Technical Report

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Discussion paper on the derivation of conversion factors for new sources and forms of nutrients

European Food Safety Authority (EFSA)

Abstract

The European Commission requested that EFSA update its "Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources" regarding the scientific principles and data requirements for applicants in order to derive a conversion factor (CF) 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. To that end, EFSA held an open consultation through an Expert Survey on key points to consider for derivation of conversion factors, to be followed by a workshop with scientific experts and representatives of bodies in charge of setting conversion factors for new sources and forms of nutrients to share and exchange views on the principles and data requirements in this scientific field. The outcome will inform EFSA's update of its Guidance. The present document takes into consideration the outcome of the Expert Survey. It outlines the background, existing scientific principles, methodologies, and data requirements, illustrated with practical examples of past assessments of new sources of nutrients in the context of specific applications and highlights additional key points to consider for which EFSA invites scientific input through a workshop.

Key words: Nutrients, new sources or forms, conversion factors, guidance

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1 Background

The European Commission requested that EFSA 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 (CF) for proposed new sources or forms of nutrients (e.g. different chemical forms or vitamers, nutrient metabolites) to be authorised for addition to foods, including food supplements. The conversion factor should indicate the extent to which the proposed new sources or forms of nutrients 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.

- **Mandate and Terms of Reference** as provided by the European Commission.

To that end, EFSA's Nutrition and Food Innovation (NIF) Unit held an open consultation through an Expert Survey on key points to consider for deriving conversion factors, to be followed by a workshop on the scientific principles and data requirements in this scientific field.

- The Expert Survey invited 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. The outcome was used to prepare this Discussion paper to shape and optimise the workshop discussion, as well as to identify scientific experts in the field who were subsequently invited to the workshop. A summary of the comments received can be found in Annex A.

- A workshop gathering scientific experts and representatives of bodies in charge of setting conversion factors for new sources or forms of nutrients will be held to share and exchange views on the scientific principles and data requirements in this scientific field. The workshop will help to define the scientific principles, methodologies and data needs that EFSA will apply when deriving a conversion factor for new sources or forms of nutrients.

The outcome will inform EFSA's update of its Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources (EFSA ANS Panel, 2021).

This Discussion paper takes into consideration the outcome of the Expert Survey. It outlines the background, existing scientific principles, methodologies and data requirements (EFSA guidance), illustrated with practical examples of past assessments of new sources of nutrients in the context of specific applications, and highlights additional key points to consider for which EFSA invites input through the workshop.

The purpose of the workshop is to exchange views regarding conceptual and methodological principles relevant to the revision of the guidance. Specifically, the workshop will seek to collect input from participants on the following themes:

1. *Selection of the reference nutrient source for comparison with the novel source*
2. *Requirements for nutrient metabolites to be considered also as nutrient sources*
3. *Conditions under which chemical data or data from in vitro studies could be considered sufficient to estimate relative bioavailability and a conversion factor*
4. *Human studies to estimate relative bioavailability and a conversion factor*
5. *Implications of the conversion factor for Dietary Reference Values (DRVs) for nutrient adequacy and the Tolerable Upper Intake Level (UL) of the nutrient*

This Discussion paper provides background information on each theme as well as a series of key questions to be addressed during the workshop.

The workshop program will be structured in one session covering the key themes. The session will consist of a brief presentation in which the themes will be introduced, followed by a discussion of the questions associated with the relevant themes. The session will end with reflections collected and key messages from the workshop synthesised.

The intention of the workshop is to collect the views of the participants and possible orientations about the questions raised (in a Workshop report) that will be considered by the NDA Panel in revising the guidance on the scientific principles and data requirements for deriving conversion factors for proposed new sources or forms of nutrients.

2 Conversion factors (CF)

Conversion factors for vitamins and minerals are needed to calculate more precisely the content of such vitamins and minerals in foods, particularly for labelling purposes, but also for assessing the adequacy and safety of nutrient intakes. For practical application, there is a need for a limited number of conversion factors for a nutrient source, e.g., a single value for all food categories and population groups or possibly a separate conversion factor for some food categories (e.g., food supplements) and for adults or infants and young children.

The EFSA guidance (EFSA ANS Panel, 2021) establishes data requirements for the scientific assessment of both safety and bioavailability of new nutrient sources. 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 will review and extend this guidance to include the scientific principles, methodologies, and data needs for the scientific assessment of all new forms of nutrients and for deriving conversion factors for proposed new sources or forms of nutrients.

To assist in revising the guidance to include scientific principles, methodologies, and data needs for deriving conversion factors, the following issues will be considered for the Expert Survey and workshop.

3 General approach to estimating conversion factors

Conversion factors are estimated from data on the comparative bioavailability of the nutrient from the novel source versus a reference source. The relative bioavailability of the nutrient from the novel source is estimated by comparing bioavailability of the nutrient from the novel source against a similar amount of the nutrient from the reference source (ideally an equimolar amount) under identical experimental conditions. This may be based on studies in humans, in animals, in in vitro gastro-intestinal models (bioaccessibility or cellular uptake/absorption studies), or from studies of dissociation under conditions similar to the human gastrointestinal tract.

Methodologies and data requirements may reflect the chemical similarity of the sources. For example, vitamins may have chemically related compounds having vitamin activity ('vitamers'), i.e. capable of meeting the nutritional requirement for the vitamin. As relative vitamin activity ('equivalence') may vary between vitamers (Gregory, 2012; Jacobsen et al., 2019), the approach to assessing bioavailability and estimating conversion factors may need to reflect this.

- **General guidance** on methodologies and data requirements for nutrient bioavailability is presented in section 2.5 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

4 Food categories and population groups to which conversion factors should apply

The application should address to which extent the nutrient is bioavailable from the novel source in the target population, e.g., adults, the general population or certain defined population subgroups, and also at the use levels in food categories, e.g., foods intended for the general population, foods for specific groups (FSG) or food supplements, in which the source is intended to be added/used.

- General guidance on proposed uses and use levels of the nutrient source is presented in section 2.3 of the EFSA guidance (EFSA ANS Panel, 2021).

Examples:

- **Calcium-l-methylfolate and (6S) -5-methyltetrahydrofolic acid glucosamine salt** (collectively called 5-MTHF hereafter) was proposed for use in all food categories and population groups (e.g. infants, children, adults including pregnant or lactating women; healthy subjects, patients with a disease) (EFSA ANS Panel, 2013; EFSA NDA Panel, 2022).
- **Nicotinamide riboside chloride** was proposed for use as a source of niacin in food supplement capsules at levels up to 300 mg/day for the general healthy adult population, including pregnant and lactating women (EFSA NDA Panel, 2019).

5 The reference nutrient source

For many nutrients, a number of sources may be authorised already and potentially eligible as a reference for deriving a conversion factor.

The reference source should be a form of the nutrient naturally present in foods or an authorised nutrient source for which the relative bioavailability versus one or more forms of the nutrient naturally present in foods is known. If there are multiple forms of the nutrient naturally present in foods a form with a significant contribution to dietary intake is preferable. It should be covered by existing endpoints for establishing DRVs for nutrient adequacy and tolerable upper intake levels (UL). While a reference source with high bioavailability may be preferable this is not a characteristic of some naturally occurring nutrients in foods.

Absolute bioavailability of the nutrient from the reference source is not needed; rather, it is the comparative bioavailability of the nutrient from the novel source versus that of the nutrient from the reference source that is needed to establish a conversion factor.

The relationship between intake of the nutrient from the reference comparator and biomarkers of nutrient intake, status or effect should be defined. Ideally, there should be a linear relationship between intake of the nutrient from the reference comparator and the relevant biomarkers. However, this may not be true for nutrients for which the percentage absorption is dose-dependent.

- **General guidance** on methodologies and data requirements for nutrient bioavailability is presented in section 2.5 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

Example:

- For **novel sources of folate** an appropriate comparator might be folic acid, which is an authorised source 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 dietary folate equivalents (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 conversion factor 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, 2022).
- **For novel sources of vitamin D** an appropriate comparator might be vitamin D3 (cholecalciferol), which is an authorised source, and naturally present in foods. This form makes a significant contribution to dietary intake and covered by existing endpoints for establishing DRVs for nutrient adequacy and tolerable upper intake levels (UL). The relationship of vitamin D3 intake to serum 25(OH)D, a reliable marker of vitamin D status, is well established, and vitamin D3 was considered an appropriate comparator for estimating relative bioavailability of the novel source, calcidiol to be >1 in adults (EFSA 2021).

6 Information on the characteristics of the nutrient sources to be provided

Both the novel source and the reference source should be sufficiently characterised for a scientific assessment with respect to the factors which may have an impact on bioavailability, e.g., physicochemical characteristics, water soluble or lipophilic compounds, nutrients released in the gastrointestinal tract or nutrient source absorbed intact.

Equivalence of different chemical forms of the nutrient in terms of capacity to meet the requirement for the nutrient may need to be considered (Melse-Boonstra et al., 2017; Moltedo et al., 2021).

➢ **Detailed guidance** on data requirements for characterisation of nutrient sources for assessment of both safety and bioavailability is presented in section 2.1 and Appendix A and D of the EFSA guidance (EFSA ANS Panel, 2021).

7 Nutrient metabolites as nutrient sources

The concept of bioavailability is described in the relevant legislation as 'available to be used by the body', which, for a nutrient, is considered to mean 'available to be used to exert its function as a nutrient'. Thus, it is necessary to consider whether the metabolite has the same physiological effect(s) as the authorised form of the nutrient, in terms of nutrient function at physiological intake levels (i.e., is it covered by existing endpoints for nutrient adequacy?). Equivalence of different chemical forms in terms of capacity to meet the requirement for the nutrient may need to be considered (Melse-Boonstra et al., 2017; Moltedo et al., 2021). From a safety perspective it is also necessary to consider whether the metabolite may have the same adverse effects at high intakes (i.e., is it covered by existing endpoints for Tolerable Upper Intake Levels (UL)?). In order to address these issues an understanding of the physicochemical characteristics and absorption, distribution, metabolism and excretion (ADME) properties of the metabolite and authorised form of the nutrient is needed.

Examples:

- **Calcidiol vs vitamin D**. Calcidiol has same physiological effect(s) as authorised chemical forms (e.g., vitamin D3), in terms of nutrient function at physiological intake levels. Oral

administration of calcidiol increases serum 25(OH)D, the endpoint for establishing nutrient requirements. 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. Calcidiol has same physiological effect(s) as authorised chemical forms (e.g., 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 endpoint hypercalcemia/hypercalciuria for establishing a UL (EFSA NDA Panel, 2021).

- **Nicotinamide riboside chloride**. Nicotinamide riboside chloride is considered a source from which nicotinamide, a form of the vitamin niacin, is released in the gastrointestinal tract 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) is a main metabolite from nicotinamide but is without vitamin function, i.e., absorbed into blood but not available to act as a precursor of nicotinamide adenine dinucleotide (NAD+) in cells (EFSA NDA Panel, 2019).

8 Minimum data requirements for estimating comparative bioavailability and a conversion factor

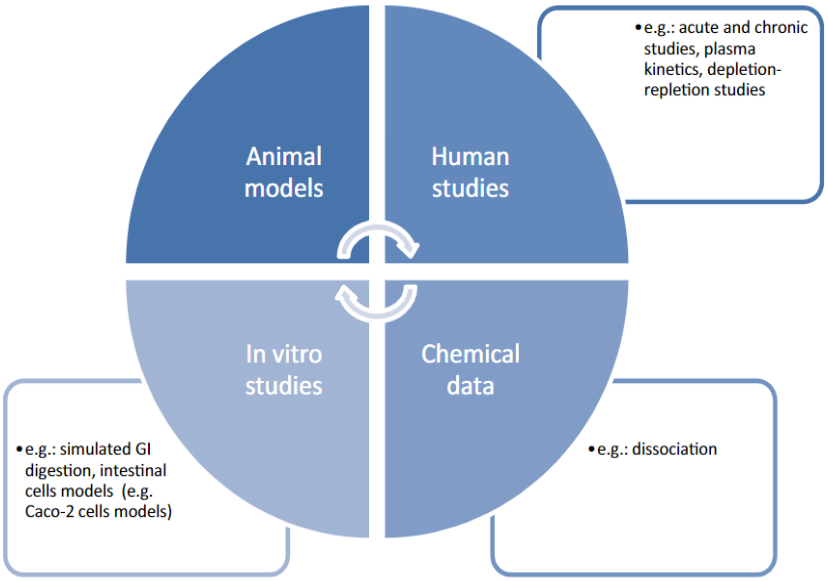
There is no pre-established rule as to how many or which types of studies are needed for establishing a conversion factor (CF). This is because the approach used may depend on the physicochemical characteristics and absorption, distribution, metabolism and excretion (ADME) properties of the novel source, as well as what is already known about the bioavailability of the nutrient. It may also be influenced by the target population, e.g., vulnerable population groups.

The scientific opinions on nutrient sources assessed by the former ANS Panel and the NDA Panel provide examples as to the type and quality of the studies that may be needed for estimating relative bioavailability of nutrients from sources in the context of specific applications. These opinions have generally not estimated a value for relative bioavailability of the nutrient from novel sources as this was not required by the mandate; rather, they have addressed the question of whether the nutrient is bioavailable, or in some cases whether bioavailability is similar, lower, or greater than from the comparator authorised source.

Methodologies and data requirements may reflect the chemical similarity of the sources. For example, for different salts of the same chemical form of a nutrient, data on dissociation under gastrointestinal conditions may be sufficient to predict relative bioavailability of a nutrient. However, for different forms of a nutrient, measurement of biomarkers of status or effect of the nutrient in chronic comparative bioavailability studies in humans may be required to assess relative bioavailability of a nutrient from a new proposed source. It is noted that for estimation of relative bioavailability of a nutrient from a novel source using comparative studies vs a reference source there may be conditions where a wider range of data (e.g., chemical data or in vitro data) may be sufficient but not for estimation of absolute bioavailability where data from human bioavailability studies are generally needed.

If the data are considered insufficient to estimate relative bioavailability of the nutrient from the novel source a conversion factor may not be established.

- **General guidance** on methodologies and data requirements for nutrient bioavailability is presented in section 2.5 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021). Various approaches can be used for assessing the bioavailability of a nutrient (as illustrated in Figure 2 of the EFSA Guidance).



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. For some of these food categories the target population may be a vulnerable group and/or the food may be the only source of nutrition (e.g. infant formula, FSMP, TDR). This requires greater certainty on the relative bioavailability and conversion factor of new sources or forms of the nutrient proposed for use in these food categories. In these circumstances, human data in a relevant population would be considered essential for the assessment. It is noted that it may not be possible to obtain such data in the target population as ethical concerns may limit clinical studies in vulnerable and especially paediatric populations. The study population should be representative of the target population, or a population from which extrapolation of the results to the target population is biologically appropriate, as justified by a scientific rationale.

Example:

- **For 5-MTHF** a DFE conversion factor of 1.7 for 5-MTHF (relative to natural folates) was established based on studies using folic acid as comparator in adults. 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 infants 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, 2022).

9 Hierarchy of the evidence for estimating comparative bioavailability and a conversion factor

There is no pre-defined hierarchy of evidence for establishing a conversion factor. Assessment should be done in line with the principles of the EFSA guidance on bioavailability (EFSA ANS Panel, 2021), i.e. comparative bioavailability assessment and adaptation of the methodological choices for such an assessment are to be considered on a case-by-case basis.

The application should include available data on ADME of the nutrient for the reference source and novel source, as well as a scientific justification of relevant biomarkers of intake and status.

- **General guidance** on methodologies and data requirements for nutrient bioavailability is presented in section 2.5 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

9.1 Chemical data

Chemical data may be sufficient if they can predict the fate of the source in the human body once it is ingested, e.g., if dissociation under gastrointestinal conditions is the key process for bioavailability it may predict relative bioavailability of a nutrient from different salts of the same chemical form of a nutrient with similar dissociation characteristics. If dissociation characteristics are similar, a conversion factor of 1 may be acceptable. However, if dissociation characteristics are different, chemical data alone may not be sufficient to establish a conversion factor (but may be used as supportive evidence). While it is recognised that the food matrix may have a significant effect on bioavailability of nutrients this should be controlled for in comparative studies of the novel and reference sources.

- **General guidance** on use of dissociation tests for nutrient bioavailability is presented in section 2.5.1 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

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 gastrointestinal 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 approved calcium forms (EFSA ANS Panel, 2016).

9.2 *In vitro* studies

Data on the release of the nutrient component from a source (in vitro bioaccessibility) coupled with uptake into/translocation across intestinal cell models under simulated gastrointestinal digestion conditions using in vitro systems may be considered sufficient to estimate comparative bioavailability. This would be relevant when there is evidence that bioavailability critically depends on these steps. The conditions for such assays should be standardised, e.g., by use of the INFOGEST method (Egger et al., 2015; Sulaiman et al., 2021; Brodkorb et al., 2019). If these characteristics are similar, a conversion factor of 1 may be acceptable. However, if these characteristics are different, such in vitro data alone may not be sufficient to establish a conversion factor (but may be used as supportive evidence).

- **General guidance** on use of in vitro systems for nutrient bioavailability is presented in section 2.5.1 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

Example: No example available.

9.3 Animal models

Although animal models have known limitations in predicting bioavailability in humans, they can provide useful data on the bioavailability of a new proposed source or form of the nutrient with respect to established ones. Therefore, they may be used as supportive evidence.

Relevant animal models would be mammals except ruminants (e.g., rats, mice, pigs, dogs, cats, guinea pigs, hamsters, primates, rabbits) as well as birds (e.g., hens). The choice of animal model should be made on a case-by-case basis taking into account what is known about the ADME characteristics of the nutrient in the animal species compared to humans.

- **General guidance** on use of animal models for nutrient bioavailability is presented in section 2.5.1 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

Example:

- **Fe EDTA**: pig data as supportive evidence - a study of iron absorption from ferric sodium EDTA 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. The authors reported that the iron that was absorbed was incorporated into haemoglobin (EFSA ANS Panel, 2010).

9.4 Human studies

Studies in humans have the highest predictive value on the bioavailability of a new proposed source with respect to established ones. Suitable study designs include randomised two-period crossover studies (with an adequate wash-out period), and randomised parallel studies.

For human studies, acute and/or chronic studies in healthy (preferable) or diseased individuals may be used, ideally with equimolar dose of nutrient from novel and reference sources, and should specify conditions of consumption (empty or full stomach) and frequency (e.g. once or twice daily) that reflect the proposed use of the novel source (use in a food supplement and/or in fortified food), should be of appropriate duration, with appropriate biomarkers/parameters as outcomes (see EFSA Dietary Reference Values (DRV) opinions for well-established markers of intake, status and effect). To take into account factors that may influence bioavailability, there should be data across the dose range that is proposed for use of the source, data on the effect of the food matrix, and in relevant population and age groups representative of the target population. While bioavailability of a nutrient from a source may be influenced by the nutrient status of individuals within a population group, in the context of this guidance, the individual variability of bioavailability is not the focus. This is because it is not practical to tailor conversion factors for labelling purposes to individuals with a different nutrient status in the target population. However, the nutrient status of study subjects should be taken into consideration in the design of comparative studies of bioavailability. For example, for studies on bioavailability of iron, methods for normalisation of nutrient status are used (Cook, 1991).

- **General guidance** on use of human studies for nutrient bioavailability is presented in section 2.5.1 and Appendix D of the EFSA guidance (EFSA ANS Panel, 2021).

9.4.1 Acute/short-term studies in humans

Measurement of the concentration - time profile of the nutrient in blood in acute studies may be sufficient as a basis to assess relative bioavailability of a nutrient from a new proposed source or form following single or repeated oral administration. If concentration - time profiles of the nutrient in plasma are similar for the two sources, a conversion factor of 1 may be acceptable. However, if these profiles are different, such data alone may not be sufficient to establish a conversion factor (but may be used as supporting evidence, e.g., by providing information on pharmacodynamics of the nutrient). In order to consider whether such acute studies are appropriate, an understanding of the absorption, distribution, metabolism and excretion (ADME) properties of the novel and reference forms of the nutrient is needed.

Examples:

- **5-MTHF vs folic acid:** Acute dose studies measuring the plasma or urinary folate response were not considered sufficient to assess the relative bioavailability of 5-MTHF vs folic acid owing to the fact that, unlike 5-MTHF, folic acid needs to be reduced to folate in the gut or the liver. Owing to the necessary conversion of folic acid into folate, the contribution of folic acid to plasma/urinary folate may be delayed, or even incomplete at high intakes, as compared to 5-MTHF (EFSA NDA Panel, 2022). These studies, however, could be used as supportive evidence as far as they provide information on the pharmacodynamics of the nutrient forms.

- **5-MTHF salts:** It was noted that, while the results from the acute studies using as a comparator folic acid and plasma folate response as biomarker (a sensitive marker of recent dietary intake) cannot be considered sufficient for establishing the conversion factor for 5-

MTHF, acute studies that compare the bioavailability of folate from different salts of 5-MTHF with each other could be used to establish a conversion factor for the salts relative to each other (EFSA NDA Panel, 2022). Indeed, the relative bioavailability of 5-MTHF glucosamine salt was estimated to be similar or slightly higher than L-5-MTHF-Ca in a crossover comparative 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).

Measurement of absorption and/or utilisation of the nutrient may be sufficient as a basis to assess comparative bioavailability of a nutrient from a new proposed source or form following single oral administration. If it is not feasible to measure utilisation, retention may be acceptable. Labelling with isotopes, if feasible, permits the use of lower doses of nutrient.

Examples:

- **Iron from iron milk proteinate vs ferrous sulphate**: In a single dose comparative 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 sulphate. 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, 2022).
- **Iron from NaFeEDTA vs ferrous sulphate**: In single dose human studies with isotopically labelled foods, iron from ferric sodium EDTA is 2 to 3 times more bioavailable than iron in the form of ferrous sulphate. 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, 2010).
- **Iron from IHAT vs ferrous sulphate**: Single dose human studies, incorporation of iron into red blood cells (RBC) at 14 days post dose (EFSA NDA Panel, 2021).

9.4.2 Chronic studies in humans

Measurement of biomarkers of status or effect of the nutrient in chronic studies, may be required as a basis to assess relative bioavailability of a nutrient from a new proposed source or form, following single or repeated oral administration of a dose of the new proposed source or form. Applicants should justify/substantiate that the marker used for comparative bioavailability is appropriate.

Examples:

- **5-MTHF vs folic acid**: It was noted that the results from the acute studies using as a comparator folic acid and plasma folate response as biomarker cannot be considered appropriate for establishing the conversion factor for 5-MTHF (EFSA NDA Panel, 2022). A DFE conversion factor of 1.7 for 5-MTHF (relative to natural folates) using folic acid as comparator was established on the basis of one repeated dose intervention study of intermediate risk of bias (RoB) in healthy adults for intakes <400 µg/day (Wright et al., 2010) and three repeated dose intervention studies of low to intermediate RoB in healthy adults (Pietrzik et al., 2007; Diefenbach et al., 2013; Green et al., 2013) for intakes ≥ 400 µg/day. RBC folate concentration was considered the most reliable biomarker of folate status as it reflects long-term dietary intake.
- **Calcidiol vs vitamin D:** Calcidiol 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. Bioavailability of calcidiol relative to vitamin D3 was investigated on the basis of 6 chronic repeated dose intervention studies in healthy adults for intakes 5 to 50 µg/day in men and/or not-pregnant and non-lactating women, with or without considering background vitamin D intake or sun exposure, at latitudes relevant for Europe (37–51°N), and generally excluding

users of vitamin D supplements (Barger-Lux et al., 1998; Bischoff-Ferrari et al., 2012; Jetter et al., 2014; Cashman et al., 2012; Navarro-Valverde et al., 2016; Wittwer, 2015; Vaes et al., 2018; Kunz et al., 2016). Serum 25(OH)D concentration was considered the most reliable biomarker of vitamin D status as it reflects long-term dietary intake and is a source of the biologically active form of vitamin D (1,25(OH)2D). These studies showed that oral administration of 25(OH)D3 in adults increases serum 25(OH)D concentration more than vitamin D3. There was considerable variability in the achieved increases in serum 25(OH)D concentrations with calcidiol in comparison to vitamin D3, depending on the dose and experimental conditions in the various studies. In one study with a low RoB for the measurement of serum 25(OH)D (Cashman et al., 2012), the mean increase of serum 25(OH)D from baseline was up to 5 times higher with 20 µg/day oral 25(OH)D (EFSA NDA Panel, 2021).

10 Implications of the conversion factor for Dietary Refence Values

10.1 Dietary reference values for adequacy

Conversion factors may have implications for nutrient adequacy. A CF <1 for a novel source may need to be taken into account when assessing adequacy of the novel source under proposed conditions of use, particularly for uses where the proposed source would be the only source of the nutrient for the intended population (e.g., infant formula). It is important to consider whether the novel source is covered by existing endpoints for establishing DRVs for nutrient adequacy. Equivalence of different forms of the nutrient, in terms of their capacity to meet nutrient requirements, may need to be considered (Melse-Boonstra et al., 2017; Moltedo et al., 2021).

Example: No example available.

10.2 Tolerable Upper Intake Level (UL)

Conversion factors may have implications for tolerable upper intake levels (UL) as well as for achieving nutrient adequacy. A CF >1 for a novel source may need to be considered when assessing safety of the novel source under proposed conditions of use. If this CF applies at the high intakes in the UL range a lower UL may be needed for a novel source. It is important to consider whether the novel source is covered by an existing endpoint for establishing a UL. If not, it may be necessary to establish a different UL for the novel source.

Examples:

- **Calcidiol vs vitamin D3**: The EFSA NDA Panel considered implications of calcidiol consumption with regard to ULs for vitamin D (D2 and D3). Regarding possible adverse effects at high intakes, calcidiol increases serum 25(OH)D which is the likely mediator for endpoint hypercalcemia/hypercalciuria for establishing UL for vitamin D. Thus, safety under proposed conditions of use could be assessed from assessment of exposure relative to UL for vitamin D assuming a theoretical CF of 5 for calcidiol vs vitamin D (EFSA NDA Panel, 2021).
- **5-MTHF vs folic acid**: EFSA NDA panel considered that 5-MTHF was included in UL that was established for 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, 2022).

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Annex A – Expert Survey on the derivation of conversion factors for new sources and forms of nutrients

Summary of comments received

Background

The Expert Survey (15 December 2022 – 20 January 2023) invited 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.

Comments were received from 15 experts and addressed the scientific principles, methodologies and data needs for deriving a conversion factor for new sources or forms of nutrients.

The outcome was used to prepare a Discussion paper to shape and optimise the discussion in a workshop gathering scientific experts and representatives of bodies in charge of setting conversion factors (CF) for new sources or forms of nutrients, as well as to identify scientific experts in the field who were subsequently invited to the workshop.

Reference nutrient form/source

- If there are multiple forms of the nutrient naturally present in foods, a form with a significant contribution to dietary intake is preferable. It should be covered by existing endpoints for establishing DRVs for nutrient adequacy and tolerable upper intake levels (UL).
- While ideally there should be a linear relationship between intake of the nutrient from the reference source and the relevant biomarkers of nutrient intake, status or effect, this may not be true for nutrients for which the percentage absorption, or conversion to the active form of the nutrient, is dose-dependent.
- While a reference source with high bioavailability may be preferable, this is not a characteristic of some naturally occurring nutrients in foods.

Nutrient metabolites as nutrient sources

- It is necessary to consider whether the metabolite has the same physiological effect(s) as the reference nutrient form, in terms of nutrient function at physiological intake levels (i.e. is it covered by existing endpoints for nutrient adequacy?).
- From a safety perspective it is also necessary to consider whether the metabolite may have the same adverse effects at high intakes (i.e., is it covered by existing endpoints for Tolerable Upper Intake Levels (UL)?).
- In order to address these issues, an understanding of the physicochemical characteristics and absorption, distribution, metabolism and excretion (ADME) properties of the metabolite and reference form of the nutrient is needed.

New nutrient sources or forms targeting foods for specific groups

- Overall, there was good agreement that human data in a relevant population would be considered essential for the assessment of CF for novel sources for which the target population may be a vulnerable group and/or the food may be the only source of nutrition (e.g. infant formula, foods for special medical purposes, total diet replacements for weight control).

- It was noted that it may not be possible to obtain such data in the target population as ethical concerns may limit clinical studies in vulnerable and especially paediatric populations.

Human studies to estimate relative bioavailability and derive a CF

- Studies in humans have the highest predictive value on the bioavailability of a new proposed nutrient form/source with respect to established ones.
- For comparative bioavailability studies of novel and reference sources in humans, suitable study designs include randomised two-period crossover studies (with an adequate wash-out period), and randomised parallel studies in relevant population and age groups representative of the target population.
- As relative bioavailability of the nutrient from the novel vs reference source may be dose-related, equivalent nutrient doses (ideally equimolar doses) from novel and reference sources should be compared and studies should cover the dose range that is proposed for use of the novel source.
- Factors that may influence nutrient bioavailability should be controlled, including food matrix, conditions of consumption (empty or full stomach) and frequency (e.g., once or twice daily), and the nutrient status of study subjects.
- Acute/short term single-dose studies may be appropriate to assess relative bioavailability, e.g., measurement of concentration-time profile of the nutrient in blood. Also, measurement of absorption and/or utilisation of the nutrient may be appropriate as a basis to assess relative bioavailability of a nutrient from a new proposed source. If it is not feasible to measure utilisation, retention may be acceptable. Labelling with isotopes, if feasible, permits the use of lower doses of the nutrient.
- Chronic repeated-dose studies of appropriate duration with relevant biomarkers of nutrient status or effect may be appropriate as a basis to assess relative bioavailability.
- In order to select a suitable study design, an understanding of the ADME properties of the novel and reference forms of the nutrient is needed.

Chemical data and *in vitro* data to estimate relative bioavailability and a CF

- While chemical data and *in vitro* data are generally unsuitable to predict (absolute) bioavailability, there may be limited circumstances where they may predict relative bioavailability of a nutrient from a novel source vs reference source.
- For example, if dissociation under gastrointestinal conditions is the key process for bioavailability, chemical data may predict relative bioavailability of a nutrient from different salts of the same chemical form of a nutrient with similar dissociation characteristics.
- Data on the release of the nutrient component from a source (*in vitro* bioaccessibility) coupled with uptake into/translocation across intestinal cell models under simulated gastrointestinal digestion conditions using *in vitro* systems may be considered sufficient to estimate relative bioavailability when there is evidence that bioavailability critically depends on these steps. The conditions for such assays should be standardised, e.g., by use of the INFOGEST method (https://doi.org/10.1038/s41596-018-0119-1).

Animal models to estimate relative bioavailability and a CF

- There was general agreement that animal models have known limitations in predicting bioavailability of nutrients in humans. Data from animal models may be used as supportive evidence for estimating relative bioavailability.

Implications of the CF for DRVs for nutrient adequacy and for the UL of the nutrient

- If CF <1 for a novel source, this may need to be considered when assessing adequacy of the novel source under proposed conditions of use, particularly targeting vulnerable groups.
- If CF >1 for a novel source, this may need to be considered when assessing safety of the novel source under proposed conditions of use. If this CF applies at the high intakes in the UL range, a lower UL may be needed for a novel source. It is important to consider whether the novel source is covered by an existing endpoint for establishing a UL. If not, it may be necessary to establish a different UL for the novel source.