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Guidance on scientific principles and data requirements for the safety and relative bioavailability assessment of new micronutrient sources

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Abstract

Following the adoption of Regulation (EU) No 1169/2011 on food information to consumers, the European Commission requested EFSA to update its 'Guidance on safety evaluation of sources of nutrients and bioavailability of the nutrient from the sources' regarding the scientific principles and data requirements for the scientific assessment of all new forms of micronutrients and to derive a conversion factor for new micronutrient sources or forms of micronutrients to be authorised for addition to foods, including food supplements. This guidance outlines the scientific principles that the NDA Panel will consider for the assessment of the safety and the quantification of the relative bioavailability of new sources of micronutrients, which applicants are requested to consider when preparing their applications. It also outlines the data requirements for dossiers. Applicants should integrate the data presented in different sections to provide their overall considerations on how the information provided supports the safety of the new micronutrient source and the quantification of its relative bioavailability compared to a reference source under the proposed conditions of use. As preparatory work for the development of this guidance, EFSA launched an Expert Survey and held an online workshop on 9th March 2023 inviting scientific input from stakeholders and scientific experts, the report of which is now available online in the EFSA's webpage.

KEYWORDS

conversion factor, food labelling, micronutrient source, minerals, relative bioavailability, safety assessment, vitamins

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1 | INTRODUCTION

The steps taken by EFSA to address this mandate are shown in Appendix A. These include an Expert Survey inviting scientific input from stakeholders and scientific experts on key points to consider for the derivation of conversion factors for new sources or forms of nutrients and an online scientific workshop on the topic held by the EFSA on 9th March 2023. All documents relative to the workshop, including the event report, can be found on the EFSA website.[1]

A draft of this guidance was endorsed by the NDA Panel on 31 January 2024 and was open for public consultation from 15 February to 14 April 2024. The draft guidance has been amended in view of the comments received, which have all been addressed and are published in a technical report (Annex A).

1.1 | Background and Terms of Reference as provided by the European Commission

1.1.1 | Background

The EFSA Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources (Revision 1) (EFSA ANS Panel, 2021) acknowledges that the use of chemical substances as sources of vitamins and minerals in food (hereafter "nutrients") is regulated in the European Union (EU) by the establishment of positive lists of substances, annexed to the relevant sectoral legislation, i.e.:

Directive 2002/46/EC on food supplements;[2]
Regulation (EC) No 1925/2006 on 'fortified' foods.[3]
Regulation (EU) No 609/2013 on food for specific groups,[4] covering infant formula and follow-on formula; processed cereal-based food and baby food; food for special medical purposes; and total diet replacement for weight control.

According to the above-mentioned legislations, the chemical substances used as sources of nutrients which may be added to food, including food supplements and foods for specific groups, should be safe and bioavailable, a property which is described, in the relevant legislation, as 'available to be used by the body'. The scope of those legislations includes all forms of nutrients and is not limited to nutrient sources only.

However, the EFSA guidance establishes data requirements for the scientific assessment of the safety and bioavailability of new nutrient sources only. To cover all forms of nutrients in line with the relevant sectoral legislations, it is necessary that the EFSA Guidance establishes data requirements for the scientific assessment of all new forms of nutrients.

Furthermore, in relation to bioavailability, "*it is acknowledged that it is not always possible to determine directly whether the nutrient from the proposed source is available to be used by the body, and therefore, a range of surrogate tests are proposed as examples that will generate data to be used in assessing the bioavailability of the nutrient from the proposed source. These data should allow a comparison between the behaviour of the proposed source and one or more sources of the same nutrient, already permitted for use in foods*" (EFSA ANS Panel, 2021).

As for Article 31(2) of Regulation (EU) No 1169/2011 on the provision of food information to consumers, *"the Commission may adopt, by means of delegated acts, in accordance with Article 51, conversion factors for the vitamins and minerals referred to in point 1 of Part A of Annex XIII, in order to calculate more precisely the content of such vitamins and minerals in foods. Those conversion factors shall be added to Annex XIV"*. In addition, Article 32(3) states that, *"when provided, the declaration on vitamins and minerals shall, in addition to the form of expression referred to in paragraph 2, be expressed as a percentage of the reference intakes set out in point 1 of Part A of Annex XIII in relation to per 100 g or per 100 ml"*.

The Commission thus needs EFSA's scientific advice not only on whether new proposed sources of nutrients are bioavailable (i.e. 'available to be used by the body'), but also on the extent to which they are bioavailable as compared to native forms of the nutrient naturally present in foods or as compared to an authorised nutrient source or form for which the relative bioavailability versus one or more forms of the nutrient naturally present in foods is known. To that end, EFSA is requested to define the principles for the scientific assessment and data requirements for applicants in order to provide a conversion factor for proposed new sources or forms of nutrients that allows expressing their contribution to the reference intakes of those nutrients.

[1] https://www.efsa.europa.eu/en/events/workshop-derivation-conversion-factors-new-sources-or-forms-nutrients.

[2] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

[3] 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.

[4] 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.

1.1.2 | Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) 178/2002, the European Commission asks EFSA to update its Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources (EFSA ANS Panel, 2021) regarding the scientific principles and data requirements for applicants in order to establish data requirements for the scientific assessment of all new forms of nutrients and to derive a conversion factor for proposed new sources or forms of nutrients to be authorised for addition to foods, including food supplements. The conversion factor should indicate the extent to which the proposed new nutrient sources or forms are bioavailable as compared to native forms of the nutrient naturally present in foods or as compared to an authorised nutrient source for which the relative bioavailability versus one or more forms of the nutrient naturally present in foods is known.

1.2 | Interpretation of the Terms of Reference

In line with the definition given in the background provided by the EC and relevant legislation, and in agreement with the mandate requestor, the Panel interprets that this guidance should focus on micronutrients (i.e. vitamins and minerals), and specifically on new substances that are proposed as sources of vitamins and minerals in food supplements,[5] foods for the general population[6] and/or foods for specific groups,[7] including new forms of micronutrients. The scope of the document is limited to the implementation of the aforementioned legislation (thereafter referred to as the 'relevant European legislation').

1.3 | Definition of terms

In the context of this guidance document:

- The term **micronutrient** is used to denote vitamins and minerals. There may be different chemical forms of a micronutrient.[8]
- **Micronutrient source** means a chemical substance (e.g. a single molecule or simple mixture[9]) which is, or contains, a micronutrient (in its native form or in a new form) that is available to be used by the body for specific micronutrient-dependent functions.
- **Chemical substance** means a chemical element and its compounds in the natural state or as the result of a manufacturing process. There are three main types of chemical substances, namely (a) mono-constituent substances, (b) multi-constituent substances and (c) unknown or variable composition, complex reaction products or of biological materials (UVCB) substances.[10]
- The term **native form of a micronutrient** is used to denote a micronutrient in a form that is naturally present in foods and/or beverages, including drinking water. In this guidance, the use of the term is restricted to native forms that substantially contribute to the dietary intake of the micronutrient and excludes synthetic forms and forms naturally occurring in foods in marginal amounts.
- The term **new (micronutrient) source** means a substance that is currently not authorised as a source of vitamins or minerals in relevant European legislation. New sources may contain micronutrient and non-micronutrient components.
- **Reference (micronutrient) source** refers to the micronutrient source that is used as comparator in relative bioavailability studies. It can be a native form of the micronutrient, or a source authorised for use in the relevant European legislation for which the relative bioavailability versus one or more native forms of the micronutrient is known, including synthetic forms of micronutrients.
- Forms of a micronutrient that are not native forms as defined in this guidance and/or are not currently authorised sources of vitamins or minerals in the relevant European legislation are referred to as **new forms of a micronutrient**. These include forms that differ from native or authorised forms regarding their chemical structure (e.g. micronutrient metabolites), their physical characteristics (e.g. engineered nanomaterials), or both.

[5]Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

[6]Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, p. 26–38.

[7]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.

[8]For example, cholecalciferol, ergocalciferol and calcidiol are different chemical forms of vitamin D; iron hydroxide adipate tartrate (engineered nanomaterial), ferrous sulfate and elemental iron are chemical forms of iron with different physicochemical characteristics.

[9]A simple mixture is defined as a chemical mixture whose components can be fully characterised (EFSA NDA Panel, 2021a).

[10]https://echa.europa.eu/support/substance-identification/what-is-a-substance.

- The term **dissociation** covers breakdown in whatever form (e.g. dissociation of salts, complexes, chelates; ester hydrolysis; etc).
- **Bio-accessibility** is defined as the extent to which a micronutrient is released from its matrix in the gastrointestinal (GI) tract, becoming available for absorption.
- The concept of **bioavailability** is defined in the relevant European legislation as 'available to be used by the body' which, for a micronutrient, is considered to mean 'available to be used for specific micronutrient-dependent functions in the body'. It describes the capability of a micronutrient source to contribute to the physiological requirements for that micronutrient.
- **Relative bioavailability** denotes the bioavailability of the micronutrient from the new source as compared to a reference source. For chemically related compounds having vitamin activity ('vitamers' i.e. capable of contributing to the physiological requirements for the vitamin), the term **bioequivalence** is often used.
- **Relative bioavailability studies** are those used to quantify the bioavailability of a micronutrient from a new source compared to a reference source.
- **Conversion factor (CF)** is used to quantify the relative bioavailability of a micronutrient from a new source for labelling purposes.
- **Health-based guidance value (HBGV)** is an 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) (EFSA Scientific Committee, 2021).
- **Tolerable upper intake level (UL)** is the maximum level of total chronic daily intake of a nutrient (from all sources) which is not expected to pose a risk of adverse health effects to humans (EFSA NDA Panel, 2022b).[11]

2 | OBJECTIVES AND SCOPE

This guidance is intended to assist applicants in preparing applications for the authorisation of new substances proposed as sources of micronutrients through an understanding of the data requirements for:

a. the evaluation of their safety;
b. the evaluation and quantification of the relative bioavailability of the micronutrient from the new source.

Examples drawn from previous and ongoing assessments are used in this guidance to illustrate the approach of the NDA Panel in the scientific assessment of new sources, which could help applicants in preparing their applications.

This guidance focuses on micronutrients (i.e. vitamins and minerals), and specifically on new substances that are proposed as sources of vitamins and minerals in food supplements,[12] foods for the general population[13] and/or foods for specific groups,[14] including new forms of the micronutrient.

It is outside the scope of this guidance to establish dietary reference values (DRVs), including ULs, for vitamins or minerals.

It is within the scope of this guidance to provide a detailed description of the scientific principles and data requirements that need to be considered when assessing the safety of new micronutrient sources, and in particular:

a. the bioavailability of new sources (i.e. their capability to be used by the body for specific functions and contribute to the micronutrient requirements) and
b. the relative bioavailability of new sources in comparison to a reference source for the purpose of deriving a CF for labelling purposes.

Quantification of the bioavailability of a micronutrient from a new source in absolute terms (i.e. quantification of the fraction of the micronutrient that, upon ingestion, is absorbed and utilised by the body for specific micronutrient-dependent functions) is complex and not needed to establish a CF, and it is therefore out of the scope of this guidance.

It is also not within the scope of this guidance to provide detailed instructions on the design of scientific studies, on data analysis or interpretation of the results, which rely on general scientific knowledge.

[11] https://www.efsa.europa.eu/sites/default/files/2024-05/ul-summary-report.pdf.

[12] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

[13] 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.

[14] 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.

This document is based on the experience gained by the EFSA ANS and NDA Panels on the assessment of new substances proposed as sources of micronutrients and cannot foresee all new substances that may be submitted in the future for that purpose. Consequently, it is intended that the guidance will be kept under review and will be amended and updated as appropriate in the light of experience gained from the assessment of additional applications.

The present guidance should be read in conjunction with the Guidance on the scientific requirements for an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283 (EFSA NDA Panel, 2024), as the scientific principles for the safety assessment of novel foods also apply to the safety assessment of substances proposed as new sources of nutrients, including micronutrients.

The present guidance and the new version of the guidance on novel foods (EFSA NDA Panel, 2024) apply as of 1 February 2025.

3 | STRUCTURE OF THE GUIDANCE

This guidance document is structured in two main sections:

- **Section 4**, which summarises the **scientific principles** that the NDA Panel will consider for the scientific assessment of new sources of micronutrients, and which applicants are requested to consider when preparing their applications and
- **Section 5**, which depicts **data requirements** for dossiers for new sources of micronutrients.

It is anticipated that, owing to the diversity of the new sources that could be proposed, not all data requested in Section 5 may be pertinent for each application. Whenever that is the case, proper justification should be provided by applicants for the absence of the requested data in the dossier.

4 | SCIENTIFIC PRINCIPLES

It should be noted that the scientific principles outlined in the most recent applicable guidance for the safety assessment of novel foods (EFSA NDA Panel, 2024) also apply to the safety assessment of substances proposed as new sources of nutrients, including micronutrients.

The present guidance focuses on the principles that will be used by the NDA Panel to specifically assess the safety and bioavailability of new sources of micronutrients in their native form and of new form(s) of a micronutrient (e.g. micronutrient metabolites, new vitamers). Examples of previous evaluations from the EFSA ANS and/or NDA Panels will be used in footnotes for clarity.

4.1 | Safety assessment

4.1.1 | Tiered approach for toxicity testing

For the safety assessment of new micronutrient sources, the scientific principles are not different from the safety assessment of novel foods (EFSA NDA Panel, 2024).

A critical consideration for the safety assessment of new sources is the anticipated behaviour or degree of dissociation within the GI tract. The degree of dissociation (outcome of the dissociation test) would help to determine the toxicological data needed for the safety assessment (see Figure 1), as follows:

a. If the dissociation test data demonstrate that the new source is extensively and readily dissociated in the GI tract, the safety assessment will rely on toxicological information on the resulting components, as follows:

    i. If the products (micronutrient and non-micronutrient components) of dissociation that occurs in biological milieus already have established HBGVs,[15] including ULs for the micronutrient component, these are used as the basis for risk assessment and no further toxicological data are needed.
    ii. If the non-micronutrient components of the new source do not have established HBGVs, toxicological data are required in line with the tiered approach described in relevant sectoral guidance on novel foods (EFSA NDA Panel, 2024). From these data, a toxicological reference point (RP) is established, from which either a HBGV could

[15]Example: In an application for **ferrous sodium EDTA (ethylenediaminetetraacetic acid)** as new source of iron, acceptable daily intake (ADI) for EDTA had been established. However, from the ADI of 2.5 mg/kg body weight (bw)/day established for calcium disodium EDTA, a value of 1.9 mg EDTA/kg bw/day could be calculated. The ANS Panel concluded that ferric sodium EDTA as a source of iron was of no safety concern at the proposed use levels as long as it does not lead to an exposure to EDTA above 1.9 mg EDTA/kg bw per day (EFSA ANS Panel, 2010b).

be derived for comparison with the exposure estimate, or a comparison could be performed of the exposure estimate with the RP (margin of exposure approach).[16] Toxicological data in line with the tiered approach described in relevant sectoral guidance on novel foods would also be required for the nutrient components of the new source that do not naturally occur in food or the body.[17]

iii. In cases where no ULs for the micronutrient have been established[18] (e.g. ULs have been established for different chemical forms of the same micronutrient),[19] which is often the case for new form(s) of a micronutrient, it may be necessary to acquire data on absorption, distribution, metabolism and excretion (ADME). Such data would help to predict whether the same adverse effects are expected to occur as with the form(s)/sources of the micronutrient that are covered by the UL (i.e. whether the same endpoints considered for the derivation of the UL are applicable).[20] If the identity, compositional characteristics and ADME data do not provide conclusive evidence on the type of potential adverse effects expected at high intake levels or suggest different adverse effects as compared to those covered by the UL for the micronutrient,[21] data from long-term human intervention studies may be needed to assess the safety of the new source under the proposed use(s) and use level(s).

b. If data from the dissociation tests indicate that the new source does not extensively or readily dissociate in the lumen of the GI tract, then the possibility that the new source is absorbed at least partly unchanged from the GI tract cannot be ruled out, and the tiered approach to toxicological testing as described in the novel foods guidance (EFSA NDA Panel, 2024) should be applied.

A decision tree to decide on the approach for ADME and toxicity testing of new substances proposed as new sources of micronutrients is presented in **Figure 1**.

[16]Example: In the evaluation of an application for **calcium phosphoryl oligosaccharides** (POs) as source of calcium, Tier 1 toxicity testing was provided for the non-micronutrient component of the source (POs). The ANS Panel concluded that, although the toxicity database was too limited to derive an ADI, there was no safety concern for its use as a source of calcium added for nutritional purposes to food, food supplements and foods for special medical purposes at the proposed use and use levels due to the high margin of exposure (EFSA ANS Panel, 2016).

[17]Example: synthetic retinoids as source of preformed vitamin A.

[18]ULs may have not been established for some nutrients (e.g. vitamin C, vitamin K, potassium, vitamin B_{12}), either because the available data was insufficient or inadequate to do so or because no clearly defined adverse effects from the nutrient have been identified at high intakes. However, the chemical forms of the nutrient that were considered in the UL assessment are covered by the UL (i.e. doses at which no adverse effects have been reported can be used instead). For the most updated UL values, please refer to: https://www.efsa.europa.eu/sites/default/files/2024-05/ul-summary-report.pdf.

[19]For example, the nutrient component **calcium** in **calcium phosphoryl oligosaccharides** (a calcium salt) is expected to be absorbed, distributed and eliminated in a manner similar to other dietary sources of calcium (EFSA ANS Panel, 2016). A UL for calcium from all dietary sources has been established (EFSA NDA Panel, 2012). Conversely, the nutrient component **5-MTHF (5-methylterathydrofolate)** in 5-MTHF glucosamine and L-5-MTHF-Ca salts, although it is naturally occurring in foods and significantly contributes to nutrient requirements together with other natural folates, is not covered by the UL, which has been established for folic acid (EFSA NDA Panel, 2023a).

[20]For example, **calcidiol monohydrate** (25-hydroxyvitamin D3) has the same physiological effect(s) as vitamin D3 in terms of possible adverse effects at high intakes. Oral administration of calcidiol increases serum 25(OH)D, which is the likely mediator for the critical endpoint (i.e. hypercalcemia/hypercalciuria) used for establishing a UL (EFSA NDA Panel, 2023c).

[21]For example, no UL has been established for natural folates, including 5-MTHF salts that were proposed as new nutrient sources, but for folic acid (i.e. synthetic folate). 5-MTHF salts are covered by the UL for folate (adverse effects of natural sources of folate were addressed in the UL opinion, which concludes that no adverse effects can be expected at the observed levels of intake from dietary sources). However, the UL for folic acid has been established on an endpoint for adverse effects (i.e. masking haematological signs of vitamin B12 deficiency) that cannot be expected from 5-MTHF salts based on ADME data in humans, and no information is available on the chronic adverse effects of natural folates at intakes close to the UL for folic acid (EFSA NDA Panel, 2023b).

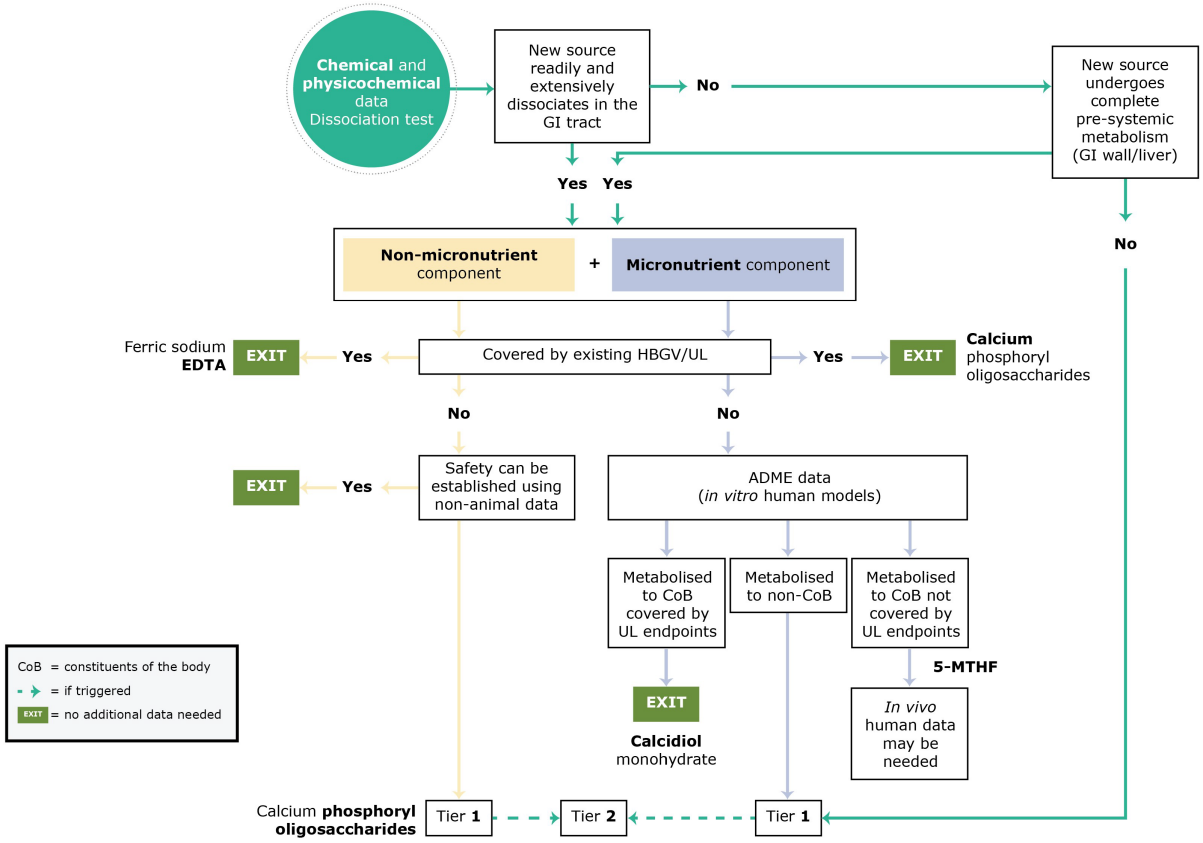

FIGURE 1 Decision tree for ADME and toxicity testing for the safety assessment of new substances proposed as sources of micronutrients. In the examples provided, the component of interest of the new source is highlighted in **bold**. ADME, absorption, distribution, metabolism and excretion; CoB, constituents of the body; EDTA, ethylenediaminetetraacetic acid; GI, gastrointestinal; HBGV, health-based guidance value; MTHF, methyltetrahydrofolate; UL, tolerable upper intake level.

4.1.2 | Processing contaminants and genotoxic and/or carcinogenic residuals

Substances assessed for their intended use as sources of micronutrients, including processing contaminants contained therein, shall not exhibit genotoxic or carcinogenic activities.

4.2 | Relative bioavailability of the micronutrient from the new source and derivation of a conversion factor for labelling purposes

4.2.1 | General approach

The first aspect to be established for micronutrients in general, and for new forms of micronutrients in particular (e.g. metabolites, new vitamers), is their bioavailability (i.e. whether the micronutrient component from the new source can be available to be used for specific micronutrient-dependent functions in the body).[22]

The relative bioavailability of the micronutrient from the new source is estimated by comparing its bioavailability against a similar amount (ideally an equimolar amount) of the micronutrient from an appropriate reference source under identical experimental conditions. This may be based on studies in humans, in animals, in in vitro gastro-intestinal models (bio-accessibility or cellular uptake/absorption studies) or on studies of dissociation under conditions similar to the human GI tract.

Such comparative studies should allow to derive a CF for the new source. However, there is no pre-established rule as to how many or which types of studies are needed for establishing a CF. This is because the approach used may depend on the chemical/physicochemical characteristics and ADME properties of the new source, as well as what is already known about the bioavailability of the micronutrient. It may also be influenced by the target population and the food matrices to which the new source is intended to be added. Which data, or combination of data, is the most appropriate to establish the

[22]For example: **Nicotinamide riboside chloride** is considered a source from which nicotinamide, a form of the vitamin niacin, is released in the GI and is bioavailable, i.e. absorbed into blood and available to act as a precursor of nicotinamide adenine dinucleotide (NAD+) in cells (EFSA NDA Panel, 2019). In contrast, **1-methyl nicotinamide (chloride)**, a main metabolite of nicotinamide, has no vitamin function, i.e. it is absorbed into blood but not available to act as a precursor of NAD+ in cells (EFSA NDA Panel, 2019).

relative bioavailability of a new source remains a matter of scientific judgement based on all available data and weighing of the evidence and should be decided on a case-by case basis.

Methodologies and data requirements may reflect the chemical/physicochemical similarity of the new and the reference source, as well as the novelty of the chemical form of the micronutrient, as shown in **Figure 2**.

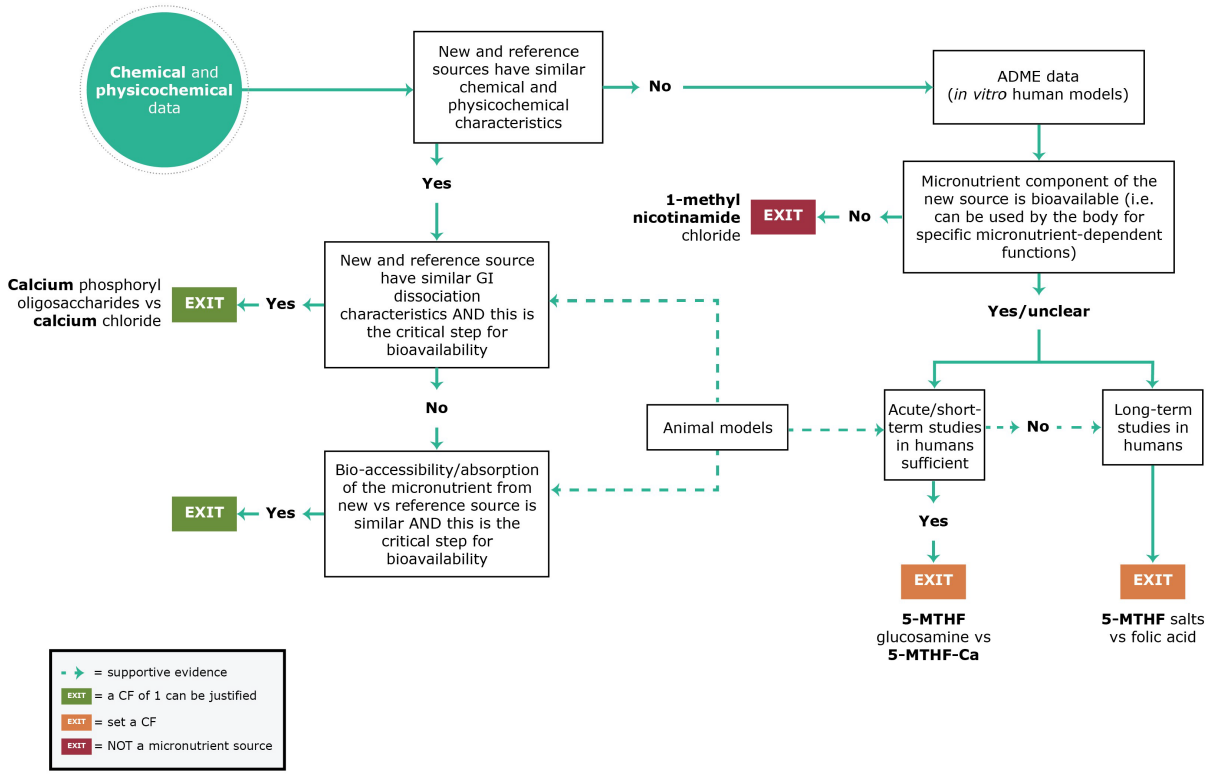

FIGURE 2 Decision tree for relative bioavailability testing of new substances proposed as sources of micronutrients.
In the examples provided, the component of interest of the new source is highlighted in **bold**. ADME, absorption, distribution, metabolism and excretion; CF, conversion factor; GI, gastrointestinal; MTHF, methyltetrahydrofolate.

The scientific opinions on new sources assessed by the former ANS Panel and the NDA Panel provide examples as to the type of studies that may be needed for estimating relative bioavailability of micronutrients from new sources in the context of specific applications. These opinions have generally not estimated a value for relative bioavailability of the micronutrient from new sources as this was not required by the mandate; rather, they have addressed the question of whether the micronutrient is bioavailable, or in some cases whether bioavailability is similar, lower, or greater than from the reference source. Further guidance on the data, or combination of data, that may be most appropriate to quantify the relative bioavailability of a new source versus a reference source is given in the following Sections (4.2.2–4.2.5 and subsections thereof).

4.2.2 | Data sources

4.2.2.1 | *Chemical and physicochemical data*

Chemical and physicochemical data may be sufficient if they can predict that the dissociation characteristics of the new source and the reference source(s) under identical experimental GI conditions are similar. Such predictions should allow assuming, with reasonable confidence, that a similar physicochemical form of the micronutrient from both the new and the reference source will be absorbed and utilised in a similar manner.[23] In such a case, a CF of 1 can be justified.[24] However, if the chemical forms of the micronutrient or the dissociation characteristics are different from the reference source,

[23]For example, **calcium phosphoryl oligosaccharides** is a calcium salt of phosphoryl oligosaccharides that is highly soluble and readily dissociates into phosphorylated oligosaccharides and calcium cation (Ca2+) in the gastrointestinal tract. The calcium is expected to be absorbed, distributed and eliminated in a manner similar to other dietary sources of calcium. Dissociation tests under simulated GI conditions demonstrated high solubility of calcium from this source, comparable to that of calcium chloride and greater than calcium lactate. It was concluded that calcium from calcium phosphoryl oligosaccharides is at least as bioavailable as the currently authorised calcium forms (EFSA ANS Panel, 2016).

[24]For example, it can be assumed that magnesium ascorbate will dissociate in the acid conditions of the stomach. Based on the predicted dissociation of magnesium ascorbate, the bioavailability of magnesium and ascorbic acid from magnesium ascorbate would be expected to be similar to other dissociable forms of magnesium and ascorbic acid in the GI tract (EFSA ANS Panel, 2009).

chemical and physicochemical data alone are unlikely to be sufficient to conclude on a similar absorption and utilisation of the micronutrient and to establish a CF (but may be used as supportive evidence).

While it is recognised that the food matrix may have a significant effect on the bioavailability of micronutrients, this should be controlled for in relative bioavailability studies that assess the new source against the reference source(s).

4.2.2.2 | *In vitro ADME data*

In vitro data on the release of the micronutrient component from a source and its solubilisation during simulated GI digestion (in vitro bio-accessibility), coupled with its uptake into/translocation across intestinal cell models under simulated GI digestion conditions using in vitro systems, may be considered sufficient to estimate relative bioavailability for similar chemical forms of a micronutrient. This approach would be relevant only when there is evidence that bioavailability critically depends on digestion, release from the food matrix and solubilisation in the GI tract as well as intestinal uptake and further transport across the gut epithelium. If in such cases, in vitro bio-accessibility and intestinal absorption of the micronutrient from the new and the reference sources are similar, a CF of 1 may be justified. If not, such in vitro data alone may not be sufficient to establish a CF (but may be used as supportive evidence).

For new form(s) of a micronutrient (e.g. metabolites, new vitamers), in vitro data may not be sufficient to assess relative bioavailability of the micronutrient from the new source vs the reference source. However, an understanding of the chemical/physicochemical and ADME characteristics is required to conclude on whether the new form is bioavailable[25] (i.e. available to be used by the body for specific micronutrient-dependent functions) and to anticipate the role of acute/short-term vs longer-term studies in humans to assess relative bioavailability of the micronutrient from new source.

4.2.2.3 | *Animal data*

Animal models, despite their known limitations in predicting bioavailability in humans, can still provide useful data for comparing the bioavailability of the micronutrient from a new source to a reference source. Therefore, they may be used as supportive evidence.[26] Relevant animal models comprise mammals (e.g. rats, mice, pigs, ferrets, dogs, guinea pigs, hamsters, rabbits, primates), except ruminants. The selection of the animal model should be made on a case-by-case basis, considering the existing knowledge regarding the ADME characteristics of the micronutrient in the chosen animal species compared to humans.

4.2.2.4 | *Human data*

Human intervention studies are the most reliable to estimate the bioavailability of the micronutrient from a new source vs a reference source. They are required to quantify the relative bioavailability of a new source when chemical and physicochemical data (Section 4.2.2.1) and in vitro ADME data (Section 4.2.2.2) cannot establish similar bioavailability (i.e. justifying a CF of 1) of the micronutrient from the respective sources. This is likely to be the case for new form(s) of a micronutrient.

Acute/short-term and/or longer-term studies in healthy (preferable) or diseased individuals may be used. Human studies should use conditions (e.g. empty or full stomach) and frequency (e.g. once or twice daily) of consumption that reflect the proposed use of the new source (use in a food supplement and/or in fortified foods), should measure appropriate biomarkers/parameters, and be of appropriate duration, which depends on the respective micronutrient and the selected biomarkers/parameters. Such studies should ideally compare equimolar doses of the micronutrient from the new and reference sources, as relative bioavailability of the micronutrient from the new vs the reference source may be dose related.[27]

To account for factors that may influence relative bioavailability of the new vs the reference source, there should be data:

a. across the dose range that is proposed for use of the new source,
b. on the effect of the food matrix, particularly for new sources of micronutrients intended to be added to a range of foods (i.e. food fortification). Bioavailability of the micronutrient from the new vs the reference source should be compared in the intended foods as consumed,
c. in populations and age groups representative of the target population for which the new source is proposed.

[25]For example:

- **Calcidiol monohydrate** has same physiological effect(s) as native forms of the micronutrient (e.g. vitamin D3), in terms of micronutrient function at physiological intake levels. Serum 25(OH)D is the major circulating metabolite of vitamin D3 in the body and is a source of 1,25-dihydroxyvitamin D, the biologically active form of vitamin D. Oral administration of calcidiol increases serum 25(OH)D, the endpoint for establishing nutrient requirements (EFSA NDA Panel, 2016, 2021b).
- **Nicotinamide riboside chloride** is considered a source from which nicotinamide, a form of the vitamin niacin, is released in the GI and is bioavailable, i.e. absorbed into blood and available to act as a precursor of nicotinamide adenine dinucleotide (NAD+) in cells (EFSA NDA Panel, 2019). In contrast, **1-methyl nicotinamide (chloride)**, a main metabolite of nicotinamide, has no vitamin function, i.e. it is absorbed into blood but not available to act as a precursor of NAD+ in cells (EFSA NDA Panel, 2019).

[26]For example, in a previous application (EFSA ANS Panel, 2010b), a study in pigs (Candela et al., 1984) demonstrating that the iron in **ferric sodium EDTA** dissociates from the chelate and is released into the luminal inorganic iron pool was used as supportive evidence for bioavailability of iron from the new proposed source of the micronutrient. The authors reported that the iron that was absorbed was incorporated into haemoglobin.

[27]For example, it has been observed in long-term studies that the relative bioavailability of calcidiol monohydrate vs vitamin D_3 per unit dose administered (assessed in relation to their effect on serum 25(OH)D concentrations) differs depending on the dose of vitamin D_3 used as reference (EFSA NDA Panel, 2023c).

Foods for specific groups (FSG) of the population include foods intended for infants and young children, foods for special medical purposes (FSMP) and total diet replacements (TDR) for weight control. In some cases, foods within these categories may be the only source of nutrition for the target population (e.g. infant formula, FSMP, TDR). This requires greater certainty on the relative bioavailability and CF for the new source proposed for use in these food categories, and human data is required to that end. It is noted that it may not be possible to obtain such data in the target population as ethical concerns may limit experimental studies in specific, especially paediatric, populations. In such cases, applicants should justify by a scientific rationale that extrapolation of the results obtained in other population groups to the target population is biologically plausible.[28]

While bioavailability of a micronutrient from a source may be influenced by the nutrient status of individuals within a population group, in the context of this guidance, the inter-individual variability of bioavailability is not the focus. This is because it is not practical to tailor CFs for labelling purposes to individuals with a different nutrient status in the target population. However, the nutrient status of the study subjects should be taken into consideration in the design of relative bioavailability studies. For example, for studies on bioavailability of iron, methods for normalisation of nutrient status are used (Cook et al., 1991).

Measurement of the concentration – time profile of the micronutrient in blood in **acute/short-term human intervention studies** may be sufficient as a basis to assess relative bioavailability of a new source following single or repeated oral administration.[29] If concentration – time profiles of the micronutrient in plasma are similar between the new and the reference source, a CF of 1 may be justified.[30] However, if these profiles are different, such data alone are not sufficient to establish a CF (but may be used as supporting evidence, e.g. by providing information on the kinetics of the micronutrient from the new and reference sources).

Measurement of absorption and/or utilisation of the micronutrient following single oral administration may be sufficient as a basis to assess the relative bioavailability of a micronutrient from a new source. If it is not feasible to measure utilisation (e.g. by monitoring biomarkers of status or function), retention may be acceptable.[31]

For new forms of a micronutrient, measurement of biomarkers of status or function **in long-term human studies** may be required to assess the relative bioavailability of the micronutrient from the new source. For example, vitamins may comprise chemically related compounds ('vitamers') having vitamin activity, i.e. capable of contributing to the vitamin requirement to a different extent (Gregory & Jesse, 2012; Jakobsen et al., 2019). Relative bioavailability can be assessed by comparing the effect of oral administration of different 'vitamers' on a relevant biomarker of status or function ('bioequivalence'). The selection of the biomarker of status or function to be measured in human studies is critical and should be duly justified (Section 4.2.4).

4.2.3 | Selection of the reference (micronutrient) source

When establishing a CF for a micronutrient, the absolute bioavailability of the micronutrient from the reference source is not needed. Instead, the relative bioavailability of the micronutrient from the new source compared to the reference source is required.

The reference source selected for comparison in relative bioavailability studies should be:

a. a source of a native form of the micronutrient or
b. a substance authorised as source of the micronutrient for which the relative bioavailability versus one or more native forms of the micronutrient is known.

For many micronutrients, several sources may already be authorised and could potentially serve as a reference for deriving a CF.

[28] A dietary folate equivalents (DFE) conversion factor of 1.7 for **5-MTHF** (relative to natural folates) was established for all population groups, including infants, based on studies in adults using folic acid as comparator. While there were no suitable studies in infants, the Panel assumed a similar bioavailability between 5-MTHF and folic acid in infants as it was considered unlikely that the bioavailability of 5-MTHF in this population group is lower than the one of folic acid. The Panel considered that this ensures that folate from 5-MTHF is provided to infants in at least the labelled amounts and considered this a conservative approach (EFSA NDA Panel, 2022a).

[29] For example, acute studies that compared the bioavailability of folate from **different salts of 5-MTHF** with each other could be used to establish a CF for the salts relative to each other using serum folate concentrations as marker of intake, whereas longer-term studies (e.g. 16 weeks duration) were needed to derive a CF for 5-MTHF salts versus folic acid using RBC (red blood cells) folate concentration as marker of folate status (EFSA NDA Panel, 2022a).

[30] Indeed, the relative bioavailability of 5-MTHF glucosamine salt was estimated to be similar or slightly higher than that of L-5-MTHF-Ca in a crossover relative bioavailability study in human volunteers by measurement of the concentration – time profile of the folate in plasma following a single dose of 400 μg of the folate source (EFSA ANS Panel, 2013).

[31] The following examples illustrate this point:

- **Iron from iron milk proteinate vs ferrous sulfate:** In a single dose relative bioavailability study with isotopically labelled foods in human adults, relative bioavailability of iron from iron milk proteinate was estimated to be 87% of that from ferrous sulfate. The dual stable isotope technique was used to estimate erythrocyte incorporation of the isotopes from a single dose of the labelled sources 14 days post dose and for calculation of the fractional iron absorption (EFSA NDA Panel, 2022c).
- **Iron from NaFeEDTA vs ferrous sulfate:** In single dose human studies with isotopically labelled foods, iron from ferric sodium EDTA is two to three times more bioavailable than iron in the form of ferrous sulfate. Fe absorption was measured in adult human subjects consuming different cereal foods fortified with radiolabelled FeSO4 or ferric sodium EDTA (NaFeEDTA), based on erythrocyte enrichment at 14 days post dose (EFSA ANS Panel, 2010b).
- **Iron from iron hydroxide adipate tartrate (IHAT) versus ferrous sulfate:** Single dose human studies, incorporation of iron into red blood cells (RBC) at 14 days post dose (EFSA NDA Panel, 2021c).

If there are multiple chemical forms of the micronutrient naturally present in foods, a form with significant contribution to the dietary intake in the target population (i.e. a native form of the micronutrient) is preferred to be used as a comparator. For some micronutrients, however, it may not be feasible to use chemical forms of the micronutrient naturally present in foods to assess relative bioavailability (e.g. iron and folate). In such cases, authorised sources for which the relative bioavailability versus native forms of the micronutrient is known are more commonly used as reference sources (e.g. ferrous sulfate and folic acid, respectively). An important consideration, however, is that the authorised chemical form used as a reference is covered by existing endpoints for establishing DRVs for nutrient adequacy and safety (UL).[32,33]

Similarly, when there are multiple chemical forms of the micronutrient naturally present in foods, it is preferable to select a reference source with high bioavailability, and the choice should be justified.

The choice of the reference source for use in relative bioavailability studies must be described and justified by the applicant. Both the proposed new source and the reference source should be sufficiently characterised to allow a scientific assessment with respect to the factors influencing bioavailability (e.g. chemical and physicochemical characteristics, water soluble or lipophilic compounds, or micronutrients released in the GI or absorbed intact).

4.2.4 | Selection of biomarkers in human studies

The shape of the curve for the relationship between the intake of the micronutrient from the reference source and biomarkers of nutrient intake, status or function should be defined and considered for the selection of the biomarker. Ideally, there should be a linear relationship between the intake of the micronutrient from the reference source and the selected biomarker. However, for micronutrients for which the percentage absorption or conversion to the active form is dose-dependent, a linear relationship may not be observed between the intake of the micronutrient and available biomarkers. In that case it is particularly important to test the new source vs the reference source across the dose range that is proposed for use of the new source (Section 4.2.2.4).

Applicants are referred to EFSA opinions on DRVs for vitamins and minerals for well-established biomarkers of intake, status and/or function, although the Panel acknowledges that new biomarkers could have become available or may become available in the future. Applicants should justify/substantiate that the biomarker used in relative bioavailability studies is appropriate.[34]

4.2.5 | Derivation of a conversion factor for labelling purposes

Conversion factors for vitamins and minerals are needed to calculate more precisely the content of micronutrients in foods, to be used for food labelling in relevant European legislation. To derive a CF, quantification of the relative bioavailability of the micronutrient from the new source compared to the reference source is needed.

It is important to consider whether the chemical form of the micronutrient in the new source is covered by existing endpoints for establishing DRVs for nutrient adequacy, and to establish bioequivalence in terms of its capacity to meet nutrient requirements (Melse-Boonstra et al., 2017; Moltedo et al., 2021). Depending on the proposed use and use levels of the new source, a CF < 1 may have implications for nutrient intake adequacy. Conversely, a CF > 1 may have implications for the safety assessment of the new source (Section 4.4.1).

[32]For **new chemical forms of folate** an appropriate comparator might be folic acid, which is an authorised source covered by endpoints for deriving DRVs (adequacy and safety) and for which the relative bioavailability versus natural food folates is known, albeit with considerable uncertainty. Folic acid is assumed to be linearly related to responses of biomarkers of intake and status at intakes < 400 μg/day and was considered an appropriate comparator for deriving a DFE conversion factor of 1.7 for 5-MTHF (relative to natural folates) at these levels of intake. However, because of its non-linear relationship with biomarker responses at higher intakes, folic acid was not considered a suitable comparator at these doses, possibly > 400 μg/day. **Note**: a CF of 2.0 for 5-MTHF (relative to natural folates) was established for ≥ 400 μg/day based on expert judgement, although with greater uncertainty than for intakes < 400 μg/day (EFSA NDA Panel, 2022a).

[33]For **new chemical forms of vitamin D**, an appropriate comparator might be vitamin D_3 (cholecalciferol), which is a native form of the micronutrient that has been authorised for addition to foods and use in food supplements in relevant legislation. This form makes a significant contribution to dietary intake and it is covered by existing endpoints for establishing DRVs for nutrient adequacy and safety (UL). The relationship between vitamin D_3 intake and serum 25(OH)D, a reliable marker of vitamin D status, is well established. Vitamin D_3 was considered an appropriate comparator for estimating the relative bioavailability of calcidiol monohydrate, a new chemical form of vitamin D (EFSA NDA Panel, 2023c).

[34]The following examples are used to clarify this point:

- **5-MTHF versus folic acid**: Although acute single dose studies comparing the concentration – time profile of the folate in plasma were sufficient to establish the bioavailability of different 5-MTHF salts to each other and establish a CF of 1 (similar chemical forms of the micronutrient), acute studies comparing the relative bioavailability of 5-MTHF versus folic acid using plasma folate response as biomarker were not considered appropriate by the NDA Panel for establishing a CF for 5-MTHF (different chemical forms of the micronutrient). Long-term, repeated dose human intervention studies using RBC folate concentration as biomarker of status were used instead because **RBC folate** concentration was considered the most reliable biomarker of folate status, as it reflects long-term dietary intake (EFSA NDA Panel, 2022a).
- **Calcidiol monohydrate versus vitamin D_3:** Serum 25(OH)D concentration was considered the most reliable biomarker of vitamin D status as it reflects long-term dietary intake, is a source of the biologically active form of vitamin D 1,25$(OH)_2$D and has been used and endpoint to set DRVs for nutrient adequacy (EFSA NDA Panel, 2021b, 2023c).

The minimum data requirements to derive a CF for a new source vs a reference source may vary depending on the chemical, physicochemical and ADME characteristics of both (new and reference) sources, the biomarker of status or effect that is selected for the assessment, and the proposed conditions of use, including the proposed use(s) and use level(s), and the target population. While chemical and physicochemical data, with or without in vitro ADME data, may be sufficient to establish a CF under certain conditions for similar chemical forms of a micronutrient, human studies are generally required to assess the relative bioavailability of different chemical forms of a micronutrient (Section 4.2).

As previously mentioned, human studies should cover at least the target population (or allow extrapolation of the results to the target population), the range of anticipated intakes of the new form(s) of the micronutrient (ideally in equimolar comparison with the reference form of the micronutrient) and the proposed conditions of consumption (e.g. times daily, full/empty stomach), and should be of sufficient duration to allow a scientific evaluation.[35]

If the data are considered insufficient to estimate the relative bioavailability of the micronutrient from the new source, a CF may not be established.

4.3 | Exposure to the new (micronutrient) source and resulting intake of the micronutrient

The exposure assessment, in relevant European populations and at the proposed use(s) and use level(s) of the new source, should include an estimation of the anticipated intake of:

a. the new source
b. the micronutrient from the new source
c. the micronutrient from all sources

In case of new sources added to food(s) (e.g. food fortification), the exposure estimates of the new source are determined based on consumption data for the food(s) in which the source is intended to be added and by summing the contribution of each food. If the new source is naturally present in foods (albeit in low amounts or in amounts not significantly contributing to nutrient requirements), and/or is intended for use in food supplements, background intake of the new source and/or intake from food supplements should also be estimated and added.[36] The resulting intake of the micronutrient from the new source is subsequently calculated **by applying the CF** derived for the new source. The intake of the micronutrient from all sources should then be calculated for all population groups.

A different paradigm applies to new sources intended for use in FSG.[37] In this case, exposure estimates may not be applicable to the whole European population but only to those who are likely to use these food products, and the incorporation of the proposed new source is expected to be in such a way that a pre-defined daily intake of the micronutrient is achieved. In addition, the food to which the new source is intended to be added could be the only source of nutrition for the target population (e.g. infant formula, certain FSMPs, TDR), and thus estimates of the background intake of the new source and the micronutrient are limited to those foods and population groups.

4.4 | Outcome of the assessment

4.4.1 | Safety assessment

The Panel will not establish a HBGV for a new source, or for the non-micronutrient component(s) of the source. Instead, the Panel will rather base its conclusions on a comparison between the estimated exposure to the proposed new source and a RP derived from the safety dataset provided. However, if a HBGV has already been defined for other uses of the same substance (e.g. new source already authorised as a food additive), the Panel considers it is appropriate to compare the estimated exposure to this established HBGV.

[35]For example:

- **5-MTHF vs natural folates**: A DFE conversion factor for 5-MTHF (relative to natural folates) using folic acid as comparator and RBC folate response as biomarker of status was established based on one repeated dose intervention study in healthy adults for intakes < 400 μg/day (DFE = 1.7, as for folic acid) and three repeated dose intervention studies in healthy adults for intakes ≥ 400 μg/day (DFE = 2, from the observation that the effect of 5-MTHF on RBC concentrations was, on average, 1.2 times higher than the effect of folic acid). Studies lasted ≥ 12 weeks (EFSA NDA Panel, 2022a).
- **Calcidiol monohydrate vs vitamin D_3.** A CF for calcidiol monohydrate using cholecalciferol (vitamin D_3) as comparator and serum 25(OH)D concentrations as biomarker of status was derived based on 10 repeated dose randomised controlled trials (RCTs) mostly in European populations including adult males and females (healthy subjects and individuals with osteopenia, osteoporosis, hypovitaminosis D and geriatric patients hospitalised for acute illness) and lasting from 2.5 to 6 months (criteria for inclusion was at least 6 weeks, the time estimated for serum 25(OH)D concentration to stabilise after the start of the intervention). Calcidiol monohydrate and vitamin D3 were given daily or weekly, and doses of vitamin D ranged from 5 to 38 μg/day as calcidiol and from 20 to 62.5 μg/day as vitamin D_3. The derivation of the CF took into account the conditions of use (up to 10 μg/day in food supplements in subjects 11 years of age) in two ways: (a) relative bioavailability was higher as the dose of calcidiol decreased and (b) studies with a supplementation pattern less frequent than weekly were excluded owing to the uncertainties associated to the extrapolation of the results from these studies to the health effects of daily doses of vitamin D (EFSA NDA Panel, 2021b, 2023c).

[36]For example, **calcidiol, proposed as a new source of vitamin D,** is naturally present in foods, albeit in low amounts. Background intake of calcidiol from the diet was considered in intake estimates, and the resulting intake of vitamin D (as calcidiol) from the new source and the background diet was subsequently calculated by applying the conversion factor derived for calcidiol (EFSA, 2024).

[37]Foods for specific groups (FSG) include foods intended for infants and young children, foods for special medical purposes (FSMP) and total diet replacement (TDR) for weight control as laid down in Regulation (EU) No 609/2013.

In the absence of an existing HBGV, the Panel would consider the margin of exposure (MoE) approach for evaluating the data generated for the new source in Tier 1 (and Tier 2, when applicable) of the toxicity testing strategy (Section 4.1.1 and Figure 1). The MoE is defined as the ratio between the RP and the anticipated exposure to the new source for a given population group. In order to conclude on whether the MoE is sufficient to establish the safety of the new source under the proposed conditions of use, the Panel will consider, on a case-by-case basis, aspects such as uncertainties arising from the quality and the completeness of the safety database and potential higher sensitivities of vulnerable population groups.

If the new source is considered to be safe for the proposed use(s) and use level(s) after the safety assessment, the Panel will assess whether the type of adverse effects that could be expected from the micronutrient component of the new source at high intakes are similar to those covered by the endpoint(s) used to derive the UL for the micronutrient. If that is the case, the Panel will compare the estimated intake of the micronutrient from all sources (i.e. including the new source) with the established UL for the micronutrient but will not derive a UL for the micronutrient or the new source.[38] If a CF > 1 applies to high intakes in the UL range, a lower UL may need to be established for the new source.[39]

If the micronutrient component of the source does not have established ULs, or the type of adverse effects that could be expected at high intakes are not covered by the endpoint(s) used to derive the UL, a UL may need to be derived for the new source.

4.4.2 | Relative bioavailability and derivation of a conversion factor

When evaluating new form(s) of a micronutrient, the Panel will conclude on whether the new form(s) is(are) available for specific functions in the body in vivo in humans, and thus on whether the proposed new source is indeed a micronutrient source or not. Only for sources of new form(s) with a demonstrated nutrient function and for sources of native forms of a micronutrient, the Panel will then consider whether a CF for the new source can be derived from the data provided (i.e. whether its capacity to meet nutrient requirements can be quantified). If not, the implications of the consumption of the new source under the proposed use(s) and use level(s) in relation to DRVs for adequacy and safety (UL) cannot be assessed.

It is anticipated that the level of certainty in the data that is needed to derive CFs for new sources proposed for use in FSG (e.g. foods which are intended to be the only source of the micronutrient) will be higher (and thus data requirements to derive such values), particularly if the CF is expected to be < 1 owing to the implications that this may have on the capacity of new sources to meet nutrient requirements.

5 | DATA REQUIREMENTS FOR DOSSIERS

Data requirements are similar for applications on new sources of micronutrients (i.e. vitamins and minerals) and for applications on new sources of macronutrients and/or other substances. The only difference is that, for new sources of micronutrients, relative bioavailability needs to be quantified, whereas for new sources of macronutrients and/or other substances, bioavailability needs to be demonstrated but not quantified.

For Sections 5.1–5.4, applicants are requested to refer to the most recent applicable guidance for the safety assessment of novel foods (EFSA NDA Panel, 2024).

5.1 | Identity of the substance proposed as new source of micronutrient(s)

5.2 | Production process

5.3 | Compositional data

5.4 | Specifications

[38] **Calcidiol monohydrate versus vitamin D_3**: The EFSA NDA Panel considered the implications of the consumption of calcidiol monohydrate supplements under the proposed conditions of use with regard to ULs for vitamin D (covering vitamins D_2 and D_3). Regarding possible adverse effects at high intakes, calcidiol increases serum 25(OH)D, which is the likely mediator for the endpoint (hypercalcemia/hypercalciuria) used for establishing ULs for vitamin D. Thus, the safety of calcidiol monohydrate under the proposed conditions of use could be assessed by estimating the intake of calcidiol from all sources (including background intake), the intake of vitamin D from all sources (including calcidiol monohydrate by applying a CF), and by comparing the estimated intakes of the nutrient with the ULs for vitamin D for all population groups (target population) for which the use of calcidiol monohydrate supplements was intended (EFSA NDA Panel, 2021b).

[39] **5-MTHF versus folic acid**: A CF of 1.2 for 5-MTHF relative to folic acid was estimated for supplements providing intakes ≥ 400 μg/day. It is noted that this CF is different from the CF of 1 for 5-MTHF relative to folic acid for addition to fortified foods and to food supplements providing intakes < 400 μg/day (EFSA NDA Panel, 2022a, 2023b).

5.5 | Proposed uses and use levels and anticipated intake

5.5.1 | Target population

It is assumed that the target population for new sources of micronutrients intended to be added to foods is from 4 months of age onwards, and for FSMP is the general population (no age restriction). For infant formula, the target population is from birth to 6 months of age; for follow-on formula, from 6 to 12 months of age; for processed cereal-based food and baby food, from 4 to 36 months of age; and for TDR, from 18 years of age onwards. For food supplements, the target population is to be defined by the applicant, subject to applicable EU legislation.

5.5.2 | Proposed uses and use levels

This section should provide a justification for the use of the proposed new source (not just a general justification for the micronutrient), accompanied by information on the types of products in which the new source is intended to be added/used. The information provided in this section will form the basis for the exposure assessment.

New sources intended for use in food supplements (Directive 2002/46/EC)

For new sources that are intended for use in food supplements, the anticipated daily intake (amount/day) of the source should be provided, alongside the corresponding anticipated daily intake of the micronutrient (amount/day):

- e.g. the new source is intended to be used in food supplements at a typical/maximum intake level of X mg source per day which corresponds to a recommended typical/maximum of Y mg of the micronutrient per day.

If different use levels are anticipated for different population subgroups, these should be specified in detail.
Information on the pattern of consumption and conditions of use should also be provided, e.g. once daily, twice daily, full/empty stomach, etc.

New sources intended for use in foods for specific groups (Regulation (EU) No 609/2013)

For new sources that are intended for use in FSG of the population (i.e. infant and follow-on formula, processed cereal-based baby food and baby food, FSMP, TDR for weight control), the proposed use levels of the new source should be provided, alongside the corresponding use levels of the micronutrient:

- e.g. the new source is intended to be added to FSG [specify legal food category under Regulation (EU) No 609/2013] at a typical/maximum level of X mg source per 100 g or 100 mL of the food, which corresponds to a typical/maximum of Y mg of the micronutrient per 100 g or 100 mL of the food.

The applicant should provide an indication of the proposed daily intake of the food product.
For micronutrients added to foods for which regulatory limits define the minimum/maximum concentration/amount of the micronutrient:

- e.g. the new source is intended to be added to foods [specify food] at a minimum/ maximum level of [specify concentration/amount] which corresponds to the minimum/maximum level of the micronutrient established for these foods as defined by e.g. Commission Delegated Regulation (EU) 2016/127,[40] Commission Delegated Regulation (EU) 2016/128,[41] Commission Delegated Regulation (EU) 2017/1798[42] and Commission Directive 2006/125/EC.[43]

New sources intended for use in foods (Regulation (EC) No 1925/2006)

If the new source is intended for use in foods according to Regulation (EC) No 1925/2006, the applicant should provide the following information in a tabulated format:

[40]Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. OJ L 25, 2.2.2016, p. 1–29.

[41]Commission Delegated Regulation (EU) 2016/128 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for food for special medical purposes. OJ L 25, 2.2.2016, p. 30–43.

[42]Commission Delegated Regulation (EU) 2017/1798 of 2 June 2017 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for total diet replacement for weight control. OJ L 259, 7.10.2017, p. 2–10.

[43]Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16–35.

a. the food categories in which the new source is proposed to be added. Food categories can be specified according to the EFSA Food Additive Intake Model (FAIM) tool[44] or the Dietary Exposure (DietEx) tool.[45] All intended uses should be expressed with the use of a unique classification system (i.e. either FAIM tool categories or DietEx tool categories). Codes and names of the proposed food categories should be provided as in the examples in Tables 2 and 3 below.

TABLE 2 Example of proposed uses and use levels according to the FAIM tool.

FAIM tool code	FAIM tool category	Maximum level
01.7.2	Ripened cheese	100 mg/kg

TABLE 3 Example of proposed uses and use levels according to DietEx tool.

FoodEx code	FoodEx category	Maximum level
A00EY	Cereal bars	10 mg/100 g

When selecting the FAIM tool categories, please refer to the instructions available on the website[46] particularly in relation to the unspecified food categories displayed in the FAIM tool. When using DietEx tool, the applicant is advised to use broad FoodEx categories instead of overly specific ones (e.g. yoghurts in general rather than certain types of yoghurts; biscuits in general rather than certain types of biscuits). Selection of overly specified food categories may pose difficulties for national authorities when it comes to the authorisation process of the new source:

b. the proposed maximum use levels (i.e. maximum concentrations) of the new source, and of the micronutrient(s) from the new source, in each food category as consumed (e.g. expressed as mg/kg or mg/100 g or mg/100 mL);
c. if the new source is proposed in different forms (e.g. dried, frozen, powder), the food categories and maximum use levels should be proposed for each form of the new source as requested in points (a) and (b). It should be specified whether the different forms of the new source are meant to be used singularly and/or in combination in a specific food category.

5.5.3 | Anticipated intake of the new source and corresponding intake of the micronutrient(s) from the new source

Based on the information provided in Sections 5.5.1 and 5.5.2, estimations of anticipated daily intakes of the new source and the corresponding intakes of the micronutrient(s) from the new source are required, both per kg bw and in absolute amounts. The applicant should provide estimates of the mean/median and high (95th percentile) anticipated daily intakes of the new source and corresponding micronutrient(s) for each target population group, including specific population groups such as infants, children, pregnant and lactating women, where appropriate.

The FAIM tool or the DietEx tool are available to applicants to perform the chronic intake estimate of the new source when added to foods. When estimating the intake, the applicant should consider all food categories to which the new source is intended to be added for a conservative scenario. Both FAIM and DietEx tools use individual consumption data from the EFSA Comprehensive Food Consumption Database to generate estimates (mean and 95th percentile) for population groups (infants, young and other children, adolescents, adults) throughout several EU countries. It is noted that the DietEx tool uses more refined food categories as compared to the FAIM tool, which leads to a more refined intake estimate of the new source. If the available toxicological data, human data, data on chemical composition or literature review raise concerns regarding an acute effect, the applicant should also consider acute intake estimates of the new source.

Summary statistics from the EFSA Comprehensive European Food Consumption Database provide valuable estimates of intake and are available on the EFSA website in the form of spreadsheets, both for chronic and acute consumption. Detailed information on the database and guidance on its use have been published (EFSA, 2011). Anticipated daily intakes for mean and high-percentile consumers can be calculated through the combination of the intended use level in each food category with mean and high chronic consumption values from the database, respectively.

The NDA Panel proposes a tiered approach where the first step makes use of the summary statistics of the EFSA Comprehensive Food Consumption Database at the maximum proposed use levels for each food category (Tier 1). In some

[44]The FAIM tool was developed by EFSA for estimating chronic exposure to food additives in the regulatory framework of food additives Regulation (EU) 1333/2008. Considering that the exposure assessment of food additives and intake assessment of micronutrient sources share common principles, the FAIM tool can also be used for the intake assessment of new (micronutrient) sources. The FAIM tool is available at: https://www.efsa.europa.eu/en/applications/food-improvement-agents/tools.

[45]The DietEx tool was developed by EFSA for estimating chronic exposure to substances present in food, naturally present or intentionally added to foods. The DietEx tool is available at: https://www.efsa.europa.eu/en/science/tools-and-resources/dietex.

[46]https://www.efsa.europa.eu/sites/default/files/applications/FAIM-instructions.pdf.

cases, such estimates provide sufficient information, if high intake estimates are below HBGV for the new source or its non-nutrient component (e.g. acceptable or tolerable daily intake). In other cases, more refined intake estimates (from different food consumption scenarios) may be needed (Tier 2). The applicant should consider and discuss the uncertainties related to the assessment; in particular, sources of under- or over-estimation. To this end, the guidance from the EFSA Scientific Committee related to uncertainties in dietary exposure assessment should be considered (EFSA, 2007).

If the new source is intended to be used in food supplements, TDR, 'meal replacements for weight control' and/or FSMP, the applicant should not select these food categories in FAIM tool or the DietEx tool. For TDR in accordance with Regulation (EU) No 609/2013, the applicant should indicate the maximum daily intake of the new source in mg/day. For 'meal replacements for weight control', the applicant should specify the amount of the new source to be used in a single meal replacement.

When the intended uses are expressed as maximum daily intakes of the new source (e.g. in food supplements), the applicant should also provide the maximum daily intake of the new source expressed on a per kilogram bw basis for each age group of the target population. To this end, the applicant should use the mean default body weights as reported in the EFSA guidance on default body weights (EFSA Scientific Committee, 2012).

For micronutrients added to foods where regulatory limits define the minimum/maximum concentration/content of the micronutrients (e.g. FSG), a reference to these regulatory limits can be provided as an estimated intake of the micronutrient, with an indication that the addition of the new source will reach and not exceed these regulatory minimums/maximums, respectively, for any micronutrient (see Section 5.5.2).

Once the intake has been estimated for the new source, the resulting intake of the micronutrient(s) from the new source should be calculated, considering the CF that has been derived for the micronutrient from the new source.

5.5.4 | Combined intake considering other sources of the new (micronutrient) source or its main constituents

The applicant should provide intake estimates of the nutrient and non-nutrient components of the new source from other sources in relevant population groups, including (their) natural occurrence in foods (i.e. from the background diet) and intakes that may derive from other uses (e.g. as food additives).

The combined intake from all sources should be estimated by considering:

- high daily intakes (95th percentile) of the new source/its constituents from the proposed uses and maximum use levels (as estimated in Section 5.5.2);
- high daily intakes (95th percentile) of the new source/its constituents from more refined food consumption scenarios, where appropriate (Section 5.5.3);
- mean and high daily intakes from natural sources (i.e. from the background diet) derived from the literature. To this end, EFSA NDA Panel opinions on DRVs, including ULs, contain intake estimates for micronutrients from the background diet obtained from food consumption data available in the EFSA Comprehensive Food Consumption Database.[47]
- daily intake from other uses (e.g. food additives) derived from the literature, where appropriate.

Examples of combined intake estimates of the new source and its constituents, including the micronutrient(s), from all proposed uses and from other sources, including the background diet, can be found in published opinions (EFSA AFC Panel, 2008; EFSA ANS Panel, 2008, 2010a, 2010b; EFSA NDA Panel, 2022d).

5.5.5 | Estimates of the exposure to undesirable substances and other substances of possible safety concern

Exposure estimates should be provided for relevant undesirable substances and any other substances of possible safety concern identified in the compositional analysis (e.g. potential secondary plant metabolites, residues, contaminants or degradation products – Section 5.3). These substances may be present in the new source due to its source or the manufacturing process, as well as due to its use and storage.

The same approach as that used for the anticipated intake of the new source should be followed to estimate the exposure to undesirable substances/substances of possible safety concern from the new source for the proposed target population. To anticipate the exposure to these substances from the new source, the applicant should consider the maximum amount of the undesirable substances/substances of possible safety concern expected to occur in the new source (e.g. maximum limit set in the specifications or, in case that specifications are not established, the maximum level reported among the batch-to-batch analytical data) and the highest estimated daily intake (i.e. 95th percentile) of the new source for the proposed target population.

[47] https://www.efsa.europa.eu/en/topics/topic/dietary-reference-values.

When relevant, the applicant should also consider the exposure to those substances from the background diet. The exposure to undesirable substances/substances of possible safety concern from the new source (plus from the background diet when relevant) should be compared with a HBGV (e.g. ADI or TDI).

5.6 | Data on the safety of the new source and on the relative bioavailability of the micronutrient from the new source

The applicant should demonstrate that the new source is safe and that the micronutrient from the new source is available to the body for the specific micronutrient-dependent functions. The latter is particularly relevant for new forms of micronutrients (e.g. nutrient metabolites, new vitamers; see Section 4.2.1).

Applicants are requested to consult the decision trees in Figures 1 and 2 of this Guidance to decide on the tests or combination of tests that are appropriate to demonstrate both the safety of the new source and the relative bioavailability of the micronutrient from the new source, depending on its characteristics and to provide a rationale for such decision.

5.6.1 | Literature search

Prior to planning and conducting toxicity and relative bioavailability studies, applicants should consider the chemical and physicochemical characteristics of the new source, including its nutritionally and toxicologically relevant components. Additionally, a comprehensive literature review of existing data on the toxicity and bioavailability of the new source or its relevant components should be conducted, and the collected evidence should be critically appraised.

5.6.2 | Dissociation studies

The aim of this approach is to generate data which can predict the fate of the new source in the human body once it is ingested. This testing phase is, however, focussed on the initial phase of the digestive process and the tests should therefore be conducted under conditions which could mimic the process of human digestion, e.g. at a temperature of 37°C, using different buffers to simulate the different environments of the GI tract (preferably at pH 2, 4 and 6.8). Information on the rate(s) and extent(s) of dissociation under these conditions should be provided for the new source and compared to a reference source. At least three independently produced batches of the new source should be tested. Although dissociation tests have been performed for decades, no validated, standardised methods are available which can be recommended to investigate dissociation of the new source under GI conditions. Certain elements contained in OECD Guidelines for the Testing of Chemicals, Section 1 (e.g. OECD Guidelines 105, 108, 111, 112),[48] and in the European Pharmacopoeia (Council of Europe, 2023), may be relevant to conduct dissociation studies with new sources of micronutrients.

5.6.3 | Toxicity studies

The purpose of conducting toxicological studies is to identify and characterise the potential hazards of a new micronutrient source. The applicant should consult the decision tree (Figure 1) to decide on the approach for toxicological testing of new substances proposed as new sources of micronutrients, as well as the toxicological testing requirements outlined in the EFSA novel foods guidance regarding animal toxicity testing and the potential use in vitro, in silico and in chemico approaches (often referred to as New Approach Methodologies or NAMs) for replacing or reducing the use of animals (EFSA NDA Panel, 2024). Prior to planning and conducting toxicological studies, applicants should consider the chemical, physicochemical and microbiological characteristics of the new source, including its nutritionally and toxicologically relevant components.

All toxicological studies should be conducted in accordance with international guidelines (e.g. OECD) and according to the OECD principles of GLP Organisation for Economic Co-operation and Development principles of Good Laboratory Practices (OECD, 1998); see also Commission Implementing Regulation (EU) 2017/2469.

5.6.3.1 | *Genotoxicity studies*

In vitro studies should be provided to test the genotoxicity of a substance, following the tiered approach described in the EFSA novel foods guidance (EFSA NDA Panel, 2024).

[48] https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-1-physical-chemical-properties_20745753.

5.6.3.2 | *Other toxicity studies*

If conducting animal studies is deemed necessary to assess the toxicological profile of the substance proposed as a new source, the study type and study design should comply with the technical requirements outlined in Section 8 of the EFSA guidance on novel foods (EFSA NDA Panel, 2024). If new toxicological data need to be generated, ideally the respective studies could be expanded to include also toxicokinetic investigations (Section 5.6.4.2).

Human intervention studies may also be needed to assess the safety of micronutrient forms not covered by the UL (Figure 1; see Section 5.6.4.3 for data requirements).

5.6.4 | Relative bioavailability studies

Applicants are requested to consult the decision tree in Figure 2 to decide whether relative bioavailability studies are needed in addition to dissociation studies (Section 5.6.2).

5.6.4.1 | *In vitro studies*

In vitro studies can be used to inform the ADME of the new source.

The conditions for in vitro digestion assays should be standardised. Static (e.g. the INFOGEST method); (Egger et al., 2016; Brodkorb et al., 2019; Sulaiman et al., 2021) and dynamic (Minekus et al., 1995) in vitro models have been developed (Marze, 2017). In vitro models could be used on a case-by-case basis to quantify transport across the intestinal membrane and assess metabolism (EMA CHMP, 2022; OECD, 2021). Existing models include cell-based systems of various levels of complexity (e.g. MDCK, Caco-2, human small intestinal and liver organotypic 3D culture models). Such in vitro models could complement in vivo models to assess absorption and metabolism.

5.6.4.2 | *Animal studies*

As animal models are known to have a limited capability to predict relative bioavailability in humans and can only provide supportive evidence (see Section 4.2.2.3), it is recommended that these data are only provided in the context of toxicological studies when required for the safety assessment (see Section 5.6.3.2). Guidance on how to conduct in vivo ADME studies can be found in OECD Test Guideline 417.

5.6.4.3 | *Human studies*

Human intervention studies have the highest value to assess the relative bioavailability of the micronutrient from a new source vs a reference source. Suitable study designs include randomised crossover studies (with an adequate wash-out period) and randomised parallel studies.

The selection of the reference source, the dose of the micronutrient in the new and reference source, the duration of the intervention (including single vs. repeated doses), the conditions and pattern of administration, the selection of the study population, and the outcome variables investigated (concentration – time profiles of the micronutrient in plasma; biomarkers of exposure, status or function; measures of utilisation/retention; safety endpoints) should be duly justified in the context of what is known about the new source, including new forms of the micronutrient, for each of the human studies provided (see Sections 4.2.2–4.2.5). This requirement also applies to human intervention studies conducted to assess the safety of micronutrient forms not covered by the UL (Figure 1).

The minimum information requirements for human intervention studies can be found in **Appendix B**.

5.7 | Conclusions

In compiling the data in support of the safety of a new source and relative bioavailability of the micronutrient from the new source, applicants should also seek to interpret the data and draw conclusions. The significant findings of each toxicity study (both unpublished and published) should be highlighted, together with the method for the identification of the reference point, (BMDL values or the NOAEL), and any other relevant information. The reasons for disregarding any findings should be carefully explained. Where necessary, the conclusions should include an interpretation of the importance of the findings in terms of possible mechanisms underlying any effects observed, a discussion of whether these are relevant to humans and, if so, the possible importance of the extrapolation of such findings to humans. Where available, human intervention studies assessing safety endpoints should also be discussed in this context.

In terms of demonstrating that the micronutrient from the proposed source is bioavailable, and that the relative bioavailability of the micronutrient from the new source can be quantified, the applicant should seek to draw conclusions comparing the results obtained with the new source and a reference source. The conclusions should allow the determination of a conversion factor for the new source vs the reference source. The number and type of studies used for this purpose among those available, the inclusion/exclusion criteria, the methodology applied and the rationale for the derivation

of a CF should be carefully and extensively explained. The implications of the CF for the safety of the new source at the proposed uses and use levels for all relevant population groups should be clearly stated. The implications of CF <1 for nutrient adequacy when the new source is intended to be used in FSG that are the only source of nutrition and for which minimum regulatory limits for the micronutrient(s) exist should also be discussed, where appropriate.

ABBREVIATIONS

25(OH)D	25-hydroxyvitamin D
3-D	three dimensions
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AFC Panel	Scientific Panel on food additives, flavourings, processing aids and materials in contact with food
ANS Panel	Scientific Panel on food additives and flavourings
ARfD	acute reference dose
BMDL	Benchmark dose level
bw	body weight
CF	conversion factor
CoB	Constituent of the body
DFE	dietary folate equivalents
DietEx	dietary exposure
DRV	dietary reference value
ECHA	European Chemicals Agency
EDTA	Ethylenediamine tetraacetic acid
EMA CHMP	European Medicines Agency's Committee for Medicinal Products for Human Use
FAIM	Food additive intake model
FAS	full analysis set
FSG	Foods for specific groups
FSMP	Foods for special medical purposes
GI	gastrointestinal
GCP	good clinical practice
GLP	good laboratory practice
HBGV	health-based guidance value
ICH	International Conference on Harmonisation
IHAT	iron hydroxide adipate tartrate
ITT	intention-to-treat
MDCK	Madin-darby canine kidney
MoE	*margin of exposure*
MTHF	methyltetrahydrofolate
NAD	nicotinamide adenine dinucleotide
NaFeEDTA	ferric sodium ethylenediamine tetraacetic acid
NAMs	New Approach Methodologies
NDA Panel	Scientific Panel on nutrition, novel foods and food allergens
NOAEL	no observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
POs	Phosphoryl oligosaccharides
PP	per protocol
RCT	randomised controlled trial
RP	Reference point
TDI	tolerable daily intake
TDR	total diet replacements for weight control
UL	tolerable upper intake level
UVCB	unknown or variable composition, complex reaction products or of biological materials

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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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

Steps taken by EFSA

The steps taken by EFSA to address this mandate are the following:

1. The mandate was received in December 2022.
2. A Workshop was planned with the purpose of exchanging views with stakeholders and scientific experts regarding conceptual and methodological principles relevant to the revision of the guidance.
3. From 15 December 2022 to 17 January 2023, EFSA held an open consultation through an Expert Survey to invite scientific input from stakeholders and scientific experts in the field on key points to consider for the derivation of conversion factors for new sources or forms of nutrients.
4. The outcome of the Expert Survey was used to prepare a Discussion Paper to shape and optimise the workshop discussion, and to identify scientific experts in the field who were subsequently invited to the workshop.
5. The Workshop was held on 9 March 2023. All documents relative to the workshop, including the event report, can be found on the EFSA website.[49]
6. The conclusions and recommendations from the Workshop were used to prepare a first draft of this Guidance, which was discussed by the NDA Panel at its Plenary meeting on 6 July 2023.
7. Later draft versions of the Guidance were discussed at the WG on UL on 20 December 2023 and 12 January 2024.
8. The draft Guidance was endorsed by the NDA Panel for public consultation on 31 January 2024.
9. The public consultation was open from 15 February to 14 April 2024.
10. The comments received during the consultation were considered by the WG on UL to prepare the final version of the Guidance, which was adopted by the NDA Panel on 27 June 2024.

[49] https://www.efsa.europa.eu/en/events/workshop-derivation-conversion-factors-new-sources-or-forms-nutrients.

APPENDIX B

Minimum information requirements for acceptability of data and interpretation of results from human studies

A study report can be considered complete when it contains at least the information outlined in this Appendix. This Appendix has been adapted from the International Conference on Harmonisation (ICH) guideline E3 on the structure and content of clinical study reports[50] for the purpose of the assessment of the relative bioavailability of a nutrient from a proposed source vs a reference source and safety, where appropriate. Study reports which follow the full structure of ICH E3 are also acceptable.

Study reports not complying with the requirements outlined below may not allow a scientific evaluation of the study by the NDA Panel.

B.1 | Title page

The title page should include information on the nutrient source under investigation (and the reference source where appropriate), the primary outcome variable(s) studied, the method(s) of measurement used to assess the outcome variable(s) in vivo in humans, the study design (e.g. double or single-blind, two or more arms/periods, parallel or cross-over, single or multicentre), the study group(s), the study initiation and completion date, the place in which the study was conducted, the name of the sponsor, the funding source and its exact role and contribution to the study (e.g. in the design, conduct, analysis and/or reporting of the study, if any), the name of the principal investigator, the name of the author of the report and the date when the report has been signed off.

B.2 | Summary

B.3 | Table of contents

B.4 | List of abbreviations and definition of terms

B.5 | Ethical considerations

This should include information about the review and approval of the study by an ethics committee. Information about the ethical conduct of the study, about how the informed consent was obtained from participants should be provided. If a review or approval by an ethics committee was not provided, this should be specified and duly justified.

B.6 | Trial registration

It should be specified whether the study has been registered in a trial registry. If so, the trial registration number should be given. In case the study has not been registered, explanation should be given.

B.7 | General information about the study

In this section, the name/affiliation of the investigators and other people with a major role in the study (e.g. staff carrying out observations related to the outcome variable(s) under investigation), the statisticians and the authors of the report, should be provided. The section should also include information about the facilities which were used (e.g. for multicentre studies: information about the study sites and about the use of a central laboratory vs. non-central sample analyses), and on whether a contract research organisation has been tasked to carry out the work.

B.8 | Study objectives

The objective(s) of the study and the hypothesis to be tested should be specified in this section.

B.9 | Study design

This section should outline whether the study was planned e.g. as open-label, single-blind (specifying who was blinded) or double-blind study, as a single- or multicentre study (with a specification about the number of study sites). Information about the country setting, the type of control used (and the reasons why it was considered appropriate in the context of the study), the study duration and a discussion on the choice of the study design for investigating the selected outcome variable(s) should also be provided. In case the study was planned with an adaptive design, it should be specified which

[50] https://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html.

kind of adaptations at which time points were planned in the protocol and whether a Data Monitoring Committee was involved in the implementation of the plan.

B.10 | Study group

The inclusion and exclusion criteria should be described, including the criteria (and their validation) used to select subjects, if applicable. The appropriateness of the study group for the particular purpose of the study should be discussed. Any predefined criteria for excluding subjects from the study after randomisation should also be given, together with information on how these subjects were intended to be followed-up.

B.11 | Study products

A detailed description of the nutrient source[51] under investigation and the reference source used (if any), including information on the mode of administration, and the amounts used, should be provided.

B.12 | Method of assigning subjects to groups

Details on the method used to assign subjects to the study groups (randomisation or minimisation) should be given. It should be specified whether this allocation was done in a centralised or decentralised way, whether it was stratified (and if so by which factors) or whether the allocation was done in blocks. Information on the measures taken to conceal the allocation should also be described here.

B.13 | Blinding

Information on the strategy used to ensure blinding should be provided, e.g. measures taken to achieve that the study products were not distinguishable by smell, taste, colour, shape or packaging; how products were labelled (e.g. by subject individual codes or other). Information should be given on who had access to the product codes, whether there were any predefined circumstances in which the blinding could be broken and who from the team of investigators would be unblinded in case of such a need. If proper blinding could not be achieved, please discuss and justify why this was not possible. For studies with an adaptive design, it should be reported how it was ensured that the study personnel remained blinded to the interventions, especially if the pre-planned adaptation required unblinding of the data. In such a case, it should be justified why the particular adaptation made it necessary to unblind the data and why the same aim could not have been achieved with statistical methods not requiring such unblinding.

B.14 | Concomitant medication or interventions

Any concomitant medication or non-pharmacological interventions, any rescue medication allowed by the study protocol should be described here (e.g. name of medication, dose and posology; type of non-pharmacological intervention, frequency, duration).

B.15 | Compliance with the intervention and the protocol

This section should include a detailed description about the measures taken to ensure and assess compliance with the intervention and the protocol.

B.16 | Outcome variable(s) measured

Information about the predefined primary outcome variable(s), secondary outcome variable(s) and all other outcomes planned to be measured should be presented in this section.

The methods used to assess the outcome variable(s) should be specified.

This section should also include information about the timing of the measurements (e.g. flow-chart), and a justification of the appropriateness of the outcome variable(s) chosen to achieve the objective(s) of the study.

B.17 | Data quality assurance

Any measures taken with respect to the quality assurance and quality control systems implemented for data collection should be addressed here. Whenever a quality control system has been used/reported in the conduct of the studies (e.g. GCP, as relevant), the particular system should be indicated.

[51]Sufficient information should be provided to establish that the study was performed with a micronutrient source which complies with the specifications given for the new proposed source of the micronutrient that is the subject of the application.

B.18 | Pre-planned statistical analyses

This section refers to the statistical analysis planned before the implementation of the study, and should specify whether any subgroup analyses were preplanned (e.g. whether there was a priori hypothesis of a differential effect in a particular subgroup of subjects). The choice of each statistical technique should be appropriately justified. The data analysis sets (e.g. ITT, FAS, PP) should also be defined. It should be specified which of the analyses presented have been prespecified as the main analysis in case several alternative analyses for one outcome variable are planned (e.g. ITT vs PP or different models used). The reasons for the choice of the analysis should be given. If imputation of missing data is foreseen, information should be given on how it is planned to assess the robustness of the assumptions made with respect to the imputation of data. For studies for which an adjustment for multiple comparisons is needed in order to preserve the family-wise type I error rate, the preplanned approach towards adjusting for multiplicity should be specified. In case of studies with an adaptive design, the number and time-points of prespecified interim analyses, as well as the statistical methods used to conserve the type I error rate, should be given. The appropriateness of the statistical method used for the design of the study should be discussed. Finally, it should be stated which analyses were planned to be confirmatory and which ones exploratory.

B.19 | Determination of sample size

Detailed information on how the planned sample size of the study was calculated should be given here. This should include information about the expected size of the effect, the assumed standard deviation of the population, the significance level chosen, the anticipated power of the study and the statistical tests (to be performed) to which the sample size calculation relates. In addition, information should be given on whether equal or unequal allocation to groups has been accounted for in the sample size calculation (if unequal allocation is foreseen) and whether any allowance for drop-out has been made. Finally, the program used to calculate the sample size should be identified. In case of studies with adaptive design allowing for sample size re-estimation, the planned method for re-estimating sample size should be described.

B.20 | Protocol amendments, deviations and violations/deviations from the planned approaches and analyses

Non-adherence or changes made during or after the study with respect to the pre-planned approaches or pre-planned analyses should be specified here.

Any protocol amendments (i.e. a systematic change in the protocol after approval), protocol deviations and violations (i.e. unplanned unsystematic deviations from the protocol with either minor effects (deviations) or affecting the scientific integrity (violations)) should be outlined.

A protocol amendment may, for example, relate to a systematic change of the pre-established inclusion and exclusion criteria, the planned study design, addition or deletion of outcome variable(s), sample size, the planned statistical approaches or the definition of data analysis sets (e.g. ITT vs. PP). If no protocol amendments have been made, it should be confirmed that the study was carried out according to the protocol.

Protocol deviations and violations may relate, for example, to inadequate or not-timely collected informed consent, inclusion of subjects not meeting the eligibility criteria, improper breaking of the blind, improper assessment of an outcome variable, incorrect or missing tests, rescheduled or missed study visits, visits outside the permitted window, inadequate record keeping, use of not permitted medication or a non-pharmacological intervention.

Any additional exploratory analyses conducted which were not part of the (amended) protocol (e.g. unplanned subgroup analyses to inform a subsequent study) should also be recorded.

B.21 | Subject flow

A clear description of the number of subjects screened, the number of subjects recruited, the number of subjects randomised, the number of subjects who entered and completed each study phase, the number of drop-outs and the number of withdrawals should be specified. The reasons for subjects dropping-out of the study or for having been withdrawn from the study by the investigators should be stated. Information about whether and when the blind was broken (if so) should also be given here.

B.22 | Data sets analysed

This section should include a clear definition of each analysis set used for final analysis (e.g. ITT, FAS, PP), including information on the number of subjects available for each analysis at each assessment time point. In case PP analyses are presented, information should be given on the extent to which the subjects included in this analysis set could have deviated from the protocol, and the reasons why they were still eligible for inclusion in the PP analysis set. Finally, the reasons for excluding subjects from each analysis at each time point should be given.

B.23 | Baseline characteristics of the study population

In this section, baseline characteristics for all analysis sets should be given (e.g. ITT, FAS, PP, completers, other) – overall and by study centre for multicentre studies.

B.24 | Results of assessment of compliance with the intervention and the protocol

Results of the assessment of compliance with the intervention and with the protocol should be given here.

B.25 | Statistical analysis carried out

A detailed description of the statistical analysis carried out should be provided, in line with EFSA's guidance on statistical reporting[52] (EFSA, 2014). This description should include, among others, information on:

- the statistical program used (version number and operating system),
- the type of statistical tests/models used,
- the test/model selection,
- the appropriateness of the test/model used for the type of data generated
- the handling of missing data (including a detailed description of the potential missingness mechanism and of how the missing data were handled). If missing data was imputed, please describe the methods used to do so and specify which sensitivity analyses were carried out, if any,
- the variables or factors used as fixed or as random effects (if appropriate),
- the assumed covariance structure for longitudinal analyses,
- the adjustment for covariates (and justification about the covariates used),
- the handling of data stemming from multicentre trials,
- whether any issue with respect to multiple comparisons arises (in case of multiple primary outcome variables or multiple group comparisons, or if a secondary outcome variable is intended to be used as the primary efficacy criterion instead of the primary outcome variable); this should include a description of the method chosen for adjusting the analysis for multiple comparisons and information on the number of outcome variables for which the analysis has been adjusted.

B.26 | Results of the study

Results for all the outcome variables assessed and for all analysis sets investigated should be presented. The results should be given as estimates with associated confidence intervals and p-values (if corrected for multiple comparisons, both the uncorrected and the corrected results (confidence intervals and p-values accounting for multiple comparisons) should be given). Results should be presented for all groups under investigation and for each assessment time point if foreseen in the prespecified analysis plan; otherwise, descriptive statistics should be included. The information should be presented in a tabular format, and not only graphically. For multicentre trials, results or descriptive statistics for the individual centres should be presented (if prespecified). The number of subjects included in each analysis and assessment time point should be provided. In case of data imputation, the results of the related sensitivity analyses should be included. The full outputs of the statistical analyses, together with the associated codes used for programming should be given as an Annex A full list of the abbreviations used to denominate variables or factors in the programming should also be given, so that the statistical outputs are self-explanatory.

B.27 | Adverse events

In case adverse events are assessed in the study, these should be clearly reported (possibly indicating those which may be related to the intervention and those which may be not related to the intervention), together with information on the (diagnostic) criteria used to ascertain them.[53]

[52] https://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3908.pdf.

[53] For reporting of safety-related data see also ICH-E3-'Stucture and contend of study reports'.