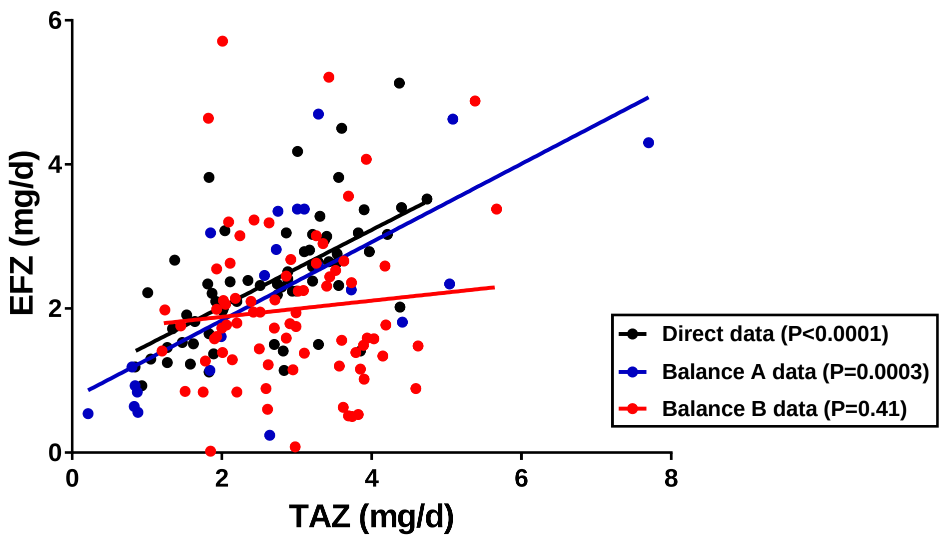


Figure 4: Data and regression lines showing relationships of EFZ and TAZ.

Figure 6 confirms the positive relationship of EFZ to body weight in the Direct data. The Balance A data suggested a positive relationship, though it was not significant. Again, the Balance B data showed no evidence of a relationship.

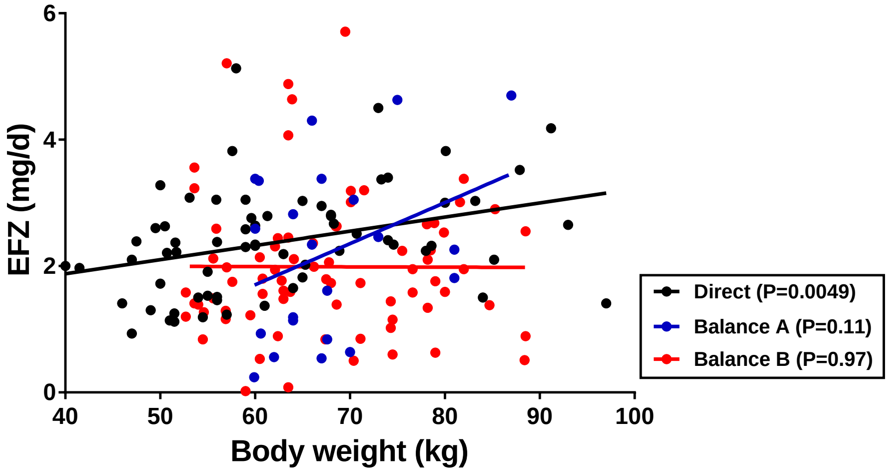


Figure 5: Data and regression lines showing relationships of EFZ and body weight.

And finally, as would be expected from the preceding information, the fitting to the Balance B data of the model used to estimate the physiological zinc requirement as a function of body weight (Section 5.1.1) produces significantly different results. An analysis comparing the model's fit to both datasets demonstrated that the weight and the (TAZ – total endogenous Zn losses) slope parameters were significantly different, with p-values of 0.044 and 0.011, respectively.

Based on the findings that the EFZ data from the Balance B studies differed in important ways from the direct measurement data the Panel decided to not include the Balance B studies in the estimation of the physiological zinc requirements.

Data from Sandstrom et al. (2000)

The EFZ data from the study of Sandstrom et al. (2000) were generally found to be implausibly high, with most values exceeding the range of values observed in the accepted studies. As with the studies described above, this is most likely attributable to use of the zinc absorption-intestinal balance method.

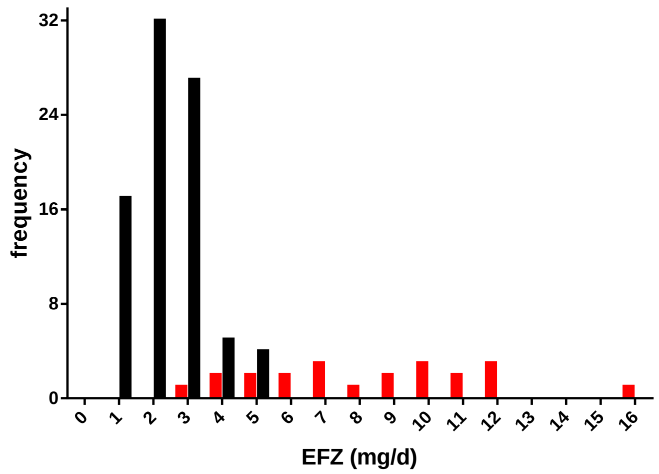


Figure 6: Frequency distribution of EFZ data from the study of Sandstrom et al. (2000) (in red) compared to the data from the included studies (in black).

APPENDIX I – DATA EXTRACTED FROM THE SELECTED STUDIES FOR ESTIMATING PHYSIOLOGICAL ZINC REQUIREMENT OF ADULTS

Study	(n) Sex	Age range (years)	Body weight Mean (range) SD (kg)	Body height Mean (range) SD (m)	BMI Mean (range) SD (kg/m^2)	BSA[a] Mean (range) SD (m^2)	EFZ [b] method	EFZ Mean (range) SD (mg/day)	Urine Zn Mean (range) SD (mg/day)	TDZ [c] Mean (range) SD (mg/day)	FAZ [d] Mean (range) SD	TAZ [e] Mean (range) SD (mg/day)	TDP [f] Mean (range) (mg/day)
Taylor et al. (1991)	(2 x 4) male	29 - 40	66 (60-70) 3.9	1.78 (1.7-1.9) 0.08	21 (19-22) 1.1	1.81 (1.7-1.9) 0.09	Balance [g]	1.6 (0.6-3.4) 1.0	0.56 (0.2-1.4) 0.40	3.2 (0.8-5.6) 2.6	0.69 (0.3-1) 0.34	1.5 (0.8-2.8) 0.74	NA
Turnlund et al. (1984)	(4) male	25 - 32	68 (60-81) 9.0	1.74 (1.7-1.8) 0.06	22 (21-24) 1.6	1.82 (1.7-2.0) 0.15	Balance	2.8 (1.8-4.3) 1.1	0.53 (0.3-1.0) 0.29	15	0.34 (0.2-0.5) 0.12	5.1 (3.3-7.7) 1.9	0
Knudsen et al. (1996)	(3) female / (5) male	23 - 27	72 (60-87) 9.1	1.81 (1.7-1.9) 0.09	22 (19-26) 2.1	1.90 (1.7-2.1) 0.15	Balance	3.0 (0.5-4.7) 1.4	0.43 [h] (0.3-0.5) 0.10	10.2 (9.4-11) 0.88	0.29 (0.02-0.5) 0.12	3.0 (0.2-5.1) 1.4	660 (NA)
Jackson et al. (1984)	(1) male	29	80	NA	NA	NA	K&W [i]	3.0	0.63	7.1	0.48	3.4	NA
Sian et al. (1996)	(20) female	17 - 27	53 (42-65) 6.2	1.58 (1.5-1.7) 0.06	21 (18-24) 1.6	1.53 (1.3-1.8) 0.11	K&W	1.8 (0.9-3.3) 0.70	0.3 [h]	6.6 (4.0-8.9) 1.6	0.32 (0.2-0.5) 0.10	2.2 (0.8-3.5) 0.92	673 (NA)
Lowe et al. (1997)	(6) female	21 - 52	57 (40-64) 8.7	1.63 (1.5-1.8) 0.08	21 (17-24) 2.4	1.61 (1.3-1.8) 0.16	comp model [j]	1.9 (1.2-2.6) 0.50	0.21 (0.03-0.4) 0.12	7.1 (5.7-8.8) 1.1	0.31 (0.1-0.6) 0.15	2.1 (1.3-3.2) 0.73	585 (NA)
Sheng et al. (2009)	(21) female	21 - 49	64 (51-97) 13	1.63 (1.4-1.8) 0.09	24 (18-35) 4.2	1.71 (1.4-2.2) 0.19	K&W	2.7 (1.4-5.1) 0.80	0.39 (0.08-0.7) 0.18	11.7 (5.6-29) 7.2	0.30 (0.1-0.5) 0.10	3.0 (1.0-4.7) 1.1	835 (250-2080)
Miller et al. (2000)	(4) female / (1) male	24 - 48	67 (47-84) 14	1.70 (1.6-1.8) 0.10	23 (19-27) 3.3	1.79 (1.4-2.0) 0.24	comp model	2.8 (1.5-4.5) 1.2	0.31 (0.06-0.5) 0.17	11.5 (8-20) 5.3	0.29 (0.2-0.4) 0.05	3.1 (2.2-4.4) 0.86	NA
King et al. (2001)	(5) male	21 - 35	74 (67-93) 11	1.77 (1.7-1.8) 0.04	23 (21-28) 3.1	1.91 (1.8-2.2) 0.15	comp model	2.7 (2.4-3.0) 0.20	0.46 (0.3-0.8) 0.22	12.2	0.26 (0.2-0.3) 0.02	3.2 (2.9-3.4) 0.22	NA
Pinna et al. (2001)	(7) male	27 - 47	78 (71-91) 8	1.78 (1.7-1.9) 0.08	25 (21-32) 3.7	1.98 (1.8-2.1) 0.10	comp model	2.8 (2.1-4.2) 0.81	0.42 (0.07-0.7) 0.20	13.7	0.20 (0.1-0.3) 0.06	2.7 (1.4-3.6) 0.82	NA
Mean (range) of males	(31) male	30.9 (21-47)	72.7 (60-93)	1.79 (1.7-1.9)	23 (19-32)	1.90 (1.7-2.2)		2.4 (0.6-4.7)	0.54 (0.07-1.4)	10.4 (0.8-20)	0.38 [k] (0.02-1)	2.8 (0.2-7.7)	NA
females	(54) female	27.5 (17-52)	59.1 (40-97)	1.62 (1.4-1.8)	22 (17-35)	1.64 (1.3-2.2)		2.3 (0.9-4.5)	0.32 (0.03-0.71)	9.0 (4.0-29)	0.31 (0.1-0.6)	2.6 (0.8-4.7)	NA

(a): BSA, body surface area (calculated with Gehan-George equation (Gehan and George, 1970))
(b): EFZ, endogenous faecal zinc
(c): TDZ, total dietary zinc
(d): FAZ, fractional absorption of zinc
(e): TAZ, total absorbed zinc
(f): TDP, total dietary phytate
(g): Balance: combination of intestinal balance and 'true' absorption measured by zinc stable isotopic labelling of diet.
(h): Some or all of the data are estimated (see text).
(i): K&W, measurements using the isotope dilution method of Kirchgessner and Weigand (Kirchgessner et al., 1980; Weigand and Kirchgessner, 1982, 1992)
(j): comp model, compartmental modelling
(k): For the calculation of an overall mean FAZ for men and women, the FAZ of the zinc-depleted subjects in the study of Taylor et al. (1991) were omitted. The overall mean FAZ is 0.30.
NA, not available
Where no range or standard deviation are shown all data had the same value.

APPENDIX J – DATA REGRESSION ANALYSIS DIAGNOSTIC RESULTS

The physiological requirement model

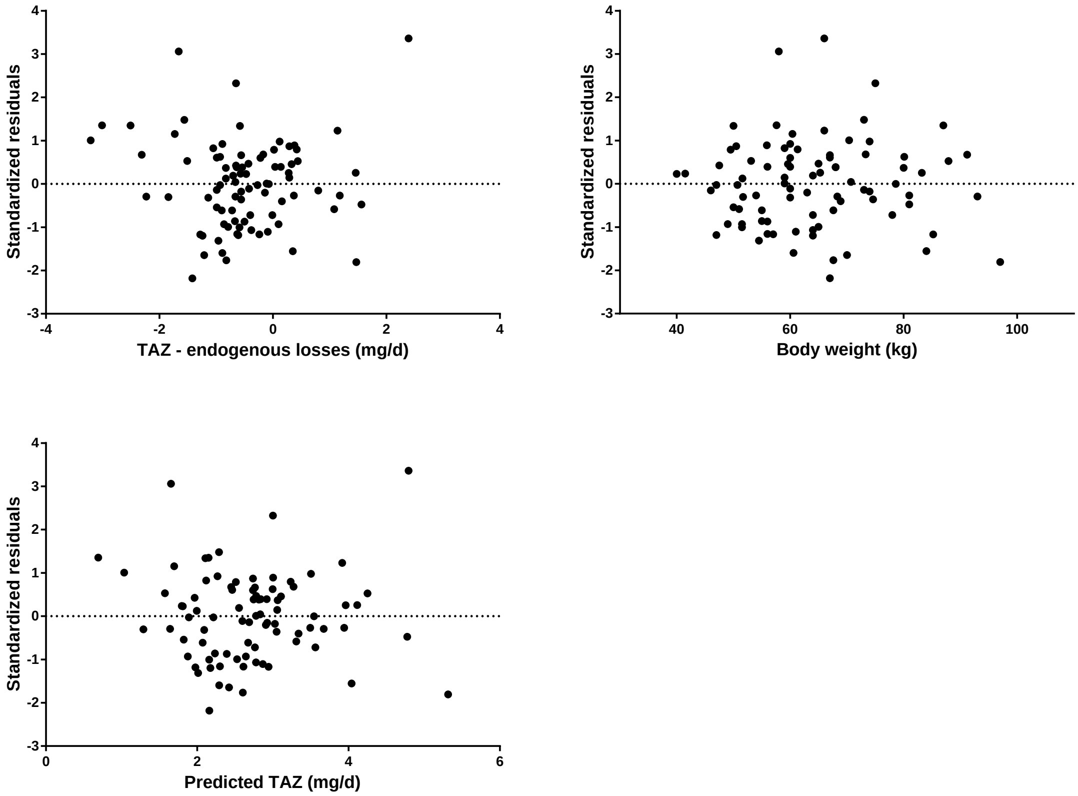


Figure 7: Residuals plotted against predictor variables and predicted values of the response variable.

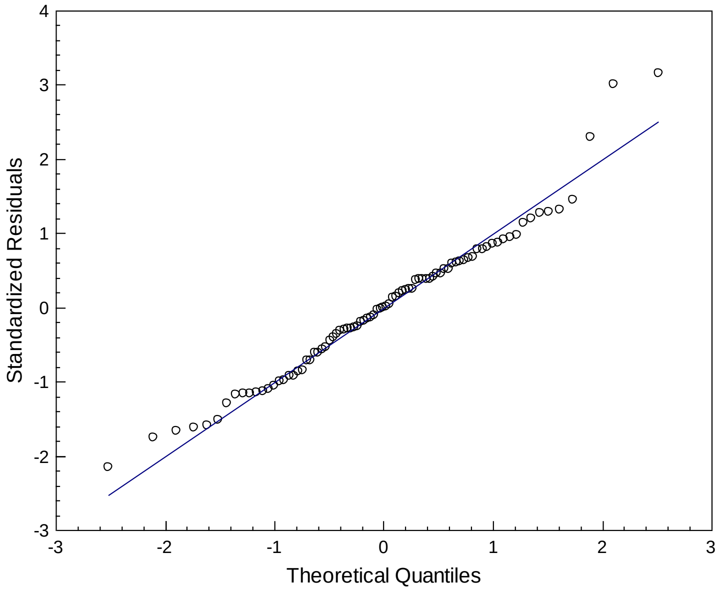


Figure 8: QQ plot of residuals

The plots show no problems with the regression assumptions, though there are two points with large standardised and studentised residuals. The externally studentised residuals for these data are 3.2 and 3.6. The point with the largest residual is also moderately influential, having a Cook's D value of 0.51. Nonetheless, all data were retained in the model.

The normality of the residuals was tested with the D'Agostino-Pearson and Shapiro-Wilk tests. P-values were 0.020 and 0.051, respectively, but the low p-values were due to one or two outlying points. When the most extreme outlier was removed, the resulting p-values are 0.33 and 0.45, respectively, indicating that the remaining data have a normal distribution.

The homoscedasticity of the residuals was tested with the Breusch-Pagan and Goldfeld-Quandt tests giving p-values of 0.74 and 0.99, respectively. Thus, the residuals exhibit constant variance.

The variance inflation factors were 1.00 indicating no problem with collinearity of variables.

In addition, there is no evidence that the model is inappropriate.

<u>The saturation response model</u>

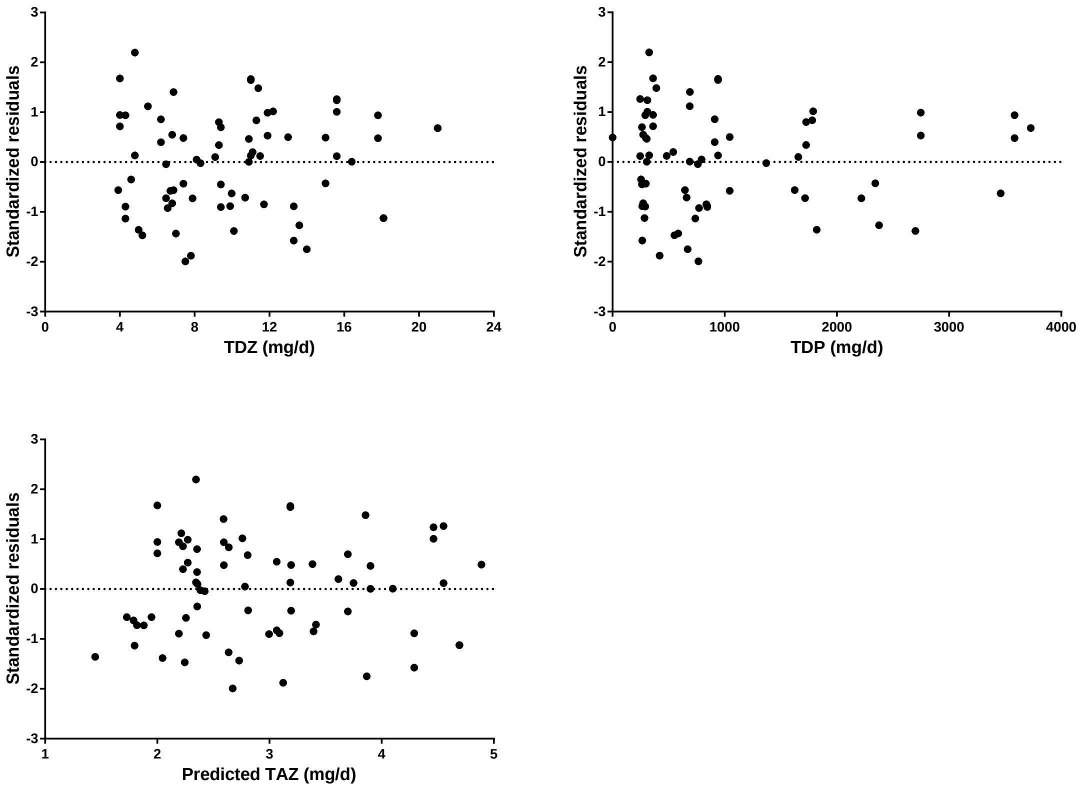


Figure 9: Residuals plotted against predictor variables and predicted values of the response variable.

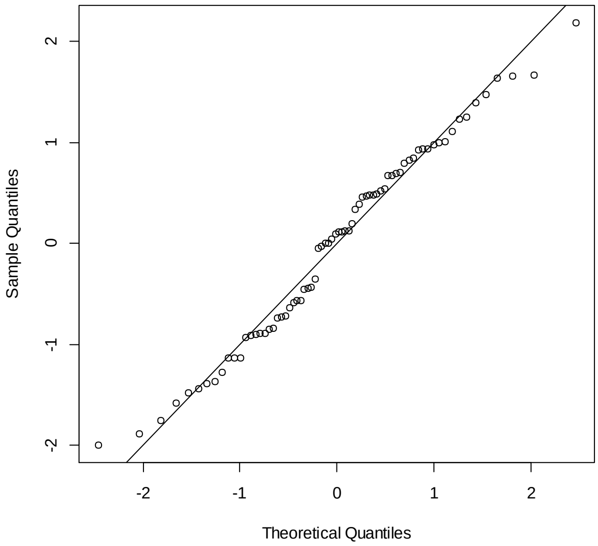


Figure 10: QQ plot of residuals

The plots show no problems with the regression assumptions, though there is a hint of decreasing variance with increasing value of TDP.

The normality of the residuals was tested with the D'Agostino-Pearson and Shapiro-Wilk tests giving p-values of 0.098 and 0.28, respectively, indicating normal distributions.

As there are no readily available tests for homoscedasticity of residuals in nonlinear regression, the variance of the residuals was examined by doing linear regression of the absolute values of the residuals against the predictor and response variables. P-values from these analyses were ≤ 0.50 indicating no problems with nonconstant variance. The appearance of larger variance at low TDP values is probably due to the larger number of data at low TDP.

Again, there is no evidence that the model is inappropriate.

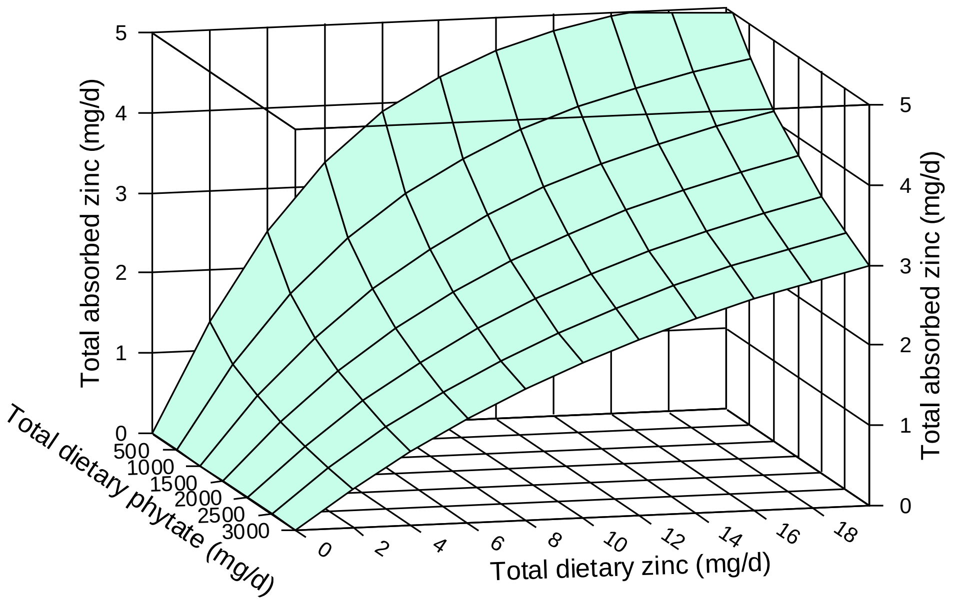
APPENDIX K – DATA EXTRACTED FROM THE SELECTED STUDIES FOR THE TRIVARIATE SATURATION RESPONSE MODEL

Study	TDZ Mean (mg/day)	TDP Mean (mg/day)	TAZ Mean (mg/day)	n	Sex
Hambidge et al. (2004)	8.3	1370	2.37	6	6M,4F
	10	3460	1.51	4	"
	10.1	2700	1.44	4	"
Knudsen et al. (1996)	10.2	660	3.0	8	5M,3F
Hunt J et al. (1992)	14	670	3.1	14	14M
	7.8	420	2.3	14	14F
Hunt et al. (1995)	13	1045	3.6	14	14F
	6.7	1045	2	14	14F
Hunt et al. (1998)	9.1	1656	2.4	21	21F
	11.1	542	3.7	21	21F
Wada et al. (1985)	16.4	688	4.1	6	6M
	5.5	688	2.7	6	6M
Lowe et al. (1997)	7.1	585	2.1	6	6F
Adams et al. (2002)	4.3	738	1.3	5	2M,3F
	5	1820	0.85	5	2M,3F
Sian et al. (1996)	5.2	552	1.6	10	10F
	8.1	794	2.8	10	10F
Pinna (1999)	4.6	254	2.2	7	7M
Turnlund et al. (1984)	15	0	5.1	4	4M
	15	2343	2.62	4	4M
Kristensen et al. (2006)	9.4	845	2.6	16	16F
	9.9	845	2.7	16	16F
	7.5	766	1.8	16	16F
Kim et al. (2007)	6.87	1623	1.7	7	7F
	6.87	690	3.2	7	7F
	6.47	1713	1.5	10	10F
	6.47	760	2.4	10	10F
Rosado et al. (2009)	3.91	645	1.48	12	12F
	6.56	771	2.03	12	12F
	7.89	2218	1.56	14	14F
	13.6	2376	2.08	14	14F
Sheng et al. (2009)	11.7	835	3.0	21	21F
Hunt et al. (2008)	4.8	326	2.4	8	19M,20F
	7.4	297	3	8	"
	10.9	305	3.9	8	"
	15.6	311	4.9	7	"
	18.1	285	4.2	8	"
	6.2	911	2.4	9	23M,21F
	9.3	1726	2.5	9	"
	11.9	2748	2.5	8	"
	17.8	3584	2.8	9	"
	21	3728	3.1	9	"
	4.3	292	1.8	8	8F
	6.8	273	2.7	6	6F
	9.4	263	3.5	4	4F

Study	TDZ Mean (mg/day)	TDP Mean (mg/day)	TAZ Mean (mg/day)	n	Sex
	13.3	265	3.9	4	4F
	15.6	246	4.6	4	4F
	4.8	326	3.3	8	19M,20F
	7.4	297	3.4	8	"
	10.9	305	4.1	8	"
	15.6	311	5	7	"
	18.1	285	4.2	8	"
	6.2	911	2.6	9	23M,21F
	9.3	1726	2.7	9	"
	11.9	2748	2.7	8	"
	17.8	3584	3	9	"
	21	3728	3.1	9	"
	4.3	292	2.6	8	8F
	6.8	273	3.3	6	6F
	9.4	263	4	4	4F
	13.3	265	3.6	4	4F
	15.6	246	5.1	4	4F
Chung et al. (2008)	11	941	3.91	9	9M
	4	361	2.41	9	9M
	11	941	3.9	9	9M
	4	361	2.73	9	9M
	11	941	3.24	9	9M
	4	361	2.31	9	9M
Hunt and Beiseigel (2009)	11.5	483	3.8	10	10F
	11.3	1781	3.0	10	10F
	11.4	391	4.5	10	10F
	12.2	1789	3.2	10	10F

M, males, F, females

1886 **APPENDIX L – THREE-DIMENSIONAL REPRESENTATION OF FIGURE 1**



1887

ABBREVIATIONS

Afssa	Agence française de sécurité sanitaire des aliments
AI	Adequate Intake
AR	Average Requirement
BMI	Body mass index
COMA	Committee on Medical Aspects of Food Policy
CV	Coefficient of variation
D	Day
D-A-CH	Deutschland- Austria- Confoederatio Helvetica
DH	UK Department of Health
DRI	Dietary Reference Intake
DRV	Dietary Reference Value
EC	European Commission
EAR	Estimated Average Requirement
EFSA	European Food Safety Authority
EFZ	Endogenous faecal zinc
EU	European Union
EURRECA	EURopean micronutrient RECommendations Aligned
F	female
FAO	Food and Agriculture Organization
FAZ	Fractional absorption of zinc
IAEA	International Atomic Energy Agency
IOM	US Institute of Medicine of the National Academy of Sciences
IZiNCG	International Zinc Nutrition Consultative Group
LTI	Lower/Lowest Threshold Intake
M	male
MRE	Metal-response element
MTF	MRE-binding transcription factor

NNR	Nordic Nutrition Recommendations
PRI	Population Reference Intake
RDA	Recommended Dietary Allowance
REE	Resting energy expenditure
RNA	Ribonucleic acid
SCF	Scientific Committee on Food
TAZ	Total absorbed zinc
TDP	Total dietary phytate
TDZ	Total dietary zinc
UNICEF	United Nations Children's Fund
WHO	World Health Organization