

Background Information  
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**DOCUMENT:**

**The DRI Development Process:  
Issues Related to the Adjustment for Uncertainty**

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# **Background Paper**

## **The DRI Development Process: Issues Related to the Adjustment for Uncertainty**

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### **Introduction**

In 1994, the Institute of Medicine included the establishment of Tolerable Upper Levels (ULs) as a component of the overall effort to the development of Dietary Reference Intakes (or DRIs) (IOM, 1994). The identified principles for developing such values rest upon the acknowledged decision-making steps of classic risk assessment (IOM, 1998). As such, the UL development process put in place by the IOM acknowledged the need to “adjust” or “correct” for uncertainty. Uncertainty of different kinds is an invariable companion of risk assessment. The identification of the various reasons for uncertainty and the method of dealing with it are prerequisites for increasing the credibility of the assessment result, for helping the users of the DRIs in decisions for managing risks associated with nutrient intake, and for stimulating research aimed at closing gaps of knowledge or in developing models for quantitative assessment (Edler et al., 2002).

This paper is concerned primarily with uncertainty as a component of establishing upper levels of intake. However, while it has not been formally addressed, interest exists in the uncertainty that surrounds the components of the DRIs associated with adequate intakes, and there is some evidence that efforts to adjust for uncertainties have taken place. For example, for several age/gender groups the Adequate Intake for vitamin D is based on available data multiplied by a factor of 2 to, in a sense, account for uncertainties in the data and in turn allow the needs of the age/gender group to be covered regardless of exposure to sunlight (IOM, 1997, pp 268-275).

Despite the desirability of taking uncertainty into account in developing DRI quantitative values, a clear organizing set of principles for making such adjustments is lacking. It is likely that such adjustments may need to be carried out on a case-by-case basis as a general matter, but it is still worthwhile to ask the question of whether general guiding principles can be identified and whether there are ways to avoid making the use of uncertainty adjustments seem arbitrary and capricious.

For the purposes of this paper, the uncertainty referred to is that associated with limitations of the database or knowledge. There are the same sources of uncertainty in extrapolations, for example in the extrapolation of findings from test animals to humans or from average

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<sup>1</sup> World Health Organization (2006): *A model for establishing upper levels of intake for nutrients and related substances; Report of a joint FAO/WHO technical workshop on nutrient risk assessment*. World Health Organization, Geneva, Switzerland. [www.who.int/ipcs/methods/en](http://www.who.int/ipcs/methods/en).

humans to sensitive subgroups. While the focus of this paper is the uncertainty associated with the existing database, occasional references to the adjustment by extrapolation are included, primarily for background.

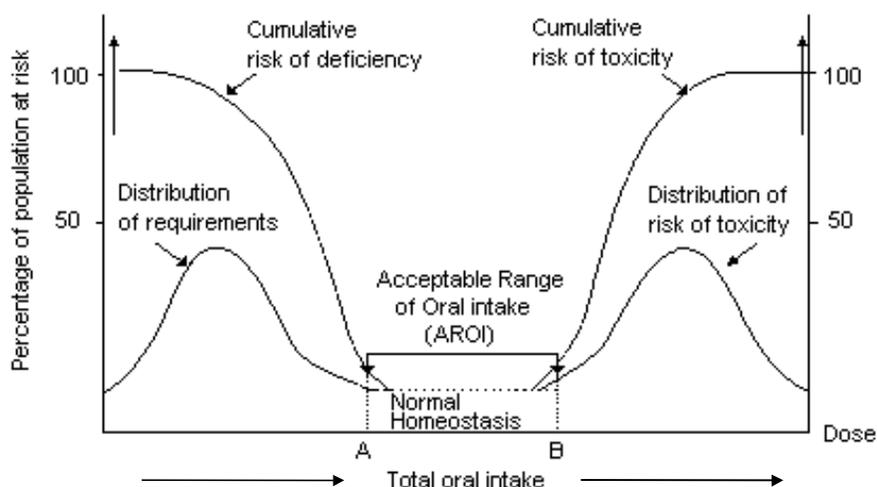
Finally, it is important that uncertainty should be differentiated from variability (NRC, 1994). Uncertainty may arise with inadequate data, with selection of relevant parameters, and with the judgement of the severity of the observed effect as well as, of course, with the necessary extrapolation steps between species and for differences in body size. Variability, on the other hand, will always exist and is a consequence of the distribution of exposure, of susceptibility to toxic effects in the population due to age, development, sex, disease, or genetic heterogeneity in homeostatic or metabolic pathways. While uncertainty because of lack of knowledge and data can be reduced by additional research, variability can not and must be taken into account so as to provide for the most robust estimates, but the process and considerations are separate from those associated with adjusting for uncertainty due to data deficiencies.

## **Background: Non-nutrients versus nutrients**

There are established guidelines for the extent and design of toxicity studies necessary for the approval of a chemical, such as a food additive or a pesticide, onto a positive list (SSC, 2000). Moreover, some consensus exists on the use of uncertainty factors to allow for deficiencies in the database, such as the absence of a NOAEL or of chronic studies in animals (SSC, 2000). However, no such agreement exists for the assessment of risks to humans in connection with nutrients. In addition, human studies with nutrients often have limitations: generally, they are performed either in a group of healthy volunteers or in groups at risk of or afflicted with certain diseases, comprise mostly a restricted age group or one sex only, and are often of short duration. The restricted data gathered from such studies are subject to the uncertainty described above with respect to extrapolation to the average human and to age groups that have not been tested.

Extrapolation from data derived from studies done in animals to humans normally is performed by applying an uncertainty factor (safety factor) of 100 to a No Observed Adverse-Effect Level (NOAEL) identified in the most sensitive animal species. This factor of 100 is composed of a factor of 10—to allow for differences between the animal and an average human—multiplied by a factor of 10 to allow for differences between average humans and sensitive subgroups (WHO, 1987). An uncertainty factor is “*a product of several single factors by which the NOAEL or LOAEL of the critical effect is divided to derive a tolerable intake (TI or UL). These factors account for adequacy of the pivotal study, interspecies extrapolation, interindividual variability in humans, adequacy of the overall database, and nature of toxicity. The term uncertainty factor was considered to be a more appropriate expression than safety factor since it avoids the notion of absolute safety and because the size of this factor is proportional to the magnitude of uncertainty rather than safety. The choice of UF should be based on the available scientific evidence*” (IPCS, 1994).

Although the use of these uncertainty factors has been reviewed and validated for the risk assessment of chemicals by numerous authors (SSC, 2000), their routine application in the risk assessment of nutrients is not possible. Toxicity of some nutrients has been observed in test animals at dose levels (expressed in mg/kg of body weight/day) that are close to or only slightly above the nutritional need. The acceptable range of oral intake (AROI) of an essential nutrient is represented by a trough in the U-shaped dose–response curve that spans requirements for essentiality to toxic levels (Figure 1). The distribution of the nutrient requirement and of the risk of toxicity in a population is also depicted schematically in Figure 1.



**Figure 1:** Percentage of the population at risk of deficiency and toxic effects through oral intake of a nutrient (modified from IPCS, 2002)

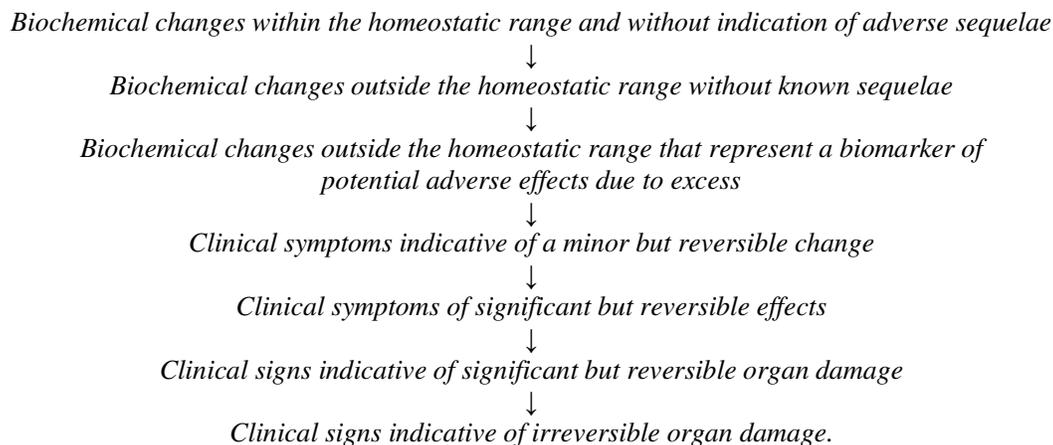
The normal physiological range of intake, in which homeostatic regulatory mechanisms are sufficient, is between the lower margin (point A in Figure 1), which usually is equivalent to the recommended daily allowance (RDA; defined as the intake at which only 2.5% of the population under consideration are at risk of deficiency) and the higher margin (point B in Figure 1), which could be the benchmark dose (BD) at which for example 2.5% of the population will be at risk for minimal adverse effects. In the absence of sufficient data on dose–response relationships and on homeostasis, a UL often is used to set the higher margin of the AROI (IPCS, 2002). The breadth and location of the trough on the dose–response curve are subject to the variability in populations both for requirement and for the susceptibility to the toxicity.

The routine use of the uncertainty factors employed for the setting of a tolerable upper limit for a chemical is not appropriate in the case of essential (indispensable) nutrients. It can result in a tolerable upper intake level that is less than the nutritional requirement. In the risk assessment of nutrients both the requirement for a nutrient and its variability in the population and the potential adverse effect due to excessive consumption must be considered simultaneously (Renwick et al., 2004). Adjustment for uncertainties may be required on both sides of the dose–response curve. In summary, uncertainty is even greater in the case of assessment of risks for human health posed by the consumption of nutrients than it is in the risk assessment of chemicals or of contaminants. For the latter substances, systematic toxicity data usually exist from animal and/or in-vitro experiments, and standard approaches have been developed to address uncertainty (SSC, 2000; IPCS, 2002).

## Uncertainty surrounding adverse health effects

Crucial in the hazard identification process is the recognition of one or more potential adverse effects, of relevance to human health, that may be associated with exposure to a nutrient. If a nutrient presents more than one hazard to human health, a risk assessment for each hazard may be required. An adverse effect is: *“a change in morphology, physiology, growth, development or life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. Decisions on whether or not any effect is adverse require expert judgement”* [emphasis added] (IPCS, 1994; SSC, 2000).

A decision about the adversity of an observed effect has to be made. That is, it is necessary to differentiate between adaptive and truly adverse reactions (Dybing et al., 2002). Observed effects of high nutrient intakes can range from biochemical effects (e.g. enzyme activity) without functional significance to clinical effects that signify irreversible impairment of organ function. A possible ranking of indicators of adverse effects has been given by Renwick et al. (2004):



Biochemical effects without functional significance should not be regarded as adverse effects (IPCS, 2002). Scientific judgement is required to decide where precisely to locate the adversity of an effect in the ranking.

However, with some nutrients, observed effects can be difficult to categorise as either beneficial or adverse. In the case of biotin, for instance, the administration during three weeks of 2100 µg/day (i.e. >40-fold the adequate intake) to healthy adults resulted in the increased expression of 139 genes and the decreased expression of 131 genes in ex-vivo cultured peripheral blood mononuclear cells. There was a substantial increase in the expression of the gene encoding cytochrome P450 1B1, which activates procarcinogens and promutagens (Wiedmann et al., 2004). Pharmacological concentrations of biotin in the culture medium of NCI-H69 small cell lung cancer cells increased the expression of oncogenes (Scheerger and Zempleni, 2003). Currently, the effects of deficiency of biotin on intermediary metabolism, immunosuppression, teratogenesis, cell signalling and gene regulation are continuously better characterized and can help in defining adequate intakes (Hassan and Zempleni, 2006), while the administration of high doses (up to 15 mg/day) to healthy, diabetic or hyperlipidemic subjects was shown to have beneficial effects on blood glucose and serum lipids (Fernandez-Mejia, 2005) without reflecting dietary needs for biotin. Biotin was considered not to cause adverse effects in humans by either the IOM (1998) or the SCF (2001).

Chemical hazards usually are identified from a series of in-vitro or in-vivo animal studies that are designed to address different endpoints or target systems and that follow established guidelines for the conductance of such studies (Barlow et al., 2002). With ethical limitations, the same study guidelines can be applied to nutrients. Because the margin between the level of nutrient requirement and the level of nutrient toxicity may be narrow, for example, nutrient doses may need to be spaced more narrowly. Both the different bioavailability and the different toxicity of different forms of nutrients must be taken into account, as well as the method of exposure (diet, drinking water, by gavage as a bolus, or parenterally) and the relevance of such studies for human toxicity. The administration of high nutrient doses to test animals may have secondary effects on other nutrients, e.g. their bioavailability, that would not be observed in humans. Some animal models are inappropriate for the risk assessment of nutrient toxicity in humans, either because absorptive or metabolic functions are different or because adverse effects in humans, e.g. neurobehavioural effects or allergic reactions, cannot be elicited adequately in animals, (Barlow et al., 2002; Renwick et al., 2003).

For some nutrients (e.g. preformed vitamin A from liver, fluoride or copper from drinking water), excessive intakes via conventional foods are known to cause adverse health effects in healthy or susceptible persons. For many nutrients, however, the question of adverse effects has arisen only with increasing food fortification measures or the intake of food (dietary) supplements. In some

instances, these practices have increased nutrient intakes to levels far above those possible from the consumption of conventional foods.

Human data are preferable over animal data in identifying hazards and in the assessment of risks to human health from exposure to nutrients. But hazard identification for nutrients in humans often has to rely on observational reports of single cases or on the reporting of adverse effects from intervention or therapeutic studies performed with the goal of proving a benefit from the administration. Even in well-conducted randomised placebo-controlled interventional studies, several factors are likely to cause uncertainties about the relevance of such studies for the assessment of nutrient toxicity: typically only one nutrient dose level is tested, the additional intake from the diet is not reported, and adverse effects are not systematically investigated.

The establishment of causality between reported effects and the administered nutrient is another source of uncertainty, even when applying the Bradford-Hill criteria (1965). Factors such as selection of the study group according to sex, age, (risk of) disease, genetic variability or other inclusion or exclusion criteria raise uncertainties about the applicability of the data to the average human.

All adverse health effects identified in appropriate animal studies should be qualitatively described, along with the nature of those effects (Barlow et al., 2002). It is advisable to collect, organise and evaluate all information pertaining to the capacity of a nutrient to cause one or more types of adverse health effects in humans (Renwick et al., 2004). Uncertainty in hazard identification can to some extent be minimised by a-priori application of a strictly structured approach. Such an approach is based on a comprehensive search of data from human, animal and in-vitro studies, ranking of the evidence according to strength and statistical significance, and detailed explanation of the judgements made in identifying hazards.

## **Uncertainty surrounding dose-response assessment**

### ***Exposure assessment***

Exposure assessment is part of the risk assessment and is needed for the dose–response evaluation and for the risk characterisation. A qualitative and quantitative description of the likely levels and the duration of the exposure to the hazard source or sources is necessary. The nature and size of the human populations and the routes, magnitude, frequency and duration of exposure are included in the assessment

In the case of nutrients, an evaluation of the total exposure to a specific nutrient—predominantly oral intake from food, beverages, water, and supplements and, eventually, from drugs—must be performed in addition to the test doses of the same nutrient administered in experimental studies. The variability and distribution of the background dietary intake of the tested nutrient can introduce considerable uncertainties in the dose–response assessment and must be adequately documented.

Food consumption data for populations usually are gathered from observational protocols, not in an experimental controlled setting. They correspond to a crude estimate of intake, and their validity depends on the assessment methodology. Nutritional habits and food choices can be assessed reliably on an individual basis. For populations, the distribution curve of the exposure does not permit the estimation of the exposure for high percentiles of the curve (high consumers) or for special groups at risk due to variability in sensitivity (Kroes et al., 2002).

Food composition data banks of different origins, which are used to calculate nutrient intakes on the basis of food consumption data, vary in precision and completeness. They may lack data about some processed foods and meals and certain nutrients. Missing parameters for certain nutrients do not necessarily signify absence of the nutrient. Instead, they may be due to low analytic sensitivity. Changes in nutrient content due to the preparation of a food and differences due to degree of ripeness, selection of plant cultivars/animal strains, climatic and geographic influences and storage are possible and will have an impact on the precision of the estimation of the nutrient intake. The quality of food composition data banks is, moreover, dependent on the number of samples analysed and the sensitivity and validity of the methods of analysis. Results can vary between different laboratories. The data should include means and/or medians and ranges or percentiles to enable the calculation of the potential distribution of the nutrient intake from a food.

Apart from true total diet studies in which duplicates of food consumed are analysed, all intake calculations and most quantitative food consumption assessments contain some degree of uncertainty that can result in both under- and overestimation of intake. A careful evaluation of the quality of the exposure data will decide on the necessity and magnitude of adjustments.

Another source of uncertainty in exposure assessment is the combination of intake data from individual dietary records with standard average population body weights.

When different forms of a nutrient differ in bioavailability, i.e. when “*the fraction of the dose that is transferred from the site of administration into the general circulation as the parent compound*” differs (Renwick, 1993a), the estimated external dose of exposure to a nutrient (intake) ideally should be adjusted by the assessment of a biomarker of exposure (e.g. the daily excretion into the urine of a specific metabolite or the steady-state blood concentration of the nutrient). That would decrease uncertainty of the exposure assessment and consequently of the dose–response assessment (Kroes et al., 2002). However, this possibility is limited in the case of some nutrients, such as vitamins A, E, and D and copper and zinc, for which simple biomarkers of exposure determined in urine and/or blood do not exist.

### **Identification of the dose with/without adverse effect**

After having identified one or several hazards (endpoints), the relevant critical data sets from animal and human experimental or epidemiological studies must be selected that allow the identification of 1) no-observed-adverse-effect levels (NOAEL) or, if not available, 2) lowest-observed-adverse-effect levels (LOAEL), or 3) the definition of a benchmark dose (BD)..

The NOAEL is the highest intake of a nutrient at which the adverse effect(s) of concern has (have) not been observed. It is identified from the dose–response data for the critical effect, usually the effect of relevance to humans that is produced at the lowest dose levels.

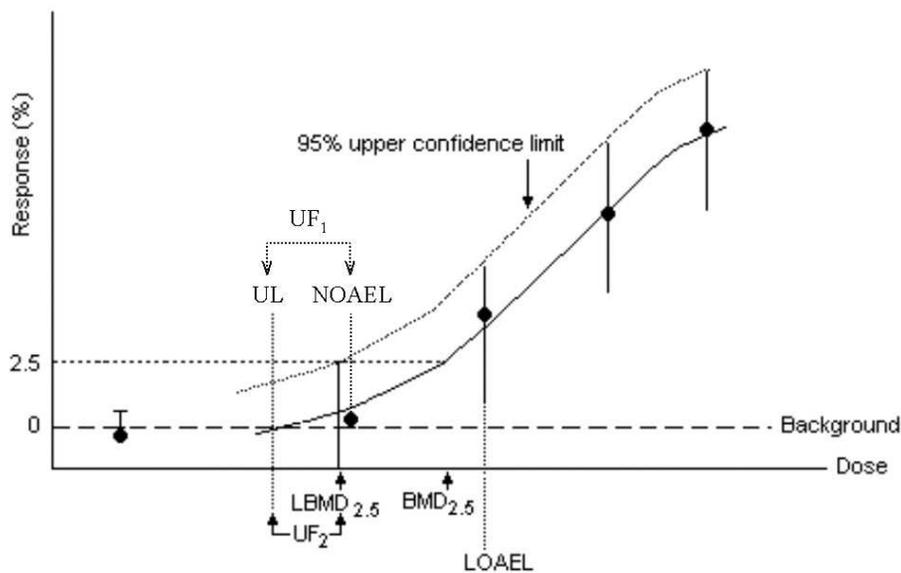
If the data are not adequate to demonstrate a NOAEL, then a LOAEL (the lowest intake at which an adverse effect has been demonstrated) may be used (Renwick et al., 2004). If different adverse effects have been observed for a nutrient, the NOAELs and LOAELs for these different endpoints can differ. The NOAEL corresponding to the lowest dose for eliciting an adverse health effect is chosen to identify the critical effect and then used for the derivation of a tolerable upper intake level (UL).

The identification of both a NOAEL and a LOAEL is affected by a number of uncertainties related to the quality of the animal study (sensitivity of the toxicological endpoint and the methods used to measure it, the size of the group studied, the increment between doses) and the steepness of the dose–response curve. These factors are decisive for the NOAEL to represent the true no-adverse-effect-level (NAEL) (Renwick et al., 2003). A concept of a threshold for a response is the basis for the identification of both a NOAEL and a LOAEL. From a biological perspective, a threshold should be seen as a certain dose range, above which a substantial change in response may occur, and one that takes into account the variability in homeostatic regulation within the population. Another factor that can have an impact on the dose threshold is the length of exposure to a nutrient. Chronic exposure to dose levels that do not elicit an adverse effect on short-term exposure can lead to accumulation in the body or at the cellular level and induce a toxic response when a critical level is surpassed (Dybing et al., 2002). NOAELs and especially LOAELs are, as a rule, imprecise because they depend on the study design: that is, the group size, the sensitivity of the detection method, and the spacing of the doses given.

The benchmark dose approach takes into account the entire dose–response curve and the variation in response within the studied population. The U.S. Environmental Protection Agency (EPA) has introduced a benchmark dose level as “*a statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect ... compared to background*”. A regression function is fitted on the response data to estimate the dose at which adverse effects start to arise or at which a specified percent change in the level of the chosen endpoint occurs, e.g. an increase of 2.5%, 5%, or 10% over background (BD2.5, BD5 or BD10). The lower 95% confidence limit of the chosen BD or critical effect dose is used to account for uncertainties of the database. This lower bound is called the benchmark dose lower confidence limit (LBMD) or simply the benchmark dose (BD). The BD may be used for the development of an intake limit, e.g. a UL, by applying uncertainty factors (Crump, 1984; Edler et al., 2002; IPCS, 1994; 2002).

Figure 2, adapted from IPCS (2002), illustrates the principle of the benchmark approach in relationship to NOAELs and LOAELs.

In this figure,  $BMD_{2.5}$  is the benchmark dose at which 2.5% of the individuals experience an adverse effect over the background level.  $LBMD_{2.5}$  is the lower 95% confidence interval of the  $BMD_{2.5}$ , i.e. the dose at which no more than 2.5% of the individuals experience the adverse effect estimated with 95% certainty. The position of the NOAEL and the LOAEL also are indicated in Figure 2.  $UF_1$  is the uncertainty factor applied to the NOAEL for deriving a UL (tolerable upper intake level), and  $UF_2$  is the uncertainty factor applied to the  $LBMD_{2.5}$  to derive the upper bound of the AROI. When an LMBD cannot be calculated, the upper bound of the AROI can be considered to be represented by the UL. Assumptions have been made that the BD of a chemical calculated from the lower 95% confidence limit for a 10% increased risk (BD10) is comparable to the LOAEL and that the BD derived on the basis of an increased risk at 5% (BD5) corresponds to the NOAEL. However, more recent data analyses indicate that the BD10 is closer to the NOAEL (Herrman and Younes, 1999). It is not known if this applies also to nutrients.



**Figure 2:** Theoretical representation of the lower part of the dose–response curve for an adverse effect in a population with the upper 95% of confidence limit of response (modified from IPCS, 2002)

The benchmark approach has been used rarely in the risk assessment of nutrients, because the available data must be suitable for modelling and measurements at three or more dose levels are required. The approach has been used to explore the relationships between drinking water fluoride, urine fluoride, and serum fluoride and dental fluorosis in Chinese children from two villages with six categories of fluoride content in drinking water. The LBMD for the prevalence of dental fluorosis was found to be 1.01 mg fluoride/L (Xiang et al., 2004). The data from the studies of Dean et al. (1942) on the relationship between dental fluorosis in 12- to 14-year-old children and the fluoride content of their drinking water also lend themselves to benchmark modelling.

The three graphs in Figure 3 show benchmark dose modelling with the data of Dean et al. (1942). These graphs illustrate clearly how the choice of the endpoint influences the result. The study of dental fluorosis included 4429 children between the age of 12 and 14 years from 13 cities with different fluoride concentrations in the drinking water (0.09 to 2.55 mg/L). The fluoride concentration in the drinking water is used as a surrogate for the fluoride intake, because at that time drinking water was the main source of fluoride. Dental fluorosis was divided into seven degrees of severity: normal, questionable, very mild, mild, moderate and severe. The investigators considered the first two grades not to represent fluorosis. Following this judgement (Figure 3a), the LBMD is determined as 0.559 mg fluoride/L. When dental changes of the grades of mild and more are considered to represent the

adverse effect, the LBMD is 1.499 mg fluoride/L (Figure 3b), and it is 2.205 mg/L when only changes graded as moderate and more are taken to be critical (Figure 3c).

Other methods for a quantitative dose–response analysis, such as categorical regression for non-cancer toxicity, or dose–response extrapolation to provide a quantitative risk estimate for non-threshold effects, or probabilistic approaches to derive tolerable intake levels or physiologically-based toxicokinetic ((PBTk) modelling to evaluate target organ doses following exposure to a substance by any route (Edler et al., 2002) have not been used in the risk assessment of nutrients as yet.

NOAELs, LOAELs and BMDs are adjusted for differences and variabilities in the susceptibility of individuals within and between species and for uncertainties in the data sets by numerical values called “safety factors”, “default values”, “uncertainty factors”, “assessment factors” or “correction factors”. The lower the degree of confidence in the scientific basis of the data and the greater the gaps in knowledge of the differences in kinetic and dynamic functions in different species, the greater the uncertainty factors to be chosen will have to be.

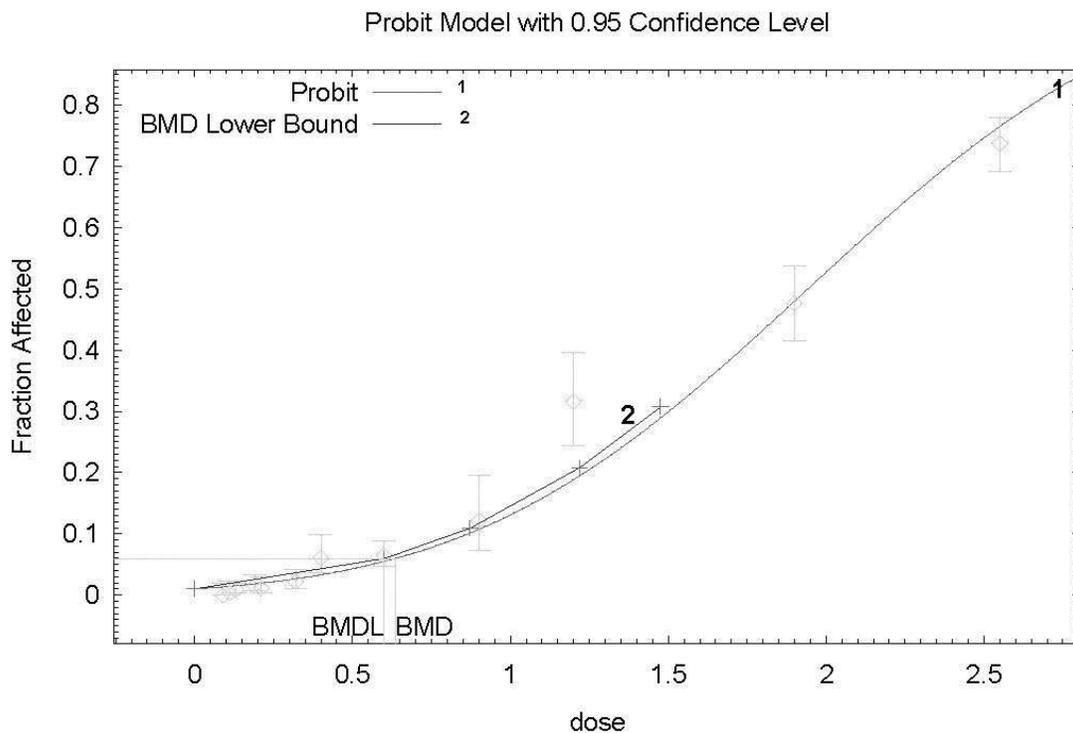


Figure 3a

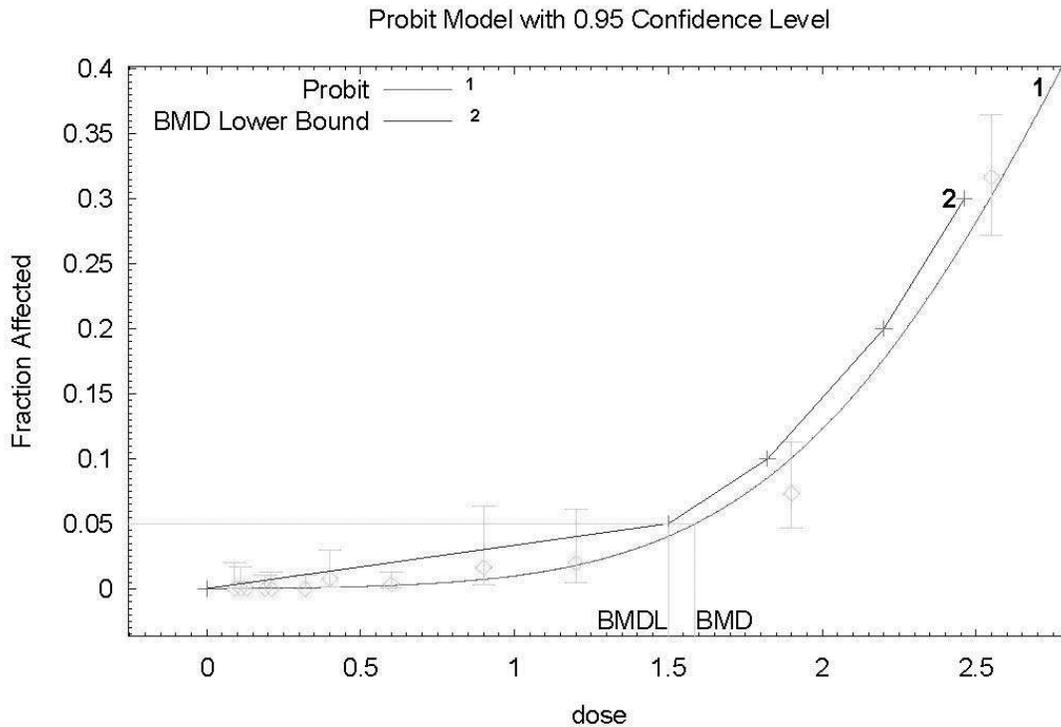


Figure 3b

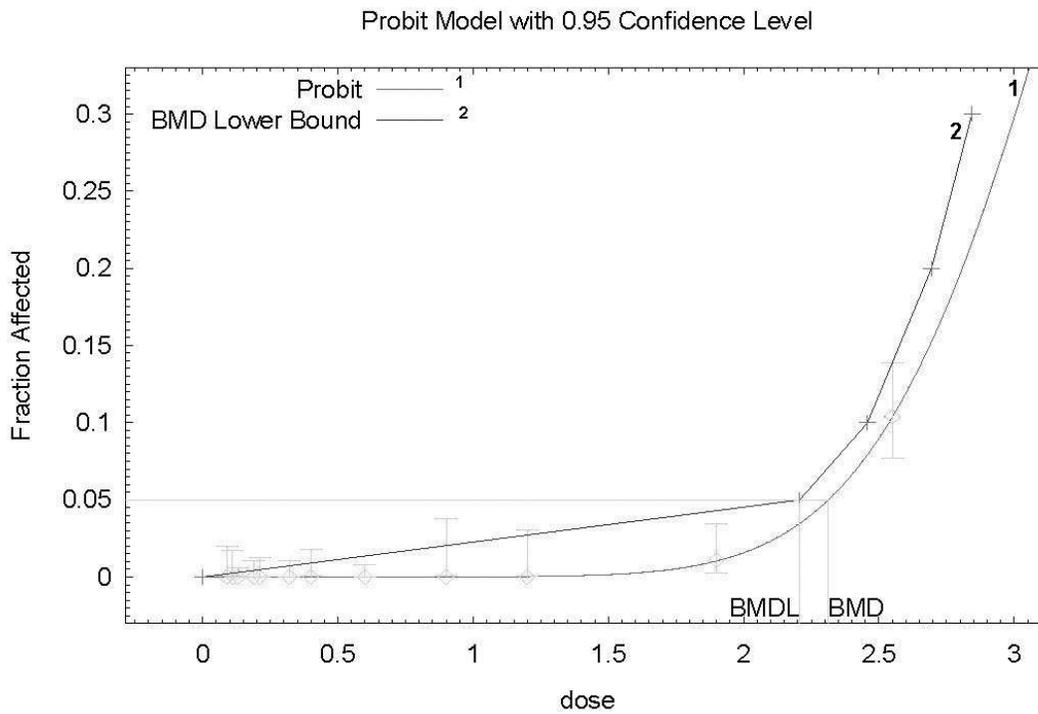


Figure 3c

**Figure 3:** Benchmark dose approach to define the fluoride concentration in drinking water associated with a 5% increase of risk for three different degrees of severity of dental fluorosis in children (data from Dean et al., 1942).  
 a) the 95% benchmark dose lower confidence limit is 0.559 mg fluoride/L for dental fluorosis of degree

> questionable according to Dean; b) the  $LBMD_{0.5}$  is 1.499 mg fluoride/L for dental fluorosis of degree > mild according to Dean; c) the  $LBMD_{0.5}$  is 2.205 mg fluoride/L for dental fluorosis > moderate according to Dean (Probit Model Rev. 2.1)

The uncertainties to be taken into account include:

- Problems in estimating total exposure to a nutrient from all sources, especially in human epidemiological studies but also in intervention studies, lack of quantification of (dietary) intake in addition to the study dose. These uncertainties apply to both the modelling systems used and the reliability of analytical measurements of exposure markers;
- Problems of reliability of data due to study design, conductance of the study and statistical evaluation: too small and too short studies; selection of a particularly sensitive or insensitive study population; insufficient assessment of compliance and of adverse effects as opposed to the expected beneficial effects;
- Insufficient availability of data: animal and/or in-vitro data only; one dosage studies as compared to multiple dose studies;
- Differences in bioavailability of different forms of the test substance and the influence of food matrices on bioavailability;
- Influence of age, sex, genetic polymorphisms, or medication;
- Biological mechanisms causing the observed adverse effect;
- Relevance of data on the nature of an adverse effect gathered in animals for humans;
- Gaps in knowledge of the variability in kinetics and dynamics of a nutrient between species;
- Clinical significance of observed/measured effects and reversibility of effects.

## Dealing with uncertainties

### Non-nutrients

#### ***Extrapolation and adjustment for uncertainty of data***

Methods of dealing with uncertainties in connection with identified risks from chemical or environmental hazards to protect public health by defining “safe”, “tolerable” or “acceptable” daily intakes (ADI) for non-carcinogenic substances were suggested 50 years ago. Lehman and Fitzhugh (1954) first proposed ADIs for additives and contaminants to be derived from a chronic animal NOAEL (or NOEL = no effect level) in mg/kg diet by dividing the NOAEL by 100. This factor was to account for both interspecies (animal → human) differences and intraspecies variability in sensitivity. This approach was adopted also for pesticide residues by the WHO Expert Committee for Pesticide Residues (Lu, 1979). One 10-fold factor is applied to convert a sub-threshold dose in mg/kg body weight/day for a population of test animals to a sub-threshold dose for average humans, whereas the second 10-fold factor is supposed to convert the dose for a group of average humans to a sub-threshold dose for sensitive individuals.

The overall adequacy of the factor of 10 for both interspecies and intraspecies variability was justified by subsequent reviews of numerous experimental data (Dourson and Strara, 1983; Calabres, 1985; Hattis et al., 1987; Sheehan and Gaylor, 1990; Lewis et al., 1990; Renwick, 1991; Calabrese et al., 1992; Naumann and Weideman, 1995; Dourson et al., 1996; Renwick and Lazarus, 1998), including children (Dourson et al., 2002).

Additional uncertainty factors ranging between 2 and 10 have been proposed to adjust for deficiencies in the database (Beck et al., 1993; IPCS, 1994; Vermeire et al., 1999):

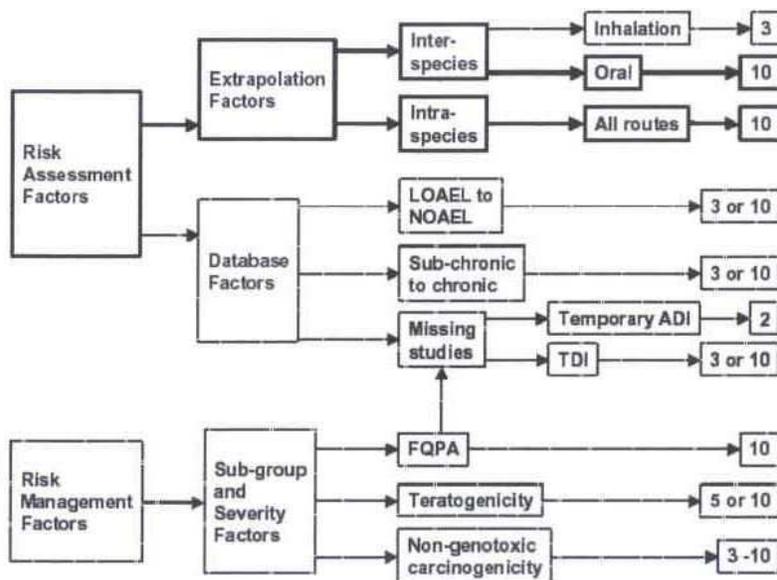
- a factor between 3 and 10 when only a LOAEL is available;
- an uncertainty factor between 3 and 10 for extrapolation of a subchronic NOAEL to a chronic NOAEL when data from two animal species are available;

- an additional factor of 2 when only one animal species has been tested.

In Figure 2 an additional uncertainty factor  $UF_3$  could be introduced for the conversion of a LOAEL to a NOAEL.

Because the individual uncertainties have been deemed to be independent of each other, the overall uncertainty factor is the product of multiplying all the individual uncertainty factors (Dourson and Stara, 1983). This total independence of uncertainty factors has been questioned (Calabrese and Gilbert, 1993). The choice of the magnitude of additional uncertainty factors requires scientific judgement on the strength of the available evidence (Dourson et al., 1996). The reasoning for the decision should be clearly and explicitly described in order to avoid the impression that additional uncertainty factors are policy driven. Uncertainty factors greater than 10,000 should not be applied, because they would signify an insufficient database for a reliable risk assessment (IPCS, 1994).

Figure 4 (taken from SCC, 2000) illustrates the different uncertainty factors applied by different agencies to establish acceptable levels of human exposure to chemical, environmental or microbiological agents based on animal databases. The factors shown with continuous lines are those usually used in the EU for the assessment of food additives and pesticides. Other factors may be used for other types of chemicals, e.g. contaminants, and by authorities and bodies outside the EU. The lower part of Figure 4 illustrates that extra factors can be applied to address the severity of the effects, such as teratogenicity or non-genotoxic carcinogenicity for risk management reasons. An example of the latter is the protection of special subgroups, such as infants and children, under the Food Quality Protection Act (FQPA) in the United States.



**Figure 4:** Schematic representation of uncertainty factors applied to establish acceptable levels of human exposure based on data derived from animal studies

### **Replacement of default uncertainty factors**

The 10-fold factors to adjust for interspecies and intraspecies differences are applied to a wide variety of compounds regardless of their structure and metabolic fates. They also are applied to different effects on organs of different species regardless of differences in kinetic and dynamic processes among species. Although the overall adequacy of these factors has been ascertained, they should be replaced by more specific factors that account for both kinetic and dynamic aspects of a compound in different species when such knowledge becomes available (Renwick, 1991; 1993b).

It was proposed to divide each of these 10-fold factors into two components for the separate evaluation of differences in toxicokinetics and toxicodynamics. Toxicokinetics include the considera-

tion of the rate and extent of absorption of a substance; its distribution, rate and pathway of bioactivation; and its rate, route and extent of elimination. Toxicodynamics consider the toxic entity (either parent compound or metabolite) and its molecular target and the sensitivity of the target tissue as well as activating, protective or repair mechanisms. The interspecies differences in toxicokinetics generally are greater than toxicodynamic differences and can be attributed, in part, to differences in body weight. A generic kinetic default factor of 4.0 ( $10^{0.6}$ ) was proposed in the absence of compound-specific data, which would be multiplied by a default factor of 2.5 ( $10^{0.4}$ ) to give the interspecies factor of 10. The 10-fold factor for intra-human variability can be divided in a similar manner to allow for variability in kinetic and dynamic processes as a default value for compound-specific data (Renwick and Lazarus, 1998).

The International Programme on Chemical Safety (IPCS, 1994) has adopted these principles with the modification that the uncertainty factor of 10 for intraspecies variability be divided evenly into 3.16 ( $10^{0.5}$ ) for both kinetics and dynamics. This modification is supported by data for the kinetics of 60 compounds in humans (Renwick and Lazarus, 1998). Sensitive humans are those with kinetic and dynamic characteristics such that their internal dose on exposure (kinetics) is >3.16-fold away from the population mean and their individual internal dose threshold for response is >3.16-fold lower than the population mean (Renwick, 1999). The prevalence of individuals in a population who would not be covered by the standard default factors for kinetics and dynamics depends on the variability of the distribution of the relevant parameters.

The ultimate uncertainty factor applied for interspecies and intra-human variability will be the result of the multiplication of the compound-specific factors for kinetics and dynamics, if available from animal and human data, respectively. Such a factor could be named a correction factor (Edler et al., 2002) or an adjustment factor (IPCS, 2002) instead of uncertainty factor.

Recently probabilistic approaches to risk assessment have been proposed, where both the uncertainties and variabilities in hazard characterization and exposure distribution are integrated into the model and their relative contribution to the overall uncertainty of the result can be estimated (van der Voet and Slob, 2007). This methodology allows to give an idea of the effect that an intentional modification of one source of uncertainty could have.

## Nutrients

The risk assessment of nutrients follows the general principles of risk assessment with the restriction that the derived tolerable upper intake level (UL) “*judged to be unlikely to pose a risk of adverse health effect to almost all individuals in the general population*” (SCF 2000) must not be lower than the nutritional requirement or the recommended intake. This restriction will influence the choice of uncertainty factors to correct for inter- and intra-species variability and for deficiencies in the available databases. NOAELs or, in the absence of appropriate data, LOAELs should be derived from human studies if possible. If they are identified from studies in animals, the relevance of observed adverse effects for humans must be evaluated.

It is illustrative to compare the approach used for uncertainty adjustment among different groups. Other than the IOM, several institutions, authorities, or authors have undertaken systematic risk assessments of nutrients according to protocols they have developed and published. The IOM approach is described first below and is followed by others which are listed by year of publication. Four are summarised in Appendix 1 of this discussion paper.

### ***Food and Nutrition Board, Institute of Medicine, National Academy of Sciences USA, 1997–2004***

As mentioned earlier, as part of the the DRI development task, “*Tolerable Upper Intake Levels*” (UL), i.e. “*the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population*”, were derived.

Reviews of observational and experimental studies published mostly in peer-reviewed journals and analysis of the evidence were performed; scientific judgement was used to determine the basis for establishing values. The possibility was considered and rejected that the methodology used to derive ULs might be reduced to a mathematical model that could be generally applied to all

nutrients. A standard risk assessment procedure was followed for each nutrient individually, and two types of uncertainty were acknowledged: those related to data and those associated with inferences that are required when directly applicable data are not available. In the risk characterisation, scientific uncertainties associated with both the UL and the intake estimates were described.

ULs were preferably derived from identified NOAELs, taking into account causality, relevance of experimental data (animal versus human, route of exposure, duration of exposure), mechanism of toxic action, quality and completeness of the database, and the identification of distinct and highly sensitive subgroups.

In the dose–response assessment, human data were preferred over animal data, and the routes and durations of exposure chosen were those most relevant for a toxic response in humans. The choice of uncertainty factors was determined by scientific judgements on the interindividual variation in sensitivity (between 1 and 10); for extrapolation from animal data to humans (up to 10); for using a LOAEL in the absence of a NOAEL, taking into account the severity and incidence of the adverse effect and the steepness of the dose–response (up to 5); and to correct for a subchronic NOAEL in the absence of a chronic NOAEL.

ULs were derived for the age categories for which the data were available. When data were not available on children and adolescents, ULs were determined by extrapolating from the UL for adults based on body weight differences using the formula:

$$UL_{child} = (UL_{adult}) (weight_{child} / weight_{adult}),$$

except in the case of niacin, vitamin B<sub>6</sub>, folate, and choline, for which a formula based on metabolic size was used:

$$UL_{child} = (UL_{adult}) (weight_{child} / weight_{adult})^{0.75}.$$

### ***Conseil Supérieur d'Hygiène Publique de France, 1995***

All vitamins and the minerals zinc, iron, selenium, and fluoride were assessed. A review of the literature was undertaken to identify NOAELs or LOAELs from human and animal studies. The documented doses of a nutrient in studies were ranged according to magnitude and adverse effects observed: NOAELs or LOAELs were identified. Both NOAELs and LOAELs identified from human studies were divided by a safety factor of 10 to derive a “*safety threshold dose*” for intake in addition to the intake from a normal diet. In exceptional cases, where the safety threshold dose calculated in this way would be lower than the recommended daily allowance (RDA), this recommended dose was chosen as the safety threshold dose.

### ***Scientific Committee on Food/European Food Safety Authority, 2000–2005***

The framework of general principles for evaluation of the adverse effects of micronutrients in humans and for establishing upper levels of intake that are unlikely to result in adverse effects in the general population was formulated as a guideline in 2000. It was based on available reports in the literature.

ULs were stated not to be recommended levels of intake; but they were to apply to the general population throughout the life stage (excluding those receiving the nutrient under medical supervision), including sensitive individuals. However, certain identifiable subgroups (e.g. those with genetic predisposition or certain disease states) were to be excluded while evaluating each nutrient. To the extent possible, ULs for age and life-stage groups were set. The usual steps of risk assessment were followed. Uncertainties in the database were to be described in the risk characterisation.

Scientific judgement was applied on the adversity of an effect and on the causality between nutrient and effect, the relevance of experimental data, and the mechanisms of adverse effects. Selection of data was to give preference to human data, and, in the absence of human data, to the animal species with biological responses most like those of humans and with the most relevant route of exposure.

Low uncertainty factors were chosen with higher quality data and for adverse effects that are extremely mild and reversible. Uncertainty factors were applied for interindividual variation and sensitivity (between 1 and 10); for extrapolation from animal to human; for LOAEL to NOAEL (dependent on the slope of the dose–response curve); and for a subchronic NOAEL to a chronic NOAEL.

If no data were available to derive ULs for extrapolation to different age groups, it was suggested that extrapolations should be made on the basis of known differences in body size, physiology, metabolism, absorption and excretion. The extrapolation from an adult UL to ULs for children and adolescents was regularly based on body weight differences. Reference weights were given for males and females of nine age groups. Scaling of an adult UL to children was done on a body weight basis for niacin, vitamin B<sub>6</sub>, folic acid, fluoride, copper, molybdenum, and selenium and on a metabolic body size basis (body weight<sup>0.75</sup>) for vitamin A, E, iodine, zinc, and boron.

In those cases where no UL could be established because of insufficient data, the risk characterisation included an indication on the highest level of intake where there is reasonable confidence in data on the absence of adverse effects.

### ***Expert Group on Vitamins and Minerals (EVGM, 2003) of the Food Standards Agency, UK***

The terms of reference of the expert group were somewhat different from those used by both the IOM and the SCF/EFSA, namely to establish principles on which controls for ensuring the safety of vitamin and mineral supplements sold under food law can be based and to recommend maximum levels of intakes of vitamins and minerals from supplements if appropriate, after having reviewed the levels of individual vitamins and minerals associated with adverse effects. Both animal and human studies were evaluated, including acute toxicity and single dose studies.

No single scheme of risk assessment was considered satisfactory, and each nutrient was assessed individually. Where adequate data were available, safe upper levels (SUL) of lifetime intake of a nutrient by the general population were established both per day and per kg body weight per day (using a reference body weight of 60 kg for adults). In the absence of sufficient data, guidance levels for safe intakes that would not be expected to cause adverse effects were defined. Uncertainty factors were applied for extrapolation from a LOAEL to a NOAEL (usually 3), for database deficiencies (subchronic exposure, few subjects only), and for the severity of the adverse effect. The magnitude of the factors was determined by scientific judgement, taking care to arrive at safe levels above the lower advisory levels of intake.

The available margin for additional intake exposure from supplements or new sources of fortification was calculated by subtracting the actual intake from dietary and other sources from the defined safe upper level (SUL).

SULs can be applied to children by scaling for body weight or body surface area as appropriate, unless it is specifically indicated that children are particularly vulnerable to the effect concerned or have a greater requirement.

### ***Non-governmental assessment***

Two reports are mentioned, both of which were both published in 1997 (Hathcock, 1997; Shrimpton, 1997). Both applied the principles of risk assessment to establish safety limits while considering evidence for benefits from intakes of certain nutrients at levels above recommended dietary intakes.

#### ***Council for Responsible Nutrition (Hathcock, 1997)***

From a review of the available literature, NOAELs for all vitamins and for calcium, phosphorus, magnesium, chromium, copper, iodine, iron, manganese, molybdenum, selenium, and zinc were identified as well as LOAELs for vitamin A, D, nicotinamide, nicotinic acid, vitamin B<sub>6</sub>, iron, selenium, and zinc.

NOAELs are proposed to be considered as safe levels of intake, whereas LOAELs are considered to be not safe for everyone: they may require the application of a safety factor to calculate a safe level of intake.

The author suggested that a nutrient safety limit be calculated as an intermediate between the LOAEL and the recommended intake when no NOAEL can be identified.

*European Federation of Health Product Manufacturers Associations (Shrimpton, 1997)*

The goal was to base the upper safe level of intake from all sources of a vitamin or mineral well below that at which significant adverse effects have been responsibly reported. From a review of the available literature, two types of levels of intake were suggested: an upper safe level of long-term consumption and an upper limit of short-term consumption. With the exception of phosphorus, chromium, iron, and manganese, the upper safe levels of long-term consumption are identical to the NOAELs identified by Hathcock (1997). In contrast, the upper limits for short-term consumption suggested for vitamin B<sub>6</sub>, iron, selenium, zinc are somewhat lower than the LOAELs in the mentioned reference.

## **Comparisons among approaches for dealing with uncertainties**

The differences in the results of the risk assessments performed by the Institute of Medicine, the Scientific Committee on Food/European Food safety Authority, the Conseil Supérieur d’Hygiène Publique de France and the Food Standards Agency are due to differences in procedure, particularly in adjustments made because of uncertainties and in scientific judgements. These differences can be summarized as follows :

- *selection of the database* (in some cases to be explained by the non-availability of data at the time of assessment). This applies to vitamin A, vitamin D, vitamin E, niacin (nicotinamide), vitamin B<sub>6</sub>, vitamin C, calcium, phosphorus, magnesium, iron, fluoride, manganese, nickel, and zinc. The criteria for the selection, evaluation and ranking of available data need to be discussed;
- *the selection of the critical adverse effect*. This applies to calcium, phosphorus, fluoride, and nickel. This is mainly a question of scientific judgement. The reasons and justification of this judgement need to be described in detail;
- *the identification of a LOAEL or NOAEL*. This applies to vitamin E, vitamin B<sub>6</sub>, folic acid, vitamin C, calcium, magnesium, fluoride, iodine, nickel, selenium, and zinc. This closely related to the two items above;
- *the establishment of a UL*. This applies to vitamin D, vitamin E, niacin, vitamin B<sub>6</sub>, magnesium, fluoride, iodine, copper, molybdenum, selenium, zinc, and boron. Although the same database was used at least partially for nicotinic acid, vitamin B<sub>6</sub>, magnesium, iodine, copper, molybdenum, selenium, copper and boron, scientific judgement and weighing of the evidence are responsible for these discrepancies;
- *the scaling method chosen to establish specific ULs for subgroups*. It is not clearly apparent from the reports on what basis the choice for the scaling method was made. A nutrient-specific approach should be considered;
- *the selection of uncertainty factors*. This applies to vitamin E, vitamin C, iodine, copper, molybdenum, selenium, and boron—even when the same database was used and the same NOAEL or LOAEL was identified

The selection of uncertainty factors by different workgroups for adjustment for different types of uncertainty is summarized Box 1. The information in Box 1 can create the false impression that the choice was arbitrary, and this was indeed the focus of criticism. In each case the deliberations of the different scientific workgroups and the justification for individual uncertainty factors can be found in the report. It cannot be ignored, however, that for some nutrients the choice of an uncertainty factor was driven less by scientific judgement than by aiming for a UL above the recommended intake.

**Box 1. A summary of the selection of uncertainty factors by four different assessors**

A NOAEL from human studies was identified 39 times. The following uncertainty factors were applied:

19 times a UF of	1
3 times a UF of	1.2, 1.5 or 1.8
7 times a UF of	2
1 times a UF of	2.5
4 times a UF of	3
5 times a UF of	10.

The high uncertainty factor of 10 to establish a UL from a human NOAEL was applied for vitamin K, vitamin B<sub>2</sub>, pantothenic acid, biotin and selenium because of assumed great variability of sensitivity in humans.

Thirty LOAELs from human studies were identified. The following uncertainty factors were applied:

4 times a UF of	1
5 times a UF of	1.5
1 times a UF of	1.6
4 times a UF of	2
6 times a UF of	3
1 times a UF of	4
5 times a UF of	5
4 times a UF of	10

An uncertainty factor of 10 was applied for vitamin D, vitamin E and vitamin B<sub>6</sub> as a routine procedure.

Nine NOAELs from animal studies were identified. The following uncertainty factors were applied to establish a UL:

2 times a UF of 30	(interspecies extrapolation: 10; intraspecies variability: 3)
2 times a UF of 60	(interspecies extrapolation: 10; variability in kinetics: 6)
4 times a UF of 100	(interspecies extrapolation: 10; intraspecies variability: 10)
1 times a UF of 300	(interspecies extrapolation: 10; intraspecies variability: 10; adverse reproductive effects: 3).

In the case of boron, for which the IOM, EFSA and EGVM all based their assessment on the same animal data, intraspecies variability was taken into account twice by an uncertainty factor of 6 and once by an uncertainty factor of 3 in addition to the uncertainty factor of 10 for extrapolation from animals to humans.

Six LOAELs were identified from animal studies. The following uncertainty factors were applied to establish a UL:

1 times a UF of	36	(LOAEL → NOAEL: 2; animal → human: 3; intraspecies variability: 3; subchronic → chronic: 2)
1 times a UF of	100	(animal → human: 10; intraspecies variability: 10)
3 times a UF of	300	(LOAEL → NOAEL: 3; animal → human: 10; intraspecies variability: 10)
1 times a UF of	1000	(LOAEL → NOAEL: 10; animal → human: 10; intraspecies variability: 10).

A comparison of the numbers of ULs defined by the Institute of Medicine and by the Scientific Committee on Food/European Food Safety Authority, two institutions who basically applied the same procedure and the same definition of a UL, is given in Box 2.

Box 2. Comparison of ULs derived by SCF/EFSA with ULs by FNB of IOM		
	SCF/EFSA	FNB
Number of ULs defined*	18	29
Number based on human NOAEL	10 (56%)	11 (38%)
UF applied	1-3	1-3.3
Number based on animal NOAEL	2 (11%)	3 (10%)
UF applied	60-100	30-300
Number based on human LOAEL	6 (33%)	13 (45%)
UF applied	1-5	1-10
Number based on animal LOAEL	-	2 (7%)
UF applied	-	36-300

\* nicotinic acid (human LOAEL) and nicotinamide (human NOAEL), vitamin A (human NOAEL and LOAEL for adults and infants), vitamin D (human NOAEL for adults and infants) and fluoride (human LOAEL for >8 y and human NOAEL for <8 y) counted separately

It is shown that there is a difference in the number of nutrients evaluated – 33 by SCF/EFSA and 37 by IOM – which only partly explains the difference in the number of ULs, together with a greater reliance on human data by the SCF/EFSA than by the IOM. The uncertainty factors applied to human data are mostly small and quite similar, while the uncertainty factors applied to animal data are much larger and have a wider range, which was to be expected. Both institutions do not provide a systematic listing of identified sources or types of uncertainty with a qualitative estimate of their relative importance for the numerical result – let alone a quantitative estimate

## Summary and suggestions

The risk assessment of nutrients – and it may assumed also the assessment of nutrient requirements - , even if performed according to established guidelines, is influenced by uncertainties that predominantly are due to the restricted availability of data and a relative lack of systematic studies with the desirable characteristics: a range of well defined doses, sufficient duration, and a design that assesses the occurrence of a priori defined adverse effects or validated biomarkers of such effects. In spite of existing guidelines for the risk assessment procedure, there also is a lack of consensus on how to proceed at those points in the process at which scientifically based decisions have to be made—e.g. in the selection of the decisive and reliable studies, the identification of the critical adverse effect, the choice of uncertainty or adjustment factors, and the scaling to adjust for differences in body size.

Nutrients are a heterogeneous group of substances with widely differing properties with regard to absorption, elimination and biologic functions in the body, and different organs and cell types. Nutrients occur in different forms that can have different bioavailability. However, differences in bioavailability are almost never considered in the risk assessment; or one form only is taken into account.

The resultant overall uncertainty is largely responsible for the variability in the outcome of the risk assessments performed by different scientific workgroups. Considering the extent of uncertainty due to both data insufficiency and differences in approach, the degree of conformity in the magnitude of established upper tolerable intake levels is surprisingly high.

Nonetheless, for greater transparency and better understanding both of the process of the assessment and the significance of the outcome, a systematic identification of sources and types of uncertainty and an estimate of the direction and magnitude of their influence on the overall outcome

should be part of each assessment. In some instances, this could be done quantitatively by introducing identified uncertainties as a function into mathematical models.

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