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TECHNICAL REPORT

Outcome of a public consultation on the Draft Scientific Opinion of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) on Dietary Reference Values for folate[1]

European Food Safety Authority[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Food Safety Authority (EFSA) carried out a public consultation to receive input from the scientific community and all interested parties on the Draft Scientific Opinion on Dietary Reference Values (DRVs) for folate, prepared by the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) and endorsed by the Panel for public consultation at its Plenary meeting on 26 June 2014. The written public consultation for this document was open from 22 July to 14 September 2014. EFSA received comments from six interested parties. EFSA and its NDA Panel wish to thank all stakeholders for their contributions. The current report summarises the outcome of the public consultation, and includes a brief summary of the comments received and how the comments were addressed. The NDA Panel prepared an updated version of the Scientific Opinion on Dietary Reference Values for folate, taking into account the comments received. The Opinion was discussed and adopted at the NDA Plenary meeting on 30 October 2014, and is published in the EFSA Journal.

KEY WORDS

folate, Dietary Reference Value, Average Requirement, public consultation

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[2] Correspondence: nda@efsa.europa.eu

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BACKGROUND

Scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition; for example, such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food report on nutrient and energy intakes for the European Community dates from 1993.

In 2005, the European Commission asked EFSA to review and if necessary update such advice to ensure that Community action in the area of nutrition is underpinned by the latest scientific advice. To this end, EFSA has been requested to consider the existing Population Reference Intakes for nutrients and certain other dietary components.

The Scientific Opinion on general principles for deriving and applying Dietary Reference Values, and the Scientific Opinions on Dietary Reference Values for water, for fats and for carbohydrates and dietary fibre were published in 2010. The Scientific Opinions on Dietary Reference Values for protein and for energy were published in 2012 and 2013, respectively. The work on Opinions on Dietary Reference Values for micronutrients is ongoing.

TERMS OF REFERENCE

In line with EFSA's policy on openness and transparency, and in order for EFSA to receive comments from the scientific community and stakeholders, EFSA shall release the Draft Scientific Opinion on Dietary Reference Values for folate for public consultation. The comments resulting from the public consultation shall be published in a technical report.

Before its adoption by the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel), the Draft Scientific Opinion on Dietary Reference Values for folate needs to be revised, taking into account the comments received during the public consultation.

CONSIDERATION

1. Introduction

Upon request from the European Commission and following previously published Scientific Opinions on Dietary Reference Values (DRVs) for macronutrients, energy, water, and several micronutrients, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) developed a Draft Scientific Opinion on DRVs for folate. In line with EFSA's policy on openness and transparency, and in order for EFSA to receive comments on its work from the scientific community and stakeholders, EFSA engages in public consultations on key issues. Accordingly, the Draft Scientific Opinion was published on EFSA's website for comments (22 July to 14 September 2014) (see Appendix A). The NDA Panel prepared an updated version of the Scientific Opinion, taking into account the comments received. The updated Scientific Opinion was discussed and adopted at the NDA Plenary meeting on 30 October 2014, and is published in the EFSA Journal (EFSA NDA Panel, 2014a). EFSA is committed to publishing the comments received during the public consultation, as well as a short report on the outcome of the consultation.

2. Screening and evaluation of comments received

2.1. Comments received

EFSA received 42 comments, of which one identical comment was made seven times and another identical comment was made four times, from six interested parties, including two national competent authorities, a national scientific advisory body, a University Hospital, an industry association and an interest group.

Table 1: List of organisations submitting comments

Organisations
Chilean Food Quality and Safety Agency
Food Supplements Europe
National Food Agency, Sweden
Health Council of the Netherlands
Saarland University Hospital
Alliance for Natural Health International

A summary of the comments is given below, and all written comments received are listed in Appendix B. The numerous comments related to policy or risk management aspects were considered to be outside the scope of the consultation, and are not covered in this report.

2.2. Nature of specific comments

The main issues raised in the comments received are summarised below. The NDA Panel has reviewed all comments carefully and has updated the Scientific Opinion on Dietary Reference Values for folate accordingly. The updated Scientific Opinion is published in the EFSA Journal.

2.2.1. Folate chemistry

Comment received

– The Panel was asked to add calcium-L-5-methyl-THF as a source of folate.

Panel consideration of comment received

– In Section 2.2.1 of the Scientific Opinion the chemistry of various folate forms is described. As calcium-L-5-methyl-THF (i.e. the calcium salt of (6*S*)-5-methyl-tetrahydrofolic acid) is also a synthetic form of the vitamin, the referral in this section to pteroylmonoglutamic acid may be misleading. This aspect has been clarified in the Opinion as follows: "In contrast, folic acid, one of the synthetic forms of the vitamin, is a fully oxidised monoglutamate and is the most chemically stable form". The Panel notes that calcium-L-5-methyl-THF is already mentioned as a source of the vitamin which may be added to foods and food supplements in Section 3.1 of the Opinion.

2.2.2. Folate analytical methodology

Comment received

– The Panel was asked to define an analytical 'gold standard' for the measurement of folate in blood and food.

Panel consideration of comment received

– The choice of an analytical method for folate depends on whether total folate or individual folate derivatives are of interest (Pfeiffer et al., 2010). The section discusses advantages/disadvantages of each type of method and clearly states their comparability and reliability. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.3. Deficiency

Comment received

– It was suggested to refer to the impact of deficiency on neural tube defects (NTDs), endothelial function and cardiovascular disease.

Panel consideration of comment received

– The Panel considers that Section 2.2.2.1 of the Opinion was intended to address clinical signs and symptoms of folate deficiency. Any relationship with (long-term) health outcomes is discussed in Section 5.2 of the Opinion. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.4. Excess

Comments received

– The Panel was asked to define the Tolerable Upper Intake Level (UL) by different age and life-stage groups. It was also suggested to reference recent studies on excess folate intake and the adverse effect on neurological symptoms related to cobalamin deficiency, though no references were mentioned in the comment.

– It was stated that unlike folic acid, L-5-methyl-THF does not mask cobalamin deficiency.

– The importance of considering intake of polyglutamic and monoglutamic folates separately when assessing the risk of reduced folates was stated.

Panel consideration of comments received

– The Panel notes that the reference to the UL opinion by the SCF (2000) is already contained in Section 2.2.2.2 of the Opinion, and that a range of UL values for children is given. The Panel points out that it is not in the Terms of Reference of the present Opinion to review the evidence for the setting of the UL. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

– As stated in Section 2.2.2.2 of the Opinion, the SCF (2000) set a UL of 1 mg/day for folic acid, based on the need to avoid the masking of cobalamin deficiency. The Panel notes that the safety of calcium-L-5-methyl-THF has been evaluated based on the assumption that the same UL would be applied to the combined intake of folic acid and calcium-L-5-methyl-THF (expressed as folate) (EFSA, 2004). The same applies to the evaluation of the safety of 5-methyl-tetrahydrofolic acid, glucosamine salt (EFSA ANS Panel, 2013). It was considered that no change in the Scientific Opinion was needed in relation to this comment.

– The understanding of the Panel is that in this comment the term "monoglutamic folate" refers to folic acid; the risk of excess folic acid intake is discussed in the Opinion. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.5. Physiology and metabolism

Comments received

– It was pointed out that dihydrofolate is omitted from Figure 1. It was noted that dihydrofolate reductase is the key enzyme in the conversion of dihydrofolate to tetrahydrofolate.

– It was pointed out that the importance of pH was not mentioned in relation to folate transport and enzymatic deconjugation.

– The derivation of the dietary folate equivalents (DFE) definition was questioned, and concern was expressed for the methodology used to develop this definition. Strong concerns about the use of the DFE definition were also expressed.

– It was stated that studies using plasma folate concentration to assess folate bioavailability were inadequate, and that the target parameter to assess bioavailability should rather be red blood cell folate.

– It was stated that studies on the bioavailability of reduced folate supplements were insufficiently described.

– The importance of considering folate bioavailability when setting DRVs was mentioned. It was suggested to consider the effect of cooking techniques and technological treatments on the bioavailability of dietary folate.

Panel consideration of comments received

– The Panel agrees that the metabolism of folic acid through dihydrofolate should be given in the figure; Figure 1 in the Opinion has been revised in order to include the metabolic steps of conversion of folic acid to dihydrofolate and tetrahydrofolate (catalysed by the enzyme dihydrofolate reductase).

- The Opinion mentions that the active absorption of folate in the intestine is pH-dependent. This statement has been expanded in Section 2.3.1.1 to include further information on the optimal pH for the activity of γ-glutamyl carboxypeptidase and intestinal folate absorption.

- The derivation of the DFE definition, including its limitations, is clearly described in Section 2.3.1.3 of the Opinion. Taking into account further evidence published after derivation of the DFE definition by IOM (1998), it is clearly stated in Section 2.3.1.5 of the Opinion that the Panel accepts this definition "in the absence of better data", with the intention of taking into account the fact that food folate has a lower bioavailability compared to folic acid added to foods, or consumed as a supplement. It was considered that no change in Section 2.3.1.5 of the Scientific Opinion was needed in relation to this comment, while the section on Recommendations for research was expanded to stress this aspect.

- It is already mentioned in Section 2.3.1.4 of the Opinion that one study (Brouwer et al., 1999) also measured red blood cell folate to assess relative folate bioavailability. The Panel considers, however, that the period of the study (four weeks) was rather short and may have affected the suitability of this marker for assessing relative bioavailability. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- As supplements containing reduced folates are not among the main contributors to folate intake, the Panel deliberately kept information on their relative bioavailability brief. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- The Panel agrees that considerable losses of folate can occur as a result of food processing and cooking (DeSouza and Eitenmiller, 1986; McKillop et al., 2002). Related information has been added to Section 3.1 on dietary sources. The text of Section 2.3.1.2 of the Opinion has been slightly modified to clarify the impact on folate bioavailability.

2.2.6. Biomarkers

Comments received

- It was stated that the analysis of folate concentration in both red blood cells and serum is preferable for the assessment of folate status.

- The Panel was asked for justification of using maximal lowering of plasma total homocysteine concentrations to indicate folate adequacy.

- The Panel was asked how serum folate concentrations of between 6.8 (being above the cut-off for folate deficiency) and 10 nmol/L (being below the cut-off for folate adequacy) were judged.

- Disagreement was expressed with the definition of folate adequacy based on a red blood cell folate concentration $\geq$ 340 nmol/L. It was mentioned that evidence related to prevention of chronic diseases should be taken into account and which would support a higher cut-off for red blood cell folate concentration to indicate folate adequacy. However, no references were provided to support this statement.

Panel consideration of comments received

- In Section 2.4.3 of the Opinion, it is already mentioned that "for assessment of folate status, multiple measurements of serum folate should be taken over a period of several weeks or a single measurement should be combined with other biomarkers of folate status." The Panel considered the results of the study by Kauwell et al. (2000) together with those by Milne et al. (1983) in which both serum and red blood cell folate were measured. Despite the differences in the

protocols and execution of these studies, their results were in close agreement. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- Folate is a cofactor in homocysteine metabolism and is considered to be the main nutritional determinant of plasma total homocysteine concentrations in healthy people. However, other B-vitamins (cobalamin, vitamin B6 and riboflavin) and choline/betaine are also involved in homocysteine metabolism. Thus, plasma total homocysteine lacks specificity for folate, and on its own is not suitable for use in assessing folate status, but it can provide some information on folate function with regard to trans-methylation. In other words, plasma total homocysteine concentration can be considered as an indicator of folate (and related B-vitamin) adequacy. The results of randomised controlled trials on homocysteine lowering have so far not consistently supported the role of homocysteine in disease aetiology in order for long-term health outcomes to be considered for defining such adequacy. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- Serum folate concentrations between 6.8 and 10 nmol/L are indicative of folate insufficiency, as they are associated with plasma total homocysteine concentrations that have not yet levelled off. Sections 5.1 and 6.1 have been slightly modified so as to emphasise that the Panel considers that the cut-offs for folate adequacy, i.e. serum folate ≥ 10 nmol/L and red blood cell folate ≥ 340 nmol/L, are used as criteria for deriving the requirement for folate.

- Folate adequacy was defined based on the relationship between plasma total homocysteine and folate, and the cut-offs determined for serum folate (≥ 10 nmol/L) and red blood cell folate (≥ 340 nmol/L) are associated with the levelling off of plasma total homocysteine concentration (Selhub et al., 2008). The use of these cut-offs for assessment of folate status of populations was also recommended by a WHO Technical Consultation on folate and cobalamin deficiencies (de Benoist, 2008). Evidence on the relationship of biomarkers of folate status with long-term health outcomes has not been considered for defining the serum and red blood cell folate cut-offs for folate adequacy. The reason for this approach was that after a thorough review of the published literature the Panel concluded that only limited evidence exists for the relationship between folate and cardiovascular disease-related outcomes, and that folate intake/status has not consistently been associated with cancer and cognition-related outcomes (see Section 5.2. of the Opinion).

2.2.7. Genotypes

Comments received

- It was suggested to add information on the methylene-tetrahydrofolate reductase (MTHFR) 1298A→C polymorphism.

- Disagreement was expressed with the statement that the impact on health of the methionine synthase and methionine synthase reductase polymorphisms is inconclusive, and it was stated that there is evidence for an increased risk of cancer in affected individuals. However, no references were provided to support this statement.

Panel consideration of comments received

- The Opinion has been revised to cover also the MTHFR 1298A→C polymorphism.

- A number of studies have not found an association between methionine synthase 2756A→G polymorphism and the risk of colorectal cancer (Ma et al., 1999; Le Marchand et al., 2002; Ulrich et al., 2005; Koushik et al., 2006; Morita et al., 2013), and one study reported that the GG genotype was protective against cancer development in the sigmoideum and rectum (Ulvik et al., 2004). The results for methionine synthase reductase 66A→C variant and colorectal cancer risk are not conclusive; an increased risk was suggested by one study (Koushik et al., 2006), whereas

others failed to detect any relationship (Le Marchand et al., 2002). It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.8. Dietary sources

Comment received

– It was suggested to include tofu and algae as rich sources of folate.

Panel consideration of comment received

– It has already been mentioned in Section 3.1 of the Opinion that legumes are among the principal folate sources, and the Panel considers that this includes tofu. The Panel was unable to retrieve information on the folate content of algae. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.9. Criteria on which to base Dietary Reference Values

2.2.9.1. Indicators of folate requirement

Comments received

– Disagreement was expressed with the cut-off for red blood cell folate concentration used, as this would only ensure prevention of "gross deficiency" and would fail to account for disease prevention and optimum health. Disagreement was also expressed with the use of data that did not account for polymorphisms affecting folate metabolism and for the occurrence of multiple polymorphisms.

– It was suggested that individuals with the 677TT genotype of the MTHFR gene have an additional requirement of 100 µg DFE/day and (apparently unpublished) results from a logistic regression analysis were provided to support this statement. However, no reference was provided.

– Disagreement was expressed with the use of the study by Kauwell et al. (2000) for the setting of DRVs for adults; it was stated that the depletion phase of this study was not long enough for subjects to have reached a steady state, and that subjects still had adequate folate status after depletion.

Panel consideration of comments received

– The Panel adopted the cut-offs for folate adequacy defined as serum folate ≥ 10 nmol/L and red blood cell folate ≥ 340 nmol/L as suitable criteria for deriving the requirement for folate intake. Of note is the fact that these cut-offs have also been recommended for assessment of folate status of populations by the WHO Technical Consultation on folate and cobalamin deficiencies (de Benoist, 2008). The Panel did not use evidence on the relationship between folate intake and long-term health outcomes for deriving the requirement for folate because the Panel concluded, after a thorough review of the published literature, that only limited evidence exists on the relationship between folate intake and cardiovascular disease-related outcomes and that folate intake/status has not consistently been associated with cancer and cognition-related outcomes (see Sections 2.2.6. of Technical Report and 5.2. of the Opinion). In the case of NTDs, the Panel acknowledges the importance of supplemental folic acid, in addition to dietary folate, for the prevention of pregnancies affected by NTDs. However, the Panel considers that the available data on folic acid intake and NTD risk cannot be used for deriving the requirement for folate for the general (female) population. This assessment of the Panel still holds after the publication of the paper by Crider et al. (2014) (see Section 2.2.9.2 of this Technical report).

Of all the known polymorphisms of genes encoding enzymes and transport proteins in folate metabolism only the MTHFR 677C→T polymorphism has consistently been associated with adverse health effects (see Section 2.5 of the Opinion). Therefore, the Panel considered only the MTHFR 677C→T polymorphism when deriving the dietary requirement for folate.

It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- The Panel has acknowledged that individuals who are homozygous for the MTHFR 677C→T polymorphism (677TT genotype) have lower serum folate concentration of around 20–25 %, and that their response to folate intervention is also lower compared with individuals with the 677CC genotype, suggesting a higher dietary requirement for folate. For dietary folate requirements of individuals with the 677TT genotype, the Panel considered all the published studies and based its conclusion on two metabolic studies (Guinotte et al., 2003; Shelnutt et al., 2003) which showed that an intake of 400 µg DFE/day maintained serum folate and red blood cell folate above the cut-offs for folate adequacy. In addition, the Panel considered the results of a four-month intervention study which supported the view that a diet providing less than 400 µg DFE/day can maintain mean plasma folate concentrations of subjects with the MTHFR 677TT genotype at a level above the cut-off for folate adequacy (Ashfield-Watt et al., 2002). The Panel is unaware of other published evidence on this matter. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- The Panel agrees that the study by Kauwell et al. (2000) has some limitations. However, all the published metabolic studies investigating folate requirements have limitations (very small number of study participants, underestimation of folate intake due to inadequate analytical methodology, short duration of the depletion/repletion periods). In order to minimise the existing limitations, the Panel considered the results of the study by Kauwell et al. (2000) together with those by Milne et al. (1983) and Sauberlich et al. (1987) for the setting of DRVs for adults. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

2.2.9.2. Folate intake and health consequences

Comments received

- It was recommended that in addition to prevention of deficiency, the folate status required for endothelial function and prevention of "neurodegeneration" should be considered. It was also stated that the folate status required for reduction of cancer and cardiovascular disease risk should be considered.

- For NTDs, the Panel was asked to add that the neural tube closes by day 28 of pregnancy. It was pointed out that supplementation after the first month of gestation will not prevent NTDs. It was suggested to describe a number of studies that have investigated the duration and amount of folate/folic acid supplementation required to reach a red blood cell folate concentration of ≥ 906 nmol/L in women. It was stated that in early pregnancy the "optimal" plasma folate concentration to prevent NTDs is > 20 nmol/L, and that the NTD risk in women with a red blood cell folate concentration of around 1 000 nmol/L is about six-fold lower compared to women with a red blood cell folate concentration of 340 nmol/L.

Panel consideration of comments received

- After a review of the published literature, the Panel concluded that there is limited evidence for the effect of folate on the risk of cardiovascular disease (lack of dose–response relationship), and that folate/folic acid has not consistently been associated with cancer risk, and therefore the data available cannot be used for deriving the requirement for folate (see Section 5.2. of the Opinion). In relation to the effect of folic acid on endothelial function, the Panel considers that the findings

of MacKenzie et al. (2006) based on an intervention study in children with type 1 diabetes mellitus cannot be used for the setting of DRVs for healthy children. As regards "neurodegeneration", the Panel considers that no conclusions can be drawn from the study of Douaud et al. (2013) on the effect of folic acid *per se* as this intervention study used combined B-vitamin supplementation (800 µg/day of folic acid, 20 mg/day of vitamin B6 and 500 µg/day of cobalamin). It was considered that no change in the Scientific Opinion was needed in relation to this comment.

– The Panel revised Section 5.2.4 of the Opinion in order to add information on the time of closure of the neural tube (days 21–28 of embryonic life). The Panel is unaware of evidence supporting a threshold serum folate concentration of > 20 nmol/L for NTD prevention. Results of the recently published study by Crider et al. (2014) have been included in Section 5.2.4 of the Opinion.

2.2.10. Data on which to base Dietary Reference Values

Comments received

– It was suggested to set sex-specific DRVs and to take into account the folate requirement for the prevention of an NTD-affected pregnancy in women of childbearing age as a criterion for setting the DRV for this population group.

– The Panel was asked for further explanation on the selection of a coefficient of variation (CV) of 15 % to derive the Population Reference Intake (PRI) for adults, children and lactating women. It was also stated that the current assumption of using a CV of 15 % to account for differences in genotype was not based on evidence.

– Concern was expressed for the establishment of a DRV for folate, as it was felt that further research was needed to characterise the amount of folate needed for optimum health. It was pointed out that the proposed DRVs do not take into account the beneficial effects of folate on endothelial function, reduction of "neurodegeneration"/Alzheimer's disease, interactions with other nutrients, or compound effects of multiple polymorphisms.

– For pregnancy, concern was expressed over the evidence to support an Adequate Intake (AI) of 600 µg DFE/day for the entire duration of pregnancy, and it was suggested to consider setting a lower value. It was also pointed out that the decrease in plasma folate concentration in pregnancy may only be the result of haemodilution and not necessarily indicative of a low folate status.

– Support was expressed for the PRI of 500 µg DFE/day for lactation.

Panel consideration of comments received

– The Panel acknowledges that the prevention of most NTDs can be achieved by ingestion of supplemental folic acid (400 µg/day) in addition to dietary folate, but considers that these data cannot be used for deriving the requirement for folate for women of child-bearing age. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

– The Panel considered that individuals with the MTHFR 677TT genotype compared to those with the 677CC genotype have higher dietary folate requirements. Taken together, the results of two metabolic studies (Guinotte et al., 2003; Shelnutt et al., 2003) and a four-month intervention study (Ashfield-Watt et al., 2002) suggested that dietary folate intake of less than 400 µg DFE/day may be sufficient for individuals with the MTHFR 677TT genotype to maintain serum and red blood cell folate above the cut-offs for folate adequacy; however, the exact amount of folate intake required is uncertain. In order to account for these uncertainties, the Panel considered applying a CV of 15 % for deriving the PRI for folate. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- The Panel's task was to review the evidence on the dietary requirement for folate. The Panel acknowledges the limitations and uncertainty of the data on folate intake and selected biomarkers, but overall concludes that an Average Requirement (AR) and a PRI can be derived for adults, children and lactating women. The Panel agrees with the comment with respect to limitations in the data on folate intake and long-term health outcomes, and that they cannot be used for the setting of DRVs for folate, as outlined in Section 5.2 of the Opinion. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

- The Panel acknowledges that limited evidence is available for assessing folate requirements in pregnancy. The Panel considered as pertinent a metabolic study (Caudill et al., 1997) in which 630–680 µg DFE/day were administered to pregnant women during the second and third trimester and which resulted in mean concentrations of biomarkers of folate status well above the cut-offs for folate adequacy as established in non-pregnant adults. The Panel acknowledged that it is unknown whether a lower folate intake may also be sufficient. Considering these uncertainties, and in the absence of further pertinent data, the Panel proposed to set an AI for folate in pregnancy at 600 µg DFE/day. It was considered that no change in the Scientific Opinion was needed in relation to this comment.

The ARs for children and the text of Section 6.3 of the Opinion have been revised to take into account updated age-specific growth factors based on the proportional increase in protein requirement for growth (EFSA NDA Panel, 2010, 2014b). In addition, the scaling method applied to the AR for adults and the calculated folate intake of infants from birth to six months has been changed to allometric scaling on the assumption that folate requirement is related to the metabolically active body mass.

2.2.11. Recommendations for research

Comments received

- It was proposed to also highlight the need for research in relation to a cut-off for high serum folate, the contribution of ready-made foods to total folate intake, the relationship between folate and obesity and associated diseases, prevalence of folate deficiency and excess, the effect of folate on gene expression, and further clarification of folate bioavailability and DFEs.

- It was expressed that there is an urgent need for comparable dietary intake data between European countries, and also research on the folate intake required to maintain optimal plasma concentration for the prevention of NTDs.

- The need for studies to clarify the relationship between folate tissue concentration and risk of disease was expressed. It was added that these studies should independently consider folic acid supplements, fortified foods, reduced folate supplements and food folates, and that DFE should not be used.

Panel consideration of comments received

The Panel agrees with these suggestions for further research and considers that the most urgent ones are already included in the Opinion. The Panel's consideration with regard to the DFE definition is stated in Section 2.2.5 of this Technical Report. It was considered that no change in the Scientific Opinion was needed in relation to these comments.

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APPENDICES

Appendix A. EXPLANATORY TEXT PUBLIC CONSULTATION ON THE DRAFT SCIENTIFIC OPINION ON DIETARY REFERENCE VALUES FOR FOLATE

EFSA has launched an open consultation on the draft scientific opinion on dietary reference values for folate. This document proposes dietary reference values for folate for adults, infants and children, pregnant and lactating women.

In line with EFSA's policy on openness and transparency and in order for EFSA to receive comments from the scientific community and stakeholders, EFSA has launched a public consultation on the draft document developed by the NDA Panel of EFSA.

Interested parties are invited to submit written comments by 14 September 2014. Please use exclusively the electronic template provided with the documents to submit comments and refer to the line and page numbers. Please note that comments submitted by e-mail or by post cannot be taken into account and that a submission will not be considered if it is:

- submitted after the deadline set out in the call
- presented in any form other than what is provided for in the instructions and template
- not related to the contents of the document
- contains complaints against institutions, personal accusations, irrelevant or offensive statements or material
- is related to policy or risk management aspects, which is out of the scope of EFSA's activity.

EFSA will assess all comments from interested parties which are submitted in line with the criteria above. The comments will be further considered by the relevant EFSA Panel and taken into consideration if found to be relevant.

All comments submitted will be published. Comments submitted by individuals in a personal capacity will be presented anonymously. Comments submitted formally on behalf of an organisation will appear with the name of the organisation.

Appendix B. FULL LIST OF COMMENTS SUBMITTED BY MEANS OF ELECTRONIC FORM ON THE EFSA WEBSITE

CHAPTER TEXT	ORGANISATION	COMMENT TEXT
1. Introduction	Food Supplements Europe	Food Supplements Europe welcomes the work EFSA undertakes to review the DRV of many nutrients, including Folate and the opportunity to provide our comments and suggestions in this consultation.
2.1.1. Folate chemistry	Food Supplements Europe	We would like EFSA to add Calcium L-5-methyltetrahydrofolate (L-5-MTHF-Ca) another source of folate whichis the calcium salt of L-5-methyltetrahydrofolate (L-5-MTHF) and which dissociates in aqueous media readily and completely into Ca and L-5-MTHF (L-Methylfolate) ions (EFSA 2004). [EFSA, Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Calcium L-Methylfolate. EFSA Journal (2004) 135, 1-20] L-Methylfolate is the predominant form of reduced folate as naturally found in foods, the principal form of circulating folate in the body, the preferred substrate for transport into peripheral tissues and the essential form in which folates are stored in the human body (Pietrzik K. et al., 2010; Scott, 2001; Hasselwander et al., 2000). During absorption, all natural folates are converted to L-Methylfolate which is the only form of folate to enter the human circulation (Hasselwander et al., 2000). [Pietrzik K. et al., Folic Acid and L-5-Methyltetrahydrofolate Comparison of Clinical Pharmacokinetics and Pharmacodynamics, Clin Pharmacokinet, 49 (8): 535-548 (2010)] [Scott, J.,Methyltetrahydrofolate: the Superior Alternative to Folic Acid. In Krämer, K., Hoppe, P. And Packer, L. (eds) Nutraceuticals in Health and Disease Prevention. New York: Marcel Dekker Inc. (2011)] [Hasselwander O. Et al., 5-Methyltetrahydrofolate: The active form of folic acid. Functional foods 2000, 48- 59 (2006)]
2.1.2. Folate analytical methodology	Chilean Food Quality and Safety Agency	Regarding Biomarkers, we would recommend to specifically define an analytical gold standard method for measuring folate in both blood and food. Although it has been recognized that folate in red blood cells (RBC) is the best indicator to measure folate status (long term information) EFSA considered serum folate as the best indicator, over erythrocyte folate or total plasma homocysteine (non-specific). According to the available evidence, it would be recommended to use both analytical methods: serum and RBC folate, since it has not been established which one would be more beneficial. Therefore, the Chilean Expert Panel considers appropriate an official announcement to select the best indicators by the European Authority. The European Panel decided to define “deficiency” and “adequacy” of (serum) folate using cutoff points <6.8 and >10 nmol/L, respectively. There is a lack of definition for those values between 6.8 and 10nmol/L, therefore its definition is recommended. We would also recommend to describing the bases to define the categories for deficiency, adequacy and hopefully elevated values. In addition, further explanations to estimate AI from AR in pregnant women and how to handle with the physiologic fluctuations during pregnancy among trimesters are needed.
2.2.1 Biochemical function	Alliance for Natural Health International	Dihydrofolate is omitted from Fig 1, when it is the precursor to tetrahydrofolate, and dihydrofolate reductase (DHFR) is the key enzyme involved in the conversion. Critically, a rapidly growing body of evidence is showing high frequencies of DHFR, in the range 5-80% in different sub-populations, the highest frequencies being among those highly susceptible to

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		specific cancers e.g. leukemia. The 19 bp deletion of DHFR polymorphisms (with no detail of affected genotypes, such as DHFR 829C/C, 829C/T, and 829T/T) is mentioned in passing, seemingly almost as an after thought, on line 804-5.
2.2.2. Health consequences of deficiency and excess	Chilean Food Quality and Safety Agency	In this section, it is important to reference the most recent studies proving that folate excess could worsen neurological problems derived from vitamin B12 deficiency. We would recommend defining upper limits (UL) of folate intake, stratified by different age and physiological groups. EFSA has previously defined a UL of 1000µg/day of folate intake for adults and 200-800 µg/day for children aged 1 to 17 y (http://ec.europa.eu/food/fs/sc/scf/out80e_en.pdf) The Institute of Medicine has also defined folate UL in adults of 1000µg/day.
2.2.2.1 Deficiency	Alliance for Natural Health International	There is no mention of impact on neural tube defects, endothelial function or cardiovascular disease. See extract below from Verkerk, R. Toxicology, 2010 Nov 28;278(1):27-38. doi: 10.1016/j.tox.2010.02.011: "Increased risk of neural tube defects in infants born to folate deficient mothers is well established, although supplementation with folic acid will not necessarily ensure 100% elimination of such congenital abnormalities (Heseker et al., 2008). Evaluation of folate status in Germany suggests most Europeans are unlikely to meet the reference intake levels (RDAs) and are therefore folate deficient (Gonzalez-Gross et al., 2002). Inadequate intakes may contribute to increased risk of cardiovascular disease (McNulty et al., 2008) and cancer (Fairfield and Fletcher, 2002), as well as other health risks, including neural tube defects in babies born to folate-deficient mothers (Fletcher and Fairfield, 2002). Additionally, a range of polymorphisms in various genes (e.g., 5,10-methylenetetrahydrofolate reductase [MTHFR], C677T), which reduce rates of deconjugation of polyglutamate folates, appear to be widely distributed in the population (affecting some 10–30%). These individuals require higher levels of folate intake, particularly in the polyglutamate form, to normalise the metabolic disorder induced by the polymorphism (Prinz-Langenohl et al., 2009). Adequate consumption of dietary folates, such as 5-MTHF, is considered to lower the risk of cardiovascular disease, in particular by improving endothelial function in atherosclerosis (Buccianti et al., 2002; Baragetti et al., 2007). The mechanism of action is likely to be associated with 5-MTHF's role in maintaining endothelial function and vascular superoxide production by preventing peroxynitrite-mediated oxidation of tetrahydrobiopterin (BH4) which acts as a cofactor for nitric oxide synthase (eNOS), elevated levels of which are associated with atherosclerosis in humans (Antoniades et al., 2006). Ronco et al. (2007) showed that 5-MTHF, but not folic acid, stimulated the production of endothelin-1 in LDL treated human endothelial cells, suggesting that this mechanism may be involved in folate's role in the reduction of cardiovascular disease risk."
2.2.2.2 Excess	Alliance for Natural Health International	The assumption made in this section is that the risk of food folates or supplements containing reduced folates (e.g. calcium methylfolate, glucosamine-5MTHF) is similar, taking into account the DFE conversion factor of 2.0 or 2.1, without or with food, respectively. There are no data to support this. All concerns with excess have been related to consumption of the oxidised form, pteroylmonoglutamic (folic) acid, and EFSA is right to include data on circulating unmetabolised folic acid, which may play a role in generating adverse effects. It is essential that in any discussion of risk, risks associated with consumption of polyglutamic and monoglutamic folates are considered separately.
2.2.2.2. Excess	Food Supplements Europe	We would like to note that unlike folic acid, L-Methylfolate, does not mask cobalamin deficiency as it cannot correct the hematological signs of cobalamin deficiency and therefore does not interfere with the timely diagnosis of cobalamin

CHAPTER TEXT	ORGANISATION	COMMENT TEXT
		deficiency (Smulders et al. 2005, Pietrzik et al. 2010, Hasselwander et al. 2006, Scott 2011). [Smulders Y. et al., Cellular folate vitamer distribution during and after correction of vitamin B12 deficiency: a case for the methylfolate trap. British J. of Haematology, 132, 623-9 (2005)] [Pietrzik K. et al., Folic Acid and L-5-Methyltetrahydrofolate Comparison of Clinical Pharmacokinetics and Pharmacodynamics, Clin Pharmacokinet, 49 (8): 535-548 (2010)] [Hasselwander O. Et al., 5-Methyltetrahydrofolate: The active form of folic acid. Functional foods 2000, 48- 59 (2006)] [Scott, J.,Methyltetrahydrofolate: the Superior Alternative to Folic Acid. In Krämer, K., Hoppe, P. And Packer, L. (eds) Nutraceuticals in Health and Disease Prevention. New York: Marcel Dekker Inc. (2011)]
2.3.1.2. Factors influencing intestinal absorption	Alliance for Natural Health International	There is no discussion of the considerable importance of pH in both folate transport and enzymatic deconjugation. See Mason, J. B. (1990) Intestinal transport of monoglutamyl folates in mammalian systems. In: Folic Acid Metabolism in Health and Disease (Picciano, M. F., Stokstad, E.L.R. & Gregory, J. F., eds.), pp. 47–64, Wiley-Liss, New York, and Gregory J Nutr. 2001 Apr;131(4 Suppl):1376S-82S.
2.3.1.3. Dietary folate equivalents	Alliance for Natural Health International	There are no studies or data referred to in this section to support the notion that the 1.7 multiplier for DFE based on folic acid intake is scientifically valid. It is unclear what the marker of folate status was in each of the studies used by the IOM in developing the multiplier, and it is now well known that very different results emerge in different studies, and between direct (e.g. serum, erythrocyte) and indirect (e.g. homocysteine) markers. There are very considerable methodologic issues relating to bioavailability assessments of dietary folates and folic acid — and the 1.7 fold difference used by the FDA based in the IOM opinion, appears to be based largely on a single study of non-pregnant women (Am J Clin Nutr 1987; 46: 1016–28). Common methodologies have included determination of erythrocyte folate, plasma folate concentration, and determination of a marker of folate, namely plasma homocysteine (Am J Clin Nutr. 2004; 80(4): 911-8). However, there are numerous confounding factors such as the time period (end-point) over which assessments are determined, and whether the assessments included mixed diets or individual foods (Am J Clin Nutr. 2004; 80(4): 911-8). Accordingly, assessments of folate bioavailability from dietary sources have shown great variability and it would be scientifically irrational to apply the 1.7-fold DFE conversion factor to folic acid. This is especially the case given that the bioavailability of the monoglutamic, synthetic form, folic acid, is much more predictable than that of dietary folates.
2.3.1.4. Studies assessing relative folate bioavailability	Alliance for Natural Health International	This section is very inadequate. All studies referenced involve using either serum or plasma folate status to determine bioavailability. Studies measuring erythrocyte folate as a marker of tissue folate status (see Section 2.4.1.2.) may yield a considerably different view on bioavailability. Owing to folate trafficking and requirements of different cells and tissues, it is unsurprising that serum or plasma folate may be sometimes both inaccurate and misleading. Given “99 % of total body folate is in the tissues (Lin et al., 2004), with storage taking place predominantly in the liver (Duncan et al., 2013)” (lines 587-8), tissue measurement of polyglutamyl folate is an important indicator of folate status. Regarding bioavailability studies of reduced folate supplements (i.e. the calcium and glucosamine salts of 5-MTHF), there are inadequate studies other than those from the respective manufacturers. The methodologies of these studies are insufficiently detailed and it is not clear what is being measured, and where. Far more detail is required.

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2.3.1.5 Conclusions on folate bioavailability	Alliance for Natural Health International	It is incomprehensible that on such weak evidence, as admitted by the NDA panel in the draft, that the out-dated DFE unit and the proposed multipliers will be used. EFSA is supposed to uphold the highest standards of science, not simply reinforce arbitrary nutritional concepts that are based on very limited data and are now increasingly irrelevant.
2.3.1.5. Conclusions on folate bioavailability	Chilean Food Quality and Safety Agency	In this point, it is important to emphasize that bioavailability of folate has special importance in dietary recommendations along with genetics, race, gender, contraceptive methods, among others. Finally, the effects of different cooking techniques or technological treatments in the bioavailability of folate in dietary sources should be mentioned in this section, in order to be considered it in the dietary reference intakes.
2.4. Biomarkers	Health Council of the Netherlands	668-773 The Panel describes that the cut-offs for functional folate adequacy (cut-offs for plasma and red blood cell folate) are based on maximal lowering of plasma total homocystein concentrations. These cut-off values (10 and 340 nmol/L, lines 743-4) are slightly higher than those necessary for preventing folate deficiency (6,8 and 317 nmol/L, lines 682 and 697). The Panel assumes that low homocystein concentrations are needed to achieve good health, but does not present evidence to substantiate this assumption. Please present evidence that maximal lowering of homocysteine concentrations is needed to achieve optimal health. If this type of information is not available, we suggest that the committee uses the cut-offs for preventing folate deficiency.
2.4.3 Conclusion on biomarkers of intake, status and function	Alliance for Natural Health International	The widely accepted levels of folate status urgently require review and have been based around preventing deficiency not reducing the risk of disease, e.g. cardiovascular, cancer, Alzheimer's. While red blood cell (RBC) "concentrations below 317 nmol/L are indicative of 763 folate deficiency" (lines 763-4), the level for adequacy should be set well above the proposed 340 nmol/L (just 7% above above the level for deficiency), which the NDA panel considers reflects "functional folate adequacy". New studies are required to better understand these relationships and the concentrations in tissues (and RBCs) required for optimum health. Accordingly, we believe it is premature to establish a DRV for either food folate or supplemental forms, let along both together, using a crude and out-dated multiplier.
2.5 Effects of genotypes	Alliance for Natural Health International	While the relatively detailed consideration of the 677C-->T polymorphism of MTHFR is worthy, there is no mention of 1298 A>C polymorphism. Furthermore, the statement that the other less studied polymorphisms such as methionine synthase (MTRR) and methionine synthase reductase (MTR) is not justified given current data on increased cancer risk of individuals with these genotypes. Furthermore, dihydrofolate reductase polymorphisms are
3.1. Dietary sources	Chilean Food Quality and Safety Agency	It is recommended to include new foods such as Tofu or algae, which could be rich in folate (Fajardo Martín et al. Nutr Hosp. 2013;28(4):1210-1218; Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. McCance and Widdowson's The Composition of Foods).
4 Overview of Dietary Reference Values and recommendations	Chilean Food Quality and Safety Agency	We would recommend to the Panel Experts of EFSA to report the bases to use a 15% coefficient of variation (CV) to establish the "Dietary Reference Values", since other institutions have established reference values using different CVs: i.e. the US Institute of Medicine (IOM) uses a CV of 10% (1), SCF a CV of 20% (2) and The Health Council of The Netherlands a 25% CV (3). 1. IOM, 1998. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin b12, pantothenic acid, biotin, and choline. Food and Nutrition Board. National Academy Press, Washington, D. C., USA.

CHAPTER TEXT	ORGANISATION	COMMENT TEXT
		2. Scientific Committee for Food, 1993. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food, 31st Series. Food - Science and Techniques, European Commission, Luxembourg. 3. The Health Council of Netherlands a CV of 25% (Health Council of the Netherlands, 2003. Dietary Reference Intakes: vitamin B6, folic acid, and vitamin B12. The Hague: Health Council of the Netherlands, 2003; publication no. 2003/04.
4 Overview of DRVs and recommendations (all subsections)	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
4.2. Infants and children	Chilean Food Quality and Safety Agency	Table 2: Overview of Dietary Reference Values for folate for children. The meaning of the letter "e" as a superscript is missing.
5.1.1.1 Criteria (endpoints) on which to base DRVs - adults - evidence from studies not considering MTHFR genotype	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.1.1.2 Criteria (endpoints) on which to base DRVs - adults - evidence from studies considering MTHFR genotype	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.1.1.2. Evidence from studies considering MTHFR genotype	Saarland University Hospital	Additional 100 µg DFE/d is required by MTHFR 677TT carriers Klaus Pietrzik1 and Rima Obeid2 1Department of Nutrition and Food Science, Rheinische Friedrich-Wilhelms University, 53115 Bonn, Germany, k.pietrzik@uni-bonn.de. 2Department of Clinical Chemistry, Saarland University Hospital, 66421 Homburg, Germany, rima.obeid@uks.eu. The MTHFR677TT genotype (10-20% in Europeans) is an important determinant of folate level and requirement. TT carriers have lower plasma folate (2-4), are more sensitive to folate depletion, show delayed and less response to repletion,

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		and require higher doses of folic acid (FA) to achieve a metabolic level similar to that in TT carriers. The differences in plasma folate or tHcy are ≈ 25%. Independent on the genotype, it has been estimated that 100 µg/d FA can increase plasma folate by 2.13-3.3 nM (5, 6). We here propose a simplified mathematical modelling of plasma folate and folate intake dependent on the MTHFR genotype to estimate the additional intake required by TT carriers. We collected published data on means plasma folate and total folate intake from 4 independent studies for CC and TT (13 means per genotype). We then estimated plasma folate (dependent variable) at a given intake level for a given genotype (intake and genotypes are independent variables). By using a Logistic Regression Model (not adjusted for age, sex, etc) we obtained the following formula: Serum folate = 14.43 (constant)+(0.028xintake)-2.654 (for TT). R2=0.67 (adjusted=0.64). TT/CC are categorical variables. Therefore, for a mean intake (µgDFE/d) of 300, it is estimated that CC carriers can achieve =14.43+(0.028x300)=22.8nM plasma folate. In order for TT carriers to achieve 22.8nM plasma folate, a mean daily intake of 395=(22.8–14.43+2.654)/0.028 is needed. Moreover, at an intake of ~400, CC carriers are expected to achieve mean plasma folate of 25.6nM=14.43+(0.028x400). At 400 intake level, TT is estimated to achieve a mean plasma folate of 23nM =14.43+(0.028x400)-2.654. Additional 100 µgDFE is needed in order to bridge the difference in the requirements between CC and TT. Our proposed mathematical modelling of plasma folate levels and folate intakes fits very well with the observed plasma folate and estimated intake values that have been reported in several European countries. At intakes <400 µgDFE/d, 6 mean plasma folate values were < 20nM in TT subjects versus only 1 value for CC subjects. The lowest plasma folate levels necessary for prevention of folate-responsive NTDs is 20nM. When folate intakes are ≥ 400, means plasma folate were all > 20nM for TT subjects. The estimated intake values showed a strong positive correlation with the measured mean plasma folate values [r=0.789, p<0.001, n=26 observations]. At any intake level, CC had approximately 25% higher plasma folate when compared to TT. Our observations (table and figures can be provided upon request) and the evidence from numerous studies imply that the RDA should remain at 400 to cover the requirements of the majority of individuals from all genotypes. Assuming a CV of 15 % instead of 10% in order to account for TT is not evidence based. We strongly recommend keeping the RDA at 400. Our mathematical model can be applied on intakes, genotype, and plasma levels obtained from Europeans to optimize the recommended intake of folate in an age and sex specific manner and to bridge the gap between the observed and actual intakes. Selected references 1.Zhang et al., PLoS. ONE. 8, e59570 (2013). 2.Ashfield-Watt et al., Am. J Clin Nutr. 76, 180 (2002). 3. Crider et al., Am. J Clin Nutr. 93, 1365 (2011). 4.Fazili et al., Clin Chem 54, 197 (2008). 5.Berti et al., J Nutr. Metab 2012, 470656 (2012). 6.Wald et al., Lancet 358, 2069 (2001)
5.1.1.3. Conclusions on folate requirement	National Food Agency, Sweden	Line 1181-1206 The NDA panel considers that new data are available to update the AR and PRI for adults using results from a depletion-

CHAPTER TEXT	ORGANISATION	COMMENT TEXT
of adults		repletion study by Kauwell et al. (2000). The participants (60-85 y, n=30) consumed a moderately folate deplete diet for 7 wk followed by repletion diets (n=4) providing 200 or 415 µg of folate and folic acid per day. After the depletion period serum folate concentrations were in average 13.7 nmol/L and red blood cell folate in average 1487 nmol/L. After repletion, with folate from a low folate diet with orange juice and folic acid, average serum folate concentration was 14.0 nmol/L for those consuming 200 µg of folate and folic acid per day and 31.4 nmol/L for those consuming 415 µg of folate and folic acid per day. During repletion there were no differences in red blood cell folate concentrations detected among treatments (200 or 415 µg of folate and folic acid). We see some limitations in using this reference to set AR and PRI. Mainly the depletion was not long enough for participants to reach a steady state. Even after depletion the assessed folate concentrations in participants (14 nmol/L in serum and 1487 nmol/L I red blood cells) must be considered as sufficient or even excellent. For example, in the study by Daly et al (1995) the lowest risk of having a child with neural tube defects was among those having red blood cell folate concentrations above 906 nmol/L. Furthermore, the folate concentrations after depletion was high above the cut off for deficiency set by the panel, i.e. serum folate concentration below 6.8 nmol/L and red blood cell folate concentrations below 317 nmol/L. The Panel concludes that an AR for folate can be set at 250 µg DFE/day. In order to account for the additional variability as a result of the higher requirement for folate in individuals with the MTHFR 677TT genotype a CV of 15 % is applied to the AR of 250 µg DFE/day to derive the PRI of 330 µg 1431 DFE/day. References Daly LE, Kirke PN, Molloy A, Weir DG and Scott JM, 1995. Folate levels and neural-tube defects – implications for prevention. Journal of the American Medical Association, 274, 459-464. Kauwell GPA, Lippert BL, Wilsky CE, Herrlinger-Garcia K, Hutson AD, Theriaque DW, Rampersaud GC, Cerda JJ and Bailey LB, 2000. Folate status of elderly women following moderate folate depletion responds only to a higher folate intake. Journal of Nutrition, 130, 1584-1871. Milne DB, Johnson LK, Mahalko JR and Sandstead HH 1983. Folate status of adult males living in a metabolic unit: possible relationships with iron nutriture. American Journal of Clinical Nutrition, 37, 768-773. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL and Taylor PC, 1987. Folate requirement and metabolism in nonpregnant women. American Journal of Clinical Nutrition, 46, 1016-1028.
5.1.1.3. Conclusions on folate requirement of adults	Saarland University Hospital	Insufficient folate before conception continues to be a major problem in Europe Rima Obeid1, Klaus Pietrzik2 1Department of Clinical Chemistry, University Hospital of the Saarland, 66421 Homburg, Germany; rima.obeid@uks.eu.

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		2Department of Nutrition and Food Science, Rheinische Friedrich-Wilhelms University, 53115 Bonn, Germany, k.pietrzik@uni-bonn.de. The optimal levels of plasma folate in early pregnancy to prevent NTDs (>20 nM) cannot be achieved within 4 weeks of supplementation in the majority of women, unless 400-800 µg folic acid (FA) is supplemented (1,2). These concentrations are ~3 fold higher than the lowest cutoff value that is used to define classical deficiency. At whole blood folate concentrations of 340 nM, the estimated risk for NTD was 48.8 cases per 10000 births. At concentrations around 1000 nM, the risk declined to 7.9 per 10000 births (6 fold lower risk) (3). Recommendation to supplement FA pre-conceptionally failed in achieving the goal in many European countries (4-6) because of barriers related to low baseline folate, unplanned pregnancy, education level, young age, economic status and more importantly the short time interval available for NTD prevention. Therefore, subgroups of the European populations who lack the knowledge or the free access to vitamin supplements will remain vulnerable for NTDs. Plasma folate is considered a short term marker that is affected by recent intakes. However, in term of a single measurement of plasma folate in women planning for pregnancy or in pregnant women, plasma folate is the marker that indicates how much folate is available to be transported through the placenta. Therefore, if acute fluctuations in the diet can be excluded, plasma folate remains a powerful tool to screen for folate sufficiency. In term of health and disease, serum folate has a higher diagnostic value on a population level as compared to folate intake data or whole blood folate. Folate intake data are virtual values that currently lack comparability across studies and populations. In short term supplementation, doubling of total folate intake has been estimated to increase serum folate by 47% (7). Therefore, assuming a linear relationship between intake and plasma levels before reaching a steady state, the lowest baseline plasma folate that can be increased to at least 20nM before pregnancy is 13.6nM (i.e., 13.6+47%=20nM) by doubling the intake (i.e., from 330 to 660 µgDFE). Two studies used different approaches to estimate that plasma folate can be increased by approximately 3.3 (7) or 2.13 nM per 100 FA (170 µg DFEs) (8). Therefore, increasing mean intake of folate from 330 to 600 µg DFE/d for approximately 4 weeks pre-pregnancy is expected to achieve the lowest optimal protective value of 20nM only when baseline plasma folate > population median. The estimates mentioned above imply that half of the women in Europe cannot achieve the lowest protective plasma folate level even if they increased their intake from 330 to 600 µg DFE/d 4 weeks before conception. In addition, the number of women who indeed supplement FA before pregnancy is low in most European countries. Therefore, approximately 75% of European women reach the first trimester with suboptimal folate status, thus raising the question about the most effective strategy to improve folate status in the population. 1. Bramswig et al., Int J Vitam. Nutr. Res 79, 61 (2009). 2.Lamers et al., Am. J Clin Nutr. 84, 156 (2006). 3.Crider et al., BMJ 349, g4554 (2014). 4.Rofail et al., J Public Health (Oxf) 34, 90 (2012). 5. de Walle et al., Eur J Clin Pharmacol. 64, 539 (2008). 6.Barbour et al., J Hum. Nutr. Diet. 25, 140 (2012). 7.Berti et al., J Nutr. Metab 2012, 470656 (2012).

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		8.Wald et al., Lancet 358, 2069 (2001).
5.1.2 Criteria (endpoints) on which to base DRVs - infants aged 7-11 months	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.1.3 Criteria (endpoints) on which to base DRVs - children	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.1.4 Criteria (endpoints) on which to base DRVs - pregnancy	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.1.5 Criteria (endpoints) on which to base DRVs - lactation	Alliance for Natural Health International	The DRVs considered in this section are based largely on achieving given levels of folate status (e.g. RBC 340 nmol/L) which are likely to be inadequate as they reflect only levels that prevent gross deficiency and do not reflect those required to reduce disease and maintain optimum health. The fact that these data by and large precede the discovery of the importance of the MTHFR genotype and other polymorphisms affecting folate metabolism means that they are no longer relevant. No consideration is given for individuals with multiple polymorphisms.
5.2 Folate intake and health consequences (all subsections)	Alliance for Natural Health International	It is essential to consider not only folate status required to prevent symptoms of deficiency such as megaloblastic anaemia. It is imperative that other functions such as endothelial function, prevention of neurodegeneration (Alzheimer's disease), as well as reduced cancer and cardiovascular disease risk, are taken into account.
5.2.4. Neural tube defects	Food Supplements Europe	Line 1382 We would like to propose the following rewording for clarity reasons: Neural tube defects (NTD) are a group of congenital malformations (spina bifida or anencephaly) which are the result of incomplete closure of the neural tube during early embryonic development (neural tube closes 22-28 days after conception). Line 1395 We would like to offer the following scientific elements for EFSA's consideration and possible inclusion in the opinion" According to Guinotte et al. 2003, mentioned in section 5.1.1.2., it was shown that in a seven weeks folate repletion study with 800 µg/d DFE, all subjects maintain red blood cell concentration above the cut off level associated with functional folate adequacy (≥ 340 nmol/L). To note, in women with the MTHFR TT genotype it takes at least six weeks folate repletion

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		with 800 µg/d DFE to reach a preventive red blood cell folate level of ≥ 906nmol/L (Guinotte et al. 2003). [Guinotte CL, Burns MG, Axume JA, Hata H, Urrutia TF, Alamilla A, McCabe D, Singgih A, Cogger EA and Caudill MA, 2003. Methylenetetrahydrofolate reductase 677C -> T variant modulates folate status response to controlled folate intakes in young women. Journal of Nutrition, 133, 1272-1280.] In women receiving supplemental 400 µg/d folic acid, red blood cell folate level reached ≥ 906 nmol/L after 8 weeks of intervention (Lamers et al. 2006). [Lamers Y. et al., Red blood cell folate concentrations increase more after supplementation with [6S]-5-methyltetrahydrofolate than with folic acid in women of childbearing age, Am J Clin Nutr 2006;84:156–61.] Supplementation of 800 µg/d folic acid has been shown to effectively shorten the period (to 4 weeks) until the red blood cell folate concentrations reach a level of ≥ 906nmol/L (Brämswig et al., 2009). [Brämswig S, Prinz-Langenohl R, Lamers Y, Tobolski O, Wintergerst E, Berthold HK, Pietrzik K, Supplementation with a multivitamin containing 800 µg folic acid shortens the time to reach the time to reach the preventive red blood cell folate concentration in healthy women; Int J Vitam Nutr Res 79, 61 – 70 (2009)] To note, in a study of Crider et al. 2011, it was shown that with a supplemental dose of 400 µg/d folic acid, in women with the MTHFR TT genotype, it takes approximately 6 months to reach the level of ≥ 906nmol/L (Crider et al. 2011). [Crider KS et al., MTHFR 677C!T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation1–5, Am J Clin Nutr 2011;93:1365–72] As the neural tube closes by day 28 of pregnancy, when pregnancy may not have been detected, folic acid supplementation after the first month of pregnancy will not prevent neural tube defects. However, it will contribute to other aspects of maternal and fetal health (WHO 2012). [WHO. Guideline: Daily iron and folic acid supplementation in pregnant women. Geneva, World Health Organization, 2012.]
6 Data on which to base Dietary Reference Values	Saarland University Hospital	Folate intake to achieve optimal plasma levels that promote health and prevent diseases Rima Obeid (University Hospital of the Saarland), Klaus Pietrzik (University of Bonn). rima.obeid@uks.eu; k.pietrzik@uni-bonn.de. Health promotion and disease prevention are integral parts of Europe2020, the EU's 10-year economic-growth strategy.

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		Health policy will aim at keeping people healthy and active for longer, decreasing differences between member countries and increasing access to healthy choices by all people, particularly the more vulnerable one. The scope of Europe2020 implies in particular saving unborn lives. An estimated 4800 pregnancies are affected by NTDs in Europe yearly. At least 50% of these cases can be prevented by improving women folate status (1). The majority of pregnancies with NTDs are currently terminated thus raising ethical concerns on withholding effective ways to prevent folic acid (FA)-responsive cases. We stress the urgent need for an European consensus on: 1)- appropriate folate intake that ensure adequate plasma folate level, 2)- timely and appropriate definition of folate adequacy based on prevention of diseases with a substantial burden on society and individuals, 3)- comparability of dietary intake data between EU countries, 4)- evidence on intakes required to maintain optimal plasma folate (> 20nM) that can prevent NTD; 5)- sex-specific requirements arise from the fact that young women need to achieve an optimal plasma folate before conception since less than 20% of young women are using supplements that contain FA. Dutch women who were supplemented before conception had a mean plasma folate of 24nM versus 12nM in non-supplemented women (2). Serum folate >20nM (or whole blood >900nM) can protect against folate-responsive NTD (3-5). The protective effect of folate against NTD is dose-dependent and evident within the normal range (4). The concentrations of plasma folate in Europeans are 30-50% of those in Americans after 1998 (6,7). In the light of the stable, relatively high prevalence of NTDs in Europe, the low serum concentrations of folate in Europeans, and the ineffectiveness of all public health campaigns that aimed at increasing practicing or awareness of young women towards folate supplementation, it is not understandable why the population RDA should be lowered from 400 to 330µgDFE/d. Nutritional recommendations that aim at only avoiding anemia and severe deficiency are not consistent with the health promotion and disease prevention of Europe2020. The revised RDA disregards severe consequences that can occur in the normal range of serum folate. People food preference is not going to be affected by adapting different RDA. Therefore, folate intake and blood concentrations are not going to change. However, the debate continues on preventing folate-responsive NTD rather than terminating the affected pregnancies. Half of the world has issued legislations to solve the problem. Therefore, estimation of folate requirements by linking intake data with serum/blood concentrations in Europeans in order to issue sex, age and country-specific recommendations is more important than ever before. If the recommendations are far away from reality, policies to solve the problem will become more crucial, since “invest in health” and disease prevention implies preventing at least 2400 NTDs each year in Europe. 1.De-Regil et al., Cochrane.Database Syst. Rev.CD007950 (2010). 2.Steenweg-de Graaff et al., Am. J Clin Nutr. 95, 1413 (2012). 3.Obeid et al., Clin Nutr. 33, 252 (2014). 4.Crider et al., BMJ 349, g4554 (2014). 5.Daly et al., Lancet 350, 1666 (1997). 6.Pfeiffer et al., J Nutr. 142, 894 (2012). 7.Pfeiffer et al., Am. J Clin Nutr. 86, 718 (2007).
6.1 Data on which to base DRVs - adults	Alliance for Natural Health International	The proposed values do not reflect beneficial effects of folate such as effects on endothelial function (e.g. 5000 mcg/day children; Pediatrics. 2006 Jul;118(1):242-53.), effects on reducing neurodegeneration/Alzheimer’s disease (e.g. Proc Natl

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		Acad Sci U S A. 2013 Jun 4;110(23):9523-8), interactions with other nutrients and compound effects of multiple polymorphisms.
6.2 Data on which to base DRVs - infants aged 7-11 months	Alliance for Natural Health International	The proposed values do not reflect beneficial effects of folate such as effects on endothelial function (e.g. 5000 mcg/day children; Pediatrics. 2006 Jul;118(1):242-53.), effects on reducing neurodegeneration/Alzheimer's disease (e.g. Proc Natl Acad Sci U S A. 2013 Jun 4;110(23):9523-8), interactions with other nutrients and compound effects of multiple polymorphisms.
6.3 Data on which to base DRVs - children	Alliance for Natural Health International	The proposed values do not reflect beneficial effects of folate such as effects on endothelial function (e.g. 5000 mcg/day children; Pediatrics. 2006 Jul;118(1):242-53.), effects on reducing neurodegeneration/Alzheimer's disease (e.g. Proc Natl Acad Sci U S A. 2013 Jun 4;110(23):9523-8), interactions with other nutrients and compound effects of multiple polymorphisms.
6.4 Data on which to base DRVs - pregnancy	Alliance for Natural Health International	The proposed values do not reflect beneficial effects of folate such as effects on endothelial function (e.g. 5000 mcg/day children; Pediatrics. 2006 Jul;118(1):242-53.), effects on reducing neurodegeneration/Alzheimer's disease (e.g. Proc Natl Acad Sci U S A. 2013 Jun 4;110(23):9523-8), interactions with other nutrients and compound effects of multiple polymorphisms.
6.4. Pregnancy	Health Council of the Netherlands	1496-1498 The PRI for pregnant women is almost twice as high as the PRI for women who are not pregnant and its substantiation is very weak. During pregnancy blood concentrations are usually lower than when women are not pregnant due to plasma dilution. Whether these lower concentrations need to be corrected remains to be determined. The value for the PRI appears not to be based on evidence, but on lack of evidence. Recent European dietary reference value for this group range between 400 mcg/d and 550 mcg/d. The suggested EFSA-PRI of 600 mcg/d is very hard to achieve within a normal diet (see also appendix B of the draft scientific opinion). The PRI thus implies that most pregnant women should not only use additional folic acid supplements in the four weeks before and the first trimester of pregnancy, but should also use folic acid supplements in the second and third trimester. Can you substantiate this recommendation? If not, we suggest to consider setting a lower PRI value for pregnant women.
6.5 Data on which to base DRVs - lactation	Alliance for Natural Health International	The proposed values do not reflect beneficial effects of folate such as effects on endothelial function (e.g. 5000 mcg/day children; Pediatrics. 2006 Jul;118(1):242-53.), effects on reducing neurodegeneration/Alzheimer's disease (e.g. Proc Natl Acad Sci U S A. 2013 Jun 4;110(23):9523-8), interactions with other nutrients and compound effects of multiple polymorphisms.
6.5. Lactation	Health Council of the Netherlands	1502-1512 We agree with substantiation the EFSA-PRI (500 mcg DFE/d) for lactating women.
7. Conclusions	Chilean Food Quality and Safety Agency	According to the review of the international public consultation document from EFSA, this Expert Panel fundamentally recommends to update references considering recent studies on the beneficial effects to reduce neural tube defects attributed to folic acid supplementation or fortification, bioavailability and food sources of folate, analytical methodologies to determine folate and folic acid, dietary intake estimation of folate, associated polymorphisms, folate deficiency, unintended consequences of excess , among others.

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8. Recommendations for research	Alliance for Natural Health International	There is a great need for more research evaluating relationships between specific beneficial outcomes and folate status (see above). Studies involving folic acid supplements and fortified foods, reduced folate supplements and food folates should be regarded separately and the DFE should be abolished given the data supporting its use continues to be very weak scientifically.
8. Recommendations for research	Chilean Food Quality and Safety Agency	Due to the importance of this subject in the food area as well as in human health, this Expert Panel on Folate recommends to go in depth and promote further research in the future on the following topics: - Determination of cutoff value for high serum folate. - Further understanding on how the increased consumption of ready-made foods contributes to folate intake - Relationship between folate status and obesity, and i related morbidities. - Establishment of criteria to define the magnitude of public health problem of folate deficiency and ideally folate excess. - Encouragement for further studies in the field of nutrigenomics and requirements of folate and deepening the research on the bioavailability of folate and folic acid improving the definition of folate dietary equivalents.

ABBREVIATIONS

AI	Adequate Intake
AR	Average Requirement
CV	Coefficient of variation
DFE	Dietary folate equivalent
DRV	Dietary Reference Value
MTHFR	Methylene-tetrahydrofolate reductase
NTD	Neural tube defect
PRI	Population Reference Intake
SCF	Scientific Committee for Food
UL	Tolerable Upper Intake Level