GUIDELINE:

OPTIMAL SERUM AND RED BLOOD CELL FOLATE CONCENTRATIONS IN WOMEN OF REPRODUCTIVE AGE FOR PREVENTION OF NEURAL TUBE DEFECTS

World Health Organization

GUIDELINE:

OPTIMAL SERUM AND RED BLOOD CELL FOLATE CONCENTRATIONS IN WOMEN OF REPRODUCTIVE AGE FOR PREVENTION OF NEURAL TUBE DEFECTS

World Health Organization

WHO Library Cataloguing-in-Publication Data

Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects

1.Folic Acid – administration and dosage. 2.Folic Acid – blood. 3.Neural Tube Defects – prevention and control. 4.Congenital Abnormalities – etiology. 5.Nutritional Requirements. 6.Maternal Nutritional Physiological Phenomena. 7.Guideline. I.World Health Organization.

ISBN 978 92 4 154904 2 (NNLM classification: WQ 175)

Design and layout: Alberto March

■ *Suggested citation*

WHO. *Guideline: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects*. Geneva: World Health Organization; 2015.

Contents

Acknowledgements

This guideline was coordinated by the World Health Organization (WHO) Evidence and Programme Guidance Unit, Department of Nutrition for Health and Development. Dr Luz Maria De-Regil and Dr Juan Pablo Peña-Rosas coordinated the process, with technical input from Ms Amy Cordero, Dr Krista Crider and Ms Alina Flores from the National Center on Birth Defects and Developmental Disability, Centers for Disease Control and Prevention (CDC) and Dr Lisa M Rogers, Evidence and Programme Guidance Unit, Department of Nutrition for Health and Development, WHO. We would like to acknowledge the valuable technical input to this document from Ms Monica Crissel Flores-Urrutia, Dr Maria Nieves García-Casal and Mr Gerardo Zamora. We thank Dr Pierpaolo Mastroiacomo, Dr María Elizabeth Tejero Barrera and Dr Mindy Zhang for peer-reviewing a final version of this guideline.

We would also like to express our gratitude to Dr Susan L Norris from the WHO Guidelines Review Committee Secretariat and the members of the Guidelines Review Committee for their technical support throughout the guideline development process. Thanks are also due to Mr Issa T Matta from the WHO Office of the Legal Counsel, for his support in the management of conflicts of interest procedures. Ms Paule Pillard and Ms Jennifer Volonnino from the Evidence and Programme Guidance Unit, Department of Nutrition for Health and Development, provided logistic support.

WHO gratefully acknowledges the technical input of all the members of the WHO Steering Committee and the WHO guideline development group, especially of the chairs of the meetings related to this guideline, Dr Anne Molloy and Dr Lorenzo Botto. We thank the external reviewers for their contributions to the final version of this document. WHO is also grateful to staff of the National Center on Birth Defects and Developmental Disability, CDC, for their technical support during the development of the narrative and systematic reviews, as well as the modelling study that informed this guideline, particularly Dr RJ Berry, Ms Amy Cordero, Dr Krista Crider, Ms Alina Flores, Dr Heather Hamner, Dr Joseph Mulinare, Dr Joe Sniezek, Ms Becky Tsang and Ms Aliki Weakland. We would also like to thank the staff of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), for their technical input in the review on biomarkers of folate status through the Biomarkers for Nutrition and Development (BOND) programme.

Financial support

WHO thanks the National Center on Birth Defects and Developmental Disability, CDC, Atlanta, United States of America, for providing financial support for this work. Donors do not fund specific guidelines and do not participate in any decision related to the guideline development process, including the composition of research questions, membership of the guideline groups, conduct and interpretation of systematic reviews, or formulation of recommendations.

Guideline[1]: Optimal serum and red blood cell folate concentrations in women of reproductive age for prevention of neural tube defects

Executive summary

Scope and purpose

This guideline provides global, evidence-informed recommendations on blood folate concentrations in women of reproductive age for the prevention of neural tube defects (NTDs) in populations. It aims to help Member States and their partners in their efforts to make informed decisions on the appropriate nutrition actions to achieve the Millennium Development Goals (MDGs), in particular reduction of child mortality (MDG 4) and improvement of maternal health (MDG 5), through the establishment of appropriate threshold values for red blood cell folate concentrations at the population level. These values may be used to determine the need for, and guide monitoring and evaluation of the impact of, nutrition interventions aimed at improving folate status and preventing congenital anomalies.

This guideline is expected to support Member States in their efforts to achieve the global targets of the *Comprehensive implementation plan on maternal, infant and young child nutrition*, as endorsed by the Sixty-fifth World Health Assembly in 2012, in resolution WHA65.6.[2]

The guideline is intended for a wide audience, including policy-makers and their expert advisers involved in the design, implementation and scaling-up of nutrition actions for public health, as it relates to folic acid-related interventions.

A biomarker, or biological marker, generally refers to a measurable indicator of a biological state or condition. Biomarkers are often measured to examine normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention and, in the particular case of public health, to also track progress towards the achievement of public health goals.

In order to establish the utility of serum and red blood cell folate as biomarkers of the risk of having a NTD-affected pregnancy, at the population level, this guideline examined four critical questions:

1. In the absence of an intervention, what are the key genetic, biological and sociodemographic determinants of folate status (serum, plasma or red blood cell folate) in women of reproductive age?
2. What is the threshold concentration of blood folate associated with the lowest probability/risk (depending on the statistical method) of having a NTD-affected pregnancy?
3. Do blood folate concentrations respond to interventions to improve folate status in women?
4. Does the performance of the laboratory assays used to measure folate concentrations affect serum and red blood cell folate readings?

This document presents the key recommendations and a summary of the supporting evidence.

---

[1] This publication is a World Health Organization (WHO) guideline. A WHO guideline is any document developed by WHO containing recommendations for clinical practice or public health policy. All publications containing WHO recommendations are approved by the WHO Guidelines Review Committee.

[2] Resolution WHA65.6. Comprehensive implementation plan on maternal, infant and young child nutrition. In: Sixty-fifth World Health Assembly, Geneva, 21–26 May 2012. Resolutions and decisions, annexes. Geneva: World Health Organization; 2012:12–13 (http://www.who.int/nutrition/topics/WHA65.6_resolution_en.pdf?ua=1, accessed 6 February 2015).

Background

Congenital anomalies, also known as birth defects, can be defined as structural or functional abnormalities, including metabolic disorders, which occur during embryonic development and can be identified before birth, at birth or later in life. They can be caused by interacting and diverse factors, such as single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens or micronutrient deficiencies. In 2012, an estimated 270 358 deaths globally were attributable to congenital anomalies during the first 28 days of life (3.3 deaths per 1000 live births). NTDs were one of the most serious and most common anomalies. In an effort to address the emerging importance of congenital anomaly morbidity and mortality, on 21 May 2010, the Sixty-third World Health Assembly adopted a resolution[1] calling all Member States to promote primary prevention and to enhance the health of children with birth defects, by developing and strengthening vital registration and surveillance systems; promoting international cooperation; developing expertise and building capacity; and strengthening research and studies on etiology, diagnosis and prevention.

Increasing awareness of the significance of public health consequences of insufficient folate intake has emphasized the need for identification of accurate biomarkers for large-scale assessment of folate status. The information on the use of different biomarkers to monitor vitamin and mineral status worldwide is included in the World Health Organization (WHO) Vitamin and Mineral Nutrition Information System (VMNIS), hosted by the Department of Nutrition for Health and Development since its establishment in 1991, following a request by the World Health Assembly to strengthen surveillance of micronutrient deficiencies at the global level.[2] Current folate thresholds in all age groups are focused on the prevention of megaloblastic anaemia. However, blood folate concentrations in women of reproductive age need to be higher, to be sufficient to prevent NTD-affected pregnancies.

Although folate is mainly stored in the liver, folate status can be assessed in urine, serum, plasma or red blood cells, using a variety of techniques, including microbiological methods and protein-binding assays with radioactive and non-radioactive detection, such as chemiluminescence and chromatographic assays. Microbiological methods for measuring folate status were first developed in the 1950s and still form the basis for currently used assessment methods. However, as the various assays have not yet been standardized, they do not produce comparable results and there is some lack of specificity, possibly leading to diagnostic inaccuracy. Serum folate is considered an indicator of recent folate intake, whereas red blood cell folate concentrations are useful to indicate long-term folate status.

The establishment of optimal blood folate concentrations associated with preventable NTDs entails many challenges. Epidemiological information on the direct relationship between blood folate concentrations and the occurrence of NTDs is scarce, and the association may be affected by technical, genetic, biological, environmental and contextual factors that need to be considered when examining and interpreting the existing data.

---

[1] Resolution WHA63.17 Birth defects. In: Sixty-third World Health Assembly, Geneva, 17–21 May 2010. Resolutions and decisions, annexes. Geneva: World Health Organization; 2010: 32–4 (http://apps.who.int/gb/ebwha/pdf_files/WHA63-REC1/WHA63_REC1-en.pdf, accessed 6 February 2015).

[2] Resolution WHA45.33 National strategies for prevention and control of micronutrient malnutrition. In: Forty-fifth World Health Assembly, Geneva, 4–14 May 1992. Resolutions and decisions, annexes. Geneva: World Health Organization; 1992 (http://www.who.int/nutrition/topics/WHA45.33_mnm_en.pdf, accessed 6 February 2015).

Guideline development methodology

WHO developed the present evidence-informed recommendations using the procedures outlined in the *WHO handbook for guideline development*.[1] The steps in this process included: (i) identification of priority questions and outcomes; (ii) retrieval of the evidence; (iii) assessment and synthesis of the evidence; (iv) formulation of recommendations, including research priorities; and (v) planning for dissemination, implementation, impact evaluation and updating of the guideline. When feasible, the Grading of Recommendations Assessment, Development and Evaluation (GRADE)[2] methodology was followed, to prepare evidence profiles related to preselected topics, based on up-to-date systematic reviews.

The guideline development group consisted of content experts, methodologists and representatives of potential stakeholders and consumers. A WHO/United States Centers for Disease Control and Prevention (CDC) technical consultation on optimal blood folate concentrations in women of reproductive age for prevention of NTDs was convened in Atlanta, United States of America on 13–15 August 2012, in collaboration with the National Center on Birth Defects and Developmental Disabilities at CDC, to scope the priority questions regarding the genetic, biological, behavioural and contextual determinants of folate status among women of reproductive age; and the strengths and limitations of current methods used to assess indicators of folate status and folate intake, and NTD prevalence. The proposed methodological approach for retrieving, summarizing and assessing the quality of the evidence related to folate status and occurrence of NTDs; the available sources of data on the relationship between folate status (i.e. blood folate concentration and folate intake) and NTD risk from in vitro and in vivo (animal and human) studies were also discussed. A formal guideline development group meeting was convened on 23–25 September 2013 in Geneva, Switzerland, to discuss the evidence and reach group consensus on the recommendations. External reviewers were identified through a call for public comments, in order to assure proper interpretation of the recommendations, and 10 external reviewers identified through this mechanism commented on the final version of the document. Three external expert peer-reviewers also provided technical feedback on the document.

Available evidence

The methodological approach agreed by members of the guideline development group entailed a combination of narrative reviews, data analysis and modelling, as well as systematic reviews following the procedures of the *Cochrane handbook for systematic reviews of interventions*.[3]

---

[1] WHO. Handbook for guideline development, 2nd edition. Geneva: World Health Organization; 2014 (http://www.who.int/kms/handbook_2nd_ed.pdf?ua=1, accessed 2 March 2015).

[2] GRADE Working group (http://www.gradeworkinggroup.org/, accessed accessed 5 February 2015).

[3] Higgins PT, Green S, editors. Cochrane handbook for systematic reviews of interventions. Version 5.1.0 [updated March 2011] (http://www.cochrane.org/handbook, accessed accessed 6 February 2015).

Genetic, biological and sociodemographic determinants of folate status (serum, plasma or red blood cell folate) in women of reproductive age

Multiple factors influence folate status, including diet, a woman's physiological status (age, pregnancy/lactation) and contextual factors such as comorbidities and low socioeconomic status. Folate requirements are increased during pregnancy and lactation, in pathological conditions such as cancer, inflammatory conditions, and conditions where folate absorption is impaired (e.g. coeliac disease). Severe folate deficiency may cause megaloblastic anaemia. Some drugs (e.g. anticonvulsants, methotrexate and sulfasalazine) may also increase folate requirements. In the general population, the gene polymorphism methylenetetrahydrofolate reductase (*MTHFR*) *677C→T* is considered the strongest determinant of folate status in women of reproductive age.

Blood folate concentrations and risk of neural tube defects

In September 2013, an updated search was completed for an existing Cochrane systematic review examining the effects of periconceptional folic acid supplementation on prevention of congenital anomalies. No new randomized clinical trials were found to contribute information to the relationship already reported between folic acid supplementation during early pregnancy and reduced risk of NTDs. Data to determine the link between blood folate concentrations and NTDs were only available from one case–control study that included 84 NTD cases and 266 controls with blood folate concentrations considered indicative of an early stage of pregnancy. Daly et al.[1] reported a threshold concentration for red blood cell folate of 906 nmol/L, above which the risk of NTDs was reduced to <8 NTD cases per 10 000 live births in that group. More recently, a Bayesian model[2] was developed to statistically estimate the association between red blood cell folate concentrations at the time of neural tube closure (embryologic day 28) and the risk of NTDs, using existing data sources from China, with varying background prevalence of NTDs and *MTHFR* genotype. The dose–response relationship between red blood cell folate concentrations and NTD risk in this Chinese population was consistent with that described in the Irish population in the previous study.[1]

The overall quality of the evidence for the association between red blood cell folate and NTD risk is low as per the GRADE methodology.

Response of serum/plasma and red blood cell folate concentrations to nutrition interventions

Several systematic reviews of randomized clinical trials among women of reproductive age were summarized in an overview of reviews that aimed to determine the response of red blood cell folate and serum folate concentration to dietary folate intake, folate supplementation and folic acid fortification of staple foods, condiments and seasonings. The electronic search included relevant electronic databases and, in addition to the population group and the above-mentioned interventions, the inclusion criteria considered a search date not older than 3 years (i.e. 2010 onwards) and being of a high quality, as assessed by the methodology of *A Measurement Tool to*

[1] Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. JAMA. 1995;274(21):1698–702.

[2] Crider KS, Devine O, Hao L, Dowling NF, Li S, Molloy AM et al. Population red blood cell folate concentrations for prevention of neural tube defects: Bayesian model. BMJ. 2014;349. doi:10.1136/bmj.g4554.

Assess Systematic Reviews (AMSTAR).[1] When necessary, the reviews were developed or updated accordingly. It was found that serum folate in non-pregnant women of reproductive age responded to all the interventions in the short term, while red blood cell folate, which is indicative of longer-term status, responded mainly to supplementation and fortification.

Performance of laboratory assays for assessment of folate concentrations

Folate has traditionally been measured using a microbiological assay. However, since the late 1970s, commercial protein-binding assays on automated clinical analysers have often been used, but they generally underestimate folate concentrations, particularly in populations where there is a high prevalence of the *MTHFR C677T* genotype. If folate vitamers are of interest, for example for the measurement of free folic acid in serum, or of various methyl- and non-methyl-folate forms in erythrocytes, depending on the *MTHFR C677T* genotype, chromatography-based separation techniques need to be employed. These are now often coupled to mass spectrometry (high-pressure liquid chromatography [HPLC]-MS/MS), as this detection method has high sensitivity, specificity and selectivity compared to other detection methods such as fluorometric or electrochemical detection.

■ *Recommendations*

This guideline complements previously published WHO recommendations on the assessment of folate status in populations.

1. At the population level, red blood cell folate concentrations should be above 400 ng/mL (906 nmol/L) in women of reproductive age, to achieve the greatest reduction of NTDs (*strong*[2] *recommendation, low quality evidence*).
2. The above red blood cell folate threshold can be used as an indicator of folate insufficiency in women of reproductive age (*strong recommendation, low quality evidence*). Because low folate concentrations cannot explain all cases of NTDs, this threshold cannot predict the individual risk of having a NTD-affected pregnancy and thus it is only useful at the population level.
3. No serum folate threshold is recommended for prevention of NTDs in women of reproductive age at the population level (*strong recommendation, low quality evidence*). Countries interested in using this indicator may consider first establishing the relationship between both serum and red blood cell folate and use the threshold value for red blood cell folate to establish the corresponding threshold in serum.
4. Microbiological assay is recommended as the most reliable choice to obtain comparable results for red blood cell folate across countries (*strong recommendation, moderate quality evidence*).

---

[1] Shea BJ, Hamel C, Wells GA, Bouter LM, Kristjansson E, Grimshaw J et al. AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. J Clin Epidemiol. 2009;62(10):1013–20. doi:10.1016/j.jclinepi.2008.10.009.

[2] A strong recommendation is one for which the guideline development group is confident that the desirable effects of adherence outweigh the undesirable effects. Implications of a strong recommendation for patients are that most people in their situation would desire the recommended course of action and only a small proportion would not. Implications for clinicians are that most patients should receive the recommended course of action, and adherence to this recommendation is a reasonable measure of good-quality care. With regard to policy-makers, a strong recommendation means that it can be adapted as a policy in most situations, and for funding agencies it means the intervention probably represents an appropriate allocation of resources (i.e. large net benefits relative to alternative allocation of resources).

Remarks

- Reducing folate insufficiency at the population level may take time. However, reductions in NTDs may be seen, as the average red blood cell folate concentrations improve. An important consideration is that the overall reduction in NTDs will depend on the baseline folate status, time available for increasing folate status (through folate nutrition interventions) and NTD risk of each population.
- Values indicative of folate deficiency, based on the concentrations at which megaloblastic anaemia is more likely to appear, are <3 ng/mL (<6.8 nmol/L) in serum and <100 ng/mL (<226.5 nmol/L) in red blood cells.
- Although both serum and red blood cell folate concentrations are useful for monitoring interventions aimed at improving folate status, red blood cell folate is preferred, given that there is less biological variation.
- High folic acid intake has not reliably been shown to be associated with negative health effects.
- Evidence supports the use of the microbiological assay for measuring folate concentrations because of the lack of effect of the *MTHFR* gene polymorphism on the assay performance. Appropriate quality control systems for the assessment of red blood cell folate using the microbiological assay need to be in place for use in national nutritional surveillance.
- Use of different folate calibrators or different microorganisms may lead to different results among microbiological assays and laboratories and may necessitate an adjustment of the threshold value for optimal red blood cell folate.
- A threshold for public health concern on the prevalence of folate insufficiency (i.e. red blood cell folate below 400 ng/mL [906 nmol/L] in women of reproductive age) is difficult to establish at this time. Member States and their partners are advised to discuss the merits of folate nutrition interventions through fortification of staple foods or targeted supplementation.
- Owing to de novo folate synthesis in malaria parasites, red blood cell folate concentrations may be artificially high. It is suggested that measurement of folate status is not conducted immediately after febrile malaria episodes, where the parasitic load may peak.

Implications for future research

Discussions with members of the WHO guideline development group and external review group highlighted the limited evidence available in some areas, meriting further research on biomarkers of folate status, in particular in the following areas:

- interactions between red blood cell folate concentrations and tuberculosis, HIV and anti-malaria antifolate drugs;
- surveillance systems for the prevalence of NTDs and assessment of the distribution of red blood cell folate status in women of reproductive age;
- microbiological assays for the assessment of red blood cell folate that are more field-friendly, using automated devices, at a cost that is affordable for most laboratories;

- less invasive methods for the assessment of folate status;
- the distribution of red blood cell folate concentrations in women of reproductive age, and their association with NTDs, in different settings;
- population thresholds for serum folate for the prevention of NTDs;
- the effect of vitamin B_{12} on NTD risk and recurrence;
- the effect of living at higher altitudes on red blood cell folate concentrations;
- the lowest concentrations of red blood cell folate at which any potential negative health outcomes appear, if any;
- the lowest total folate intake level (dietary and/or synthetic form of this vitamin) required to reach the target optimal red blood cell or serum folate concentration at the population level that is considered to be protective against NTDs;
- optimal blood folate thresholds for reduced risk of NTD-affected pregnancy among women with overweight and obesity.

Scope and purpose

This guideline provides global, evidence-informed recommendations on blood folate concentrations in women of reproductive age for the prevention of neural tube defects (NTDs) in populations. It aims to help Members States and their partners in their efforts to make informed decisions on the appropriate nutrition actions to take to achieve the Millennium Development Goals (MDGs), in particular reduction of child mortality (MDG 4) and improvement of maternal health (MDG 5), through the establishment of appropriate thresholds for red blood cell folate concentrations at the population level. These values may be used to determine the need for, and guide monitoring and evaluation of the impact of, nutrition interventions aimed at improving folate status and preventing congenital anomalies.

This guideline is expected to support Member States in their efforts to achieve the global targets of the *Comprehensive implementation plan on maternal, infant and young child nutrition*, as endorsed by the Sixty-fifth World Health Assembly in 2012, in resolution WHA65.6 *(1)*.

The guideline is intended for a wide audience, including policy-makers and their expert advisers involved in the design, implementation and scaling-up of nutrition actions for public health, as it relates to folic acid-related interventions.

A biomarker, or biological marker, generally refers to a measurable indicator of a biological state or condition. Biomarkers are often measured to examine normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention *(2)* and, in the particular case of public health, to also track progress towards the achievement of public health goals.

In order to establish the utility of serum and red blood cell folate as biomarkers of the risk of having a NTD-affected pregnancy, at the population level, this guideline examined four critical questions:

1. In the absence of an intervention, what are the key genetic, biological and sociodemographic determinants of folate status (serum, plasma or red blood cell folate) in women of reproductive age?
2. What is the threshold concentration of blood folate associated with the lowest probability/risk (depending on the statistical method) of having a NTD-affected pregnancy?
3. Do blood folate concentrations respond to interventions to improve folate status in women?
4. Does the performance of the laboratory assays used to measure folate concentrations affect serum and red blood cell folate readings?

This document presents the key recommendations and a summary of the supporting evidence. Further details of the evidence base are provided in Annex 1 and other documents listed in the references.

Background

Congenital anomalies, also known as birth defects, can be defined as structural or functional abnormalities, including metabolic disorders, which occur during embryonic development and can be identified before birth, at birth or later in life. They can be caused by interacting and diverse factors, such as single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens or micronutrient deficiencies. In 2012, an estimated 270 358 deaths globally were attributable to congenital anomalies during the first 28 days of life (3.3 deaths per 1000 live births) *(3)*. NTDs were one of the most serious and most common anomalies. NTDs occur very early in pregnancy (neural tube closure is completed by embryonic day 28) and arise when the neural tube fails to close properly. The most common NTDs are anencephaly, encephalocele and spina bifida. Risk factors for fetal NTDs are maternal folate insufficiency, arising from low blood concentrations of vitamin B_9 (folate); maternal vitamin B_{12} deficiency; a positive family history; smoking; indoor air pollution from coal; diabetes and obesity *(4–6)*. A recent systematic review retrieved, summarized and assessed the NTD burden from 18 countries in six World Health Organization (WHO) regions *(7)*. The search was conducted on 6 February 2013 and was limited to the period after the year 2000 to estimate recent NTD trends. The burden per 1000 live births, calculated using the median, was 1.67 for total NTD burden, 1.13 for spina bifida, 0.25 for anencephaly and 0.15 for encephalocele. The review further estimated that, in low- and middle-income countries, about 190 000 neonates each year were born with a NTD.

In an effort to address the emerging importance of congenital anomaly morbidity and mortality, on 21 May 2010, the Sixty-third World Health Assembly adopted a resolution calling all Member States to promote primary prevention and to enhance the health of children with birth defects, by developing and strengthening vital registration and surveillance systems; promoting international cooperation; developing expertise and building capacity; and strengthening research and studies on etiology, diagnosis and prevention *(8)*.

Folate is the general term for a water-soluble B vitamin naturally found in foods such as leafy green vegetables, legumes, egg yolks, liver and some citrus fruits *(9)*. This vitamin is essential for normal cell growth and replication. The bioavailability of naturally occurring folate is less than that of folic acid, a synthetic compound that is used in supplements and in fortified foods *(10)*. The term folate, as used in this document, encompasses folic acid and natural food folate, unless otherwise specified.

Deficiencies of folate and vitamin B_{12} have been acknowledged as the most common causes of megaloblastic anaemia *(11)*. In addition, poor folate status is associated with other negative health outcomes; for example, inadequate maternal folate status has been linked to abruptio placentae, pre-eclampsia, spontaneous abortion, congenital heart defects, stillbirth, preterm delivery, low birth weight *(12)* and serious congenital anomalies of the brain and spine, such as NTDs *(13)*.

Although folate is mainly stored in the liver, folate status can be assessed by measurement of folate in urine, serum, plasma or red blood cells *(14)*. Microbiological methods for measuring folate status were first developed in the 1950s and still form the basis for currently used assessment methods. However, as the various assays have not yet been standardized, they do not produce comparable results and some lack specificity, possibly leading to diagnostic inaccuracy. Serum folate is considered an indicator of recent folate intake *(15)*, although a single measurement is not

recommended to differentiate between a transitory decrease in dietary folate intake and chronic deficiency states. However, repeated low values of serum folate within an individual over the course of a month are indicative of low folate status or folate depletion *(16)*. Conversely, red blood cell folate concentrations respond slowly to changes in folate intake, because the erythrocytes, which have a 120-day lifespan, only accumulate folate during erythropoiesis *(15)*. Thus, red blood cell folate concentrations are useful to indicate long-term folate status.

The establishment of optimal blood folate concentrations associated with preventable NTDs entails many challenges. Information, from both intervention trials and observational studies, on the direct relationship between folic acid intake and blood folate concentrations and the occurrence of NTDs, is scarce. These associations of intake, blood folate concentration and NTD occurrence may also be affected by technical, genetic, biological, safety and contextual factors that need to be considered when examining and interpreting the existing data**.**

Studies have shown that increased consumption of folic acid by women during the periconceptional period can significantly reduce the occurrence of NTDs, which has led to current recommendations for women to consume 400 µg (0.4 mg) of folic acid daily, to reduce their risk of having a NTD-affected pregnancy *(17, 18)*. Higher amounts of folic acid can also help reduce the risk of NTD recurrence in subsequent pregnancies *(19)*. These findings have instigated worldwide efforts to promote periconceptional folic acid intake through multiple strategies.

Interventions to improve folate status include daily or intermittent supplementation; point-of-use fortification with micronutrient powders; fortification of staple cereal grains (e.g. wheat and maize flour, rice); and dietary education to improve folate intake from natural food sources, such as beef liver, leafy green vegetables, legumes, egg yolks and some fruit (e.g. citrus fruit, papaya, strawberries). However, there is limited evidence of the success of dietary improvements for improving folate status *(20)*. Mandatory fortification of staple foods with folic acid passively improves folate status in consumers because there is no necessary change in consumption practices; however, unlike supplementation, which focuses only on at-risk populations, fortification also affects individuals for whom there is no risk of a NTD-affected birth (e.g. men, the elderly, children).

■ *Determinants of folate status*

The determinants of folate status can be complex and multifactorial, including genetic, biological and socioeconomic components. Studies have found that inheritance of a specific genetic variant, the *677C→T* allele (rs#1801133) in the gene encoding the methyltetrahydrofolate reductase (MTHFR) enzyme, is associated with lower folate status in carriers of the TT genotype compared to the CC genotype *(21)*. MTHFR catalyses the irreversible conversion of 5,10 methylenetetrahydrofolate to 5-MTHF in the methionine cycle *(22)*. Inheritance of the recessive *T* allele results in reduced enzyme activity and increased homocysteine concentrations; this is especially true under low-folate conditions *(23)*. The prevalence of the 677TT genotype varies across regions and ethnic groups *(24)*, being most common in Mexican (32%), Chinese (26%) and southern Italian populations (20%) and least common in those of African descent (0.3–0.8%) *(21, 24)*. The frequency of the 677TT genotype in Caucasians ranges from 8% to 14% in North America, to 6% to 14% in northern Europe and 15% to 20% in southern Europe *(24)*. In a study in northern China *(23)*, a prevalence of 35.1% was observed, but elsewhere in Asia the prevalence ranges from 12% to 18% *(24)*.

Biological predictors of folate status include coexisting vitamin B_6 and B_{12} status, homocysteine concentrations, and inflammation or infection. The B vitamins, folate, B_6 and B_{12}, share multiple pathways and interact with one another *(22)*. All three have roles in influencing homocysteine concentrations; therefore, homocysteine is a non-specific metabolic indicator of folate status. Inflammation and infection of the gastrointestinal tract reduce folate absorption; this reduction is considerable in malabsorption conditions such as Crohn or coeliac disease. Body mass index may also be an influencing factor but an analysis of overweight and obese adults from NHANES (National Health and Nutrition Examination Survey from the United States of America [USA]) data showed an unclear relationship *(25)*.

Contextual characteristics, such as limited access to natural dietary folate sources, infection and drug interactions, can also impact folate status. Individuals with available resources and access to fresh produce or fortified food products are more likely to have adequate folate concentrations relative to those with limited dietary diversity. Infection may decrease appetite and folate absorption, and, in the case of malaria, increase folate utilization *(26)*. A wide range of drugs fall under the class of antifolates, including, but not limited to, anti-epileptic drugs, anticancer therapies, some antibacterial drugs and some antimalarial drugs. High doses of antifolate antimalarial drugs have been shown to worsen folate status *(26, 27)*. However, it is unclear whether this is the case at current recommended treatment doses for malaria.

■ *Thresholds for folate status in populations*

Thresholds for folate deficiency in all age groups, using serum or red blood cell folate, were first proposed in 1968 *(28)*. Values were based on concentrations at which megaloblastic anaemia is more likely to appear. These thresholds were endorsed by subsequent WHO consultations in 1972 *(29)* and 1975 *(30)*, although it was acknowledged that the correlation between folate concentration and megaloblastic anaemia was not always strong. Consultation participants considered that data were urgently needed on the clinical significance of low concentrations of folate and vitamin B_{12} in non-pregnant subjects without evidence of other haematological changes, as studies at that time failed to detect any obvious impairment of health.

A WHO expert consultation examined several issues related to folate and vitamin B_{12} deficiencies, with a goal to review and decide upon the appropriate indicators for assessing micronutrient status *(31)*. The concentrations suggested for defining serum and red blood folate deficiency in all age groups were based on a point at which homocysteine concentrations became elevated among male and female individuals surveyed in the USA, as part of NHANES. However, it was acknowledged that these values represented the minimum of adequacy for metabolic functions and that they were not appropriate for the prevention of NTDs. Therefore, it is necessary to examine the current evidence among women of reproductive age, in order to establish the effective and safe concentrations of serum and red blood cell folate that would be associated with the lowest risk of having a NTD-affected pregnancy.

■ *Measurement of folate status*

Methods for measuring folate status were first developed in the 1950s and still form the basis for current assessment methods *(32)*. Folate status can be assessed by either serum/plasma folate or red

blood cell folate concentrations, using microbiological, radioisotope competitive binding, enzyme-linked, or chemiluminescent assays. Since serum/plasma folate is considered an indicator of recent folate intake *(15)*, it is not possible to differentiate between transitory drops in folate intake and chronic deficiency states. As discussed earlier, red blood cell folate concentrations are more likely to reflect long-term folate status *(15)*; however, measurement of red blood cell folate has proven to be more challenging than measurement of serum folate, in part because red blood cells first need to be haemolysed to release polyglutamate folates, which then need to be deconjugated to monoglutamates *(33)* .

The microbiological assay using *Lactobacillus casei* has been recommended since 1968 for folate measurement, as it is sensitive to multiple forms of folate species while excluding those without vitamin activity *(34, 35)*. Constant monitoring and regular use of reference preparations are necessary to check and maintain accuracy in the results, particularly at lower concentrations *(34)*. A recent study compared different microbiological assays and showed that different results were obtained, depending on the folate calibrator or microorganism used *(33)*. However, the microbiological assay lacks specificity to differentiate between different forms of folate. An assay utilizing radioisotope protein binding, previously used by NHANES between 1991 and 2006, was found to have accuracy problems, measuring ~30% lower concentrations of red blood cell folate and serum folate relative to the microbiological assay and isotope-liquid chromatography-tandem mass spectrometry (LC-MS/MS) *(36, 37)*. This is considered to be due to under-recovery of 5-MTHF, the predominant folate species in serum and red blood cells. In comparison to both the microbiological assay and the radioisotope protein-binding assay, LC-MS/MS is better able to differentiate between individual folate species. A 2010 expert round table on the use of folate assays in NHANES endorsed LC-MS/MS for serum folate but noted that use of the assay for red blood cell folate was premature at that time *(35)*.

Summary of evidence

The methodological approach agreed by members of the guideline development group entailed a combination of narrative reviews, data analysis and modelling, as well as systematic reviews following the procedures of the *Cochrane handbook for systematic reviews of interventions* *(38)*. A summary of the evidence and the findings follows, and full details may be found in the references cited.

■ *Genetic, biological and sociodemographic determinants of folate status (serum, plasma or red blood cell folate) in women of reproductive age*

The evidence to address this question was gathered from several narrative and systematic reviews:

- two narrative reviews that identified and broadly assessed the determinants of folate status and narrowed down the factors that were more likely to affect the establishment of folate thresholds *(39, 40);* these therefore needed an in-depth systematic assessment;
- a systematic review and meta-analysis of trials and observational studies assessing the association between the *MTHFR 677C→T* polymorphism and blood folate concentrations *(41)*;

- an analysis of the global distribution of genotypic frequencies of eight polymorphisms in genes of the folate/homocysteine metabolic pathway in various populations *(42)*;
- two narrative reviews on the interactions between folate and folate-dependent drugs used in malaria prevention *(26, 27)*.

Multiple factors influence folate status, including the *MTHFR 677C→T* gene polymorphism, physiological status (age, pregnancy/lactation), and contextual factors such as comorbidity and low socioeconomic status. A narrative review *(39)* summarized the history of folate as a public health issue, and its biology, as well as specific considerations for the use and interpretation of folate biomarkers across a range of clinical and population-based uses. Folate status, measured through serum folate or red blood cell folate, is altered in disease. In the clinical setting, the primary aim is to diagnose folate deficiency, and serum folate is a useful marker of folate status in this setting, although it may be variable, with concentrations influenced by recent consumption of a high-folate meal, daily or intermittent folic acid supplements, and medications. Repeated measures over time can make serum folate testing more informative. Red blood cell folate is reflective of long-term status, so may reflect status prior to disease. Increased folate requirements may be present when there are increased physiological requirements (e.g. pregnancy, lactation), pathological conditions (e.g. malignancy, inflammatory conditions, certain anaemias), or drug use (e.g. anticonvulsants, methotrexate, sulfasalazine). Decreased folate availability may occur when there is impaired folate absorption (e.g. alcohol consumption, coeliac disease) or reduced dietary intake (e.g. low intake of folic acid-fortified foods, dark green leafy vegetables, legumes, selected fruit such as orange juice) *(43)*. In conclusion, the main determinants of folate status are intake, use of antifolate medications and genetic background, including the gene polymorphism *MTHFR 677C→T*.

Genetic variation has a role in folate metabolism but the impact of a particular variant on the risk of diseases is difficult to establish, owing to the presence of many confounders in the relevant studies *(41)*. Both genetic and non-genetic variation in the population have to be considered when evaluating polymorphisms. This may be particularly relevant when studying a folate-related variant involved in health outcomes that are also associated with other nutrients, in addition to folate. The systematic review included 40 controlled trials and observational studies that reported serum, plasma, or red blood cell folate concentrations and *MTHFR C677→T* (rs1801133) genotype in healthy women *(41)*.

The meta-analysis *(41)* allowed estimation of percentage differences in blood folate concentrations between *MTHFR C677T* genotypes. Six studies that utilized the microbiological assay to measure serum/plasma and red blood cell concentrations showed a clear pattern of CC<CT<TT. A greater difference was found for CC>TT (serum/plasma 13%; red blood cell 8%), CT>TT (serum plasma 7%; red blood cell 8%) and CT>TT (serum/plasma 6%; red blood cell 9%). A reverse pattern was revealed by the results of the meta-analysis of the studies that utilized protein-binding assays for measuring red blood cell folate concentrations.

Another review *(42)* analysed the genotypic frequencies of eight polymorphisms in genes of the folate/homocysteine metabolic pathway, in 1350 Mestizo and American-Indian subjects from different regions in Mexico and 836 individuals from European, African and Asian populations of the 1000 Genomes Project. In Mexican Mestizo individuals, the prevalence of the *MTHFR C677T* risk genotype (TT) was 25%, while in American-Indian populations the prevalence was 57%. Altogether,

risk genotypes showed regional differences in Mexico, illustrating differential geographical distribution of the risk variants in the folate/homocysteine metabolic pathway relative to ethnic background. These data suggest that needs for folic acid and vitamin B supplementation may be increased in some regions of the world.

The association between the *MTHFR C677T* genotype and NTD has been investigated, with controversial results. Findings of recent systematic reviews and meta-analyses *(44)* have suggested that maternal *MTHFR C677T* genotype is a risk factor for NTD, and the strength of this association may vary across geographic regions, probably owing to differences in genetic background and environmental factors. In the general population, the gene polymorphism *MTHFR 677C→T* is considered the strongest determinant of folate status in women of reproductive age.

■ *Blood folate concentrations and risk of neural tube defects*

In September 2013, a search was conducted on electronic databases, for studies assessing folic acid intake, blood folate concentrations and NTDs. The relationship between folic acid intake during early pregnancy and reduced NTD risk has been confirmed through randomized controlled trials *(45)*, but, with the exception of the case–control study discussed below, data to determine the link between folic acid intake, blood folate concentrations and NTDs were not available. In the early stages of research on NTDs and folic acid supplementation, 400 µg (0.4 mg) was uniformly used by researchers, despite limited scientific basis for the dosage. Thus, it is unknown what risk alternate folic acid dosing regimens pose, and the blood folate concentrations associated with this risk. Given the current evidence available, ethical considerations do not allow for further human studies, and the lack of data from randomized controlled trials limits the application of systematic-review approaches to obtain summary measures of associations.

As an alternative, other studies that have studied folic acid intake–blood folate concentrations or folic acid intake–NTD risk were used to extrapolate the full relationship between intake, blood folate concentrations and outcome. This systematic search only identified one relevant case–control study, which included 84 NTD cases with blood concentrations of folate *(46)*. This study involved all women attending their first antenatal clinic in one of the three main maternity hospitals in Dublin, Ireland, from March 1986 to March 1990. Using hospital records, a systematic sample of three controls per case was taken from births without NTDs, for gestations of 23 weeks and more, in the same hospital and during the same period as the cases. From these samples (median gestational age: 15 weeks), red blood cell and plasma folate concentrations were analysed for 84 cases and 266 controls. This study reported a threshold for red blood cell folate at concentrations of 400 ng/mL (906 nmol/L; mean 1292 nmol/L), above which the risk of NTDs was lowest (8.0 NTDs per 10 000 live births). Red blood cell folate concentrations <340 nmol/L were associated with a NTD risk of 66 per 10 000 live births.

The consistency of the dose–response relationship (rapidly increasing NTD risk with decreasing red blood cell folate concentrations) in the previous findings from Ireland with dose–response relationships in other populations was confirmed by a Bayesian model recently developed to statistically estimate the association between red blood cell folate concentrations at the time of neural tube closure (embryologic day 28) and the risk of NTDs, using existing data sources in China *(47, 48)*. Two populations contributed data to this work, one from northern China and one from southern China. The studies included participants in a community intervention project to prevent

NTDs with 400 µg (0.4 mg)/day of folic acid, and participants in a population-based randomized trial to evaluate the effect of folic acid supplementation on blood folate concentrations among women of reproductive age. It was found that, at the lowest estimation of red blood cell folate concentration, there is an increased risk of NTD (e.g. 25.4 NTDs per 10 000 live births at 500 nmol/L), and at higher concentrations of red blood cells (~1000 nmol/L) there is a reduced risk of NTDS (e.g. 6 NTDs per 10 000 live births at 1180 nmol/L). It was noted that these estimated risks of NTDs at various estimated red blood cell folate concentrations were consistent with the prevalence of NTDs in the USA before and after mandatory folic acid fortification (using red blood cell folate concentrations from the USA and the Chinese dose–response relationship to estimate NTD risk). From this model, it was concluded that a population threshold for preventing NTDs could be defined as a population red blood cell folate concentration of ~1000 nmol/L, which is consistent with the previous findings *(47)*.

The overall quality of the evidence for the association red blood cell folate and NTD risk is low as per the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) methodology *(49)*.

■ *Response of serum/plasma and red blood cell folate concentrations to nutrition interventions*

A search was conducted for systematic reviews published within the past 3 years, to assess the relationship between folate indicators and various folate interventions to improve folate status. Only systematic reviews that were considered of high quality according to the approach of *A Measurement Tool to Assess Systematic Reviews (*AMSTAR) *(50)* (i.e. score 8 or higher) were considered.

For folate supplementation in non-pregnant women of reproductive age, serum/plasma or red blood cell folate was assessed in just two reviews *(20, 51)*. One review *(20)* analysed daily folate supplementation with either folic acid or 5-MTHF in women of reproductive age, and the second review *(51)* analysed intermittent iron and folic acid supplementation in women of reproductive age. The effect of daily folic acid supplementation was significant for both serum folate (4 trials, 343 women; regression coefficient 0.65, 95% confidence interval [CI] = 0.39 to 0.92) and red blood cell folate (7 trials, 486 women; regression coefficient 0.33, 95% CI = 0.23 to 0.44) *(51)*. For folate supplementation in pregnant women, serum/plasma or red blood cell folate was again assessed in one review *(52)*. The review involved daily supplementation; one group was given folic acid and the other one folic acid or 5-MTHF. There was no difference in pre-delivery serum folate concentration (8 studies, 1250 women; mean difference [MD] = 2.03, 95% CI = 0.80 to 3.27) or red blood cell folate concentration (4 studies, 427 women; MD = 1.59, 95% CI = –0.07 to 3.26) in those receiving folic acid compared to those receiving placebo *(51)*. A summary of red blood cell folate concentrations before and after mandatory fortification with folic acid in two countries (Canada and Chile) was also published. In women of reproductive age, the increase in median red blood cell folate concentrations ranged from 85 ng/mL to 184 ng/mL (193–417 nmol/L) from pre-fortification to post-fortification *(53)*. Additionally, data from NHANES *(54)* showed that the median red blood cell folate concentration increased between pre-fortification (1988–1994) and post-fortification (1990–2010) in the USA, from 674 ± 9 nmol/L to 1020 ± 7 nmol/L.

A systematic literature review included 36 controlled trials and observational studies published from January 1992 to March 2014 that reported both folate intake from natural food alone (i.e. no consumption of synthetic folic acid) and blood folate (serum, plasma or red blood cell) concentration among non-pregnant, non-lactating females aged 12–49 years *(43)*. This review retrieved and

summarized the data, in order to estimate the association between folate intake from natural food alone and blood folate concentration. For the seven studies using microbiological assay that were included in the meta-analysis, it was estimated that a 6% [95% CI = 4% to 9%] increase in red blood cell folate concentration and a 7% (95% CI = 1% to 12%) increase in serum/plasma folate concentration can occur for every 10% increase in natural food folate intake. Using modelled results, it was estimated that a folate intake from natural food of 450 µg dietary folate equivalents (DFE) per day[1] or higher could achieve the lower bound of a red blood cell folate concentration (~1050 nmol/L) associated with the lowest risk of NTDs (~6 NTDs per 10 000 live births) *(43)*. While it is known that serum and plasma folate levels are indicative of recent folate intake, whereas red blood cell folate is a measure of erythrocyte folate stores *(14)*, it is unknown whether these indicators differ in response to interventions. Serum folate in non-pregnant women of reproductive age responded to all the interventions, while red blood cell folate responded mainly to supplementation and fortification. Understanding the way in which serum/plasma folate and red blood cell folate respond to interventions will aid in the use of these indicators and guide monitoring and evaluation efforts for Member States. A review on the use of multiple micronutrient powders for home (point-of-use) fortification of foods in pregnant women *(55),* and a review on the fortification of rice with vitamins and minerals for addressing micronutrient malnutrition *(56),* were identified, but neither assessed folate status.

■ *Performance of laboratory assays for assessment of folate concentrations*

Folate has traditionally been measured using microbiological assay. However, since the late 1970s, in clinical settings where high throughput may be desired, commercial protein-binding assays on automated clinical analysers have often been used, but they generally underestimate folate concentrations, particularly in populations where there is a high prevalence of the *MTHFR C677T* genotype. If folate vitamers are of interest, for example for the measurement of free folic acid in serum, or of various methyl- and non-methyl-folate forms in erythrocytes, depending on the *MTHFR C677T* genotype, chromatography-based separation techniques need to be employed. These are now often coupled to mass spectrometry (high-pressure liquid chromatography [HPLC]-MS/MS), as this detection method has high sensitivity, specificity and selectivity compared to other detection methods such as fluorometric or electrochemical detection *(57)*.

The choice of laboratory tests depends on the type of study being carried out. In field nutritional epidemiology studies, particularly in low- and middle-income countries, the number and type of tests will be mainly limited by the specimen volume, the local laboratory infrastructure, and the availability of skilled personnel and financial resources. In population studies carried out in high-income countries with high-level laboratory facilities, the selection of laboratory tests depends on the purpose of the study, specimen volume and financial resources *(14, 18)*.

Regardless of the setting, the precision of the laboratory test largely influences the minimum detectable difference on repeat measurements, or the ability to distinguish between populations at risk of deficiency or with sufficient micronutrient status. It is therefore desirable to select laboratory tests with the highest achievable precision. Most biochemical indices do not have the required sensitivity and specificity to accurately diagnose deficiency but may be appropriate for use at the population level. A combination of findings, such as those from assessment of dietary intake with static biochemical

[1] 1 µg DFE = 1 µg food folate or 0.6 µg synthetic folate; total DFE = µg food folate + 1.7 × µg synthetic folate.

indices and functional tests, may be more informative. Most methods used to assess nutritional status have not yet been standardized, which can lead to considerable differences among laboratories and methods and may necessitate adjustment of the optimal red blood cell folate threshold value *(18)*. The need for harmonization between laboratories and methods is discussed elsewhere in this document (see "A harmonization programme for folate microbiological assays", p.20).

Recommendations

This guideline complements previously published WHO recommendations on the assessment of folate status in populations *(58–61)*.

1. At the population level, red blood cell folate concentrations should be above 400 ng/mL (906 nmol/L) in women of reproductive age, to achieve the greatest reduction of NTDs (*strong*[1] *recommendation, low quality evidence*).
2. The above red blood cell folate threshold can be used as an indicator of folate insufficiency in women of reproductive age (*strong recommendation, low quality evidence*). Because low folate concentrations cannot explain all cases of NTDs, this threshold cannot predict the individual risk of having a NTD-affected pregnancy and thus it is only useful at the population level.
3. No serum folate threshold is recommended for prevention of NTDs in women of reproductive age at the population level (*strong recommendation, low quality evidence*). Countries interested in using this indicator may consider first establishing the relationship between both serum and red blood cell folate and use the threshold value for red blood cell folate to establish the corresponding threshold in serum.
4. Microbiological assay is recommended as the most reliable choice to obtain comparable results for red blood cell folate across countries (*strong recommendation, moderate quality evidence*).

Remarks

- Reducing folate insufficiency at the population level may take time. However, reductions in NTDs may be seen, as the average red blood cell folate concentrations improve. An important consideration is that the overall reduction in NTDs will depend on the baseline folate status, time available for increasing folate status (through folate nutrition interventions) and NTD risk of each population.
- Values indicative of folate deficiency, based on the concentrations at which megaloblastic anaemia is more likely to appear, are <3 ng/mL (<6.8 nmol/L) in serum and <100 ng/mL (<226.5 nmol/L) in red blood cells.

1 A strong recommendation is one for which the guideline development group is confident that the desirable effects of adherence outweigh the undesirable effects. Implications of a strong recommendation for patients are that most people in their situation would desire the recommended course of action and only a small proportion would not. Implications for clinicians are that most patients should receive the recommended course of action, and adherence to this recommendation is a reasonable measure of good-quality care. With regard to policy-makers, a strong recommendation means that it can be adapted as a policy in most situations, and for funding agencies it means the intervention probably represents an appropriate allocation of resources (i.e. large net benefits relative to alternative allocation of resources).

- Although both serum and red blood cell folate concentrations are useful for monitoring interventions aimed at improving folate status, red blood cell folate is preferred, given that there is less biological variation.
- High folic acid intake has not reliably been shown to be associated with negative health effects.
- Evidence supports the use of the microbiological assay for measuring folate concentrations because of the lack of effect of the *MTHFR* gene polymorphism on the assay performance. Appropriate quality control systems for the assessment of red blood cell folate using the microbiological assay need to be in place for use in national nutritional surveillance.
- Use of different folate calibrators or different microorganisms may lead to different results among microbiological assays and laboratories and may necessitate an adjustment of the threshold value for optimal red blood cell folate.
- A threshold for public health concern on the prevalence of folate insufficiency (i.e. red blood cell folate below 400 ng/mL [906 nmol/L] in women of reproductive age) is difficult to establish at this time. Member States and their partners are advised to discuss the merits of folate nutrition interventions through fortification of staple foods or targeted supplementation.
- Owing to de novo folate synthesis in malaria parasites, red blood cell folate concentrations may be artificially high. It is suggested that measurement of folate status is not conducted immediately after febrile malaria episodes, where the parasitic load may peak.

Implications for future research

Discussions with members of the WHO guideline development group and external review group highlighted the limited evidence available in some areas, meriting further research on biomarkers of folate status, in particular in the following areas:

- interactions between red blood cell folate concentrations and tuberculosis, HIV and anti-malaria antifolate drugs;
- surveillance systems for the prevalence of NTDs and assessment of the distribution of red blood cell folate status in women of reproductive age;
- microbiological assays for the assessment of red blood cell folate that are more field-friendly, using automated devices, at a cost that is affordable for most laboratories;
- less invasive methods for the assessment of folate status;
- the distribution of red blood cell folate concentrations in women of reproductive age, and their association with NTDs, in different settings;
- population thresholds for serum folate for the prevention of NTDs;
- the effect of vitamin B_{12} on NTD risk and recurrence;
- the effect of living at higher altitudes on red blood cell folate concentrations;
- the lowest concentrations of red blood cell folate at which any potential negative health outcomes appear, if any;

- the lowest total folate intake level (dietary and/or synthetic form of this vitamin) required to reach the target optimal red blood cell or serum folate concentration at the population level that is considered to be protective against NTDs;
- optimal blood folate thresholds for reduced risk of NTD-affected pregnancy among women with overweight and obesity.

Dissemination, adaptation and implementation

Dissemination

The current guideline will be disseminated through electronic media such as slide presentations and the World Wide Web, through either the WHO Nutrition *(62)* and United Nations Standing Committee on Nutrition (SCN) *(63)* mailing lists, social media, the WHO nutrition web site *(62)* and the WHO Vitamin and Mineral Nutrition Information System (VMNIS) *(64)*. VMNIS was established in 1991 following a request by the World Health Assembly to strengthen surveillance of micronutrient deficiencies at the global level. Part of WHO's mandate is to assess the micronutrient status of populations, monitor and evaluate the impact of strategies for the prevention and control of micronutrient malnutrition, and track related trends over time. The Evidence and Programme Guidance Unit of the Department of Nutrition for Health and Development manages VMNIS, through WHO's network of regional and country offices, and in close collaboration with national health authorities. In addition, the guideline will be disseminated through a broad network of international partners, including WHO country and regional offices, ministries of health, WHO collaborating centres, the International Clearinghouse of Birth Defects Research and Surveillance, Centers for Disease Control and Prevention (CDC) offices, universities, United Nations agencies and nongovernmental organizations.

Particular attention will be given to improving access to these guidelines for stakeholders that face more, or specific, barriers in access to information, or that play a crucial role in the use of the guideline recommendations, for example, policy-makers and decision-makers at subnational level who disseminate the contents of the guideline, especially information on the benefits of achieving optimal concentrations of red blood cell folate in women of reproductive age. This is remarkably important in rural communities or highly isolated communities, where seeking health care is less frequent, and obtaining health care is more difficult, because of distance or transport barriers (e.g. women before and during early pregnancy). Accessing hard-to-reach population groups is extremely important during implementation stages, as it contributes to preventing or tackling health inequities. Dissemination of the guidelines and information on the benefits of achieving optimal blood folate concentrations in women of reproductive age for the prevention of NTDs helps to empower consumers, and thus contributes to creating consumer demand.

Adaptation and implementation

As this is a global guideline, it should be adapted to the context of each Member State. Establishment of harmonization programmes to build laboratory capacity and assure quality in the assessment of red blood cell folate are required as a first step in the adaptation and implementation of this guideline.

The Department of Nutrition for Health and Development maintains VMNIS. Within this system, a section on indicators has been developed to summarize the most recent guidance on assessment of the micronutrient status of populations. In addition, a WHO Global Laboratory Directory for the Assessment of Micronutrient Status has been established, to serve as a directory of laboratories that are capable of assessing biomarkers of micronutrient status for health and nutrition surveys. As a result of improved technology, and a growing awareness of the importance of micronutrients in health and development, an increasing number of biological indicators (or biomarkers) are being used to assess the vitamin and mineral status of populations, evaluate potential risk factors of health and disease, generate estimates of the global burden of micronutrient malnutrition, and evaluate the impact of public health interventions. The purpose of this directory is to help Member States and their partners identify laboratories measuring biomarkers of vitamin and mineral status at the population level, so that they may be able to further evaluate the laboratory for possible participation in their nutrition surveys.

Additionally, a global mapping of reported population folate concentrations (as measured in serum, plasma or red blood cells) in women of reproductive age was conducted.

Monitoring and evaluation of guideline implementation

A plan for monitoring and evaluation with appropriate indicators is encouraged at all stages. The impact of this guideline can be evaluated within countries (i.e. monitoring and evaluation of use at national or regional scale) and across countries (i.e. the adoption and adaptation of the guideline globally). The WHO Department of Nutrition for Health and Development, Evidence and Programme Guidance Unit, jointly with the CDC National Center on Birth Defects and Developmental Disabilities, and the International Clearinghouse of Birth Defects Research and Surveillance, and with input from international partners, will support countries for the adoption of these recommendations. VMNIS supports Member States and their partners in monitoring the vitamin and mineral nutrition status of populations, with the ultimate goal of improving their health and development, through adequate vitamin and mineral nutrition. This information system will be used to monitor guideline implementation.

The Micronutrients Database within VMNIS *(65)* is an integrated database that includes information on the micronutrient status of populations. It has the flexibility to include additional indicators of micronutrient status as they are developed, and compiles data from national and first administrative level surveys on the population status of micronutrients.

A harmonization programme for folate microbiological assays

Most biochemical methods that assess the nutritional status of individuals or communities, are not standardized. This can lead to considerable differences among laboratories and methods *(57)*. The microbiological assay is proposed to be a practical choice to obtain comparable results across countries; however, it is not yet harmonized. The method is based on assessment of bacterial growth by measuring the turbidity of samples after a suitable incubation period. Samples are added to an assay medium containing *Lactobacillus rhamnosus* and all of the nutrients necessary for the growth except for folate. The total folate level can be assessed by measuring the turbidity of the inoculated medium at 590 nm *(66)*. A recent study that compared different microbiological assays showed that different results were obtained depending on the folate calibrator or microorganism used *(33)*.

The US CDC plans to standardize a limited number of prospective regional laboratories using a prototype of a "start-up assay kit" containing microorganisms, folate calibrator and quality control material. A semi-annual certification of folate measurements can be implemented, in order to keep a record of the performance and the comparability of results from different laboratories.

Ethical considerations

This guideline proposes a threshold for red blood cell folate concentrations in women of reproductive age associated with lowest risk of NTDs. It is important that ethical aspects are considered in the adaptation and implementation of this guideline in different settings. Special attention should be given to the four principles of bioethics (respect for autonomy, non-maleficence, beneficence and justice), as they are also applicable to public health interventions.

This threshold is expected to help Member States and their partners in understanding the distribution and magnitude of folate insufficiency in women of reproductive age, to help guide public health policies. The application of this threshold for women of reproductive age may trigger the implementation of interventions at the population level for the prevention of NTDs. In the case of interventions implemented universally, there may be women and men who receive such interventions where increased folate requirements may not be clearly warranted.

The greater risk of NTDs is among disadvantaged population groups, e.g. low-income groups, less educated groups, or population groups that are discriminated against, who are usually more vulnerable to malnutrition. Public health programmes should take into account these aspects prior to implementing an intervention, and also during implementation itself *(67)*. Member States and their partners may assess where folate interventions may or may not be of benefit, after careful consideration of their context. The involvement of all stakeholders is essential. Potential ethical conflicts derived from the use of this threshold will benefit from balancing any decision using the four principles aforementioned. Individuals should always retain autonomy over any decisions relating to their own health and health interventions. Moreover, health workers must observe the principles of non-maleficence (not intentionally causing harm or injury), beneficence (the duty to benefit and avoid harm) and justice (providing services and interventions in equity). Ethical considerations are linked, conceptually and operationally, to equity and social determinants of health. Therefore, interventions will be more likely to observe ethical principles when they incorporate equity and social determinants approaches to their design and implementation. Public health interventions ideally seek the greatest attainable level of health and welfare for all their citizens, particularly those who suffer from social and economic hardship *(68)*.

Guideline development process

This guideline was developed in accordance with the WHO evidence-informed guideline development procedures, as outlined in the *WHO handbook for guideline development (69)*.

Advisory groups

A WHO steering committee (see Annex 3), led by the Department of Nutrition for Health and Development, was established in 2012, with representatives from all WHO departments with an

interest in this area of congenital anomalies and biomarkers. The steering committee guided the development of this guideline and provided overall supervision of the guideline development process. Two additional groups were formed: an advisory guideline group and an external review group.

The guideline development group was established for this guideline alone. Participants of the guideline group meetings are listed in Annex 3. Its role was to advise WHO on the choice of important outcomes for decision-making and in the interpretation of the evidence. The WHO guideline development group includes experts from various WHO expert advisory panels *(70)* and those identified through open calls for specialists, taking into consideration a balanced gender mix, multiple disciplinary areas of expertise, and representation from all WHO regions. Efforts were made to include content experts, methodologists, representatives of potential stakeholders (such as managers and other health professionals involved in nutrition surveillance) and technical staff from WHO and ministries of health from Member States. Representatives of commercial organizations may not be members of a WHO guideline group.

The WHO Nutrition *(62)* and SCN *(63)* mailing lists, which together include over 5500 subscribers, and the WHO nutrition web site *(62)* were used to identify external reviewers through a call for public comments, in order to assure proper interpretation of the recommendations. Additionally, three content experts peer-reviewed the draft guideline and provided technical input (see Annex 4).

Scope of the guideline and evidence appraisal

An initial set of questions (and the components of the questions) to be addressed in the guideline was the critical starting point for formulating the recommendation. The questions were drafted by technical staff at the Evidence and Programme Guidance Unit, Department of Nutrition for Health and Development, based on the policy and programme guidance needs of Member States and their partners.

A WHO/CDC technical consultation on optimal blood folate concentrations in women of reproductive age for prevention of NTDs was convened in Atlanta, USA on 13–15 August 2012, in collaboration with the National Center on Birth Defects and Developmental Disabilities at CDC, to review the genetic, biological, behavioural and contextual determinants of folate status among women of reproductive age; the strengths and limitations of current methods used to assess indicators of blood folate status and folate intake and NTD prevalence; and all available sources of data on the relationship between folate status (i.e. blood folate and folate intake) and NTD risk, from in vitro and in vivo (animal and human) studies. The proposed methodological approach for retrieving, summarizing and assessing the quality of the evidence related to folate status and occurrence of NTDs, as well as the priority questions and the methodological approach to estimate optimal blood folate concentrations in women of reproductive age for prevention of NTDs, were discussed with relevant stakeholders. A formal guideline development group meeting was convened on 23–25 September 2013 in Geneva, Switzerland.

Both the systematic reviews and the GRADE evidence profile *(49)* for the critical outcome were used for drafting this guideline. The draft recommendations were discussed by the WHO steering committee and at a consultation with the WHO guideline development group, held on 23–25 September 2013 in Geneva, Switzerland. The procedures for decision-making were established at the beginning of the meeting, including a minimal set of rules for agreement and decision-making. The members of the guideline development group undertook deliberations on the recommendation wording and reached

consensus with no major disagreements on the strength of the recommendation, taking into account: (i) the quality of the available evidence; (ii) values and preferences related to the intervention in different settings; (iii) the desirable and undesirable effects of the intervention; and (iv) the cost of options available to health-care workers in different settings (see Annex 2). Consensus was defined as agreement by simple majority of the guideline group members, with no dissent.

The aspects of costs, feasibility and values were taken into account and the decision was made during the guideline development group meeting. Similarly, a section on considerations for implementation were widely discussed and described in the document.

WHO staff present at the meeting, as well as other external technical experts involved in the collection and grading of the evidence, were not allowed to participate in the decision-making process. The majority of the group agreed that this is a strong/conditional recommendation.

Three content experts peer-reviewed the draft guideline (see Annex 4). A call for public comments was issued by WHO on 16 January 2015 and 17 external reviewers registered, providing a declaration of interests statement and their curriculum vitae (CVs). Ten external reviewers identified through this call reviewed the draft version of the guideline (see Annex 5). WHO staff then finalized the guideline and submitted it for clearance by WHO before publication.

Management of competing interests[1]

According to the rules in the WHO *Basic documents (71)*, all experts participating in WHO meetings must declare any interest relevant to the meeting, prior to their participation. The declarations of interest statements for all guideline development group members were reviewed by the responsible technical officer and the relevant departments, before finalization of the group composition and invitation to attend a guideline development group meeting. All guideline development group members, and participants of the guideline development meetings, submitted a declaration of interests form, along with their CV, before each meeting. Participants of the guideline development group meetings participated in their individual capacity and not as institutional representatives. In addition, they verbally declared potential conflicts of interest at the beginning of each meeting. The procedures for management of conflicts of interests strictly followed the *Guidelines for declaration of interests (WHO experts) (72)*.

Dr Lynn Bailey works at the University of Georgia as Department Head and professor of the Foods and Nutrition Department in the College of Family and Consumer Sciences. Her interests related to how changes in folate status impact maternal and child health and interact with other nutrients to maintain optimal health, as well as to the potential impact of obesity on the metabolism of folate and other micronutrients. Dr Bailey verbally declared being an author of many manuscripts and books in the area of folate nutrition and a member of the BOND (Biomarkers of Nutrition for Development) Folate Group. It was considered that she would participate fully and her interests would be documented.

[1] A conflict of interest analysis must be performed whenever WHO relies on the independent advice of an expert in order to take a decision or to provide recommendations to Member States or other stakeholders. The term "conflict of interest" means any interest declared by an expert that may affect or be reasonably perceived to affect the expert's objectivity and independence in providing advice to WHO. WHO's conflict of interest rules are designed to avoid potentially compromising situations that could undermine or otherwise affect the work of the expert, the committee or activity in which the expert is involved, or WHO as a whole. Consequently, the scope of the inquiry is any interest that could reasonably be perceived to affect the functions that the expert is performing.

Dr Anne Molloy works in the Vitamin Research Group at Trinity College, Dublin at the University of Dublin, Ireland. Her area of research involves the metabolic functions of folate and vitamin B_{12}, with a special focus on the evaluation of common polymorphisms in folate-related genes as risk factors for NTDs, and a subsequent search for phenotypic effects of gene variants that are found to be associated with NTDs. She declared being a co-author of the main reference used in the assessment of red blood cell concentrations and recused herself from the group consensus decision-making process for the selection of this as the main source of data.

Dr Christine Pfeiffer is the Chief of the Nutritional Biomarkers Branch in the Division of Laboratory Sciences at CDC's National Center for Environmental Health (NCEH). She joined CDC in 1996, where her research focuses on the development of state-of-the-art analytical methods for the measurement of nutritional biomarkers, the application of these methods to NHANES and other epidemiologic studies, and consultations on laboratory-related activities for CDC's IMMPaCt (International Micronutrient Malnutrition Prevention and Control) Programme. Dr Pfeiffer is a leading expert on folate and B-vitamin methodologies, and on the assessment and interpretation of nutritional status through biomarkers. She has authored over 100 peer-reviewed manuscripts, led the production of two major agency reports on nutritional biomarkers in NHANES, served on national and international committees, and been an invited speaker at major international conferences. It was considered that she would participate fully and her interests would be documented.

The other guideline development group members were all experts in the area of folic acid and congenital anomalies. They verbally declared their intellectual interests in the subject matter. It was considered that these interests did not preclude them from full participation in the decision-making process relevant to this guideline.

Dr Irma Silva-Zolezzi is currently employed by Nestec Ltd, Nestle Research Center, Lausanne, Switzerland. The Nestlé Research Center (NRC) is a research institution for food, nutrition and life sciences. The Nestlé R&D Network, including the Nestlé Research Center, works closely with Nestlé Business Units to drive product and process innovation and renovation. There are strategic business units for each product category, which are linked with individual Nestlé markets as well as R&D. NRC performs a variety of research activities, from fundamental studies to more applied activities. The main research pillars include nutrition and health; food science and technology; quality and safety; and sensory and consumer sciences. The Maternal Nutrition Platform aims to develop science-based product solutions and education programmes, supporting breastfeeding, and addressing malnutrition. Previously, while working as an investigator of the National Institute of Genomic Medicine (INMEGEN), Dr Silva-Zolezzi received a grant of US$ 10 000 from Nestlé. Dr Silva-Zolezzi presented on the global distribution of the *MTHFR 677C→T* polymorphism, which would not inform directly the guideline but would help as background information to contextualize the guideline. Dr Silva-Zolezzi was notified that, as an external expert, she would not participate in the rest of the meeting nor in the decision-making.

Plans for updating the guideline

Review of this guideline is planned for 2024. The Department of Nutrition for Health and Development at the WHO headquarters in Geneva, Switzerland, along with its internal partners, will be responsible for coordinating the guideline update, following the formal procedures of the *WHO handbook for guideline development (69)*. WHO welcomes suggestions regarding additional questions for evaluation in the guideline when it is due for review.

References

1. Resolution WHA65.6. Comprehensive implementation plan on maternal, infant and young child nutrition. In: Sixty-fifth World Health Assembly, 21–26 May 2012. Resolutions and decisions, annexes. Geneva: World Health Organization; 2012: 12–13 (WHA65/2012/REC/1; http://www.who.int/nutrition/topics/WHA65.6_resolution_en.pdf?ua=1, accessed 6 February 2015).
2. Naylor S. Biomarkers: current perspectives and future prospects. Expert Rev Mol Diagn. 2003;3(5):525–9.
3. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151–61. doi:10.1016/S0140-6736(12)60560-1.
4. Wallingford JB, Niswander LA, Shaw GM, Finnell RH. The continuing challenge up understanding, preventing, and treating neural tube defects. Science. 2013;339(6123):1222002. doi:10.1126/science.1222002.
5. Suarez L, Felkner M, Brender JD, Canfield M, Hendricks K. Maternal exposures to cigarette smoke, alcohol, and street drugs and neural tube defect occurrence in offspring. Matern Child Health J. 2008;12(3):394–401.
6. Li Z, Zhang L, Ye R, Pei L, Liu J, Zheng X et al. Indoor air pollution from coal combustion and the risk of neural tube defects in a rural population in Shanxi Province, China. Am J Epidemiol. 2011;174(4):451–8. doi:10.1093/aje/kwr108.
7. Lo A, Polsek D, Sidhu S. Estimating the burden of neural tube defects in low- and middle-income countries. J Glob Health. 2014;4(1):010402. doi:10.7189/jogh.04.010402.
8. Resolution WHA63.17. Birth defects. In: Sixty-third World Health Assembly, 17–21 May 2010. Resolutions and decisions, annexes. Geneva: World Health Organization; 2010:32–4. (WHA63/2010/REC/1; http://apps.who.int/gb/ebwha/pdf_files/WHA63-REC1/WHA63_REC1-en.pdf, accessed 6 February 2015).
9. World Health Organization, Food and Agriculture Organization. Vitamin and mineral requirements in human nutrition, 2nd ed. Geneva: World Health Organization; 2004 (http://www.who.int/nutrition/publications/micronutrients/9241546123/en/, accessed 6 February 2014).
10. McNulty H, Pentieva K. Folate bioavailability. Proc Nutr Soc. 2004;63(4):529–36.
11. Kaferle J, Strzoda CE. Evaluation of macrocytosis. Am Fam Physician. 2009;79(3):203–8.
12. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of folate and vitamin B_{12} deficiencies during pregnancy on fetal, infant, and child development. Food Nutr Bull. 2008;29 (2 Suppl.):S101–11; discussion S12–15.
13. Hibbard BM, Hibbard ED, Jeffcoate TN. Folic acid and reproduction. Acta Obstet Gynecol Scand.1965;44(3):375–400.

14. Pfeiffer CM, Fazili Z, M. Z. Folate analytical methodology. In: Baily LB, editor. Folate in health and diseases, 2nd ed. Boca Raton: CRC Press; 2010:517–74.

15. Chanarin I. Folate deficiency. In: Blakley RL, Whitehead VM, editors. Folates and pterins. Volume 3. Nutritional, pharmacological, and physiological aspects. New York: John Wiley & Sons; 1986:75–146.

16. Gibson R. Principles of nutritional assessment. Oxford: Oxford University Press; 2005.

17. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med. 1999;341(20):1485–90.

18. Pfeiffer CM, Schleicher RL, Johnson CL, Coates PM. Assessing vitamin status in large population surveys by measuring biomarkers and dietary intake – two case studies: folate and vitamin D. Food Nutr Res. 2012;56. doi:10.3402/fnr.v56i0.5944.

19. Integrated management of pregnancy and childbirth. Standards for maternal and neonatal care. Geneva: World Health Organization; 2007 (http://whqlibdoc.who.int/hq/2007/a91272.pdf, accessed 6 Februrary 2015).

20. Berti C, Fekete K, Dullemeijer C, Trovato M, Souverein OW, Cavelaars A et al. Folate intake and markers of folate status in women of reproductive age, pregnant and lactating women: a meta-analysis. J Nutr Metab. 2012;2012:470656.

21. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151(9):862–77.

22. Shane B. Folate and vitamin B_{12} metabolism: overview and interaction with riboflavin, vitamin B_6, and polymorphisms. Food Nutr Bull. 2008;29(2 Suppl.):S5–16; discussion S7–9.

23. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP, Gindler J et al. MTHFR 677C→T genotype is associated with folate and homocysteine concentrations in a large, population-based, double- blind trial of folic acid supplementation. Am J Clin Nutr. 2011;93(6):1365–72. doi:10.3945/ ajcn.110.004671.

24. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M et al. Geographical and ethnic variation of the 677C→T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas world wide. J Med Genet. 2003;40(8):619–25.

25. Tinker SC, Hamner HC, Berry RJ, Bailey LB, Pfeiffer CM. Does obesity modify the association of supplemental folic acid with folate status among nonpregnant women of childbearing age in the United States? Birth Defects Res A Clin Mol Teratol. 2012;94(10):749–55. doi:10.1002/ bdra.23024.

26. Nzila A, Okombo J, Molloy AM. Impact of folate supplementation on the efficacy of sulfadoxine/ pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate. J Antimicrob Chemother. 2014;69(2):323–30. doi:10.1093/jac/dkt394.

27. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005;57(1):117–45.

28. Nutritional anaemias. Report of a WHO scientific group. Geneva: World Health Organization; 1968. (WHO Technical Report Series No. 405; http://whqlibdoc.who.int/trs/WHO_TRS_405.pdf, accessed 6 February 2015).

29. Nutrional anaemias. Report of a WHO group of experts. Geneva: World Health Organization;1972 (WHO Technical Report Series No. 503; http://whqlibdoc.who.int/trs/WHO_TRS_503.pdf, accessed 6 February 2015).

30. Control of nutritional anaemia with special reference to iron deficiency. Report of an IAEA/USAID/WHO joint meeting. Geneva: World Health Organization; 1972 (WHO Technical Report Series No. 580 (http://whqlibdoc.who.int/trs/WHO_TRS_580.pdf, accessed 6 February 2015).

31. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B_{12} deficiencies. Food Nutr Bull. 2008;29(2 Suppl.):S238–44.

32. Baker H, Herbert V, Frank O, Pasher I, Hutner SH, Wasserman LR et al. A microbiologic method for detecting folic acid deficiency in man. Clin Chem. 1959;5(4):275–80.

33. Pfeiffer CM, Zhang M, Lacher DA, Molloy AM, Tamura T, Yetley EA et al. Comparison of serum and red blood cell folate microbiologic assays for national population surveys. J Nutr. 2011;141(7):1402–9. doi:10.3945/jn.111.141515.

34. Anderson BB, Cowan JD. Effect of light on the *Lactobacillus casei* microbiological assay. Am J Clin Pathol. 1968;21(1):85–7.

35. Yetley EA, Pfeiffer CM, Phinney KW, Fazili Z, Lacher DA, Bailey RL et al. Biomarkers of folate status in NHANES: a roundtable summary. Am J Clin Nutr. 2011;94(1):303S–12S. doi:10.3945/ajcn.111.013011.

36. Fazili Z, Pfeiffer CM, Zhang M. Comparison of serum folate species analyzed by LC-MS/MS with total folate measured by microbiologic assay and Bio-Rad radioassay. Clin Chem. 2007;53(4):781–4.

37. Fazili Z, Pfeiffer CM, Zhang M, Jain RB, Koontz D. Influence of 5,10 methylenetetrahydrofolate reductase polymorphism on whole-blood folate concentrations measured by LC-MS/MS, microbiologic assay, and bio-rad radioassay. Clin Chem. 2008;54(1):197–201.

38. Higgins J, Green S, editors. Cochrane handbook for systematic reviews of interventions version 5.1.0 [updated March 2011]. Oxford: The Cochrane Collaboration; 2011 (http://www.cochrane.org/handbook, accessed 6 February 2015).

39. Bailey L, Stover P, McNulty H, Fenech M, Gregory J, Mills J et al. Biomarkers of Nutrition for Development (BOND) – folate review. Personal communication, 2015.

40. Christensen K, Rozen R. Genetic variation: effect on folate metabolism and health. In: Bailey L, editor. Folate and health and disease. Boca Raton: CRC Press; 2007:75–110.

41. Tsang BL, Devine OJ, Cordero AM, Marchetta CM, Mulinare J, Mesereau P et al. Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies. Am J Clin Nutr. 2015;Mar 18. pii: ajcn099994. [Epub ahead of print].

42. Binia A, Contreras AV, Canizales-Quinteros S, Alonzo VA, Tejero ME, Silva-Zolezzi I. Geographical and ethnic distribution of single nucleotide polymorphisms within genes of the folate/homocysteine pathway metabolism. Genes Nutr. 2014;9(5):421. doi:10.1007/s12263-014-0421-7.

43. Marchetta CM, Devine OJ, Crider KS, Tsang BL, Cordero AM, Qi YP et al. Assessing the association between natural food folate intake and blood folate concentrations: a systematic review and Bayesian meta-analysis of trials and observational studies. Nutrients. 2015;7:2663-86. doi: 10.3390/nu7042663.

44. Yan L, Zhao L, Long Y, Zou P, Ji G, Gu A et al. Association of the maternal MTHFR C677T polymorphism with susceptibility to neural tube defects in offsprings: evidence from 25 case-control studies. PLoS One. 2012;7(10):e41689. doi:10.1371/journal.pone.0041689.

45. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Pena-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev. 2010(10):CD007950. doi:10.1002/14651858.CD007950.pub2.

46. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. JAMA. 1995;274(21):1698–702.

47. Crider KS, Devine O, Hao L, Dowling NF, Li S, Molloy AM et al. Population red blood cell folate concentrations for prevention of neural tube defects: Bayesian model. BMJ. 2014;349:g4554. doi:10.1136/bmj.g4554.

48. Clarke R, Derrik B. Editorials: Folate and prevention of neural tube defects. Tracking red blood cell concentrations will help guide policy decisions about fortification. BMJ. 2014;349:g4810.

49. GRADE Working Group (http://www.gradeworkinggroup.org/, accessed 6 February 2015).

50. Shea BJ, Hamel C, Wells GA, Bouter LM, Kristjansson E, Grimshaw J et al. AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. J Clin Epidemiol. 2009;62(10):1013–20.

51. Fernandez-Gaxiola AC, De-Regil LM. Intermittent iron supplementation for reducing anaemia and its associated impairments in menstruating women. Cochrane Database Syst Rev. 2011(12):CD009218. doi:10.1016/j.jclinepi.2008.10.009.

52. Lassi ZS, Salam RA, Haider BA, Bhutta ZA. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. Cochrane Database Syst Rev. 2013;3:CD006896. doi:10.1002/14651858.CD006896.pub2.

53. Berry RJ, Mulinare J, Hammer HC. Folate acid fortification. In: Bailey L, editor. Folate in health and disease. Boca Raton, Florida: CRC Press; 2010:179–204.

54. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M et al. Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988–2010. J Nutr. 2012;142(5):886–93. doi:10.3945/jn.111.

55. Suchdev P, De-Regil L, Walleser S, Vist G, Peña-Rosas J. Multiple micronutrient powders for home (point of use) fortification of foods in pregnant women: a systematic review. WHO e-Library of Evidence for Nutrition Actions. Geneva: World Health Organization; 2011. (http://apps.who.int/iris/bitstream/10665/44748/1/9789241502559_eng.pdf, accessed 9 February 2015).

56. Peña-Rosas J, Ashong J, Muthayya S, De-Regil L, Laillou A, Guyondet C et al. Fortification of rice with vitamins and minerals for addressing micronutrient malnutrition. Personal communication, 2015.

57. Pfeiffer C, Schleicher R, Caldwell K. Biochemical Indices. In: Caballer OB, editor. Enclyclopedia of human nutrition. 1, 3rd ed. Waltham, MA: Academic Press; 2013:156–74.

58. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva: World Health Organization; 2012 (http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf, accessed 6 February 2015).

59. Guideline: Daily iron and folic acid supplementation in pregnant women, Geneva: World Health Organization; 2012 (http://apps.who.int/iris/bitstream/10665/77770/1/9789241501996_eng.pdf?ua=1, accessed 6 February 2015).

60. Guideline: Intermittent iron and folic acid supplementation in non-anaemic pregnant women. Geneva: World Health Organization; 2012 (http://apps.who.int/iris/bitstream/10665/75335/1/9789241502016_eng.pdf?ua=1, accessed 6 February 2015).

61. Weekly iron–folic acid supplementation (WIFS) in women of reproductive age: its role in promoting optimal maternal and child health. Position statement. Geneva: World Health Organization; 2009 (http://www.who.int/nutrition/publications/micronutrients/weekly_iron_folicacid.pdf, accessed 6 February 2015).

62. World Health Organization. Nutrition (http://www.who.int/nutrition/en/, accessed 6 February 2015).

63. United Nations Standing Committee on Nutrition (http://www.unscn.org/, accessed 6 February 2015).

64. World Health Organization. Vitamin and Mineral Information Service (VMNIS) (http://www.who.int/vmnis/en/, accessed 6 February 2015).

65. World Health Organization. Vitamin and Mineral Information Service (VMNIS). Micronutrients database (http://www.who.int/vmnis/database/en/, accessed 6 February 2015).

66. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol. 1997;281:43–53.

67. Marchetta CM, Hamner HC. Blood folate concentrations among women of childbearing age by race/ethnicity and acculturation, NHANES 2001–2010. Matern Child Nutr. 2014 ; 17 June epub ahead of print. doi:10.1111/mcn.12134.

68. Fuller-Deets M, Dingwall R. The ethical implications of options for improving the folate intake of women of reproductive age. Nottingham: University of Nottingham; 2007 (http://www.food.gov.uk/sites/default/files/multimedia/pdfs/ethicalfolate.pdf, accessed 6 February 2015).

69. Handbook for guideline development, 2nd ed. Geneva: World Health Organization; 2014 (http://www.who.int/kms/handbook_2nd_ed.pdf?ua=1, accessed 2 March 2015).

70. Expert advisory panels and committees, Geneva: World Health Organization; 2010 (http://www.who.int/rpc/expert_panels/Factsheet_EAP2010.pdf, accessed 6 February 2015).

71. Governing body documentation. Basic documents. Governance. Geneva: World Health Organization; 2009 (http://apps.who.int/gb/bd/, accessed 6 February 2015).

72. Guidelines for declaration of interests (WHO experts). Geneva: World Health Organization; 2010.

Annex 1. GRADE "Summary of findings" table

Red blood cell folate concentration in women of reproductive age				
Methodologists: De-Regil LM, Peña-Rosas JP **Patient or population:** Women of reproductive age **Settings:** All settings (low-, middle-,-high-income countries) **Exposure:** Red blood cell folate concentration **Comparison:** Women with births with neural tube defects vs controls				
Outcomes	**Threshold associated with maximum reduction of neural tube defects**		**Quality of the evidence (GRADE*)**	**Comments**
	Threshold	Number of participants (studies)		
Red blood cell folate in nmol/L (ng/mL) for lowest risk of neural tube defects	906 (400)	350 women: 84 cases and 266 controls (1 study)	⨁⨁⨀⨀ Low[1]	The NTD rate associated to this threshold was 0.8 (95% CI 0.4 to 1.5) per 1000 births in the study hospital.
Plasma folate in nmol/L (ng/mL) for lowest risk of neural tube defects	15.9 *(7)*	328 women: 81 cases and 247 controls (1 study)	⨁⨁⨀⨀ Low[1]	The NTD rate associated to this threshold was 0.8 (95% CI 0.4 to 1.5) per 1000 births in the study hospital.

CI, confidence interval.

*GRADE Working Group grades of evidence:
High quality: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate quality: We have moderate confidence in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low quality: Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect.
Very low quality: We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of the effect.

[1] Only one case–control study with good methodological quality and no serious limitations in the study design and the execution. No serious indirectness. Serious imprecision likely, owing to the small number of cases. The quality of evidence was upgraded because of the clear dose–response gradient (inverse) between red blood cell folate concentrations and risk of neural tube defect. Consistency is supported by indirect evidence with mathematical modelling *(47)*.

Annex 2. Summary of guideline development group members' considerations for determining the strength of the recommendations

Quality of evidence:	• The only high-quality case–control study identified was conducted in pregnant women rather than non-pregnant women of reproductive age. There were few events and wide confidence intervals. • Although there is limited direct evidence, indirect data from a rigorous mathematical modelling exercise showed consistent results. • Data come from few populations around the world (China, Ireland, United States of America).
Values and preferences:	• Blood withdrawal may not be acceptable in some settings. • There is likely to be a highly protective effect for reducing neural tube defects (NTDs) in women who are able achieve higher folate concentrations. • Many countries may not have the capacity to monitor the occurrence of NTDs or measure red blood cell folate at the population level and this capacity would need to be improved.
Trade-off between benefits and harms:	• It is important to have thresholds that reflect the risk of folate-preventable NTDs and avoid confusion with concentrations associated with megaloblastic anaemia. • The overall benefit to the population of achieving this threshold would depend on the baseline incidence of NTDs, and the overall size of effect will depend on success in reaching the target population. • Increasing folate concentrations may provide other benefits to the population other than reducing NTDs, such as reducing folate-related anaemia. • No solid evidence of harm. One study in Pune, India showed higher maternal folate concentrations predicted greater adiposity (fat mass and body fat per cent) and higher insulin resistance, and lower vitamin B_{12} concentrations predicted higher insulin resistance in their offspring. Children born to mothers with low vitamin B_{12} concentrations but high folate concentrations were the most insulin resistant.

Costs and feasibility:	• There are costs associated with scaling up the use of the red blood cell folate measurements in national surveys, and the extent of resources required to standardize the methodologies in different laboratories for microbiological assays with adequate techniques, trained human resources and equipment is unclear. • There are also costs associated with setting up neural tube defect surveillance systems. • Improving the laboratory capacity and surveillance systems is feasible but it requires coordination of efforts by stakeholders and financial resources, which may be constrained in some low- and middle-income countries.

Annex 3. WHO steering committee, WHO guideline development group, WHO Secretariat and external resource experts

WHO steering committee

Dr Luz Maria De-Regil
Epidemiologist
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Dr Mario Merialdi
Coordinator, Research, Evidence and Norms
Department of Reproductive Health and Research

Dr Juan Pablo Peña-Rosas
Coordinator
Evidence and Programme Guidance
Department of Nutrition for Health and Development

WHO guideline development group

(*Note*: the areas of expertise of each guideline group member are given in italics.)

Dr Deborah Ash
FHI 360
USAID's Food and Nutrition Technical Assistance Project III (FANTA).
Dar es Salaam, United Republic of Tanzania
Epidemiology, nutrition and HIV

Dr Lynn Bailey
Department of Food and Nutrition
University of Georgia
Athens, United States of America
Folate metabolism and assessment

Professor Beverley-Ann Biggs
Department of Medicine
University of Melbourne
Parkville, Australia
Micronutrient supplementation, clinical infectious diseases

Dr Lorenzo Botto
International Clearinghouse for Birth Defects Surveillance and Research
Department of Pediatric Genetics
University of Utah
Salt Lake City, United States of America
Genetics, neural tube defects

Dr Robert Clarke
Clinical Trial Service Unit and Epidemiological Studies Unit
University of Oxford
Oxford, United Kingdom of Great Britain and Northern Ireland
Epidemiology, clinical trials, B vitamins

Ms Melanie Galvin
The Micronutrient Initiative
New Delhi, India
Epidemiology, community health, micronutrients

Ms Gowri Gopalakrishna
Academic Medical Center
University of Amsterdam
Amsterdam, The Netherlands
Clinical epidemiology, biostatistics, methods

Dr Ling Hao
China Office
US Centers for Disease Control and Prevention – Global AIDS Program (CDC-GAP)
Beijing, China
Nutrition epidemiology, laboratory, folate

Dr Ibrahim Khatib
Department of Community Medicine, Faculty of Medicine
Jordan University of Science & Technology
Irbid, Jordan
Public health, nutrition metabolism, assessment of micronutrient status

Dr Anne Molloy
Trinity Biomedical Research Institute
Trinity College Dublin
Dublin, Ireland
Biochemistry, one-carbon metabolism, B-vitamins

Professor Malcolm E Molyneux
College of Medicine
University of Malawi
Blantyre, Malawi
Malaria, international tropical diseases research and practice

Dr Alexis Nzila
Chemistry Department
King Fahd University of Petroleum and Minerals (KFUPM)
Dhahran, Saudi Arabia
Biochemistry, parasitology, antimalarial resistance

Dr Christine Pfeiffer
Nutritional Biomarkers Branch, Division of Laboratory Sciences
National Center for Environmental Health
US Centers for Disease Control and Prevention
Atlanta, United States of America
Analytical methods, nutrition biomarkers

Professor HPS Sachdev
Pediatrics and Clinical Epidemiology
Sitaram Bhartia Institute of Science and Research
New Delhi, India
Clinical epidemiology, infant and young child feeding, global health

Dr Patrick Stover
Division of Nutritional Sciences
Cornell University
Ithaca, United States of America
Biochemistry, nutritional sciences, B vitamins

Dr Parag M Tamhankar
Genetic Research Center
National Institute for Research in Reproductive Health (NIRRH)
Mumbai, India
Genetics, molecular biology, neural tube defects

Dr Nelly Zavaleta
Instituto de Investigación Nutricional
Lima, Peru
Nutrition, maternal and infant health, micronutrient interventions

WHO Secretariat

Dr Karla Botello-Ortiz *(rapporteur)*
Intern
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Ms Hala Boukerdenna *(rapporteur)*
Intern
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Ms Monica Crissel Flores-Urrutia
Intern
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Dr Maria de las Nieves García-Casal
Senior Consultant
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Dr Susan L Norris
Technical Officer
WHO Press
Department of Knowledge Management and Sharing

Dr Lisa Rogers
Technical Officer
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Ms Becky Tsang
Intern
Evidence and Programme Guidance
Department of Nutrition for Health and Development

Mr Gerardo Zamora
Consultant, Equity and Implementation Research
Evidence and Programme Guidance
Department of Nutrition for Health and Development

WHO regional offices

Dr Neena Raina
Regional Adviser, Child and Adolescent Health
WHO Regional Office for South East Asia
New Delhi, India

External resource experts

Dr Robert J Berry
National Center on Birth Defects and Developmental Disabilities
Centers for Disease Control and Prevention
Atlanta, United States of America

Ms Amy Cordero
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Dr Krista S Crider
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Ms Alina Flores
Prevention Research Branch
Division of Birth Defects and Developmental Disabilities
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Dr Marianne Klemp
Health Economics and Drugs Unit
The Norwegian Knowledge Centre for the Health Services
Oslo, Norway

Professor Berthold Koletzko
Division of Metabolic and Nutritional Medicine
Dr von Hauner Children's Hospital
University of Munich Medical Centre
Munich, Germany

Dr Nicole Fichtner Dowling
Division of Birth Defects and Developmental Disabilities
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Dr Joseph Mulinare
Carter Consulting, Inc.
Atlanta, United States of America

Dr Daniel J Raiten
Office of Prevention Research and International Programs and Endocrinology, Nutrition, and Growth Branch
Center for Research for Mothers and Children
Eunice Kennedy Shriver National Institute of Child Health and Human Development
Bethesda, United States of America

Dr Irma Silva-Zolezzi
Nutrition & Health Department
Nestec Ltd, Nestle Research Center
Lausanne, Switzerland

Dr Joseph Sniezek
Division of Birth Defects and Developmental Disabilities
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Dr Mary Serdula
Division of Nutrition, Physical Activity, and Obesity
National Center for Chronic Disease Prevention and Health Promotion
US Centers for Disease Control and Prevention
Atlanta, United States of America

Dr Kevin Sullivan
Department of Epidemiology
Rollins School of Public Health, Emory University
Atlanta, United States of America

Ms Aliki Pappas Weakland
Prevention Research Branch
Division of Birth Defects and Developmental Disabilities
National Center on Birth Defects and Developmental Disabilities
US Centers for Disease Control and Prevention
Atlanta, United States of America

Annex 4. Content expert peer-reviewers

Dr Pierpaolo Mastroiacovo
Alessandra Lisi International Centre on Birth Defects and Prematurity
Centre of the International Clearinghouse for Birth Defects Surveillance and Research
Rome, Italy

Dr María Elizabeth Tejero Barrera
Laboratory of Nutrigenetics and Nutrigenomics
National Institute of Genomic Medicine (INMEGEN)
Mexico City, Mexico

Dr Mindy Zhang
Global Micronutrient Laboratory
Nutritional Biomarkers Branch
Division of Laboratory Sciences
National Center for Environmental Health
US Centers for Disease Control and Prevention
Atlanta, United States of America

Annex 5. External reviewers (from call for public comments)

Dr. Cutberto Espinosa Lopez
Directorate of Epidemiology
Ministry of Health, Mexico

Ms Michelle Gibbs
Biosecurity Science, Food Science & Risk Assessment Directorate
Regulation and Assurance Branch
Ministry for Primary Industries
Wellington 6140, New Zealand

Dr Vijaya Kancherla
Department of Epidemiology
Center for Spina Bifida Research, Prevention and Policy
Rollins School of Public Health
Emory University
Atlanta, United States of America

Dr Guillermo Melendez
Hospital General de México "Dr. Eduardo Liceaga"
Delegación Cuauhtémoc,
CP 14610, Mexico City, Mexico

Dr Sirimavo Nair
Department of Foods and Nutrition,
Faculty of Family and Community Sciences,
The MS University of Baroda,
Vadodara, Gujarat, India

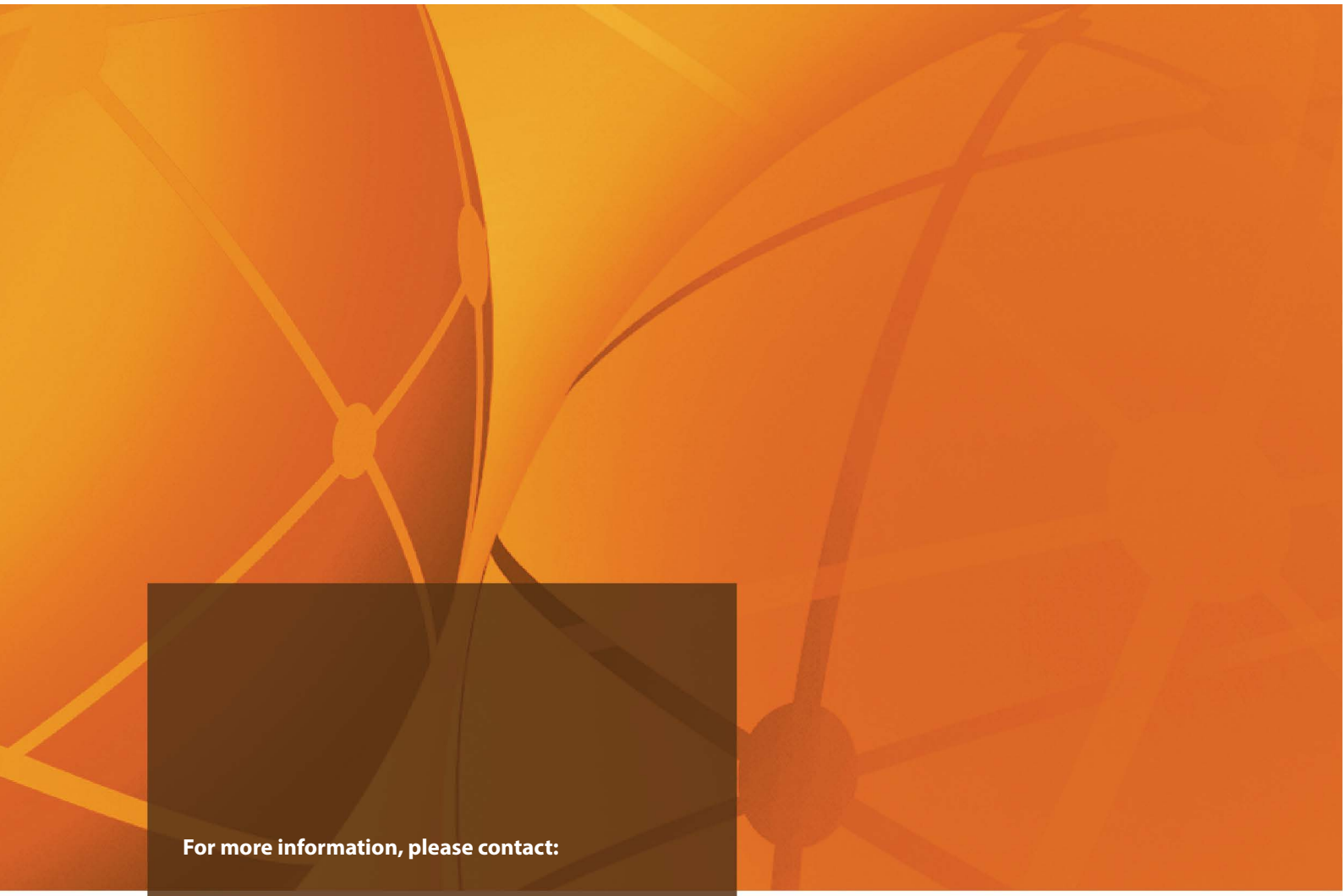
Dr Rima Obeid
Aarhus Institute of Advanced Studies
Aarhus University
Høegh-Guldbergs Gade 6B, building 1632
Aarhus, Denmark

Mr Moshood Olanrewaju Omotayo
Division of Nutritional Sciences
Cornell University
Ithaca, United States of America

Ms Lawal Remilekum Olubunmi
Special Care Baby Unit,
Nursing Services
National Hospital A buja
Abuja, Nigeria

Dr Helena Pachón
Hubert Department of Global Health
Emory University
Atlanta, United States of America

Ms Rusidah Selamat
Nutrition Division
Ministry of Health
62590 Putrajaya, Malaysia



For more information, please contact:

Department of Nutrition for Health and Development
World Health Organization
Avenue Appia 20, CH-1211 Geneva 27, Switzerland
Fax: +41 22 791 4156
E-mail: nutrition@who.int
www.who.int/nutrition

ISBN 978 92 4 154904 2

World Health Organization