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SCIENTIFIC OPINION

# Guidance for establishing and applying tolerable upper intake levels for vitamins and essential minerals

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**Abstract**

Vitamins and essential minerals are micronutrients that are required for the normal functioning of the human body. However, they may lead to adverse health effects if consumed in excess. A tolerable upper intake level (UL) is a science-based reference value that supports policy-makers and other relevant actors in managing the risks of excess nutrient intake. EFSA's principles for establishing ULs for vitamins and minerals were originally developed by the Scientific Committee on Food in 2000. This guidance from the EFSA Panel on Nutrition, Novel Foods and Food Allergens provides an updated framework for UL assessments. A draft was published in 2022 and underwent a 2-year piloting period. The present document incorporates revisions based on the experience gained through its practical implementation. It covers aspects related to the planning of the risk assessment (problem formulation and definition of methods) and its implementation (evidence retrieval, appraisal, synthesis, integration, uncertainty analysis). As in the previous framework, the general principles developed for the risk assessment of chemicals in food are applied, i.e. hazard identification, hazard characterisation, intake assessment, risk characterisation. Specific to nutrients are their biochemical and physiological roles and the specific and selective mechanisms that maintain the systemic homeostasis and accumulation of the nutrient in the body. Such considerations must also be taken into account when conducting risk assessments of nutrients.

**KEYWORDS**

dietary reference value, mineral, tolerable upper intake level, UL, vitamin

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# 1 | BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

## 1.1 | Background

Article 6 of Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods and Article 5 of Directive 2002/46/EC on the approximation of the laws of the Member States relating to food supplements provide that maximum amounts of vitamins and minerals added to foods and to food supplements respectively, shall be set.

The above-mentioned provisions lay down the criteria to be taken into account when establishing these maximum amounts that include the upper safe levels (ULs) of vitamins and minerals established by scientific risk assessment based on "generally accepted scientific data, taking into account, as appropriate, the varying degrees of sensitivity of different groups of consumers".

To set maximum amounts of vitamins and minerals in fortified foods and food supplements, the Commission would like to ask the European Food Safety Authority (EFSA) to review the previous opinions of the Scientific Committee on Food (SCF) or the NDA Panel on the ULs for vitamin A,[1] folic acid[1]/folate, vitamin D[1], vitamin E[1], vitamin B6, iron[1], manganese[1] and β-carotene[1] to take into account recent scientific developments and evidence.

In this context, EFSA should first review the guidelines of the SCF[1] for the development of tolerable upper intake levels for vitamins and minerals (adopted on 19 October 2000).

Tolerable Upper Intake Levels should be presented separately for the age group from 4/6 months onwards until 3 years of age and the general population group from 3 years onwards, taking into account, as appropriate, the varying degrees of sensitivity of different consumer groups. As foods intended for the general population are also consumed by young children, young children should be considered as a potentially sensitive consumer group.

## 1.2 | Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to:

1. Update the guidelines of the SCF for the development of Tolerable Upper Intake Levels for vitamins and minerals in the light of available recent scientific and methodological developments.
2. Review existing scientific evidence and provide advice on Tolerable Upper Intake Levels for the following vitamins and minerals including their currently authorised forms for the addition to fortified foods and food supplements for the general population and, as appropriate, for vulnerable subgroups of the population:

- vitamin A
- folic acid/folate
- vitamin D
- vitamin E
- iron
- manganese
- β-carotene
- vitamin B6.

For nutrients for which there are no, or insufficient, data on which to base the establishment of a UL, an indication should be given on the highest level of intake where there is reasonable confidence in data on the absence of adverse effects.

# 2 | INTRODUCTION

Vitamins and essential minerals (which include essential trace elements) are micronutrients that are crucial for the normal functioning of the human body and must be obtained from the diet.[2] Like other chemical substances present in foods, micronutrients may lead to adverse health effects if consumed in excess. The concept of a UL refers to the maximum daily

[1]SCF (2000). Scientific Committee on Food. Guidelines of the Scientific Committee on Food for the Development of Tolerable Upper Intake Levels for Vitamins and Minerals. In: Scientific Committee on Food, Scientific Panel on Dietetic Products, Nutrition and Allergies (2006). Tolerable Upper Intake Levels for Vitamins and Minerals. European Food Safety Authority. SCF (2001). Scientific Committee on Food. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Magnesium. In: Scientific Committee on Food, Scientific Panel on Dietetic Products, Nutrition and Allergies (2006). Tolerable Upper Intake Levels for Vitamins and Minerals. European Food Safety Authority.

[2]Vitamin D is an exception as it can be produced via UVB-radiation in the skin.

intake from all dietary sources (i.e. food and beverages, fortified foods and food supplements) above which a nutrient may cause adverse health effects. It supports policy-makers and other relevant actors in managing the risks of excess nutrient intake.

Examples of the application of a UL include:

- the setting by risk managers of maximum amounts of micronutrients that can be added to foods or used in food supplements;
- the evaluation by risk assessors of the safety of a new nutrient source prior to its marketing authorisation;
- the safety assessment by risk assessors, public health authorities or other health professionals of the intake of micronutrients by individuals or populations.

In 2000, the Scientific Committee on Food published guidelines for establishing ULs for vitamins and minerals (SCF, 2000a). The guidelines outlined general principles for the evaluation of adverse effects of micronutrients in humans and for establishing ULs. In 2010, the NDA Panel published principles for deriving and applying dietary reference values (DRVs) and integrated the concept and definition of UL as part of DRVs for nutrients. Other DRVs include the average requirement (AR), population reference intake (PRI) and lower threshold of intake (LTI), which describe the distribution of the requirement for a nutrient. When the average requirement cannot be determined for a vitamin or an essential mineral, an adequate intake (AI) can be proposed (EFSA NDA Panel, 2010).

This guidance provides an updated framework for establishing ULs for vitamins and essential minerals based on the experience gained and relevant scientific developments in the field. The principles are illustrated by examples taken from the most recent EFSA risk assessments of vitamins and essential minerals.[3] The guidance also provides explanations on the interpretation and potential applications of ULs.

In general, the principles developed for the risk assessment of chemicals in food (FAO/WHO, 2009) also apply to nutrients. The four steps of the risk assessment process are illustrated in Figure 1. However, specific to nutrients are their biochemical and physiological roles and the specific and selective mechanisms that maintain the systemic homeostasis and regulate the accumulation of the nutrient over a range of intakes. Nutritional requirements also need to be considered, i.e. there is a level of intake below which the risk of deficiency or sub-optimal function arises (EFSA NDA Panel, 2010).

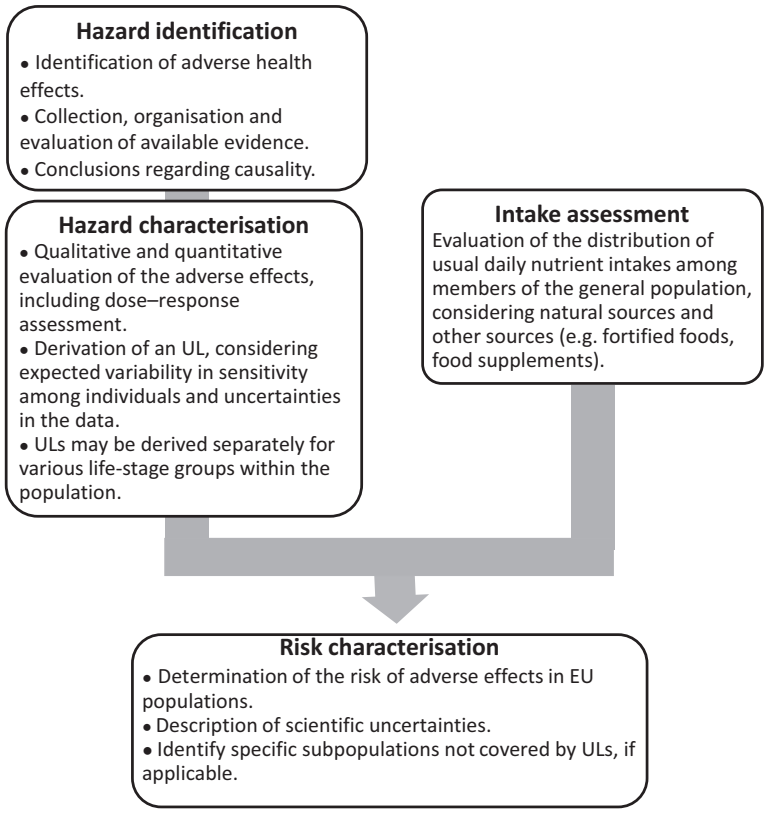


**FIGURE 1** Four-step process of nutrient risk assessment.

[3]Selenium (mandate number M-2020-0158); copper (mandate number M-2020-0087); vitamin A, including β-carotene, folate, vitamin D, vitamin E, iron, manganese, vitamin B6 (mandate number M-2021-00058).

## 3 | DATA AND METHODOLOGIES

Several reports on ULs for nutrients and methodological guidance documents from other competent authorities have been consulted in preparing the present document (Australian Government Department of Health, 2015; FAO/WHO, 2009; FAO/WHO, 2020; NASEM, 2017; NASEM, 2022, 2023; OHAT-NTP, 2019; WHO/FAO, 2006; WHO/IPCS, 2002).

Guidance documents from EFSA were considered, including those addressing the application of the systematic review methodology in food and feed safety assessments (EFSA, 2010), the protocol development for EFSA generic scientific assessments (EFSA Scientific Committee, 2023a), the biological relevance of data (EFSA Scientific Committee, 2017a), the use of the weight of evidence approach (EFSA Scientific Committee, 2017b), the use of the benchmark dose approach in risk assessment (EFSA Scientific Committee, 2022), the appraisal and integration of evidence from epidemiological studies (EFSA Scientific Committee, 2024), the analysis of uncertainty in scientific assessments (EFSA Scientific Committee, 2018) and the derivation of health-based guidance values for regulated products that are also nutrients (EFSA Scientific Committee, 2021b).

The revision of the guidance was also informed by the feedback collected through an expert workshop organised by EFSA, held on 28–29 September 2021, on data and methodologies for establishing ULs for vitamins and minerals (EFSA, 2022). In addition, a dedicated workshop on human-to-human scaling approaches for the derivation of ULs was organised on 2 February to 1 March 2023. The proceedings of the workshop are available in Annex A.

A draft of the guidance was published in January 2022 and subsequently piloted between 2022 and 2024 in EFSA's assessments of ULs for vitamin B6, manganese, vitamin D, vitamin A and β-carotene, iron and vitamin E (EFSA NDA Panel, 2022, 2023e, 2023a, 2023b, 2024b, 2024c). The present document has been enriched based on the experience gained during that period.

In line with EFSA's policy on openness and transparency, and for EFSA to receive comments from the scientific community and stakeholders, the draft Guidance was released for public consultation from 8 July 2024 to 25 August 2024.[4]

## 4 | DEFINITION OF A TOLERABLE UPPER INTAKE LEVEL AND ASSOCIATED TERMINOLOGY

**Tolerable upper intake level (UL):** the maximum level of total chronic daily intake of a nutrient (from all dietary sources) which is not expected to pose a risk of adverse health effects to humans.

A UL is a health-based guidance value for nutrients (EFSA Scientific Committee, 2021b). A UL is normally established for the nutrient from all dietary sources, i.e. food (including fortified foods), beverages (including water) and food supplements. In some cases, the UL may be restricted to specific sources (see Section 5.1). A UL does not take into account adverse effects of acute bolus dosages.

'Tolerable intake' in this context connotes what is physiologically tolerable and can be established based on an assessment of risk, i.e. the probability of an adverse health effect occurring at a specified level of intake. The UL is not a recommended level of intake. As the intake increases above the UL, the risk of adverse health effects increases.

The critical concepts that underpin the definition of a UL are defined below:

**Adverse health effect** (thereafter called **adverse effect):** an effect is considered 'adverse' when 'leading to a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences' (EFSA Scientific Committee, 2017a; FAO/WHO, 2009).

**Biomarker of effect:** 'a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognised as associated with an established or possible health impairment or disease' (EFSA Scientific Committee, 2017a; WHO/IPCS, 1993). Its biological relevance depends on its relation to the mode of action and the linkage with the adverse effect or the relevant adverse outcome pathway (EFSA Scientific Committee, 2017a). In the context of nutrient risk assessment, the observable effects of high nutrient intake can range from biochemical or physiological changes without functional significance (e.g. certain changes in enzyme activity) to irreversible clinical outcomes. Some changes that occur before clinical manifestations could be used as surrogate or predictive markers of subsequent adverse health effects, i.e. biomarkers of effect (see Section 5.2).

**Total chronic daily intake:** average daily nutrient intake over a substantial part of the lifespan, also referred to as the 'usual' or 'habitual' intake of a nutrient. ULs protect from the risks associated with the consumption of nutrients over long periods of time (Section 9.1). Occasional, short-term and/or limited exceedances of the UL will not necessarily result in adverse effects.

**Risk of adverse effect:** probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent (WHO/ICPS, 2004). In the context of a nutrient risk assessment, 'risk' refers to the probability of an adverse effect at a given level of nutrient intake. A theoretical representation of the risk of adverse effects associated with the intake of a given essential micronutrient and the corresponding DRV values is depicted in Figure 2.

[4] https://open.efsa.europa.eu/consultations.

**Threshold:** Regarding the effects of 'excess' nutrient intake, no risk of adverse effects is expected unless a threshold of intake is exceeded. Thresholds for any given adverse effect vary among members of the population, i.e. there is a distribution of individual thresholds within the general population (inter-individual variability in sensitivity). Therefore, ULs should be established by defining a point in the distribution of thresholds that would not lead to adverse effects in the whole population.

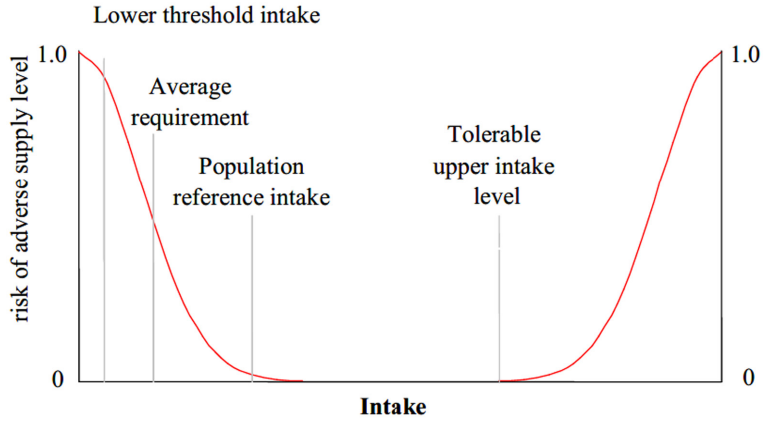


**FIGURE 2** Relationship between individual intake and (cumulative) risk of adverse effects due to 'insufficient' or 'excess' intake. At intakes between the population reference intake (PRI) and the tolerable upper intake level (UL), the risk of inadequacy and the risk of excess are both very low. At intakes below the PRI and above the UL, the risk of adverse effects increases. The definition of an UL assumes the existence of a threshold dose below which the risk of adverse effects due to excess of the nutrient is null, while up to 100% of the population would be affected by the adverse effect of excess when intakes reach a sufficiently high level. It is acknowledged, however, that this model is theoretical and that, in practice, a small residual risk below the UL can never be ruled out in view of the inherent limitations of data.

**Target population:** ULs should be protective for all members of the general population[5] throughout their lifetime.

Adverse effects of excess nutrient intake may be influenced by the changes associated with growth, development and ageing that occur during an individual's lifespan. Therefore, where necessary and to the extent possible, ULs are derived for each separate life-stage group, e.g. infants, children, adults, older adults and women during pregnancy or lactation. Sex-specific values should be established where relevant. The population groups that have been used by the NDA Panel for setting DRVs are proposed as a default (Appendix A). However, the age ranges used for each micronutrient can be adapted on a case-by-case basis depending on the available data.

Even within relatively homogeneous life-stage groups, there is a range of sensitivities to adverse effects. The derivation of ULs accounts for the expected variability in sensitivity among individuals to be protective for the general population. However, the UL may exclude sub-populations with distinct vulnerabilities to adverse effects of nutrient 'excess' due to specific genetic predisposition or other factors (e.g. specific (chronic) medical conditions or use of certain medications). Including those sub-populations would result in ULs that are significantly lower than needed to protect most people of the general population against adverse effects of high nutrient intakes. Sub-populations needing special protection are better served through public health screening, healthcare providers, product labelling or other individualised strategies.[6] The exclusion of such sub-populations must be considered on a nutrient-by-nutrient basis and is an area of scientific judgement and of risk management. It must be based on evidence that the specific genetic predisposition, medical condition or medication can alter the adverse effect(s) of the nutrient under review. In practice, the exclusion of a sub-population from a UL should take into consideration whether individuals from that group can be identified (e.g. through screening, diagnosis).

The UL is not applicable to sub-populations who are receiving the nutrient under medical supervision.[7]

[5]In principle, DRVs are meant for the 'general healthy population' (EFSA NDA Panel, 2010). Yet, the term 'healthy' is imprecise and, due to an ageing population and growing burden of chronic diseases, a significant proportion of the general population may suffer from a variety of conditions. Therefore, the term 'healthy' is omitted from the definition of the target population. Sub-populations with distinct vulnerabilities to adverse effects of a micronutrient may be excluded on a case-by-case basis (see text).

[6]For instance, the safe levels of intake established for iron do not apply to patients with haemochromatosis (EFSA NDA Panel, 2024b) (see Section 6.4.3 for the definition of a safe level of intake); the ULs for vitamin E (α-tocopherol) do not apply to individuals receiving anticoagulant or antiplatelet medications (e.g. aspirin), individuals on secondary prevention for CVD or individuals with vitamin K malabsorption syndromes (EFSA NDA Panel, 2024d).

[7]For instance, the safe levels of intake established for iron do not apply to individuals with iron deficiency anaemia who are on iron treatment (EFSA NDA Panel, 2024b) (see Section 6.4.3 for the definition of a safe level of intake); the ULs for vitamin E (α-tocopherol) do not apply to individuals with ataxia with vitamin E deficiency (AVED) or cholestatic liver disease who are on vitamin E treatment (EFSA NDA Panel, 2024d).

# 5 | PROBLEM FORMULATION AND DEFINITION OF METHODS

The assessment questions underlying a UL evaluation are the following:

- What is the maximum level of total chronic daily intake of the nutrient (from all sources) which is not expected to pose a risk of adverse health effects to humans? (*Hazard identification and characterisation*)
- What is the daily intake of the nutrient from all dietary sources in EU populations? (*Intake assessment*)
- What is the risk of adverse effects related to the intake of the nutrient in EU populations, including related uncertainties? (*Risk characterisation*)

The UL evaluation follows EFSA's scientific assessment process (EFSA, 2020) (Figure 3). As a first step, a protocol is developed to clarify the aim and scope of the assessment (problem formulation) and defines the methods to address the problem. For each UL evaluation, the problem formulation requires the exposure of interest to be specified (Section 5.1) and the relevant endpoints to be identified (Section 5.2), along with the sub-populations of interest, where appropriate (Section 5.3). The assessment questions are broken down into sub-questions that are specific to the nutrient under evaluation. The evidence needs and the methods used to address each sub-question are defined (Section 5.4).

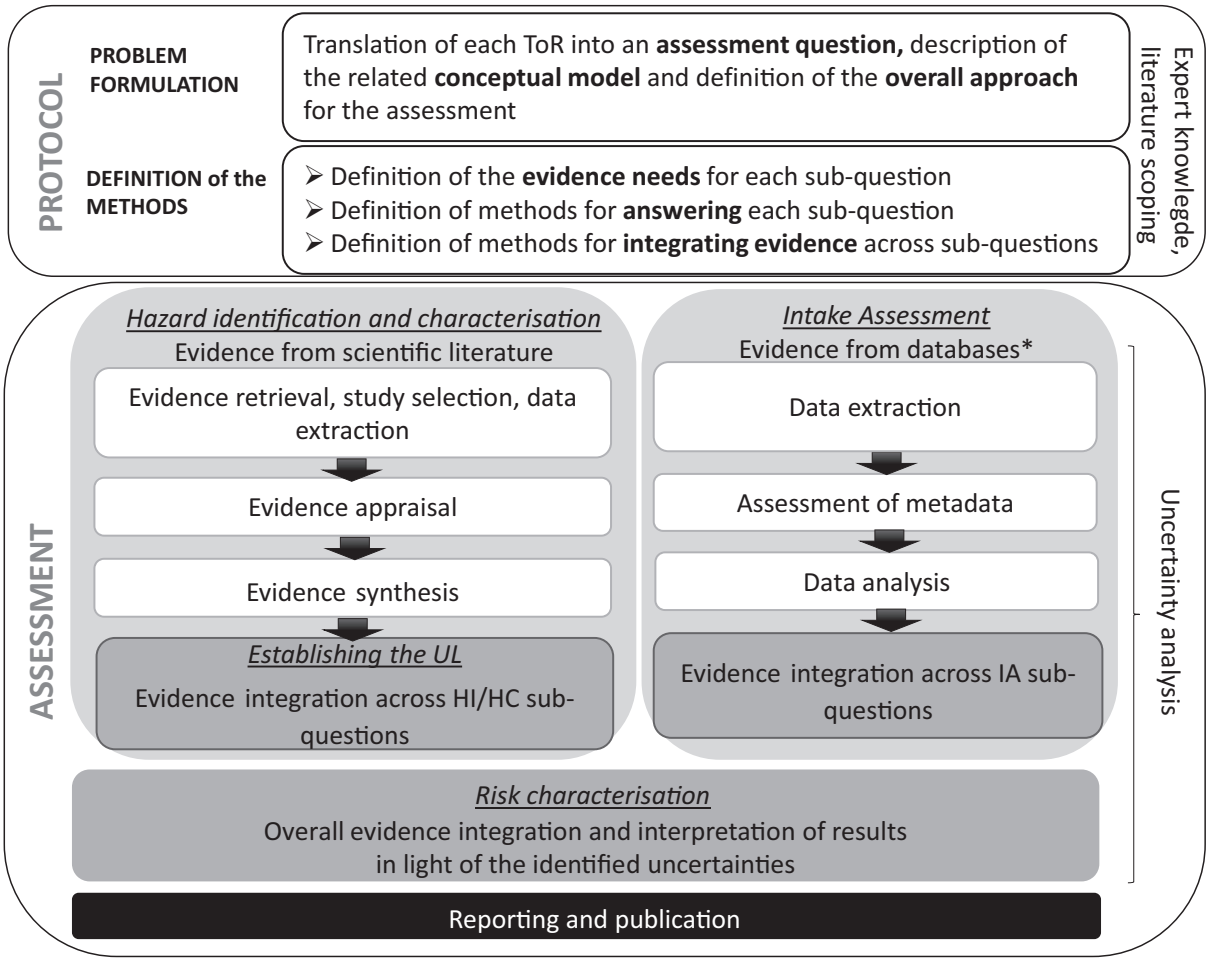


**FIGURE 3** EFSA nutrient risk assessment process. *Data may also be extracted from published intake assessment reports. HC, hazard characterisation; HI, hazard identification; IA, intake assessment; ToR, Terms of Reference; UL, tolerable upper intake level.

## 5.1 | Determination of the exposure of interest

The UL relates to the total chronic daily intake of the nutrient *from all dietary sources*. In practice, a nutrient may exist in a variety of chemical forms within the diet, which may exhibit different properties with regard to absorption, distribution, metabolism, excretion (ADME) and biological functions within the body. Thus, relevant information on the chemical forms of the nutrient and their sources should be considered to define the exposure of interest for the risk assessment. Specific considerations may be required with regard to the bioavailability of the nutrient (or its specific chemical forms), given that this may influence the nature and severity of any adverse effects.

It may be feasible to define a priori the focus of the risk assessment on a specific chemical form of the nutrient (or selected forms), or a particular source of the nutrient from which the specific chemical form originates (e.g. food supplements). In

some cases, the need to derive a UL for specific chemical forms of the nutrient (or dietary sources thereof) may emerge during the process of hazard identification and characterisation. Examples include folate and magnesium, for which ULs specifically apply to folic acid and 5-methyl-tetrahydrofolate salts (EFSA NDA Panel, 2023a), as well as readily dissociable magnesium salts and compounds such as magnesium oxide (SCF, 2001), when these are added to foods or consumed as food supplements. With regard to niacin, separate ULs were established for nicotinamide and nicotinic acid because of their different adverse effect profiles (SCF, 2002).

The chemical forms of a nutrient that are authorised for addition to foods and/or for use in food supplements are those listed in Annex II of Regulation (EC) No 1925/2006,[8] in the Annex to Regulation (EC) No 609/2013[9] and in Annex II of Directive 2002/46/EC.[10] The addition of new forms of vitamins or minerals to the aforementioned Annexes requires an evaluation by EFSA of the safety and bioavailability of these new forms of the micronutrient. Whether the established UL for a micronutrient also applies to its new form(s) is considered in the evaluation of new micronutrient sources (EFSA NDA Panel, 2024a).

## 5.2 | Identification of relevant endpoints

The generic chain of potential events accompanying increasing intake and body content of nutrients is illustrated in Figure 4. The nature of the endpoints relevant to establishing a UL can be diverse, ranging from initial changes in response to excess nutrient intake, to clinical signs and/or symptoms of toxicity or disease endpoints (Appendix B).

Specific to nutrients, the identification of relevant endpoints from homeostatic and adaptive responses to excessive intakes is recognised as a useful approach for nutrient risk assessment ('biological-based model') (EFSA Scientific Committee, 2021b; WHO/FAO, 2006; WHO/IPCS, 2002). Relevant endpoints can be early biochemical changes or biological markers for which a mechanistic pathway can be discerned, and which can be characterised and validated as predictive of adverse effects (e.g. a biomarker of effect).

Figure 4 illustrates the generic chain of potential intake–responses that may occur with increasing chronic intake and body content of nutrients and their metabolites. The physiological regulators and mediators of homeostasis, as illustrated in the left half of the figure, refer to the mechanisms of absorption, distribution, metabolism and excretion (ADME) involved in maintaining a constant body content. As intakes increase, the homeostatic mechanisms become overwhelmed. An increasing body content elicits responses involving, among others, altered metabolism and speciation, and increased deposition of the nutrient and/or its metabolites in tissues (in many instances the liver is the key organ involved in both homeostasis and adaptation). The extent of these responses varies depending on the nutrient in question. Prolonged excessive intake results in overload and adverse effects. Initially these features are reversible, as adverse biochemical and physiological changes are likely to reverse in response to a reduced intake and/or due to adaptive mechanisms in the tissue. However, if a high intake is maintained, phenomena arising from abnormal metabolite production, excess tissue deposition and ultimately ectopic deposition, with resultant tissue and organ damage, and organ failure, will occur. The latter are associated with clinical features, the reversibility of which is uncertain, and which may contribute to overt clinical disease. The time periods over which the different endpoints appear are highly variable; they can extend over decades and often the events occur concurrently.

[8]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. 26–38.

[9]Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

[10]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 (Text with EEA relevance). OJ L 183, 12.7.2002, p. 51–57.

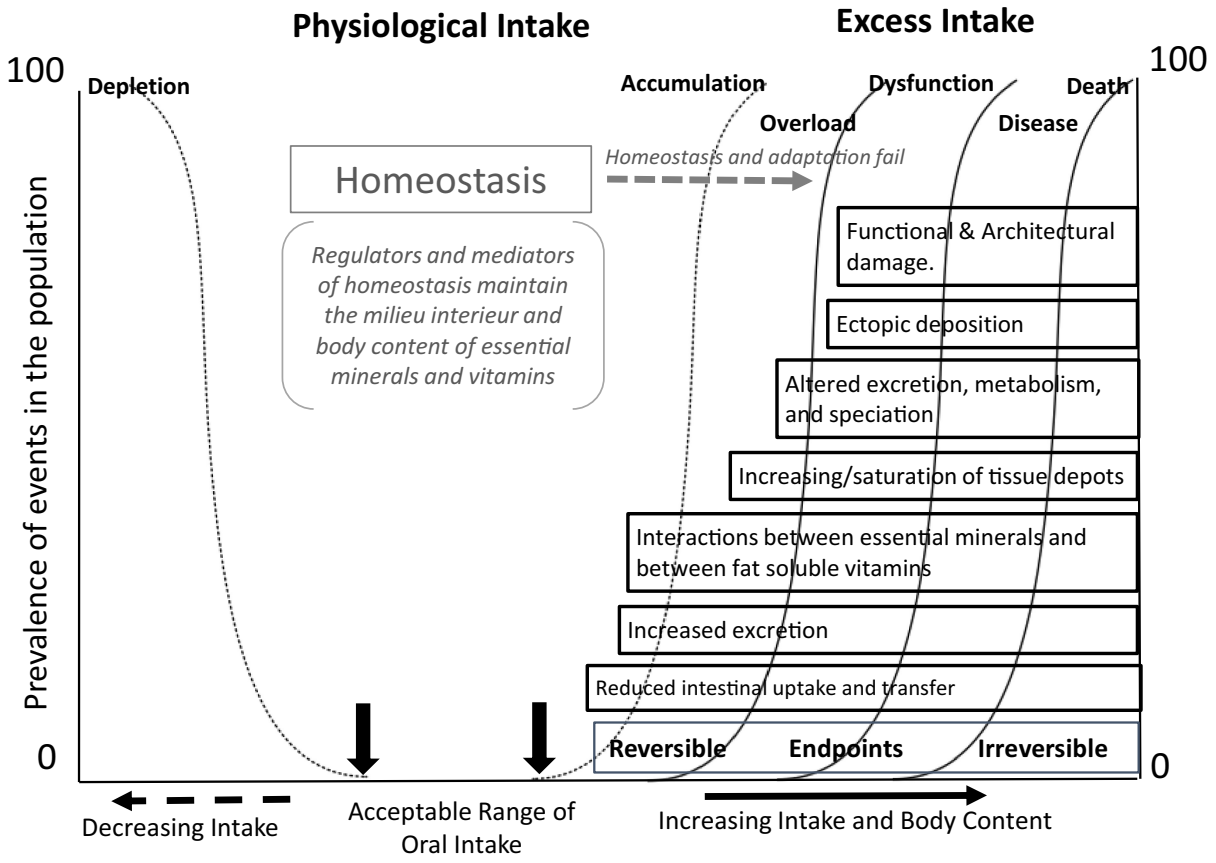


**FIGURE 4** The generic chain of potential intake–responses accompanying increasing chronic intake and body content of nutrients and their metabolites. The boxes describe potential physiological adaptation mechanisms and pathological responses to increasing intakes and the increasing body content of the nutrient being considered. This figure illustrates a schema for the integration of evidence on adverse effects and pathophysiological sequelae which in turn would aid the identification of endpoints as candidate biomarkers and an appreciation of the mechanisms of adverse effects (i.e. mode of action). *(Figure adapted from (*EFSA Scientific Committee, 2021b*))*.

A ranking of biological and toxicological endpoints, based on their severity and potential value in risk assessment, has also been proposed (Renwick et al., 2004; WHO/FAO, 2006). Appendix B provides an overview of endpoints evaluated in recent EFSA opinions on ULs for micronutrients.

Guiding questions for the identification of relevant endpoints are outlined in Figure 5. Prior knowledge of the biological responses resulting from excess nutrient intake is needed to identify relevant endpoints. Evidence typically comes from experimental and/or observational studies in humans. Animal data can also be helpful to identify target organs and pathologies or to describe the sequential development of toxicological endpoints and/or adaptation.

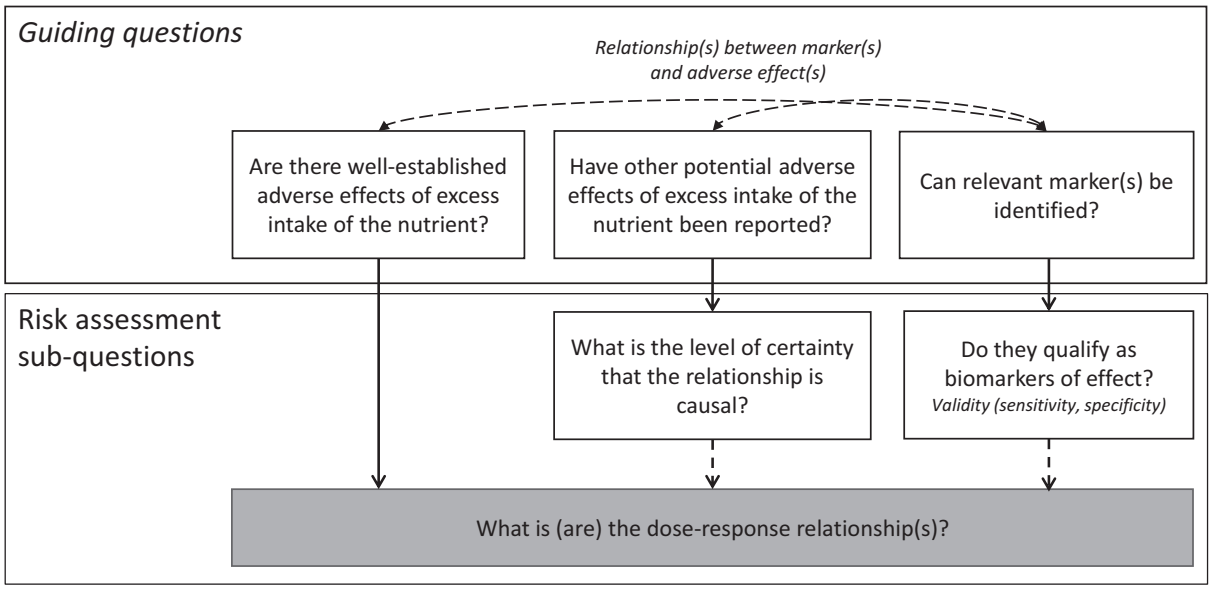


**FIGURE 5** Guiding questions for the identification of relevant endpoints for the risk assessment and the formulation of risk assessment sub-questions. White boxes address the hazard identification, while the grey box addresses the hazard characterisation.

## 5.3 | Identification of relevant sub-populations

ULs should be protective for all members of the general population, throughout their lifetime (i.e. infants, children, adolescents, adults, older adults, pregnant and lactating women). Prior knowledge should be used to identify any life-stage groups of the population or sub-populations particularly relevant for the assessment (e.g. in relation to specific endpoints[11]).

A UL may exclude sub-populations with distinct vulnerabilities due to genetic predisposition or other factors (e.g. specific medical conditions or use of certain medications) (Section 4). Including these sub-populations would result in ULs that are significantly lower than needed to protect the majority of people against the adverse effects of high intakes. This may be identified as part of the problem formulation for individual nutrients, based on prior knowledge. The rationale for the exclusion of specific sub-populations must be documented and reported in the risk characterisation (Section 8).

## 5.4 | Definition of assessment sub-questions and related methods

The assessment questions are subdivided into a series of sub-questions and the methods to address them are defined (Table 1). At the protocol stage, it is beneficial to clarify the logical relationships between the assessment sub-questions (i.e. the definition of a conceptual model) and to ascertain the relative priority of these sub-questions. Sub-questions identified as having higher priority require a greater degree of effort to be answered. This is reflected in the greater burden that the process for data collection, extraction, appraisal/validation, synthesis, integration and uncertainty analysis will consequently bear (EFSA Scientific Committee, 2023a). Sub-questions directly addressing the identification and characterisation of hazards in humans constitute the core of the UL assessment and are typically addressed through systematic reviews. Other methodologies may be employed in addressing the remaining sub-questions.

**TABLE 1** Examples of assessment sub-questions for the evaluation of a tolerable upper intake level

| Risk assessment step | Sub-question | Examples of methods to answer sub-questions[a] |
|---|---|---|
| **HI/HC** | What is the ADME of NUTRIENT X in humans? | Narrative review |
| **HI** | Is there a causal relationship between NUTRIENT X intake and endpoint Y in humans? | Systematic review |
| **HI** | What is the evidence for a relationship between NUTRIENT X intake and endpoint Y in experimental animals? | Narrative review |
| **HI** | What is (are) the potential mode(s) of action underlying the relationship between NUTRIENT X intake and endpoint Y? | Narrative review |
| **HC** | What is the intake–response relationship between NUTRIENT X intake and endpoint Y in humans? | Intake–response modelling |

[11]For instance, the teratogenic effect of preformed vitamin A (EFSA NDA Panel, 2024c).

| Risk assessment step | Sub-question | Examples of methods to answer sub-questions[a] |
|---|---|---|
| **IA** | What is the daily NUTRIENT X intake from all dietary sources in EU populations? | Intake assessment |

Abbreviations: ADME, absorption, distribution, metabolism, excretion; HC, hazard characterisation; HI, hazard identification; IA, intake assessment.

[a]Guidance on protocol development for EFSA generic scientific assessments (EFSA Scientific Committee, 2023a). The choice of the method to answer each sub-question is made on a case-by-case basis. For instance, a systematic review of animal data may be conducted in some cases, e.g. when human data are expected to be insufficient for hazard identification and/or characterisation.

*Prioritisation of sub-questions to be addressed through systematic reviews*

The systematic review method employs a standardised approach to identify and critically appraise relevant research, and to collate, report and analyse data from available studies (EFSA, 2010). The core steps of the systematic review process are depicted in Appendix C. It requires the formulation of a well-structured question, which specifies the relevant population, intervention/exposure, comparator and outcome (e.g. according to the PICO/PECO framework), as well as eligible study designs. The eligibility criteria for studies relevant to UL assessments are presented in Appendix D. The protocol defines the methods that will be used to conduct each step: searching for and selecting studies (evidence retrieval and screening for inclusion or exclusion), collecting data (data extraction), assessing methodological quality of included studies (evidence appraisal), synthesising data (e.g. meta-analysis) (EFSA, 2010; EFSA Scientific Committee, 2023a).

As the process of conducting systematic reviews is resource-intensive, endpoints that are expected to play a critical role in establishing a UL are prioritised (e.g. based on scoping literature searches). The rationale for the prioritisation should be clearly stated and documented.

The selection of priority endpoints should be informed by a consideration of the following:

- The nature of the relationship between the adverse effects and the endpoints in question. Priority endpoints may be clinical outcomes or other relevant endpoints (e.g. biomarker of effect) (Section 5.2).
- The availability of experimental and/or observational data. Sufficient evidence should be available to conclude on the relationship between the intake of the nutrient and the selected endpoint(s) and, ideally, to characterise the intake–response relationship.

Systematic reviews are typically restricted to human studies as they provide the most pertinent data for hazard identification and hazard characterisation. In certain instances, systematic reviews of animal evidence may be conducted, for example, when human studies are expected to be insufficient (e.g. a lack of data to characterise an intake–response relationship) or unavailable (e.g. to investigate specific toxicity endpoints such as reproductive toxicity).

*Use of existing systematic reviews*

A de novo systematic review may not be necessary if a relevant systematic review already exists. The degree to which existing systematic reviews can be used varies depending on several factors, including the alignment between the research question and the assessment question, the methodological quality of the review and the time span covered by the review. The decision as to whether an existing systematic review should be used for the risk assessment, and to what extent, is made on a case-by-case basis.[12]

*Sub-questions addressed through other methods*

Some sub-questions may target animal and/or mechanistic data that are gathered as supportive evidence for the hazard identification. Narrative reviews are usually considered sufficient to address these sub-questions.

In regard to the intake assessment, EFSA typically relies on data drawn from the EFSA comprehensive European food consumption database and the EFSA food composition database (FCDB). Nevertheless, additional data sources may be necessary to address data gaps (e.g. on the contribution of fortified foods and food supplements to the total intake of micronutrients) (Section 7).

*Definition of lines of evidence*

As the body of evidence (BoE) relevant to the assessment of a UL is often complex (i.e. multiple exposure-effect relationships; multiple study designs; multiple species), it can be beneficial to organise it into lines of evidence. This enables the different steps of the assessment (e.g. prioritisation of the risk of bias (RoB) appraisal, step-wise uncertainty analysis) to be tailored and facilitates the integration of the evidence in order to answer the assessment sub-questions (EFSA Scientific Committee, 2017b). Box 1 provides an illustration of the manner in which lines of evidence were defined in the assessment

[12]This has been formalised by other committees through the definition of criteria for the identification of 'qualified systematic reviews' (Arnesen et al., 2020; NASEM, 2023).

of the UL for selenium (EFSA NDA Panel, 2023c). These principles have been applied to the assessment of the UL for other vitamins and minerals (EFSA NDA Panel, 2023a, 2023b, 2023c).

**Box 1 Definition of lines of evidence: example of the assessment of the UL for selenium**

For assessment of the UL for selenium, several sub-questions (sQ), which addressed specific exposure–health outcome relationships, were defined for the hazard identification (EFSA NDA Panel, 2023c). Within each sQ, randomised controlled trials and prospective cohort/case-cohort studies were organised in separate lines of evidence (LoE), which were then classified in the following hierarchical order:

- **Standalone (main) line of evidence**: Studies on disease endpoints. These studies could, on their own, answer the sQ directly.
- **Standalone (surrogate) line of evidence**: Studies on endpoints which are surrogate measures of the disease risk. These studies also could, on their own, answer the sQ, on the assumption that a sustained increase in the surrogate measure over time would eventually lead to an increased risk of disease. However, the Panel is aware of the uncertainty inherent in this assumption and this will be considered in the overall uncertainty analysis for each sQ.
- **Complementary line of evidence**: Studies on endpoints which are relevant to the disease but less direct than those included in standalone LoE (e.g. risk factors, upstream indicators, other biologically related endpoints). These studies, on their own, cannot answer the sQ but can be used as supporting evidence to the standalone LoEs.

**Table**. Examples of standalone and complementary lines of evidence.

| Health outcome | Type 2 diabetes | Hypertension | Thyroid diseases |
|---|---|---|---|
| **Standalone main LoE** | Incidence of type 2 diabetes | Incidence of hypertension | Incidence of hypothyroidism<br>Incidence of hyperthyroidism |
| **Standalone surrogate LoE** | Measures of glucose tolerance | Measures of blood pressure | Measures of thyroid hormones |
| **Complementary LoE** | • Indices of insulin sensitivity/ beta-cell function<br>• Measures of insulin sensitivity | | |

## 6 | HAZARD IDENTIFICATION AND CHARACTERISATION

Following the planning phase, the hazard identification and characterisation steps are implemented (Figure 3).

### 6.1 | Evidence retrieval, study selection, data extraction

Systematic reviews are the preferred method for addressing sub-questions regarding the relationship between high intakes of a nutrient and adverse effects (and/or related biomarkers) (Section 5.4). In case of a de novo systematic review, the process is implemented according to the methods specified in the protocol (Section 5.4). The relevant studies are retrieved and selected by applying the pre-defined search strategy and eligibility criteria. The number of studies selected for inclusion at each stage of the screening process are reported in the scientific opinion (e.g. in a flow chart).

For each eligible study, the relevant characteristics and findings are extracted in a standardised format (e.g. evidence tables). This typically includes the study design, key elements (e.g. population, intervention/exposure, comparator, outcomes (endpoints), setting and duration), results and aspects relating to the internal validity of the studies (e.g. confounders, randomisation).

### 6.2 | Evidence appraisal

The appraisal of the internal validity or risk of bias (RoB) of eligible studies is a key element of the uncertainty analysis. Internal validity refers to the extent to which a piece of evidence provides an unbiased estimate of the causal association between exposure and outcome, i.e. the extent to which the study results reflect the 'truth' among the study population (EFSA Scientific Committee, 2020). For a given study, assessment of internal validity refers to the evaluation of its design and conduct, particularly in terms of the likelihood, magnitude and direction of possible biases.

The internal validity of individual studies (RoB) is evaluated using a critical appraisal tool (CAT). CATs are structured checklists that facilitate the identification of potential threats to the internal validity of studies by employing a set of criteria

(EFSA Scientific Committee, 2020). Specific tools are available to appraise RoB relevant to different study designs (e.g. NTP OHAT CAT (OHAT-NTP, 2015), Cochrane RoB-2 (Sterne et al., 2019), Cochrane ROBINS-E (Higgins et al., 2024), NESR RoB-NObs (NESR, 2019). Such tools facilitate the formulation of RoB judgements on RoB domains identified as critical for each study design. The tool developed by NTP OHAT has the advantage of proposing a unique framework applicable to the various study designs relevant for UL assessment and was used in previous UL assessments (EFSA NDA Panel, 2023d, 2023e, 2023a, 2023b, 2024b, 2024c).

The process of appraising RoB through the use of structured CATs is a time-consuming and resource-intensive endeavour. Based on available resources, it may be necessary to restrict the RoB appraisal to the lines of evidence identified as the most critical for the conclusions of the assessment.

The outcome of the critical appraisal is reported in the scientific opinion. Risk of bias is among the critical sources of uncertainty considered in the formulation of causal inferences for the hazard identification (Section 6.4.1).

## 6.3 | Evidence synthesis

The amount and diversity of studies available on a specific sub-question determines the type of evidence synthesis that is appropriate (i.e. narrative synthesis, visual presentation, meta-analysis, intake–response modelling).

When several studies report on the same endpoint, results can be displayed in descriptive forest plots. The effect measures and confidence intervals of individual studies are provided, along with key study characteristics, e.g. variables which may contribute to the heterogeneity of the results (Table 2). Descriptive forest plots are a valuable tool for visually presenting evidence on a specific endpoint and for assessing the consistency of results across studies.

The evidence may be synthesised through a meta-analysis to estimate a pooled effect size (estimated average effect size) and related confidence interval. Strengths of meta-analyses include their ability to increase the statistical power and the precision of effect estimates and to provide a summary of the strength and consistency of the evidence, which are important elements in judging on a causal relationship between the exposure and the relevant endpoint (Section 6.4.1). The decision to combine study results should consider whether the studies are sufficiently similar in terms of study populations, interventions/exposures and outcomes to allow a meaningful interpretation of the summary estimate. As studies relevant for nutrient risk assessment are often disparate and rarely specifically designed to investigate the nutrient-endpoint relationship under assessment, this requires careful consideration. Meta-analyses of very diverse studies can be misleading and narrative synthesis and/or a visual presentation of the evidence are more appropriate approaches in such cases.

The issue of heterogeneity requires careful consideration and interpretation, particularly in cases where there is variation in the direction of effect or associations. Examples of methodological and contextual sources of heterogeneity are provided in Table 2. A statistical test for heterogeneity is available ($\chi^2$ or chi-squared test), which assesses whether observed differences in results are compatible with chance alone (Deeks et al., 2023). This test can be performed with a minimum of three studies; however, due to its low power, caution should be exercised when interpreting the results in the presence of a limited number of studies or a small sample size. A greater number of studies is necessary to characterise sources of heterogeneity, e.g. through subgroup analyses or multivariable meta-regression. The use of prediction intervals from random-effects meta-analyses represents a valuable approach for the presentation of the extent of between-study variation.

If the nature and extent of the data allow, data modelling should be used for the characterisation of the intake–response between the nutrient intake and the occurrence/level of the endpoint of interest. Intake–response meta-analyses can be valuable in describing the shape of the relationship (e.g. linear or non-linear; monotonic or not) and for its quantification. The choice of the modelling method must be made on a case-by-case basis, depending on the nature of the data. This requires considerations of multiple elements, including:

- the study design, i.e. controlled experimental data versus observational data (which typically require adjustment for potential confounders and accounting for potential modifiers);
- the type of endpoint (e.g. biological parameter, measure of incidence) and the type of the response variable (i.e. dichotomous, categorical, count, continuous);
- the use of individual vs. aggregated data;
- the interpretability and usability of the model for the purpose of risk assessment (i.e. determination of a reference point, see Section 6.4.2).

The selection of an appropriate approach requires the input of technical support and expertise, taking account of methodological developments in the field (Vinceti et al., 2020). Mechanistic data can help to interpret the biological plausibility of the intake–response shape.

Sensitivity analyses should be conducted where possible to examine the influence of specific assumptions, methodological choices and individual studies on the results of the analyses.

**TABLE 2** Examples of methodological and contextual sources of heterogeneity across studies.

| **Methodological sources of heterogeneity** *variability in study design and conduct* | **Contextual sources of heterogeneity** *variability in the populations studied, the interventions/exposures involved and the endpoint measured* |
|---|---|
| • Study design<br>• Study duration<br>• Method/tool/diagnostic criteria applied to measure the outcome<br>• Method/tool used to measure the intake/exposure<br>• Metrics used to estimate the effect or association (e.g. hazard ratios, risk ratios, odds ratios)<br>• Risk of bias | • Characteristics of study participants (e.g. age, sex, health status, ethnicity)<br>• Variability in the intake/exposure (e.g. dose, form, timing, frequency, compliance)<br>• Variability in the endpoint (e.g. severity) |

## 6.4 | Evidence integration and conclusions

The evidence collected is integrated to identify critical effects (Section 6.4.1) and intake–responses which can be used as a basis for establishing the UL (Section 6.4.2). Alternative approaches are needed when the evidence is insufficient to establish a UL (Section 6.4.3) or when no hazard is identified (Section 6.4.4).

### 6.4.1 | Hazard identification

The process of hazard identification consists of the identification of the type and nature of adverse effects that an excess intake of the nutrient in question can cause (Figure 1).

Some adverse effects of nutrients are well-established in the scientific literature. These are identified at the problem formulation step (Section 5.2). In such cases, the assessment focuses on the characterisation of the intake–response relationship (Section 6.4.2).

For other nutrient–endpoint relationships, judgement about causality is required. This must account for the uncertainties identified in the eligible BoE. A weight of evidence approach is necessary, integrating data from all relevant lines of evidence. Consistent findings across different study designs, supportive evidence from complementary lines of evidence (see Box 1), evidence of intake–response relationships, give added weight to the hazard identification.

In past UL evaluations (EFSA NDA Panel, 2023c, 2023a, 2024b), the OHAT-NTP framework for formulating hazard identification conclusions (OHAT-NTP, 2019) has been used and adapted:

- The initial level of certainty assigned to the BoE on the nutrient–endpoint relationship under review is based on the study design. In accordance with the OHAT framework, a rating of 'high' confidence is assigned to human controlled trials (HCTs), a rating of 'moderate' confidence is assigned to prospective cohort studies (PCs) and a rating of 'low' confidence is assigned to case series/reports (OHAT-NTP, 2019).
- This initial rating is subsequently downgraded on the basis of factors that decrease certainty in the results (i.e. RoB, unexplained inconsistency, indirectness or lack of applicability, imprecision and publication bias) and upgraded for factors that increase certainty in the results (i.e. large magnitude of effect, evidence for an intake–response association, consistency across study designs/populations/animal models or species and consideration of residual confounding or other factors that increase the certainty in the causal nature of the relationship) (Table 3).
- As probability is the preferred means for expressing uncertainty (EFSA Scientific Committee, 2018), the 'confidence ratings' assigned by OHAT were translated into 'levels of certainty' expressed as approximate probability ranges. These correspond to four levels of certainty: 'high' (> 75%–100% probability), 'moderate' (> 50%–75% probability), 'low' (> 15%–50% probability) and 'very low' (0%–15% probability). This standard scale facilitates the formulation of experts' judgement about the causality of the relationship and convey their level of certainty in the evidence.
- The overall conclusion is formulated by considering the consistency of the evidence across study designs (i.e. consistent evidence could result in a higher level of certainty on the causality of a positive relationship), as well as mechanistic or mode of action data (i.e. strong support or no support for biological plausibility could result in higher or lower certainty on the causality of the positive relationship, respectively).

The formulation of hazard identification conclusions is inherently a matter of scientific judgement. The value of this framework lies in its reliance on a reproducible and transparent methodology for expressing uncertainty in both the evidence and the methods employed.

**TABLE 3** Approach applied to assign the final level of certainty in a causal relationship.

| Initial level of certainty for a causal relationship by study design | Factors decreasing certainty | Factors increasing certainty | Final level of certainty for a causal relationship[a] |
|---|---|---|---|
| **High:**<br>> 75%–100% probability HCTs<br>**Moderate:**<br>> 50%–75% probability<br>PCs/NCCs (*assessing the exposure prior to the endpoint)*<br>**Low:**<br>> 15%–50% probability<br>Case series/case reports<br>**Very low:**<br>0%–15% probability | • RoB across studies (limitations to internal validity)<br>• Unexplained inconsistency (heterogeneity)<br>• Indirectness<br>• Imprecision<br>• Publication bias | • Large magnitude of the effect (or a strong association/response)<br>• Intake–response (monotonic or not)<br>• Residual confounding<br>(i) Studies report an effect and residual confounding is toward the null<br>(ii) Studies report no effect and residual confounding is away from the null<br>• Consistency (across endpoints in standalone LoEs) | **High:**<br>> 75%–100% probability<br>**Moderate:**<br>> 50%–75% probability<br>**Low:**<br>> 15%–50% probability<br>**Very low:**<br>0%–15% probability |

*Adapted from* OHAT-NTP *(2019)*.

Abbreviations: HCT, human controlled trial; LoE, line of evidence; NCC, nested case–control study; PC, prospective cohort study; RoB, risk of bias.

[a]As an example, a 'high level of certainty' means that, based on the available evidence, experts are 75%–100% certain that intake of the nutrient is causally associated with the adverse effect of interest.

## 6.4.2 | Hazard characterisation

The hazard characterisation comprises the qualitative and quantitative evaluation of the nature of the adverse effects associated with a nutrient. This includes an intake–response assessment, i.e. determining the relationship between the nutrient intake (dose) and the adverse effect. It allows the derivation of a threshold of intake above which adverse effects may occur, i.e. the UL. When the intake–response is unknown, the threshold is approximated through the selection of a reference point (RP) to which uncertainty factors are applied.

### 6.4.2.1 | *Selection of a reference point*

Based on the conclusions of the hazard identification step, endpoints which are biologically relevant for the assessment are considered for the identification of a RP. Key considerations for the assessment of intake–responses are outlined in Box 2.

**Box 2 Key considerations for the assessment of intake–responses**

For each relevant endpoint:

- Map data on intake–response within studies (multiple dose studies) and across studies (i.e. consistency across studies which investigated similar intake levels).
- Consider whether data are suitable for intake–response modelling; data modelling may be applied to an individual study or several studies (e.g. using intake–response meta-analysis techniques).
- To the extent possible, integrate the total intake of the nutrient (e.g. including from background intake) into the assessment of the intake–response.
- Consider the impact of the frequency and duration of intake.
- Consider the impact of the chemical forms and sources of intake.
- In case a biomarker of intake is used, consider its specificity (e.g. can it be affected by the underlying condition?) and the possibility to predict dietary intake therefrom (e.g. availability of validated prediction equations).
- Consider the characteristics of the study populations and study settings; discuss external validity/generalisability of results.
- Discuss uncertainties related to e.g. the intake range covered by available data, the reliability of methods to measure intake and response.

Whenever possible, intake–response modelling should be used. The determination of the RP from an intake–response model will require case-by-case considerations, depending on its nature and interpretation. The general principles of the benchmark dose (BMD) approach can be considered, where: (1) the use of all available dose (intake)-response data is recommended; (2) the specification of a response level considered as adverse is required; (3) the dose (intake) of concern is estimated from the fitted dose (intake)–response curve associated with the specified response level; (4) the RP identification is based on the dose (intake) identified in step 3 taking into account the associated uncertainties (EFSA Scientific Committee, 2022; EFSA Scientific Committee, 2024).

The methodology described in the EFSA guidance on BMD was developed for setting RP for chemicals in the context of animal experimental data (EFSA Scientific Committee, 2022). The implementation of the recommended methodology

requires adaptations when dealing with nutrients.[12] The definition of a benchmark response (BMR) as a relative change in response in the exposed vs. unexposed groups cannot be directly used since minimum intakes are required for nutrients. In addition, the nature of the data might be different (e.g. aggregated data on humans), multiple studies could be available, the use of a biomarker as a surrogate for the endpoint could be needed. All these circumstances occurred when deriving the UL for vitamin D in infants (EFSA NDA Panel, 2018). This example provides one illustration of an application of the approach to derive RPs based on intake–response modelling (Appendix E).

Notably, since the UL should be protective for all individuals in the target population, inter-individual variability in the response must be taken into consideration. This may be addressed through the model (e.g. using bounds of the prediction interval of the response) or by other approaches, such as simulation methods (e.g. EFSA's assessment of the UL for vitamin D in infants, Appendix E). Alternatively, an uncertainty factor may be applied to the RP derived from the predicted mean or median response to cover for inter-individual variability (Section 6.4.2.2).

When data are not suitable for intake–response modelling or knowledge is insufficient to set a level of the response that can be considered biologically relevant, a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL) may be identified and used as the RP. Careful consideration should be given in this case to the uncertainties stemming from the design of the study (e.g. NOAEL/LOAEL must be one of the intake levels in the studies, which is dependent on the intake levels selected by the investigators) and lack of quantification of the dose (intake)–response curve.

The determination of UL based on either the intake–response models or NOAEL (or LOAEL) approach implies that experimental or observational data of sufficient quality are available over a range of intakes which encompasses levels eliciting adverse effects.

(EFSA Scientific Committee, 2022; FAO/WHO, 2009, 2020)(EFSA NDA Panel, 2018) Where several adverse effects (or related markers) are identified, a critical effect needs to be selected (e.g. the effect occurring at the lowest dose). This needs expert judgement and integration of the totality of the evidence, considering the reliability, relevance and consistency of the data available (weighing of the evidence) (EFSA Scientific Committee, 2017b).

The rationale for the RP used as a basis for the UL should be documented, including information on the underlying assumptions and uncertainties.

#### 6.4.2.2 | *Application of uncertainty factors*

Following the identification of a RP, adjustments for uncertainty are applied to establish a UL which is protective for the general population. The overall uncertainty factor (UF) covers for the expected variability in sensitivity among individuals and accounts for the uncertainty associated with extrapolating from the observed data to the general population.

The UL is derived as follows:

$$UL = \frac{RP}{UF},$$

where RP is the selected reference point and UF is the overall uncertainty factor (Figure 6). The greater the uncertainty, the larger the uncertainty factors and the lower the UL, which represents a lower estimate of the threshold above which the risk of adverse effects may increase (Figure 6).

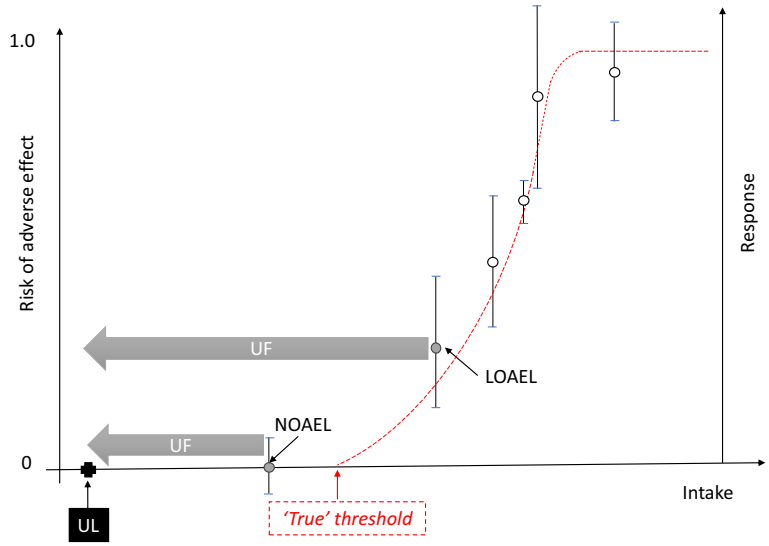


**FIGURE 6** Illustration of the derivation of an UL based on a NOAEL or a LOAEL as a reference point. The dashed line represents the unknown 'true' intake–effect relationship. The response may be a measure of biological response or a measure of risk in the studied population. Dots represent the empirical data collected. Data are used to identify a reference point, e.g. a NOAEL or a LOAEL in order to approximate the 'true' threshold dose. Either the NOAEL or LOAEL is divided by an UF to establish the UL, i.e. the maximum level of total chronic daily intake of a nutrient which is not expected to pose a risk of adverse health effects to humans. UFs can differ depending on the reference point (NOAEL or LOAEL). LOAEL, lowest observable adverse effect level; NOAEL, no observable adverse effect level, UF, uncertainty factor; UL, tolerable upper intake level.

The following sources of variability and uncertainty are generally considered: human inter-individual variability, use of a LOAEL in the absence of a NOAEL, duration of the exposure and (for animal data) inter-species differences. Depending on the available body of evidence, one or more of these elements may be relevant and combined in the overall UF. Default UFs recommended by the EFSA Scientific Committee for chemical risk assessment are presented in Box 3 (EFSA Scientific Committee, 2012).

**Box 3 Default uncertainty factors recommended by the EFSA Scientific Committee**

For chemical risk assessment, default UFs are recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2012).

- *Human inter-individual variability*: in the absence of data to characterise inter-individual variability, a default uncertainty factor of 10 is proposed.
- *Use of a LOAEL in the absence of a NOAEL*: where a NOAEL is not available, an uncertainty factor may be applied to account for the uncertainty in deriving a UL from a LOAEL. There is no default UF to take into account the absence of a NOAEL. The Scientific Committee recommended a case-by-case approach based on the available dataset. The size of the uncertainty factor involves a judgement based on the severity and incidence of the observed effect at the LOAEL, and the steepness (slope) of the intake–response.
- *Extrapolation for exposure duration*: an UF may be required if there is concern about extrapolating results obtained over the duration of the available studies to chronic (long-term) exposure, e.g. whether the critical effect is expected to occur at a lower dose if the study duration was extended. A default UF of 2 for extrapolation from sub-chronic to chronic exposure is applicable to toxicity studies in rodents. There is no default value for the extrapolation of exposure duration in human studies.
- *Extrapolation from experimental animals to humans*: when the RP is identified from animal data, an UF is applied to account for inter-species variation in toxicokinetics and toxicodynamics. Where relevant specific data on kinetics and/or dynamics are available, a physiologically based kinetics (PBK) modelling approach is recommended to set this factor (chemical specific adjustment factor). In the absence of such data, a default factor of 10 is applied.

For nutrients, a case-by-case approach that takes into account the essential role of the nutrient and homeostatic mechanisms is required, to avoid establishing ULs that run the risk of causing nutrient deficiency or sub-optimal function (EFSA NDA Panel, 2010; WHO/FAO, 2006).

However, applying conservative default UFs could result in establishing ULs that run the risk of causing nutrient deficiency or sub-optimal function (EFSA NDA Panel, 2010; WHO/FAO, 2006). A case-by-case approach is required, which takes into account the essential role of the nutrient (intake requirements) and homeostatic mechanisms (Figure 4). The following elements are typically considered:

- The number, size and diversity of available human studies, which can cover part of the variability inherent in the population.
- Actual data on the inter-individual variability in the kinetics and dynamics of the nutrient in humans and current knowledge of homeostatic and adaptive mechanisms and ADME processes (Figure 4).
- The severity and nature of the selected critical effect, i.e. a lower UF may be applied if the effect is mild and reversible (e.g. early marker, as discussed in Section 5.4).
- Information on kinetics and dynamics that indicate a risk of accumulation of the dose and/or effects with longer-term exposure (EFSA NDA Panel, 2023d).
- In case the RP is derived from animal data, available nutrient-specific data on kinetics and/or dynamics in the experimental species compared to humans (EFSA FAF Panel, 2019; EFSA Scientific Committee, 2012; Smeraldi et al., 2020).

Table 4 illustrates the case-by-case approach applied for the selection of UFs based on examples from recent risk assessments. The selection of UFs is a matter of scientific judgement based on the available information and considerations listed above. A rationale summarises the key considerations which underpin the selection and is documented in the scientific opinion.

**TABLE 4** Examples of uncertainty factors applied in EFSA's risk assessments of vitamins and essential minerals and their rationale.

| | Reference point | | | Overall UF | Rationale[a] | HBGV, in adults |
|---|---|---|---|---|---|---|
| **Vitamin D** (EFSA NDA Panel, 2023e) | Human | LOAEL | 250 µg/day | 2.5 | • To account for the use of LOAEL as RP.<br>• Persistent hypercalciuria is an early marker of adverse events and is reversible with vitamin D and/or calcium supplements withdrawal.<br>• UL of 100 µg/day supported by a large body of evidence from RCTs where no evidence for persistent hypercalcaemia or hypercalciuria was found at supplemental doses of 100–125 µg/day. | UL 100 µg/day |
| **Vitamin B6** (EFSA NDA Panel, 2023d)6 | Human | LOAEL | 50 mg/day | 4 | • UF of 2 to account for the inverse relationship between dose and time to onset of symptoms of peripheral neuropathy.<br>• UF of 2 to account for the use of LOAEL as RP and uncertainties related to inter-individual variability in sensitivity. | UL 12 mg/day[b] |
| | Animal | LOAEL | 50 mg/kg bw/d | 300 | • UF of 2 to account for inter-species variability in TK considering similarities in vitamin B6 excretion between dogs and humans.<br>• UF of 2.5 for inter-species variability in TD.<br>• UF of 10 to account for intra-human variability in TK and TD.<br>• UF of 2 to extrapolate from sub-chronic to chronic exposure.<br>• UF of 3 to account for the use of LOAEL as RP. | |
| **Selenium** (EFSA NDA Panel, 2023c) | Human | LOAEL | 330 µg/day | 1.3 | • Data lacking to characterise the steepness of the dose–response curve. However, when compared to controls, an excess of less than 1% of the selenium supplemented participants exhibited alopecia in the SELECT, possibly indicating that the NOAEL might not be far from the LOAEL derived from that study.<br>• Alopecia is an early sign of selenium toxicity, is of mild nature and likely to be reversible.<br>• Lack of data in women but no indication that women may be more susceptible than men to selenium toxicity.<br>• The choice of an UF of 1.3 is based on expert judgement and is a pragmatic choice which allows to extrapolate the value for adults to infants and children. | UL 255 µg/day |
| **Copper** (EFSA Scientific Committee, 2023b)[c] | Human | NOAEL | 5 mg/day ~0.07 mg/kg bw/day | 1[d] | • Copper retention in the body, particularly in the liver, is an early and sensitive indicator of potential future toxicity.<br>• An HBGV based on evidence of retention as predictor of future toxicity is conservative and therefore sufficiently protective for most individuals over long-term intake. No additional uncertainty factor is considered necessary. | ADI[e] 0.07 mg/kg bw/day |

Abbreviations: ADI, acceptable daily intake; bw, body weight; HBGV, health-based guidance value; LOAEL, lowest-observed-adverse-effect-level; NOAEL, no-observed-adverse-effect-level; TD, toxicodynamics; TK, toxicokinetics; UF, uncertainty factor; UL, tolerable upper intake level; µg VDE, µg vitamin D equivalent.

[a]The selection of UFs is made on a case-by-case basis and is a matter of scientific judgement based on the available information. The rationale summarises the key considerations.

[b]The value derived from the study in humans was similar to the value derived from the study in dogs, which increased the confidence in the resulting UL. The UL was established at the midpoint of the two values and rounded down.

[c]Given its cross-cutting nature, the assessment was carried out by the EFSA Scientific Committee.

[d]Applying an overall UF of 1.0 expresses confidence that, based on the available body of evidence, intakes of the nutrient up to the identified RP are not expected to pose a risk of adverse health effects for the general population and the RP can thus be used as the UL.

[e]Regarding the similarity between ADI and UL, see EFSA Scientific Committee statement on the derivation of HBGVs for regulated products that are also nutrients (EFSA Scientific Committee, 2021b).

#### 6.4.2.3 | *Exclusion of specific sub-populations from the UL*

A UL may exclude sub-populations with particular sensitivities to the adverse effects of the nutrient, due to genetic predisposition or other factors (e.g. specific medical conditions or use of certain medications). Such sub-populations may be identified through the review of the evidence. At this step, a decision may be made to exclude these groups from the target population of the UL, following the principles outlined in Section 4. Decisions to exclude specific sub-populations should be documented and this should be stated in the risk characterisation (Section 8).

#### 6.4.2.4 | *Scaling approaches*

Data for age groups other than adults may be scarce. Therefore, it is often necessary to establish ULs for younger age groups by extrapolating from the UL for adults. Isometric (i.e. proportional to body weight) or allometric (i.e. proportional to a power of body weight) scaling are commonly used (Box 4; Annex A – Workshop report). These approaches are based on the assumption that the set of physiological and metabolic processes underlying the response to nutrient intake is common across all age groups, and that these processes differ quantitatively in a way that is mostly a matter of scale (i.e. as a function of body size).

The choice of the scaling approach is based on scientific judgement on a case-by-case basis. Several considerations are taken into account, including any evidence of increased susceptibility to adverse effects at younger ages, knowledge of differences in kinetics and dynamics between age groups, or concerns about long-term accumulation. The Panel considers that allometric scaling based on metabolic weight (defined as $BW^{0.75}$) is preferable to adjust for metabolic differences between age groups, as it accounts for the higher metabolic rate of children compared to adults. Isometric scaling is more conservative as it is directly proportional to body weight. The difference between the scaling methods decreases with increasing age (Appendix A). In particular, these scaling approaches do not account for differences in nutrient requirements, e.g. related to children's growth. How close the results of the calculations are to the physiological requirements of children must also be considered (EFSA NDA Panel, 2023e, 2023a).

**Box 4 Equations applied for scaling**

*Isometric scaling:* the UL for population group *X* is the product of the known UL for group *Y* and a factor that is the quotient between the reference body weight of group *X* and the reference body weight of group *Y*:

$$UL_X = UL_Y \times \left(\text{body weight}_X / \text{body weight}_Y\right).$$

*Allometric scaling:* the UL for population group *X* is the product of the known UL for group *Y* and a factor that is the quotient between the reference body weight of group *X* and the reference body weight of group *Y* to a given power. Allometric scaling to the power of 0.75 reflects that the metabolic rate of an organism largely depends on its lean body mass, which is an exponential function of body weight.

$$UL_X = UL_Y \times \left(\text{body weight}_X / \text{body weight}_Y\right)^{0.75}.$$

### 6.4.3 | Approaches when there are insufficient data to establish a UL

For nutrients for which there are insufficient data on which to base the UL, the European Commission has requested the Panel to provide 'an indication […] on the highest level of intake for which there is reasonable confidence on the absence of adverse effects', drawing from the totality of the available evidence. The Panel referred to this as a safe level of intake (EFSA NDA Panel, 2023b, 2024b).

A safe level of intake is defined when there is a lack of data to characterise the intake–response relationship or to identify a suitable RP that allow to derive (or approximate) a threshold of intake above which the risk of adverse effects begins to increase. Different from a UL, a safe level of intake is based on data which characterise levels of intake *up to* which no adverse effects have been observed (Figure 7). A safe level of intake may be based on the highest dose at which no adverse effects are observed in human studies (EFSA NDA Panel, 2012); on the 95th percentile of the observed daily intake in a population group (or groups) of apparently healthy individuals that is assumed to be safe (EFSA NDA Panel, 2023b); or on other conservative approaches (EFSA NDA Panel, 2024b).

Both ULs and safe levels of intake are intended to protect the general population against potential adverse effects related to 'excess' nutrient intakes. The setting of a safe level of intake indicates that more research is needed to determine, with some degree of certainty, a threshold for the adverse effect(s) of the nutrient and thereby to establish a UL. Because of the uncertainties involved, a safe level of intake has more limited applications than a UL (Section 8).

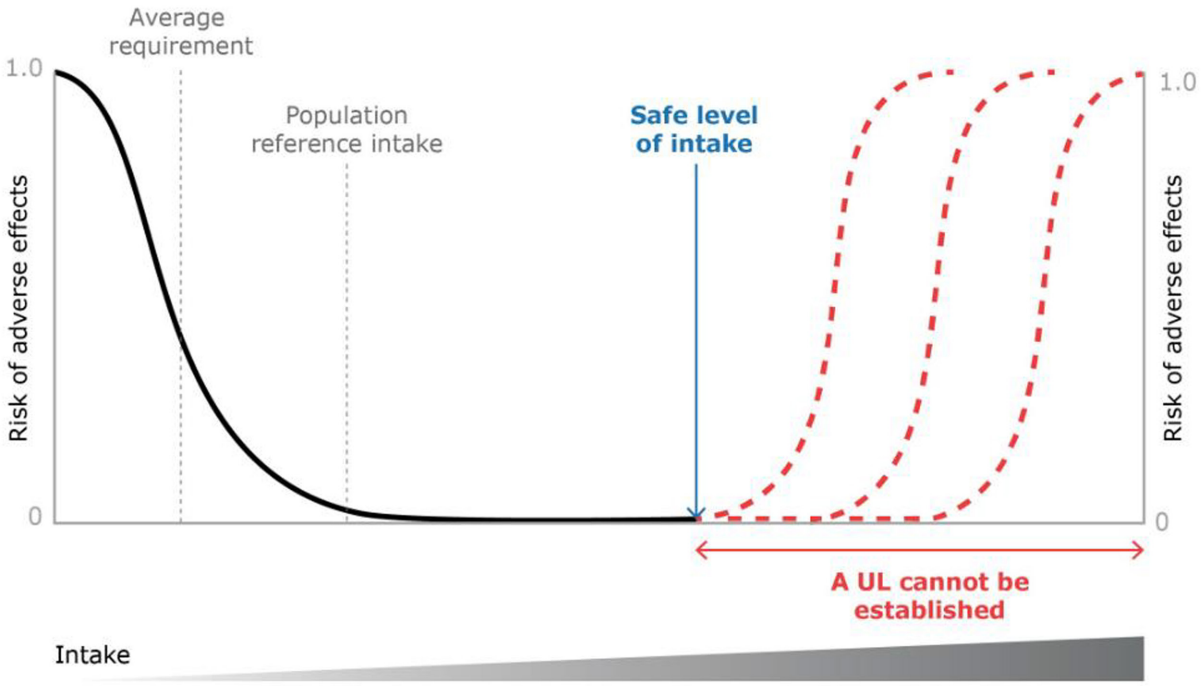


**FIGURE 7** Theoretical representation of a safe level of intake. The safe level of intake is the 'highest level of intake where there is reasonable confidence in the absence of adverse effects'. It is established when data are insufficient to establish a UL, i.e. data do not allow to approximate the threshold for adverse effect(s) with sufficient confidence. Thus, a safe level of intake reflects the high level of uncertainty around the characterisation of the dose–response(s) between the intake of the nutrient and adverse effect(s) (red dotted lines).

### 6.4.4 | Approaches when no hazard is identified

There are nutrients for which, on the basis of the available data (e.g. toxicity data, intake estimates), no adverse effects related to 'excess' intake have been identified. In such cases, a UL is not specified (i.e. UL 'not determined').[13]

## 7 | INTAKE ASSESSMENT

Intake assessment in the field of nutrients aims to characterise dietary intake by combining data on the content of the nutrient in foods and beverages with the quantity of these foods and beverages consumed. However, a comprehensive characterisation of the risks associated with the dietary intake of a nutrient requires a complete intake assessment from all dietary sources, i.e. accounting for the natural nutrient content of foods as well as any additional contributions of fortified foods and food supplements. In addition, as some nutrients have additional uses in regulated products (e.g. food additives, feed additives, pesticides), the intake resulting from these uses should also be taken into account (EFSA Scientific Committee, 2021b).

Dietary intake of nutrients can be estimated using data from food composition databases and food consumption surveys. EFSA conducts nutrient intake assessments by combining data from the EFSA Comprehensive European Food Consumption Database,[14] which includes surveys from EU Member States,[15] and data from the EFSA food composition database,[16] which provides information on the vitamin and mineral content of foods from national food composition tables. These data are used to estimate the distribution of nutrient intakes in the EU population, including the mean intake and the 95th percentile, which is taken as an estimate of the intake of high consumers. An analysis of the uncertainty associated with the intake estimates is conducted in each assessment. Key uncertainties, inherent to all nutrient intake assessments, are outlined in Box 5. Additional uncertainties specific to each nutrient may also be identified and discussed where appropriate (e.g. ambiguity due to a lack of harmonisation of the chemical forms considered or the unit in which the nutrient content is expressed).

[13] E.g. vitamin B12 (cobalamin) (SCF, 2000b).

[14] The EFSA Food consumption database is available at: https://www.efsa.europa.eu/en/data-report/food-consumption-data.

[15] Criteria for the collection of high-quality dietary information that can be used to perform nutrient intake estimations in the remit of EFSA's scientific panels are provided in the EFSA Guidance on the EU Menu methodology (EFSA, 2014).

[16] The EFSA Food composition database is available at: https://www.efsa.europa.eu/en/data-report/food-composition-data.

**Box 5 Sources of uncertainties regarding nutrient intake estimates**

Sources of uncertainty and limitations arising from the use of the EFSA Comprehensive Food Consumption Database and the EFSA Food Composition Database are listed below:

*Consumption data*

- Sampling strategy and response rate: the use of households as sampling units or targeted recruitment (convenience sampling), and low response rates may result in survey samples that are not representative of the general population at a national level. This could impair the generalisability (external validity) of the estimated intake.
- Inclusion of weekdays and seasons: surveys that do not cover both weekdays and weekends, or that cover only one season, may not capture habitual intake. However, most of the surveys in the Comprehensive Database, especially those conducted under the EU-Menu project (EFSA, 2014), generally cover a full year with an appropriate proportion of weekdays and weekend days.
- Methods used to collect consumption data: dietary recall (e.g. recall bias); food records (e.g. reporting errors).
- Use of standard portion sizes: may lead to overestimation or underestimation of actual consumption.
- Small number of collection days: leads to overestimation of high percentiles of chronic intake, whereas it is expected to have minimal effect on mean intakes of nutrients that are widely distributed in the diet. For foods/nutrients not consumed regularly, individual intakes could be overestimated or underestimated depending on whether consumption days are captured in the survey.
- Other systematic errors: e.g. under-reporting has been shown to be associated with participants' personal characteristics such as sex, age, educational level and body mass index (BMI).
- Fortified foods and food supplements: consumption of fortified foods rarely or inconsistently reported; limited data on the consumption of food supplements.

*Composition data*

- Representativeness of the food composition database: may be affected by a lack of update to capture changes in production processes and in product formulations. However, data cleaning, validation and gap filling steps are applied to minimise this uncertainty.
- Nutrient added to food for fortification purposes or other regulated uses (e.g. as additive): this is not always clearly indicated in the database.
- Speciation of the nutrient: data on the specific forms of a nutrient are seldomly available in food composition tables.
- Average content values for a food category: may under- or over-estimate the actual nutrient content of the products consumed by individuals. However, the impact on the mean intake estimated for a population is expected to be small.

Total diet studies (TDS) are specifically designed to assess population chronic intake assessment, usually at the national level, based on representative sampling of the whole diet, with food analysed as consumed and pooled into defined food groups (EFSA/FAO/WHO, 2011). A strength of TDS is that it most accurately represents the levels of the nutrient in the edible portion of the food at the point of consumption and takes account of losses during processing, food preparation and storage. Therefore, data from national TDS can provide useful complementary information.

The type of consumption data used also affects the overall uncertainty of the intake assessment. Intake of nutrients resulting from usual dietary intakes, intended as long-term average daily intakes, is of interest in relation to the UL. However, consumption data are collected using short-term measurements of food intake (i.e. typically a few days). The resulting intake distributions contain a bias due to within-person variability, which tends to inflate the observed intake distribution, leading to an overestimation of extreme percentiles in the observed intake distribution, e.g. 95th percentile (van Klaveren et al., 2012). Statistical modelling can be applied to improve the reliability of these estimates (Dodd et al., 2006; van Klaveren et al., 2012).

The irregular consumption of a rich source of a specific nutrient (e.g. offal for preformed vitamin A) can lead to substantial underestimation of the low percentiles and overestimation of the high percentiles of intake. A refined assessment using specific intake scenarios (e.g. considering the frequency of intake of such foods) may be required to improve the risk characterisation in such cases (EFSA NDA Panel, 2024c).

When available, biomarkers of exposure may be useful to estimate overall nutrient intake (i.e. from all sources). However, reliable biomarkers of exposure are only available for a limited number of nutrients. When these biomarkers are used, back-calculation to dietary intake using kinetic modelling may be explored (EFSA Scientific Committee, 2021b).

# 8 | RISK CHARACTERISATION

This step aims to estimate the probability of occurrence of potential adverse effects in a population by integrating the results of the hazard identification, hazard characterisation and intake assessment steps, including the related uncertainties. The expression of risk may be qualitative, quantitative or both. Associated uncertainties and data gaps should be discussed.

Typically, the UL is compared with estimates of usual intake in the EU population. If the usual intake of all individuals in a population is below the UL, no adverse effects are expected to occur. Conversely, the proportion of the population with usual intakes above the UL represents a potential at-risk group. Factors to be considered in assessing the risk of excess intake of the nutrient include:

- the accuracy of the intake data;
- the percentage of the population with usual intakes above the UL and the magnitude and duration of the exceedance;
- the nature and severity of the adverse effect, e.g. the extent to which the adverse effect is reversible if the intake is reduced to levels below the UL.

The risk characterisation should indicate whether sub-populations with distinct and exceptional sensitivities to the adverse effects of the nutrient have been excluded.

When a UL cannot be established, the identified 'highest level of intake for which there is reasonable confidence on the absence of adverse effects', i.e. a safe level of intake, is provided (Section 6.4.3). A safe level of intake is proposed when data are insufficient to characterise a dose–response relationship between the nutrient intake and the identified hazard(s) or identify a reference point. Consequently, the application of a safe level of intake for risk management is limited because:

- Intakes above the safe level of intake do not necessarily mean that there is an increased risk of adverse effects.
- Safe levels of intake cannot be used to characterise the proportion of the population at risk of adverse effects.

# 9 | APPLICATION OF ULs TO ASSESS RISKS FOR INDIVIDUALS OR POPULATIONS

## 9.1 | Application of ULs to assess risks for individuals

If an individual's usual nutrient intake remains below the UL, no adverse effects are expected to occur. At habitual intakes above the UL, the risk of adverse effects increases as the level of intake increases. However, the intake at which a given individual will develop adverse effects due to excessive intake of a nutrient is not known with certainty. In practice, the UL can be used as an upper bound for the maximum tolerable level of usual intake for individuals. The UL is not a recommended intake.

By definition, ULs allow the assessment of the risks associated with the daily consumption of nutrients over long periods of time. Occasional, short-term and/or limited exceedances of the UL will not necessarily result in adverse effects.

## 9.2 | Application of ULs to assess risks for populations

The UL is derived to protect sensitive members of the general population. Some individuals may regularly consume nutrients at or slightly above the UL without experiencing adverse effects. However, because it is not known which individuals are most sensitive, the UL must be interpreted as applying to all individuals.

Usual intake distributions (i.e. percentiles) make it possible to determine the proportion of the population that exceeds the UL, i.e. is at risk of adverse effects. The accuracy of the available usual intake estimates will affect the reliability of the assessment of the risk of adverse effects in the population. Biomarkers of intake can be helpful in assessing the dietary intake of groups of people and could theoretically be used to complement or confirm risk estimates based on dietary data (e.g. urinary sodium excretion). However, such indicators are often lacking.

# 10 | RECOMMENDATIONS

The Panel recommends:

- To explore the potential of biomarkers of effect that can be used for hazard characterisation and encourage research on the identification and validation of such markers (e.g. through systems biology and -omics);

- To follow the ongoing activities on the use of biomarkers of effect in risk assessment, including EFSA Scientific Committee's activity;[17]
- To follow the ongoing activity of the EFSA Scientific Committee on updating its guidance on default values;[18]
- To pursue the reflections on the integration of multifactorial chronic disease endpoints into the DRV framework;
- To formulate priority research needs that foster the generation of data which can fill critical gaps identified during individual risk assessments (e.g. characterisation of the kinetics and dynamics of nutrients to enable a data-based selection of UFs and scaling methods; validation of biomarkers of intake);
- To strengthen EFSA comprehensive food consumption database regarding the consumption of fortified foods and food supplements;
- To pursue dialogue with other competent bodies toward harmonised nutrient risk assessment methodologies and explore strategies for sharing resources.

## GLOSSARY

| | |
|---|---|
| Adequate intake | The value estimated when a population reference intake cannot be established because an average requirement cannot be determined. An adequate intake is the average observed daily level of intake by a population group (or groups) of apparently healthy people that is assumed to be adequate (EFSA NDA Panel, 2010). |
| Adverse effect | Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity to compensate for additional stress or an increase in susceptibility to other influences (EFSA Scientific Committee, 2017a; FAO/WHO, 2009). |
| Adverse health effect | See Adverse effect. |
| Average requirement | Level of (nutrient) intake of a defined group of individuals estimated to satisfy the physiological requirement or metabolic demand, as defined by the specified criterion for adequacy for that nutrient, in half of the healthy individuals in a life-stage or sex group (EFSA NDA Panel, 2010). |
| Bioavailability | Nutrient fraction which is absorbed and becomes available to normal metabolic and physiological processes. |
| Biomarker of effect | A measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognised as associated with an established or possible health impairment or disease (EFSA Scientific Committee, 2017a; WHO/IPCS, 1993). Its biological relevance depends on its relation to the mode of action of an adverse effect or an adverse outcome pathway (EFSA Scientific Committee, 2017a). |
| Biomarker of exposure | An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism (EFSA Scientific Committee, 2017a; WHO/IPCS, 1993). Urine, blood, faeces or nails are common media for the measurements of biomarkers of exposure. In the nutrition field, the term biomarkers of intake is used instead. |
| Biomarkers of intake | See biomarker of exposure. |
| Critical effect | Effect selected for the derivation of a health-based guidance value. |
| Dietary reference values | A set of nutrient reference values that includes the average requirement, the population reference intake, the adequate intake, the reference intake range for macronutrients and the tolerable upper intake levels. |
| Endpoint | Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment (FAO/WHO, 2009). |
| Hazard | Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent (FAO/WHO, 2009; WHO/ICPS, 2004). |

[17]Mandate No M-2023-00097 for a guidance on the use of biomarkers of effect in regulatory risk assessment of chemicals, available at: https://open.efsa.europa.eu/questions/EFSA-Q-2023-00583.

[18]Mandate No M-2024-00067 for the revision of the guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data, available at: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00409.

| | |
|---|---|
| Health-based guidance value | Umbrella term for values that are established as the result of the risk assessment of chemical substances and provides guidance on safe consumption of substances, taking into account current safety data, uncertainties in these data and the likely duration of consumption. Depending on their nature and applications, a HBGV for oral exposure may be termed tolerable upper intake level (UL) (nutrients), acceptable daily intake (ADI) (food additives, pesticides), tolerable daily intake (TDI) (contaminants) or acute reference dose (ARfD). |
| Line of evidence | A set of evidence of similar type. |
| Lower threshold intake | The level of intake below which, on the basis of current knowledge, almost all individuals will be unlikely to maintain 'metabolic integrity', according to the criterion chosen for each nutrient (EFSA NDA Panel, 2010). |
| Lowest-observed-adverse-effect level | The lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure (FAO/WHO, 2009). |
| Mechanism of action | The specific biochemical interaction through which a substance produces an effect on a living organism or in a biochemical system (FAO/WHO, 2009). |
| Mode of action | A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical events – that is, those that are both measurable and necessary to the observed effect – in a logical framework. Related term: mechanism of action (FAO/WHO, 2009). |
| Monotonic relationship | The slope of the intake–response curve does not change sign at any point along the range of doses examined. |
| Non-monotonic relationship | The slope of the intake–response curve changes sign from positive to negative or vice versa at some point along the range of doses examined (EFSA Scientific Committee, 2021a). Therefore, the curve expressing the relationship switches from increasing to decreasing or vice versa at some points along the intake range. |
| No-observed-adverse-effect level | The greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure (FAO/WHO, 2009). |
| Nutrient | A chemical (element or compound) needed for the normal growth, development and health maintenance of the organism. This includes vitamins, minerals and macronutrients. |
| Population reference intakes | The level of (nutrient) intake that is enough for virtually all healthy people in a group (EFSA NDA Panel, 2010). |
| Reference intake ranges for macronutrients | The reference intake range for macronutrients, expressed as a percentage of the daily energy intake, defined by a lower and an upper bound (EFSA NDA Panel, 2010). |
| Reference point | A defined point on an intake–response relationship for the critical effect. This term is synonymous to point of departure. Reference points include the lowest or no-observed adverse effect level (LOAEL/NOAEL). |
| Risk | The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent (FAO/WHO, 2009; WHO/ICPS, 2004). |
| Safe level of intake | When a UL cannot be determined, this represents the highest level of intake of a nutrient at which there is a reasonable confidence in data on the absence of adverse effect(s). |
| (Toxico)dynamics | Molecular, biochemical and physiological effects of chemicals or their metabolites in biological systems as the result of the interaction of the biologically effective dose of the chemical with a molecular target. |
| (Toxico)kinetics | How the body handles a chemical, as a function of dose and time, in terms of its absorption, distribution, metabolism and excretion. |

## ABBREVIATIONS

| | |
|---|---|
| 25(OH)D | 25-hydroxyvitamin D |
| ADME | absorption, distribution, metabolism and excretion |
| ADI | acceptable daily intake |
| AI | adequate intake |
| AR | average requirement |
| BMD | benchmark dose |
| BMI | body mass index |
| BMR | benchmark response |
| BoE | body of evidence |
| BW | body weight |
| CAT | critical appraisal tool |
| CDRR | chronic disease risk reduction |
| CVD | cardiovascular disease |
| DRI | dietary reference intake |
| DRV | dietary reference value |
| FAF Panel | Panel on Food Additives and Flavourings |
| FAO | Food and Agriculture Organization of the United Nations |
| FCDB | Food composition database |
| HBGV | health-based guidance value |
| HC | hazard characterisation |
| HCT | human controlled trial |
| HDI | human development index |
| HI | hazard identification |
| IA | intake assessment |
| IPCS | International Programme on Chemical Safety |
| LOAEL | lowest-observed-adverse-effect level |
| LoE | line of evidence |
| LTI | lower threshold of intake |
| MTHF | methyltetrahydrofolate |
| NASEM | US National Academies of Sciences, Engineering and Medicine |
| NCC | nested case–control study |
| NDA Panel | Panel on Nutrition, Novel Foods and Food Allergens |
| NESR | Nutrition evidence systematic review |
| NOAEL | no-observed-adverse-effect level |
| NTP OHAT | National Toxicology Programme, Office of Health Assessment and Translation |
| P95 | 95th percentile |
| PBK | physiologically based kinetics |
| PC | prospective cohort study |
| PECO | population, exposure, comparison, outcome |
| PICO | population, intervention, comparison, outcome |
| PRI | population reference intake |
| RoB | risk of bias |
| ROBINS-E | risk of bias in non-randomised studies of exposure |
| RoB-NObs | risk of bias for nutrition observational studies |
| RP | Reference point |
| SCF | Scientific Committee on Food |
| SF | scaling factor |
| sQ | sub-question |
| TD | toxicodynamics |
| TK | toxicokinetics |
| TDS | total diet study |
| ToR | Terms of Reference |
| UF | uncertainty factor |
| UL | tolerable upper intake level |
| VDE | Vitamin D equivalent |
| WHO | World Health Organization |

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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# APPENDIX A

## Population groups, reference body weights and scaling factors

Reference body weights used in DRV opinions are reported in Table A.1.

**TABLE A.1** Reference body weights.

| Population group | Age taken as reference | Reference weight (kg) | | | References |
|---|---|---|---|---|---|
| | | Males[a] | Females[a] | Males and females[b] | |
| 4–6 months[c] | 5 months | 7.5 | 6.9 | 7.2 | WHO Multicentre Growth Reference Study Group (2006) |
| 7–11 months[d] | 9 months | 8.9 | 8.2 | 8.6 | WHO Multicentre Growth Reference Study Group (2006) |
| 1–3 years[e] | 2 years | 12.2 | 11.5 | 11.9 | WHO Multicentre Growth Reference Study Group (2006) |
| 4–6 years[f] | 5 years | 19.2 | 18.7 | 19.0 | van Buuren et al. (2012) |
| 7–10 years[g] | 8.5 years | 29.0 | 28.4 | 28.7 | van Buuren et al. (2012) |
| 11–14 years[h] | 12.5 years | 44.0 | 45.1 | 44.6 | van Buuren et al. (2012) |
| 15–17 years[i] | 16 years | 64.1 | 56.4 | 60.3 | van Buuren et al. (2012) |
| ≥ 18 years | n.a. | 82.0[j] | 66.0[j] | 70[k] | EFSA Scientific Committee (2012) |

[a]Median weight-for-age at the age taken as reference.
[b]Mean of the values for males and females.
[c]≥ 4 months to < 6 months, i.e. from the 4th month birthday to the day before the 6th month birthday.
[d]≥ 6 months to < 12 months, i.e. from the 6th month birthday to the day before the 12th month (1st year) birthday.
[e]≥ 1 years to < 4 years, i.e. from the 1st year birthday to the day before the 4th year birthday.
[f]≥ 4 years to < 7 years, i.e. from the 4th year birthday to the day before the 7th year birthday.
[g]≥ 7 years to < 11 years, i.e. from the 7th year birthday to the day before the 11th year birthday.
[h]≥ 11 years to < 15 years, i.e. from the 11th year birthday to the day before the 15th year birthday.
[i]≥ 15 years to < 18 years, i.e. from the 15th year birthday to the day before the 18th year birthday.
[j]Values for adult subjects aged 18–64 years in all surveys in the EFSA Comprehensive Database.
[k]Default body weight value for adults, as recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2012).

Considering the reference body weight for males and females reported in Table A.1 and a reference body weight of 70 kg for adults (EFSA Scientific Committee, 2012), the scaling factors reported in Table A.2 can be used for the derivation of UL for infants, children and adolescents.

**TABLE A.2** Scaling factors to derive ULs for children from the UL for adults.[a]

| Age group | Isometric scaling $SF = BW_{age\ group}/BW_{adult}$ | Allometric scaling $SF = [BW_{age\ group}/BW_{adult}]^{0.75}$ |
|---|---|---|
| 4–6 months | 0.10 | 0.18 |
| 7–11 months | 0.12 | 0.21 |
| 1–3 years | 0.17 | 0.26 |
| 4–6 years | 0.27 | 0.38 |
| 7–10 years | 0.41 | 0.51 |
| 11–14 years | 0.64 | 0.71 |
| 15–17 years | 0.86 | 0.89 |

Abbreviations: BW, body weight; SF, scaling factor.
[a]$UL_{age\ group} = UL_{adult} \times$ scaling factor. For example, to scale the UL for adults down to children aged 1–3 years using isometric scaling: $UL_{1\text{-}3\ years} = UL_{adult} \times 0.17$.

# APPENDIX B

## Identification of relevant endpoints for establishing ULs

### B.1 | Endpoints evaluated and used in establishing tolerable upper intake levels in recent EFSA opinions

| | Hazard identification | | Hazard characterisation | | |
|---|---|---|---|---|---|
| | **Endpoints evaluated**[a] | **Conclusion on a positive and causal relationship with high intake** | **Critical endpoint selected [Type**[b]**]** | **Conclusion on UL** | **Additional considerations** |
| Selenium (EFSA NDA Panel, 2023c) | Signs and symptoms of selenosis + *Potential biomarkers of effects* | Well-established *No valid predictive marker* | Alopecia, as an early sign of selenium toxicity [4] | • UL for adults: established based on critical endpoint<br>• UL for pregnant and lactating women: same as non-pregnant and non-lactating women<br>• UL for infants and children: allometric scaling ($BW^{0.75}$) | |
| | Type 2 diabetes mellitus | Moderate level of certainty | | | |
| | • Amyotrophic lateral sclerosis<br>• Skin cancer<br>• Neuropsychological development in children | Insufficient BoE | | | |
| | • Hypertension<br>• Alzheimer's dementia<br>• Thyroid diseases<br>• Prostate cancer<br>• Overall mortality | No support | | | |
| Vitamin B6 (EFSA NDA Panel, 2023d) | Peripheral neuropathy | Well-established | Peripheral neuropathy [5–7] | • UL for adults: established based on critical endpoint<br>• UL for pregnant and lactating women: same as non-pregnant and non-lactating women<br>• UL for infants and children: allometric scaling ($BW^{0.75}$) | |
| | Developmental toxicity | No support | | | |
| Folate[c] (EFSA NDA Panel, 2023a) | Cobalamin-dependent neuropathy:<br>• Progression<br>• Exacerbation | • Well-established<br>• Low level of certainty | Progression of cobalamin-dependent neuropathy [5–7] | • UL for adults: established based on critical endpoint<br>• UL for pregnant and lactating women: same as non-pregnant and non-lactating women<br>• UL for children: isometric scaling (BW)<br>• UL for infants aged 4-6 months: allometric scaling (BW)<br>• UL for infants aged 7-11 months: interpolation of the UL for infants aged 4-6 months and young children aged 1–3 years | |
| | • Cognition in individuals with low cobalamin status<br>• Colorectal cancer<br>• Prostate cancer | Insufficient BoE | | | |

| | Hazard identification | | Hazard characterisation | | |
|---|---|---|---|---|---|
| | **Endpoints evaluated**[a] | **Conclusion on a positive and causal relationship with high intake** | **Critical endpoint selected [Type**[b]**]** | **Conclusion on UL** | **Additional considerations** |
| Vitamin D (EFSA NDA Panel, 2023e) | Persistent hypercalcaemia and hypercalciuria<br>Musculoskeletal health | Well-established<br>No support at or below the LOAEL for persistent hypercalcaemia and hypercalciuria | Persistent hypercalciuria [5] | • UL for adults: established based on critical endpoint<br>• UL for pregnant and lactating women: same as non-pregnant and non-lactating women<br>• UL for children: isometric scaling (BW) | |
| Preformed vitamin A (EFSA NDA Panel, 2024c) | • Teratogenicity<br>• Hepatotoxicity<br>• Bone health | Well-established | Teratogenicity [7] | • UL for women of childbearing age: based on critical endpoint<br>• UL for other adults: same as women at childbearing age as covers for the other adverse effects<br>• UL for children and infants: allometric scaling ($BW^{0.75}$) | |
| β-carotene (EFSA NDA Panel, 2024c) | Lung cancer | Well-established | Lung cancer [7] | No UL established due to lack of adequate data to characterise a dose–response relationship | Smokers should avoid consuming food supplements containing β-carotene. Use of supplemental β-carotene by the general population should be limited to the purpose of meeting vitamin A requirements |
| Manganese (EFSA NDA Panel, 2023b) | Neurotoxicity<br>Cognition, motor function, behaviour, neurodevelopment, attention-deficit hyperactivity disorder in children | Well-established<br>Insufficient BoE | Neurotoxicity [5–7] | No UL established due to lack of adequate data to characterise a dose–response relationship | Safe levels of intake based on manganese intake from natural dietary sources among high consumers for all population groups |
| **Iron** (EFSA NDA Panel, 2024b) | • Liver toxicity<br>• Gastrointestinal effects<br>• Impaired growth in iron-replete infants and young children<br>• Type 2 diabetes mellitus<br>• Infections in infants and young children<br>• Gestational diabetes mellitus<br>• Cognitive development in infants and young children | Well-established<br>Low level of certainty<br>Insufficient BoE<br>No support | • Liver toxicity [7]<br>• Gastrointestinal effects [4–5] | No UL established due to lack of adequate data to characterise a dose–response relationship | Safe levels of intake for adults based on the presence of black stools, which reflects the presence of large amounts of unabsorbed iron in the gut. It was taken as a conservative endpoint among the chain of events that may lead to systemic iron overload (associated with liver toxicity) but not as an adverse event per se. Safe levels of intake for infants and children based on allometric scaling ($BW_{0.75}$) |

| | Hazard identification | | Hazard characterisation | | |
|---|---|---|---|---|---|
| | **Endpoints evaluated**[a] | **Conclusion on a positive and causal relationship with high intake** | **Critical endpoint selected [Type**[b]**]** | **Conclusion on UL** | **Additional considerations** |
| **Vitamin E** (EFSA NDA Panel, 2024d) | Impaired coagulation and risk of bleeding | Well-established | Bleeding time [3] | • UL for adults: established based on critical endpoint<br>• UL for pregnant and lactating women: same as non-pregnant and non-lactating women<br>• UL for children and infants: allometric scaling ($BW^{0.75}$) | ULs do not apply to individuals receiving anticoagulant or antiplatelet medications (e.g. aspirin), to patients on secondary prevention for CVD or to patients with vitamin K malabsorption syndromes |
| | Haemorrhagic stroke | BoE suggests an increased risk among people in secondary prevention for cardiovascular diseases and/or treatment with antiplatelet medications | | | |
| | Congestive heart failure | BoE suggests an increased risk following a myocardial infarction | | | |
| | Prostate cancer | Insufficient BoE | | | |
| | • Ischaemic stroke<br>• Coronary heart disease | No support | | | |
| **Copper** (EFSA Scientific Committee, 2023b)[d] | Hepatoxicity | Well-established | Copper retention, as indicative of potential hepatic sequestration and future toxicity [3] | ADI[e] for all population groups based on this endpoint | ADI[e] for copper from all dietary sources |
| | Alzheimer disease | Inconclusive BoE | | | |

Abbreviations: ADI, acceptable daily intake; BoE, body of evidence; BW, body weight; P95, 95th percentile of estimated intake.

[a] Prioritised endpoints were evaluated through de novo systematic reviews of the literature (except for β-carotene and lung cancer).

[b] Type of endpoint according to the ranking scheme proposed by Renwick et al. (2004) and adapted in WHO/FAO (2006): (1) biochemical changes within the homeostatic range and without indication of adverse sequelae; (2) biochemical changes outside the homeostatic range without known sequelae; (3) biochemical changes outside the homeostatic range that represent a marker of potential adverse effects due to excess; (4) clinical features indicative of a minor but reversible change; (5) clinical features of significant but reversible effects; (6) clinical features indicative of significant but reversible organ damage; and (7) clinical features indicative of irreversible organ damage.

[c] ULs apply to the combined intake of supplemental folate from currently authorised forms for addition to food and use in food supplements (folic acid/5-methyltetrahydrofolate salts).

[d] Given its cross-cutting nature, this mandate was assigned to EFSA Scientific Committee.

[e] Regarding the similarity between ADI and UL, see EFSA Scientific Committee statement on the derivation of health-based guidance values (HBGVs) for regulated products that are also nutrients (EFSA Scientific Committee, 2021b).

## B.2 | Specific considerations regarding multifactorial chronic diseases

Excess micronutrient intake may contribute to the risk of multifactorial chronic diseases (e.g. sodium and risk of CVD). The multifactorial nature of these diseases challenges several of the assumptions that underly the definition of an UL. This and other considerations led the National Academies of Sciences, Engineering, and Medicine (NASEM) to expand the US/Canada framework on dietary reference intakes (DRIs) by introducing a separate chronic disease reference value i.e. the 'chronic disease risk reduction intake' (CDRR) level (NASEM, 2017, 2019). The conceptual distinction made by NASEM between DRIs for adequacy and toxicity and DRIs based on chronic disease is outlined in Table B.1.

**TABLE B.1** Conceptual distinction made by NASEM between dietary reference intakes (DRIs) for adequacy and toxicity and DRIs based on chronic disease.

| DRIs for adequacy and toxicity | DRIs based on chronic disease |
|---|---|
| Needed because deficiencies (of essential nutrients) and toxicities: | Are not warranted unless sufficient evidence exists because: |
| Will affect everyone, if intake is inadequate or excessive<br>Are caused by a single nutrient<br>Are prevented by nutritional interventions | Risk to acquire chronic diseases varies by individual<br>Chronic diseases are often related to many risk factors (e.g. genetic, environmental)<br>Nutritional interventions will only partly ameliorate the risk of chronic disease |

*Source: (*NASEM, 2019*).*

As part of the workshop organised in preparation of the present guidance document, the NDA Panel held discussions on whether chronic disease endpoints could be used, in addition to classical toxicity endpoints, to derive ULs for micronutrients or whether different values should be derived based on such chronic disease endpoints (EFSA, 2022). Although most participants were in favour of including chronic disease endpoints among the endpoints that could be used to derive ULs for micronutrients, differences of use and interpretation between ULs derived from toxicity endpoints and ULs derived from chronic disease endpoints were recognised (EFSA, 2022). This could have implications regarding the measures which can be used to manage respective risks (e.g. food based dietary guidelines vs. setting of maximum amounts in food). Further reflection is needed regarding the integration of multifactorial chronic disease endpoints into the DRV framework.

# APPENDIX C

## Core steps for performing a systematic review

The core steps of the systematic review process are depicted below (Figure C.1). The principles and methods underlying each step are described in the EFSA Guidance on the application of systematic review methodology to food and feed safety assessment to support decision making (EFSA, 2010). A protocol for conducting a systematic review is always developed a priori, defining in advance the review question, scope and eligibility criteria for the inclusion of studies. It describes the methods that will be used to conduct each step: searching for and selecting studies (evidence retrieval and screening for inclusion or exclusion), collecting data (data extraction), assessing methodological quality of included studies (evidence appraisal), synthesising data (e.g. meta-analysis). During the implementation, each step is carefully documented to ensure transparency and reproducibility.

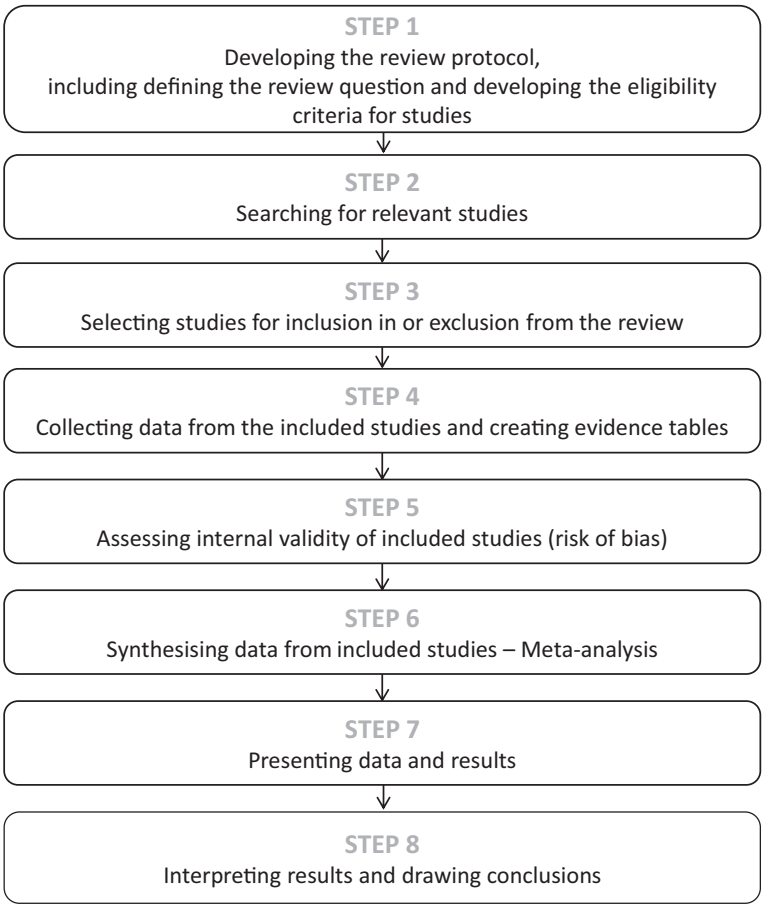


**FIGURE C.1** Core steps for performing a systematic review. Adapted from EFSA (2010) and Higgins and Green (2009)

## APPENDIX D

### Eligibility criteria for study selection

For sub-questions addressed through systematic reviews, eligibility criteria for study selection are defined in the protocol. It requires tailoring based on the specific nutrient, endpoint and population of interest, and of the availability of evidence (data-rich vs. data-poor context). The scope of the question also needs consideration: although bodies of evidence for evaluating causality and for characterising the dose–response relationship may largely overlap, they do not necessarily coincide (e.g. the body of evidence on dose–response relationship may be more restricted, as it requires quantitative estimates of intake).

Considerations made when defining eligibility criteria are outlined below. Decisions are made on a case-by-case basis, informed by expert knowledge and scoping searches. A compromise is always required between setting restrictive criteria, which may limit uncertainties (e.g. in terms of risk of bias, generalisability) but result in few eligible studies, and more permissive criteria, which may result in the inclusion of more studies, but increase associated uncertainties. The specification, at protocol stage, of separate lines of evidence (e.g. main vs. complementary) can help taking these decisions.

#### Study design

For data-rich relationships, eligible study designs may be restricted to those that provide the strongest evidence to assess causality. This typically includes controlled intervention studies (randomised and non-randomised) and prospective observational studies (cohort, nested case–control, case-cohort).[19] In case data are expected to be limited, a more inclusive strategy may be desirable, i.e. including additional study designs such as uncontrolled trials, cross-sectional studies, case–control studies, case series/reports.

If the nutrient-endpoint relationship is already well-established, the focus of the review is to characterise the dose–response. Eligible study designs may be restricted to those expected to provide the most reliable data for that purpose (e.g. regarding the quantification of nutrient intake).

#### Study duration

Inclusion/exclusion criteria based on study duration are set when an appropriate (minimum) duration of the exposure and follow-up for the outcome to occur can be defined. This is dependent on the nature of the exposure and endpoint of interest.

#### Study location

Restricting study location to countries which are comparable to EU countries in terms of health, education and economics (e.g. comparable Human Development Index (HDI[20]) may limit uncertainties regarding generalisability. However, studies conducted in different contexts may provide relevant supportive (complementary) evidence in a data-poor context (e.g. it can strengthen the weight of the evidence when data are consistent across locations).

#### Population characteristics

Eligible studies must be conducted with participants who are representative of the population addressed by the review question. This involves deciding whether specific populations should be excluded based on factors such as age, sex or other characteristics.

It is recognised that limiting eligibility to studies that exclusively enrolled 'healthy' participants is too narrow (UL must be protective for the EU general population) and difficult to implement in practice ('healthy' is an imprecise term[21]). On the other hand, recruitment criteria can vary largely across studies, depending on their objective and this may bring substantial heterogeneity and uncertainty in the body of evidence. It is thus critical to define unambiguous eligibility criteria regarding the baseline health characteristics of study participants.

Minimum exclusion criteria include:

- Studies that exclusively recruited participants with diseases or health conditions (or therapeutic management thereof) which can alter the nutrient absorption, metabolism and/or the nutrient-endpoint relationship;
- Studies in premature infants.

Studies do not always report on the medical or therapeutic status of participants to a level of detail that allows making decisions about them meeting/not meeting the inclusion/exclusion criteria for study selection at protocol level. Strategies should be defined to deal with those studies.

It may not be possible to anticipate all relevant issues regarding participants' characteristics at protocol level. Further restrictions may be applied based on the data collected. Such restrictions should be justified and documented.

[19] As human interventions are not always ethical or feasible, prospective observational studies can provide relevant complementary evidence to assess the causality of a nutrient-endpoint relationships.

[20] As defined by the United Nations Development Programme, see https://hdr.undp.org/data-center/human-development-index#/indicies/HDI.

[21] See also footnote 5.

**Exposure**

As UL concerns adverse effects from dietary exposure, eligible studies are restricted to those which investigate oral administration.

Eligible intervention studies are those comparing a supplementary dose of the nutrient vs. a placebo or lower doses. Inclusion/exclusion criteria based on the frequency of supplementation may also be established (e.g. daily, every other day, weekly) based on available knowledge on (toxico)kinetics.

Regarding observational studies, it can be justified to exclude studies which assess intake of the micronutrient from natural sources only, except in specific cases.[22] This is because the relevant range of intake to study association with 'excess' intake may not be covered and/or because not accounting for supplemental sources (e.g. food supplement) could be a major source of confounding. Criteria regarding the eligibility of observational studies which used biological measures (e.g. blood concentrations) as biomarkers of intake must also be defined, depending on their validity and reliability. Again, studies using biomarkers of intake may provide useful complementary evidence for the hazard identification. Their utility for hazard characterisation (dose–response assessment) will be limited to cases where intake can be predicted from the biomarker level with sufficient reliability.

When unreliable methods for measuring intake or related biomarkers of intake are known, exclusion criteria may be specified on this basis.

**Endpoints**

Inclusion criteria specify the relevant measures of the endpoint(s) of interest. Considerations should be made regarding acceptable measures (e.g. diagnostic criteria, composite outcomes, measurement methods). When unreliable methods are known, exclusion criteria may be specified on this basis. Eligible surrogate measures of endpoints of interest should also be defined.

**Language**

Studies published in English are included. Because of resource constraints, studies in other languages are excluded.

**Publication year**

In some cases, the search may be restricted to a specific publication period. For instance, when a pre-existing review can be used for identifying relevant studies up to a certain date.

**Publication type**

Primary research studies reported in full-text articles are included. Systematic reviews and meta-analyses are not eligible as such but can be collected for the purpose of reviewing the reference list.

Narrative reviews, expert opinions, conference abstracts, letters to editors (not reporting on original data), PhD theses and grey literature are typically excluded.

[22]Populations consuming natural sources particularly rich in the nutrient (e.g. living in seleniferous areas, frequently consuming animal liver as source of preformed vitamin A).

## APPENDIX E

### Illustration of the application of the intake–response modelling approach to the setting of the tolerable upper intake level for vitamin D in infants (≤ 1 years)

In the opinion on the tolerable upper intake level (UL) for vitamin D in infants (≤ 1 years) (EFSA NDA Panel, 2018), the NDA Panel reviewed the evidence from trials and observational studies on the relationship between vitamin D intake and the risk of hypercalciuria, hypercalcaemia, nephrocalcinosis and abnormal growth patterns and concluded that the available evidence could not be used alone to establish a UL.

The Panel used data on serum 25(OH)D concentration in relation to vitamin D intake, recognising that a 'high' concentration is not an adverse health effect per se, but can be considered as a surrogate endpoint. The Panel defined a maximum concentration of 25(OH)D of 200 nmol/L as unlikely to pose a risk of adverse effects. The intake–response relationship between serum 25(OH)D and vitamin D intake was modelled to generate the predicted mean and the associated inter-individual distribution of the 25(OH)D serum concentration of infants at different levels of intake. The latter required assumptions on the possible shape of the inter-individual variability distribution (Figure E.1A). From this, the percentage of infants expected to exceed the pre-defined threshold for the serum concentration of 25(OH)D could be estimated. A range of the response (i.e. the percentage of exceedance) considered not indicative of adverse was defined and the intake of vitamin D associated with it identified as the reference point, in line with the general principles of the BMD approach (Figure E.1A). The latter was considered to be a conservative intake value that could be used as a basis for establishing the UL for vitamin D in infants, without the application of an uncertainty factor considering that extrapolation to humans was not needed and sampling uncertainty was already accounted in the estimate of the percentage (Figure E.1B).

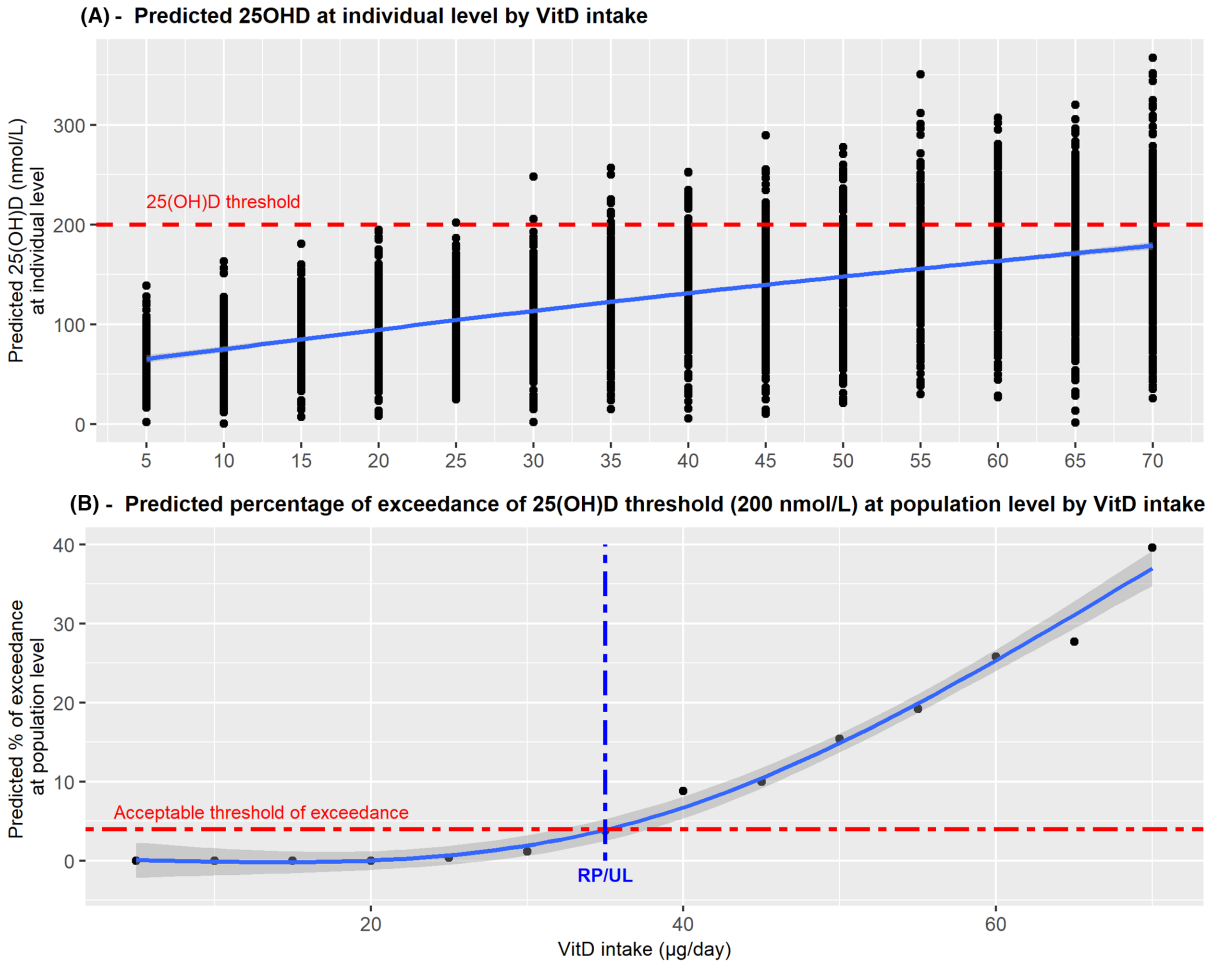


**FIGURE E.1** Illustration of the intake–response modelling approach used to establish the tolerable upper intake level for vitamin D for infants aged 6–12 months. The same approach was applied for the derivation of the tolerable upper intake level for infants aged up to 6 months. 25(OH)D, 25-hydroxy-vitamin D; RP, reference point; UL, tolerable upper intake level.

## ANNEXES

Annex A. Workshop report on human-to-human scaling approaches for the derivation of tolerable upper intake levels.
Annex B. Report of the public consultation on the draft guidance for establishing and applying tolerable upper intake levels for vitamins and essential minerals.
Annexes are available under the Supporting Information section on the online version of the scientific output.