

# **EURRECA's approach for estimating micronutrient requirements**

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## **Abstract**

In Europe, micronutrient dietary reference values have been established by (inter)national committees of experts and are used by public health policy decision-makers to monitor and assess the adequacy of diets within population groups. The approaches used to derive dietary reference values (including average requirements), vary considerably across countries and so far no evidence-based reason has been identified for this variation. Nutrient requirements are traditionally based on the minimum amount of a nutrient needed by an individual to avoid deficiency, and is defined by the body's physiological needs. Alternatively the requirement can be defined as the intake at which health is optimal, including the prevention of chronic diet-related diseases. Both approaches are confronted with, many challenges (e.g. bioavailability, inter and intra-individual variability). EURRECA has derived a transparent approach for the quantitative integration of evidence on Intake-Status-Health associations and/or Factorial approach (including bioavailability) estimates. To facilitate the derivation of dietary reference values EURRECA is developing a process flow chart to guide nutrient requirement setting bodies through the process of setting dietary reference values, which aims to facilitate the scientific alignment of the process of deriving these values.

## Introduction

In Europe, most countries have established their own national dietary reference values (DRVs). DRVs are quantitative reference values for nutrient intakes required to avoid deficiency and maintain function for healthy individuals and populations which may be used for assessment and planning of diets. DRVs are the complete set of nutrient reference values such as the adequate intake level, the lower threshold and upper intake levels. A wide range of terminologies of DRVs have been used by different (inter)national agencies. An overview is provided by Donutske-Rutten and co-workers [1]. DRVs serve as a basis for nutritional educational programs, national and/or regional nutrition policies, and food regulations such as nutrition labelling [2-5]. The variation in micronutrient DRVs across countries [3] can cause confusion among consumers, food producers and nutrition policy makers. More aligned information may change attitudes, thus influencing dietary behaviours and in turn potentially leading to a healthier population. Therefore, dietary reference values based on scientific evidence are essential for the development of public health nutrition policies [6].

Harmonization of the process of deriving DRVs is needed to align nutrition policy and public health strategies. In the context of this harmonization exercise, the EC Network of Excellence on *EUROpean micronutrient RECommendations Aligned* (EURRECA) has been established to identify and develop methodologies to standardize the process of setting micronutrient reference values.

The purpose of this paper is to describe EURRECA's strategy (methods and

potential applications) towards a uniform, transparent and evidence-based process of derivation of micronutrient dietary reference values.

## **EURRECA's evidence-based approach**

The first step in deriving dietary reference values is to determine the average micronutrient requirement for a defined population from primary research data. Several different types of studies are used to produce the evidence for deriving requirements. Table 1 summarizes the type of studies and their methodological principles and designs. In essence, the different types of study can be categorized to address either of two basic models: the association or the factorial approach.

The association approach comprises outcome measures which are related to health (such as physical function or disease) and consists of assessing/addressing the dose-response relationships between at least two of the three following components:

- (i) dietary micronutrient intake (I)
- (ii) micronutrient body status (S)
- (iii) health (H) outcomes.

The factorial approach comprises outcomes which are predominantly physiological, such as body stores. This approach is necessary where intake-status-health relationships cannot be assessed, either due to a lack of a clear

intake-status relationship (e.g. poor bioavailability) or where there is a lack of data relating to health endpoints. This factorial approach is based on

- (i) data on micronutrient losses and maintenance
- (ii) absorption/bioavailability

These measures can be affected by homeostatic mechanisms, according to the level of intake, body status and also demographic factors.

### ***EURRECA's estimation of nutrient requirement***

Established methodologies used to derive requirements do not generally integrate the two different approaches; therefore, EURRECA has developed a conceptual model that can integrate suitable quantitative evidence into one model. The aim is to offer a transparent approach to derive micronutrient requirements based on collated data relating to both the association approach and the factorial approach. The EURRECA model aims to support experts in this inferential process by making potential assumptions and uncertainties explicit. The process behind the EURRECA model is shown in Figure 1.

To illustrate the EURRECA quantitative integration of evidence, provisional requirement values (e.g. Bit B12, folate) will be derived based on

- (a) separate dose-response associations for I-S, S-H and I-H (based on systematic reviewing of the scientific literature followed by meta-analyses)
- (b) an integrated I-S-H dose-response model, where data allow
- (c) inclusion of factorial estimates and bioavailability factors where applicable

### *EURRECA Data collection process*

Best practice guidelines on micronutrient intake and status were developed in earlier EURRECA activities [7-10]. The best practice guidelines for biomarkers contains measures of status and exposure for twenty key micronutrients, including cut-off values for key biomarkers of EURRECA priority micronutrients (iron, zinc, folate, vit B12 and iodine). The guidelines also describe advantages and limitations of each measure. Table 2 provides an overview of the biomarkers identified by EURRECA as the most suitable biomarkers for a selected number of micronutrients. The prioritization process of the EURRECA micronutrients is described elsewhere [11].

The best practice guidelines were adopted in the subsequent systematic review and meta-analysis process, which were initiated to collate the evidence (for both association and factorial approaches) for determining average micronutrient requirement.

A separate systematic review was conducted for each of three main areas: I-S-H relationships, micronutrient absorption and factorial estimates, in relation to the micronutrients prioritized by the Network [11]. Each review differed slightly in the inclusion criteria and methodology (due to differences in principal review questions, and micronutrient specific issues), but all followed the basic structure detailed below.

A standard search strategy and protocol were devised, which were then tailored to each micronutrient and specific review question.

Potentially relevant studies were collected through structured and systematic electronic searches on Ovid SP Medline, Embase OvidSP) and the Cochrane Library CENTRAL database. All searches were undertaken from inception of the database, each including micronutrient specific terms and limited to 'humans'.

After finalization of the searched, references were screened and sorted on the basis of titles and abstracts to identify relevant studies and to exclude any reference clearly not meeting the purpose of the review (e.g. animal studies). A minimum of 10% of the titles and abstracts were assessed in duplicate by two independent researchers.

Potentially relevant references were collected as full-texts and assessed according to the pre-defined inclusion and exclusion criteria (e.g. healthy populations). Again, a minimum of 10% of full-texts were assessed by two independent reviewers. At this stage included languages were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek and Serbian.). The reference lists of retrieved articles and of published reviews were also checked for relevant studies.

Data were extracted onto a standardized database, including bibliographic details, methodological details, population characteristics, study groups details and outcome data. A set of indicators of internal validity specific to the study methodology was established, and information regarding these indicators was collected at the data extraction stage in order to later assess the quality of the included studies and the risk of bias.

Meta-analyses are ongoing on data from randomised controlled trials (RCTs), cohort studies and selected cross-sectional studies to examine intake-status-health dose-response relationships for the priority micronutrients (iron, zinc, folate, vitamin B12 and iodine). Additional meta-analyses are planned for the micronutrient absorption systematic reviews, and qualitative analysis has been completed for factorial estimate studies.

## **Results**

Systematic reviews are used as this methodology assesses the literature in an objective way, as opposed to an individual's opinion on the current state of knowledge (review of literature). Systematic reviews allow synthesis of the results of multiple investigations using strategies that aim to limit bias and random error, thus improving reliability and accuracy of the results. However systematic reviews are not without error (the aim is to minimise error and bias) and the methodology is not problem-free. The EURRECA experience indicates that, despite the large number search results a, few relevant studies were included in some of the reviews, especially for the selected health outcomes (which were prioritized at the start of the process –see Table 3). Though it may be perceived that certain relationships are well established, in reality there is often a lack of high quality supporting evidence in the literature. Instead there is a high prevalence of low-quality studies, an incomparable range of markers/measures used as endpoints, and there may be many

factors/confounders that play a role in each relationship. All of these points make the pooling of data very complicated.

Though data were found to be limited for health outcomes, the review results indicated that there is considerable evidence available on the intake-status relationship, albeit from different study designs. The main exception is a general lack of data for some vulnerable groups such as infants, children and adolescents.

The systematic review process of both approaches (factorial and association) has indicated that a high heterogeneity in the collated data, differences in study design, and diversity of aims and outcomes in the included studies makes analysis extremely complex. Further EURRECA found that there are very few depletion studies considering dietary achievable doses. This has resulted in the inclusion of limited reliable depletion data in the systematic reviews, with most useable data relating to high supplementary doses of micronutrients rather than dietary intake levels rendering thorough dose-response analysis relevant to dietary intake difficult.

In conclusion, the process of systematic review and meta-analysis indicates that, in most cases there is a distinct lack of high quality studies.

## **EURRECA Micronutrient Requirement Process Flow Chart**

The work undertaken to date to establish the harmonization process of deriving micronutrient requirements can be summarised in a Micronutrient Requirement Process Flow Chart (illustrated in Figure 2) outlining the ideal process for deriving dietary reference values. The aim of the EURRECA Micronutrient Requirement Process Flow Chart is to facilitate the transparent, systematic and scientific alignment of the process of setting micronutrient requirements. The framework should be considered as a guide for checking that all possibilities/options have at least been considered, rather than all being absolutely essential for deriving requirements. This framework includes the following eight steps:

***1. Establishing selection criteria for identifying micronutrient, population and health outcomes of concern.***

In this step several selection criteria (e.g. population group at risk, public health relevancy) could be used as stated by Cavelaars and co-workers [11]. In this step, it is important to question whether there is enough new evidence to warrant re-assessment of the current requirements.

***2. Setting up an expert Committee with responsibility for setting micronutrient requirements and/or recommendations.***

The composition, remit and operation of the committee should be as transparent as possible. The composition should also be appropriate to the committee's responsibilities and activities (e.g. relevant experts). Collaboration between different disciplines and stakeholders (e.g. scientists, consumers) is a preferred approach in setting up a committee

to identify nutrition policy. In instances where policy has the focus step 7 is the following step.

**3. *Establishing best practice methods to collate evidence.***

In order to robustly measure intake, status, health outcomes and incorporate other relevant information (e.g. -omics data), it is essential to identify current best practice. EURRECA identified best practice for micronutrient intake, status [7-10] and collated relevant information useful for deriving individual micronutrient requirements (<http://wiki.nugo.org/index.php/Category:Micronutrients>) [12].

**4. *Collecting available scientific data.***

Depending on the population group, micronutrient and health outcome under assessment, a systematic data collection should ideally be used for the different existing approaches (see section “EURRECA’s evidence based approach”). In order to explore nutrient needs at an individual level, metabolomics, biological networks and a ‘health space’ model [An analysis and visualization method, called the ‘health space’, that projects subjects in a multidimensional space, based on predefined multivariate parameterization of the axes. This allows separation according to the underlying relevant biological processes.][13] may be used to obtain an improved understanding of the interplay between micronutrients and health.

**5. *Integrating and summarizing the evidence into micronutrient requirements.***

Integrating and summarizing the evidence into micronutrient requirements serves to quantitative and qualitatively analyze and

summarize the data collected in the previous step to achieve the building blocks for the next step. Therefore the following analyses are conducted:

- separate dose-response models for intake-status, status-health and intake-health (based on the systematic reviewing of the association approach and related meta-analyses) and integrated I-S-H data,
- factorial estimates and bioavailability factors.

#### ***6. Deriving dietary reference values, from requirements.***

This step includes the derivation of the variation in requirements (e.g. assumed variation (mean + 2 SD as illustrated in Figure 3) or newly developed stochastic model) that reflect the different reference values and quantitatively integrates the evidence. If relevant, a scaling method has to be chosen to extrapolate requirements into reference values for age and sex groups throughout the life cycle (i.e. where no primary data are available for a population group). Moreover, additional needs for growth, pregnancy & lactation can be added on top of the former.

The integration of the evidence has to accommodate systematic variations (between study) originating from:

- differences in study quality (assessed by internal validity),
- study population (age, gender, body composition and energy needs),
- micronutrient dose (level, range, duration, mode of administration),
- Other population characteristics (growth, pregnancy, lactation, etc.)

#### ***7. Identifying the most appropriate policy option.***

This step outlines the process of integrating micronutrient recommendations (e.g. changes of intake via fortification, food based

dietary guidelines (FBDG), product reformulation) with other considerations (e.g. feasibility, acceptability, (cost) effectiveness) that may be beneficial during the decision making process. In particular, the policy options relevant to micronutrient recommendations will be mapped and the models of nutrient related consumer behaviour change identified.

***8. Implementing the chosen policy instruments and evaluating their impact.***

Policy makers are tasked with choosing policy that will maximise the likelihood of achieving a desired health outcome for the relevant population. The purpose of impact assessment (e.g. nutrient surveillance) is to assess changes in the nutrition situation that can be attributed in part or wholly to a nutrition policy. This step is not covered by EURRECA but seen as relevant in setting and applying micronutrient reference values and policy options.

## **Conclusion**

As a result of EURRECA efforts to provide an evidence-based approach to harmonize the process of setting micronutrient requirements, the following EURRECA key messages are:

- EURRECA has derived a transparent approach for the quantitative integration of evidence on:
  - Intake-Status-Health associations (innovative statistical model)
  - Factorial approach estimates (including bioavailability)

- The identification of a number of research gaps showing that deriving micronutrient requirements in a transparent and systematic way is still difficult.
- The EURRECA approach supports experts by making assumptions and uncertainties transparent.

The 8 steps described in the EURRECA Micronutrient Requirement Process Flow Chart (Figure 2) can serve as guideline and facilitate the development of dietary reference values in a transparent, systematic and sustainable way.

## **Acknowledgement**

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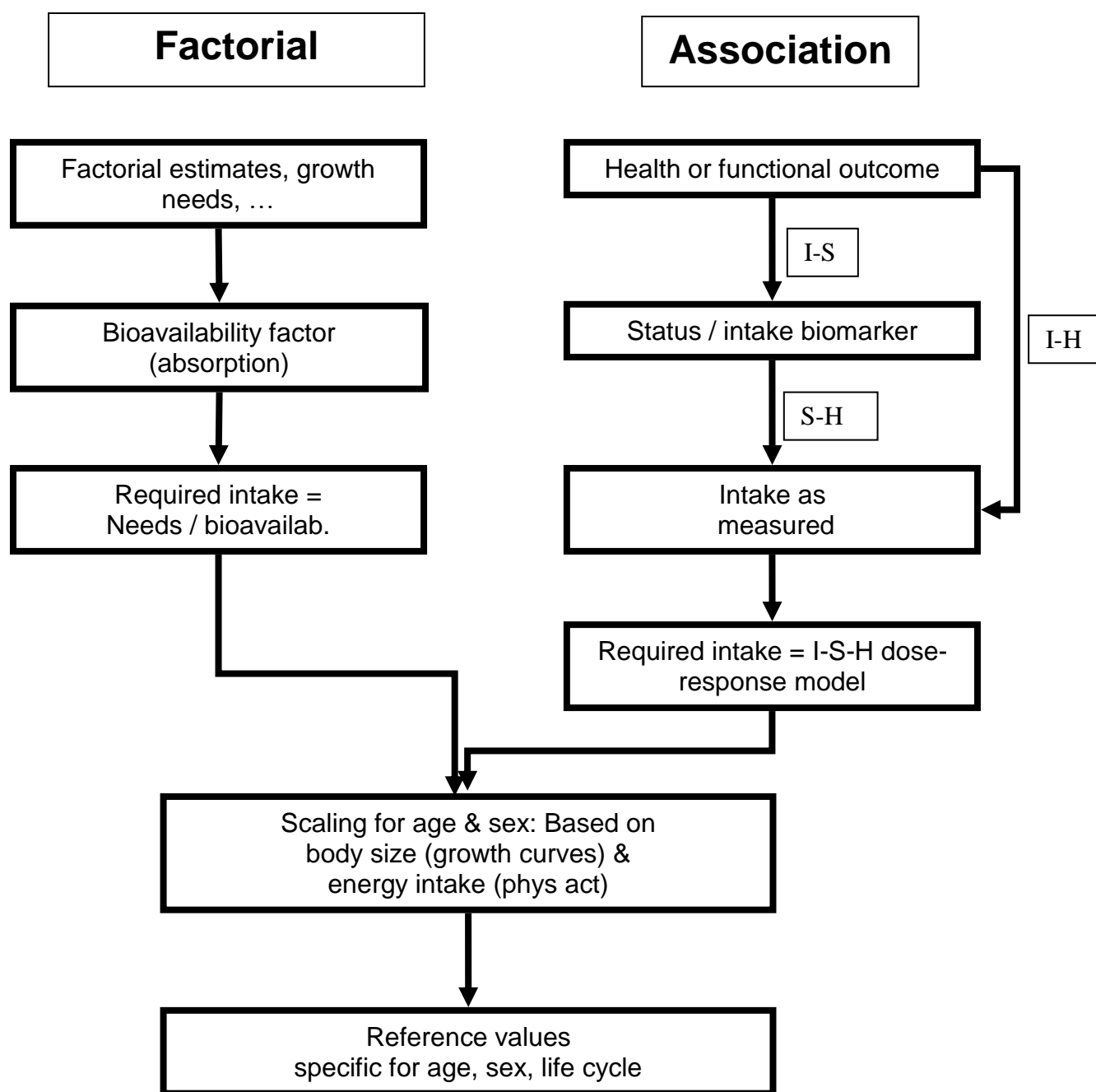


Figure 1: Scheme describing translation of available evidence to dietary reference values.

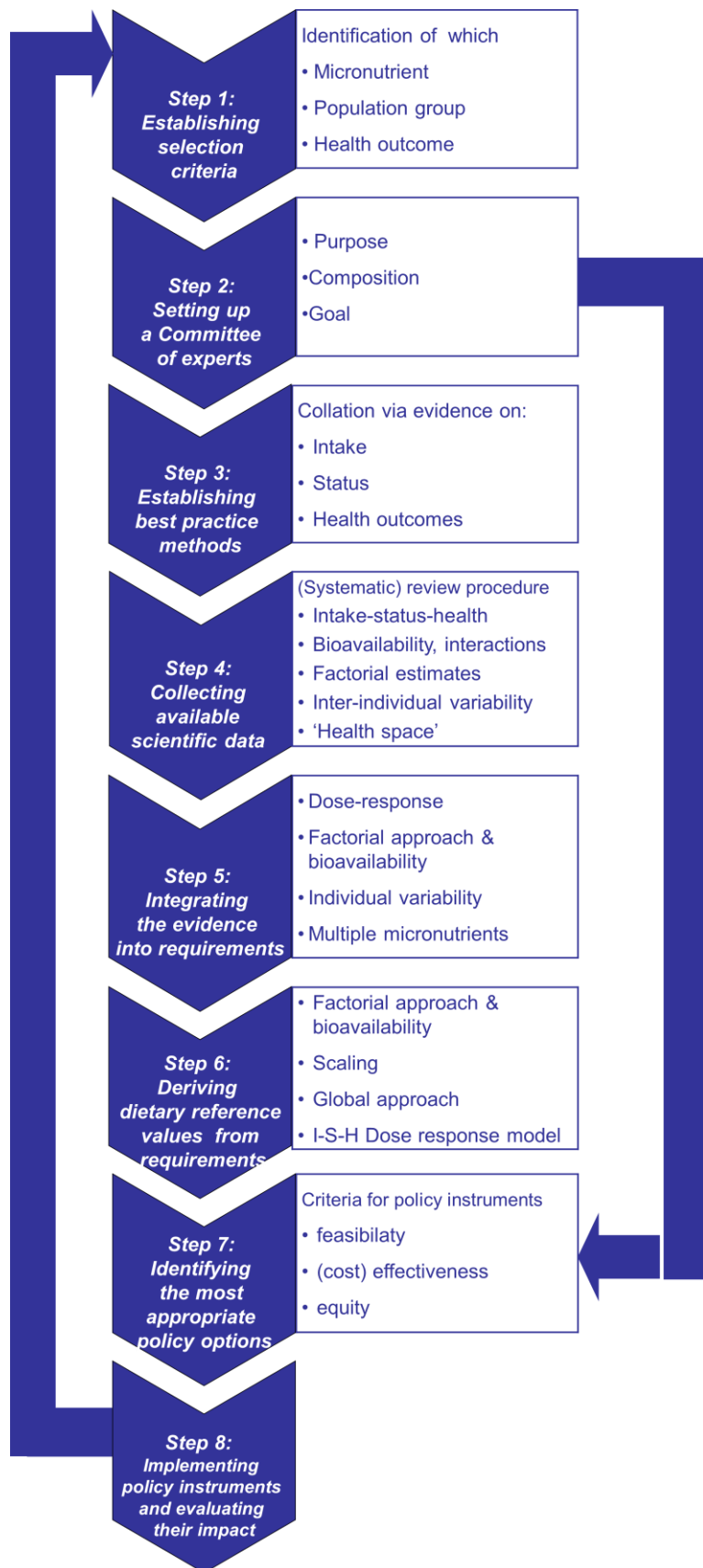


Figure 2: EURRECA Micronutrient Requirement Process Flow chart which aims to facilitate the scientific alignment of micronutrient requirements.

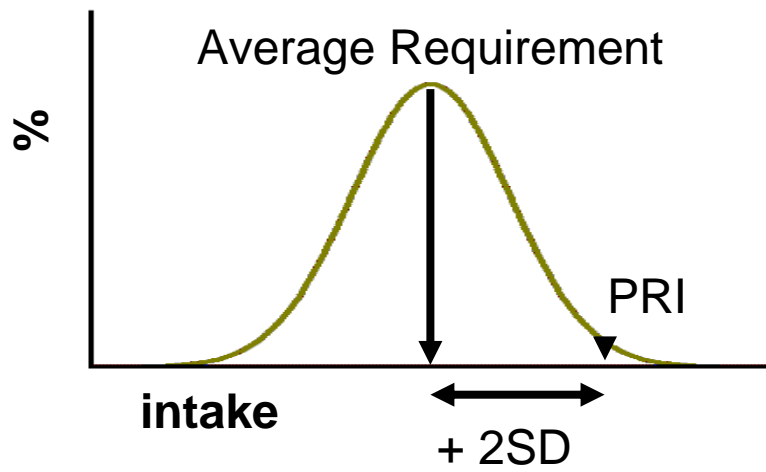


Figure 3: Different points of the dietary reference values and associated distribution (PRI: Population Reference Intake)

Table 1: Approaches and study types to estimate average micronutrient requirements

Approach	Outcome measures	Study type	Principle of method	Designs	Applied to
<b>Factorial</b>	Physical or metabolic outcome	metabolic balance studies at different levels of intake	Long term intake = Long term losses. Requirement: intake level at which balance (stable body pool, rate of absorption and excretion) can be no longer maintained.	Cross-sectional and prospective	All age groups
		growth studies, biochemical studies	Rate of accumulation of nutrients in the body (foetus, placenta, etc), content and volume breastmilk.	Prospective and cross-sectional	Fetal, infants, pregnant and lactating women
<b>Association</b>	Health outcome	deplete/replete studies	Remove nutrients from diet → symptoms occur, add back until they disappear. From these data one can deduct the nutrient requirement.	RCT	Young adults
		biochemical and biological studies	Identification of subclinical deficiencies or lack of function in relation to a certain nutrient	RCT and cross-sectional	All age groups
		epidemiological studies	Identification of (chronic) diseases (functional outcomes)	observational , intervention	Adults, elderly

For the factorial approach, reliable estimates of the bioavailability of the micronutrient are crucial

Table 2: Biomarkers for measuring nutrient status identified by EURRECA

<b>Micronutrient</b>	<b>3* biomarkers (as rated by EURRECA)</b>
Iron	Serum/plasma ferritin, sTfR, 'Body iron' (Cook method) ratio sTfr:ferritin, bone marrow examination
Iodine	Iodine excretion in 24hr or spot urine samples, serum thyroid-stimulating hormone (neonates only)
Selenium	None ( <i>2* plasma/serum/platelet/erythrocyte/urinary /toenail/hair selenium, selenoprotein P, GPx activity</i> )
Zinc	None ( <i>2* serum/plasma zinc, prevalence of stunting, prevalence of inadequate intakes (suggested by iZINCG)</i> )
Copper	None ( <i>Copper chaperone for SOD a potential 2* marker</i> )
Calcium	None ( <i>2* skeletal mineral content &amp; neutron activation</i> )
Vitamin D	Serum 25-hydroxyvitamin D
Vitamin C	Serum/plasma ascorbic acid, leukocyte ascorbic acid
Folate	Erythrocyte folate
Vitamin B12	None ( <i>2* serum/plasma total B12, methylmalonic acid, HoloTC</i> )

This table was compiled by the EURRECA Biomarkers of Status Working Party, comprised of a group of international micronutrient experts and EURRECA partners. A star rating (3\* is excellent) is used to classify the range of biomarkers available for each mineral/vitamin in relation to the limitations of the method. Since these biomarkers were needed for epidemiological analysis, mainly biochemical markers that can be obtained from blood or urine, rather than functional (e.g. immune function, cognitive function) and non-specific tests (e.g. grip-strength) are used. Only 3\* biomarkers are shown if available.

Table 3 : Health outcomes per population group and per micronutrient identified by EURRECA.

Micronutrient	<i>Infants</i>	<i>Children &amp; Adolescent</i>	<i>Pregnant &amp; lactating women</i>	<i>Adults/Elderly</i>
Iron	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Neurodevelopment</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<ol style="list-style-type: none"> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal growth</b></li> <li><b>Preterm delivery</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Preeclampsia;</b></li> <li><b>Postpartum depression</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<ol style="list-style-type: none"> <li><b>Tiredness,</b></li> <li><b>Physical performance (work capacity / aerobic capacity / work productivity),</b></li> <li><b>Immune function</b></li> <li><i>Impaired thermoregulation,</i></li> <li><i>Restless legs syndrome,</i></li> <li><i>Impaired cognitive function.</i></li> </ol> <p>(Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</p>
Zinc	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Immune response to vaccination</b></li> <li><b>Neurodevelopment</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> <li><b>Dermatitis</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal growth</b></li> <li><b>Fetus malformation</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Preeclampsia</b></li> <li><b>Preterm delivery</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Immune function</b></li> <li><b>Cognitive function</b></li> <li><b>Dermatitis</b></li> <li><i>Anorexia</i></li> <li><i>Hypogeusia</i></li> <li><i>Ischemic heart disease</i></li> <li><i>Depression</i></li> <li><i>Diabetes Mellitus</i></li> <li><i>Carcinogenesis</i></li> </ol>
Folate	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Folate-deficiency anaemia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Cancer (DNA synthesis)</b></li> <li><b>Folate-deficiency anaemia</b></li> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal malformations</b></li> <li><b>Fetal growth</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Maternal macrocytic anemia</b></li> <li><b>Preeclampsia</b></li> <li><b>Preterm delivery</b></li> <li><b>Placental abruption</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Stroke</b></li> <li><b>Cancer</b></li> <li><b>Osteoporosis</b></li> <li><i>Cognitive function (Cognitive function test scores such as MMSE, AD, depression ,etc)*</i></li> <li><i>Cardiovascular disease</i></li> </ol> <p>*As cognitive function will be covered only once the 3 primary health outcomes are done and if time allows to do so, we will be further specify what 'cognitive function' should included then.</p>
VitB12	<ol style="list-style-type: none"> <li><b>Neurodevelopment</b></li> <li><b>Megaloblastic anemia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Megaloblastic anemia</b></li> <li><b>Growth</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal malformations</b></li> <li><b>Fetal growth</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Megaloblastic anemia</b></li> <li><b>Preeclampsia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Anemia*</b></li> <li><b>Nervous system disease**</b></li> <li><b>Cognitive function ***</b></li> <li><b>Osteoporosis</b></li> <li><i>Cardiovascular disease</i></li> <li><i>Cancer</i></li> </ol> <p>* There are 4 types of anemia associated with vitamin B12 deficiency: megaloblastic anemia, pancytopenia, thrombocytopenia and leucopenia  ** The most important manifestations related to b12 are: peripheral neuropathy, degeneration of the spinal cord and ataxia  *** The most important manifestations include: dementia, depression, Alzheimer's disease, psychosis</p>
Iodine	<ol style="list-style-type: none"> <li><b>Hypothyroidism</b></li> <li><b>Endemic goiter</b></li> <li><b>Cretinism</b></li> <li><b>Cognitive function</b></li> <li><b>Neonatal mortality</b></li> </ol>	<b>FOR ALL POPULATION GROUPS</b>		

Health outcomes/endpoint were selected for each priority micronutrient based on the relevance to the micronutrient and the results of preliminary searches of the literature. Health outcomes in **bold** are the priority endpoints, depending on the available literature other health outcomes (*italic*) were included as well.

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		biochemical and biological studies	Identification of subclinical deficiencies or lack of function in relation to a certain nutrient	RCT and cross-sectional	All age groups
		epidemiological studies	Identification of (chronic) diseases (functional outcomes)	observational , intervention	Adults, elderly

For the factorial approach, reliable estimates of the bioavailability of the micronutrient are crucial

Table 2: Biomarkers for measuring nutrient status identified by EURRECA

<b>Micronutrient</b>	<b>3* biomarkers (as rated by EURRECA)</b>
Iron	Serum/plasma ferritin, sTfR, 'Body iron' (Cook method) ratio sTfr:ferritin, bone marrow examination
Iodine	Iodine excretion in 24hr or spot urine samples, serum thyroid-stimulating hormone (neonates only)
Selenium	None ( <i>2* plasma/serum/platelet/erythrocyte/urinary /toenail/hair selenium, selenoprotein P, GPx activity</i> )
Zinc	None ( <i>2* serum/plasma zinc, prevalence of stunting, prevalence of inadequate intakes (suggested by iZINCG)</i> )
Copper	None ( <i>Copper chaperone for SOD a potential 2* marker</i> )
Calcium	None ( <i>2* skeletal mineral content &amp; neutron activation</i> )
Vitamin D	Serum 25-hydroxyvitamin D
Vitamin C	Serum/plasma ascorbic acid, leukocyte ascorbic acid
Folate	Erythrocyte folate
Vitamin B12	None ( <i>2* serum/plasma total B12, methylmalonic acid, HoloTC</i> )

This table was compiled by the EURRECA Biomarkers of Status Working Party, comprised of a group of international micronutrient experts and EURRECA partners. A star rating (3\* is excellent) is used to classify the range of biomarkers available for each mineral/vitamin in relation to the limitations of the method. Since these biomarkers were needed for epidemiological analysis, mainly biochemical markers that can be obtained from blood or urine, rather than functional (e.g. immune function, cognitive function) and non-specific tests (e.g. grip-strength) are used. Only 3\* biomarkers are shown if available.

Table 3 : Health outcomes per population group and per micronutrient identified by EURRECA.

Micronutrient	<i>Infants</i>	<i>Children &amp; Adolescent</i>	<i>Pregnant &amp; lactating women</i>	<i>Adults/Elderly</i>
Iron	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Neurodevelopment</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<ol style="list-style-type: none"> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal growth</b></li> <li><b>Preterm delivery</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Preeclampsia;</b></li> <li><b>Postpartum depression</b> (Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</li> </ol>	<ol style="list-style-type: none"> <li><b>Tiredness,</b></li> <li><b>Physical performance (work capacity / aerobic capacity / work productivity),</b></li> <li><b>Immune function</b></li> <li><i>Impaired thermoregulation,</i></li> <li><i>Restless legs syndrome,</i></li> <li><i>Impaired cognitive function.</i></li> </ol> <p>(Anemia = included but is not a health outcome as such as it is defined in terms of status markers!)</p>
Zinc	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Immune response to vaccination</b></li> <li><b>Neurodevelopment</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> <li><b>Dermatitis</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal growth</b></li> <li><b>Fetus malformation</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Preeclampsia</b></li> <li><b>Preterm delivery</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Immune function</b></li> <li><b>Cognitive function</b></li> <li><b>Dermatitis</b></li> <li><i>Anorexia</i></li> <li><i>Hypogeusia</i></li> <li><i>Ischemic heart disease</i></li> <li><i>Depression</i></li> <li><i>Diabetes Mellitus</i></li> <li><i>Carcinogenesis</i></li> </ol>
Folate	<ol style="list-style-type: none"> <li><b>Growth</b></li> <li><b>Folate-deficiency anaemia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Cancer (DNA synthesis)</b></li> <li><b>Folate-deficiency anaemia</b></li> <li><b>Immune function</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal malformations</b></li> <li><b>Fetal growth</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Maternal macrocytic anemia</b></li> <li><b>Preeclampsia</b></li> <li><b>Preterm delivery</b></li> <li><b>Placental abruption</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Stroke</b></li> <li><b>Cancer</b></li> <li><b>Osteoporosis</b></li> <li><i>Cognitive function (Cognitive function test scores such as MMSE, AD, depression ,etc)*</i></li> <li><i>Cardiovascular disease</i></li> </ol> <p>*As cognitive function will be covered only once the 3 primary health outcomes are done and if time allows to do so, we will be further specify what 'cognitive function' should included then.</p>
VitB12	<ol style="list-style-type: none"> <li><b>Neurodevelopment</b></li> <li><b>Megaloblastic anemia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Megaloblastic anemia</b></li> <li><b>Growth</b></li> <li><b>Cognitive functions and Psychomotor development</b></li> </ol>	<p>Fetus</p> <ol style="list-style-type: none"> <li><b>Fetal malformations</b></li> <li><b>Fetal growth</b></li> </ol> <p>Mother</p> <ol style="list-style-type: none"> <li><b>Megaloblastic anemia</b></li> <li><b>Preeclampsia</b></li> </ol>	<ol style="list-style-type: none"> <li><b>Anemia*</b></li> <li><b>Nervous system disease**</b></li> <li><b>Cognitive function ***</b></li> <li><b>Osteoporosis</b></li> <li><i>Cardiovascular disease</i></li> <li><i>Cancer</i></li> </ol> <p>* There are 4 types of anemia associated with vitamin B12 deficiency: megaloblastic anemia, pancytopenia, thrombocytopenia and leucopenia  ** The most important manifestations related to b12 are: peripheral neuropathy, degeneration of the spinal cord and ataxia  *** The most important manifestations include: dementia, depression, Alzheimer's disease, psychosis</p>
Iodine	<ol style="list-style-type: none"> <li><b>Hypothyroidism</b></li> <li><b>Endemic goiter</b></li> <li><b>Cretinism</b></li> <li><b>Cognitive function</b></li> <li><b>Neonatal mortality</b></li> </ol>	<b>FOR ALL POPULATION GROUPS</b>		

Health outcomes/endpoint were selected for each priority micronutrient based on the relevance to the micronutrient and the results of preliminary searches of the literature. Health outcomes in **bold** are the priority endpoints, depending on the available literature other health outcomes (*italic*) were included as well.

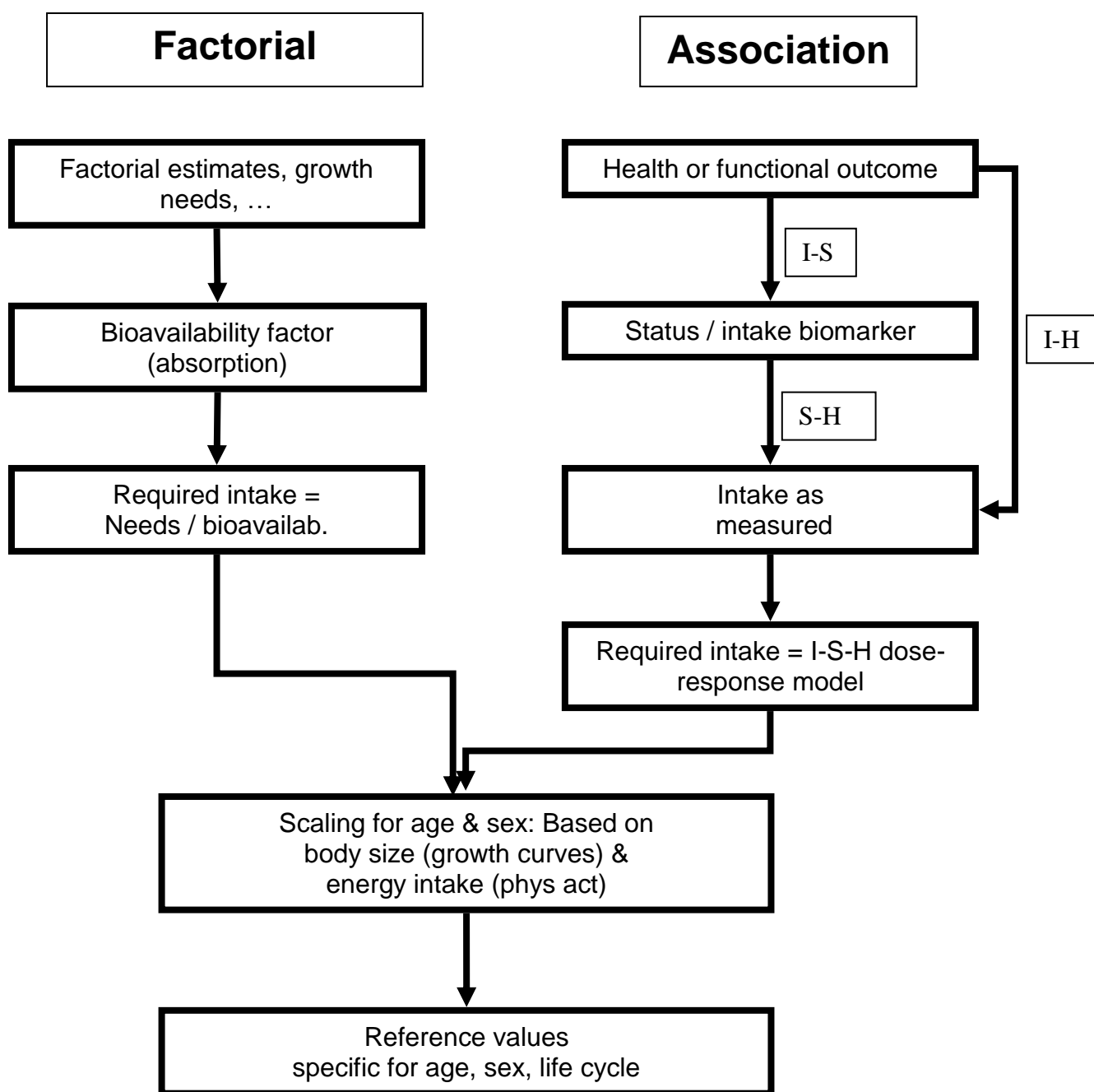


Figure 1: Scheme describing translation of available evidence to dietary reference values.

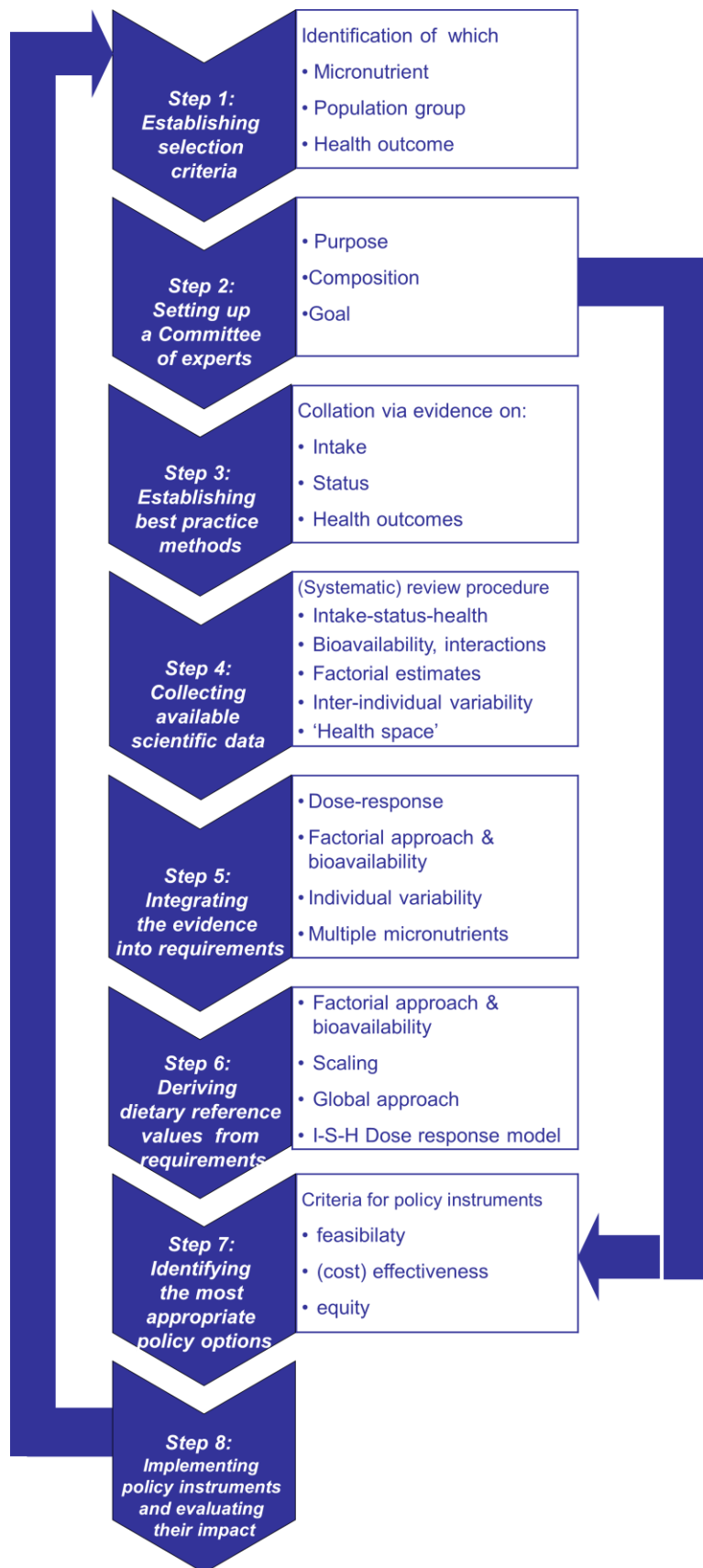


Figure 2: EURRECA Micronutrient Requirement Process Flow chart which aims to facilitate the scientific alignment of micronutrient requirements.

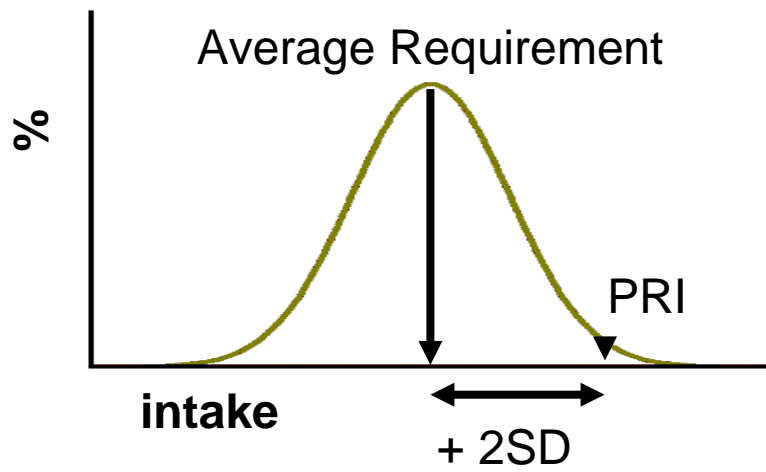


Figure 3: Different points of the dietary reference values and associated distribution (PRI: Population Reference Intake)