Periconceptional daily folic acid (400 µg) supplementation for prevention of neural tube defects

This submission was prepared by Ms Hala Boukerdenna, Dr Juan Pablo Pena-Rosas and Dr Lisa Rogers with technical input from Dr Maria Nieves Garcia-Casal.
Table of Contents

Acronyms and abbreviations ........................................................................................................................................... 3

Executive summary ......................................................................................................................................................... Error! Bookmark not defined.

I. Background and rationale for the application .............................................................................................................. Error! Bookmark not defined.

II. Background .................................................................................................................................................................. Error! Bookmark not defined.

1. Public health relevance .................................................................................................................................................. Error! Bookmark not defined.

2. Current public health interventions .............................................................................................................................. Error! Bookmark not defined.

3. Proposed public health intervention ............................................................................................................................ Error! Bookmark not defined.

III. Methods ...................................................................................................................................................................... Error! Bookmark not defined.


3. Methods for the assessment of current availability amongst Member States .................................................................... Error! Bookmark not defined.

4. Assessment of the evidence ............................................................................................................................................ Error! Bookmark not defined.

IV. Regulatory information .................................................................................................................................................. Error! Bookmark not defined.

V. Analysis of costs .............................................................................................................................................................. Error! Bookmark not defined.

VI. Current NEML availability evaluation ........................................................................................................................... Error! Bookmark not defined.

VII. Evidence on dosing, efficacy and safety ........................................................................................................................ Error! Bookmark not defined.

1. Quality of the evidence .................................................................................................................................................... Error! Bookmark not defined.

2. Summary of the evidence ................................................................................................................................................ Error! Bookmark not defined.

IX. WHO guidelines ............................................................................................................................................................ Error! Bookmark not defined.

X. Summary and recommendations .................................................................................................................................... Error! Bookmark not defined.

XI. References ..................................................................................................................................................................... Error! Bookmark not defined.
### Acronyms and abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNF</td>
<td>British national formulary</td>
</tr>
<tr>
<td>CI</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>EML</td>
<td>Essential medicines list (for adults)</td>
</tr>
<tr>
<td>FA</td>
<td>Folic acid</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GRADE</td>
<td>Grading of Recommendations Assessment, Development and Evaluation</td>
</tr>
<tr>
<td>IPTp</td>
<td>Intermittent preventive treatment during pregnancy</td>
</tr>
<tr>
<td>IR</td>
<td>Incidence rate</td>
</tr>
<tr>
<td>IRR</td>
<td>Incidence rate ratio</td>
</tr>
<tr>
<td>LMICs</td>
<td>Low and middle-income countries</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and healthcare products regulatory agency</td>
</tr>
<tr>
<td>MSH</td>
<td>Management sciences for health</td>
</tr>
<tr>
<td>NEML</td>
<td>National essential medicines list</td>
</tr>
<tr>
<td>NTD</td>
<td>Neural tube defect</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell or erythrocyte</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SP</td>
<td>Sulfadoxine/Pyrimethamine</td>
</tr>
<tr>
<td>SRA</td>
<td>Stringent regulatory authority</td>
</tr>
<tr>
<td>TGA</td>
<td>Therapeutic goods administration</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USD</td>
<td>United States Dollar</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Executive summary

This application aims to request the inclusion of a dose of 400 ug (0.4 mg) alone in the Essential Medicines List. A similar dose is already listed in combination with elemental iron but WHO recommends folic acid without iron in some settings. This application presents a comprehensive review of the supportive evidence for the effective use of periconceptional daily supplementation of 400 μg (0.4 mg) folic acid in women of childbearing age as a public health intervention for the prevention of neural tube defects (NTDs). Neural tube defects affect the brain and spinal cord, and are among the most common of the congenital anomalies. The most prevalent types of neural tube defects are anencephaly, encephalocele and spina bifida.

Evidence from two trials including 299 women summarized in a Cochrane review indicates a reduction in NTDs when folic acid supplementation was given alone compared to receiving no supplementation or placebo (average relative risk (RR) 0.32; 95% confidence interval (CI) 0.08 to 1.34).

A study on the minimum effective dose of folic acid with 173 women comparing folic acid intake and red blood cell (RBC) folate concentrations showed that 400 μg (0.4 mg) of daily folic acid leads to a median RBC folate concentration of 571μg/L \(^1\) (95% CI 481 to 654) post-treatment, which was significantly higher compared to the placebo group (mean difference (MD) 260 µg/L; 95% CI 103.81 to 416.19). Based on previous work that showed an inverse relation between the risk of NTDs and maternal RBC folate, the observed concentration would result in 47% reduction in NTDs.

Additionally, a cohort study in China showed that daily ingestion of 400 μg (0.4 mg) of folic acid during the periconceptional period reduced the risk of women having a fetus or infant with NTDs in areas of both high and low frequency of NTDs (RR 0.21, 95 % CI 0.13 to 0.32 in the northern region of China with high risk of NTDs; RR 0.84, 95% CI 0.62 to 1.14 in southern region of China with low risk of NTDs). Overall, it was estimated that daily supplementation with 400 μg folic acid during the periconceptional period would reduce NTDs by 50% globally (RR 0.5, 95 % CI 0.39 to 0.64). The preventive effect in the southern region of China, in which the baseline rate of NTDs is similar to that in the United States and elsewhere, suggests that daily intakes of 400 μg (0.4 mg) folic acid may help reduce the rate of the first occurrence of NTDs in many parts of the world.

A recent analysis shows an inverse dose-response association of NTD risk and maternal RBC folate concentrations from two studies (a community intervention with 275 women and a randomized trial with 371 women) based on the Chinese population of the above study. The analysis defines the threshold for optimal RBC folate concentration for prevention of NTDs

\(^1\) Equivalent to 1293 nmol/L
as 1000 nmol/L. These results are concordant with another study in an Irish population that indicates that RBC folate concentrations of about 906 nmol/L or greater should be the population target for preventing NTDs. At the population level, red blood cell folate concentrations above 906 nmol/L (400 ng/mL) in women of reproductive age have been associated with the greatest reduction of NTD.

In some malaria endemic areas, folic acid supplements and the antifolate drug sulfadoxine/pyrimethamine may be used concurrently for NTD prevention and malaria prevention, respectively. One study (n= 488) showed that the rates of malaria treatment failure in the placebo and 400 μg (0.4 mg) folic acid groups were identical (13.9% and 14.5%, respectively, P<0.05), while the failure rate in the 5000 μg (5 mg) folic acid group was significantly higher than in the placebo group (27.1% versus 13.9%, P=0.005). The rate of failure comparing 5mg versus 400 μg (0.4 mg) folic acid group was significantly higher (RR 1.87, 95% CI 1.35 to 3.05). A second study using the same study samples (n=467) assessed folate blood levels and correlated them with sulfadoxine/pyrimethamine drug efficacy. This study found that high daily dose of folate supplementation (5 mg) or high folate levels were independent risk factors for sulfadoxine/pyrimethamine treatment failure. A third study (n= 1035) shows that doses of folic acid ranging from 500 μg (0.5 mg) to 1500 μg (1.5 mg) do not reduce sulfadoxine/pyrimethamine efficacy. These three studies have found that 400 μg (0.4 mg) can be used without affecting the efficacy of sulfadoxine/pyrimethamine in Intermittent Preventive Treatment during pregnancy (IPTp) while providing a sufficient red blood cell folate concentrations to prevent NTDs.

WHO currently recommends all women, from the moment they being trying to conceive until 12 weeks of gestation, take a daily supplement of 400 μg (0.4 mg) folic acid to prevent NTDs (first occurrence of NTDs) and women who have previously had a fetus diagnosed as affected by a NTD, or have given birth to a baby with a NTD, should take a daily supplement of 5000 μg (5 mg) folic acid to prevent recurrent NTDs. Currently the Essential Medicine’s List includes folic acid with iron (400 μg (0.4 mg) folic acid plus 60 mg elemental iron) and high dose folic acid (1000 μg (1 mg) and 5000 μg (5 mg), but does not include the 400 μg (0.4 mg) folic acid dose without iron. For women who have difficulties in taking iron supplements, those who choose not to, or where iron is not recommended for other reasons it is important they have the option to consume folic acid alone in the recommended dose for the prevention of occurrent NTDs.

The recommendations for changes to the EML Section 27 – Vitamins and minerals, are as follows:

1. Add 400 μg (0.4 mg) folic acid tablet/capsule formulation for the prevention of NTDs during the periconceptional period.
   a. Dose
      i. 400 μg folic acid.

---

2 Equivalent to 441 μg/L
b. Frequency and duration of the supplementation

i. One tablet per day

ii. Start two months before the planned pregnancy and continue during the first 12 weeks of pregnancy

iii. This dose does not concern pregnant women who have previously had a baby with a NTD, women who have diabetes mellitus or women who are under anticonvulsant treatment. A higher dose 5000 \( \mu g \) (5 mg) is recommended for these cases.

I. Background and rationale for the application

Studies have shown that increasing the consumption of folic acid by women during the periconceptional period can significantly reduce the occurrence of neural tube defects (NTDs) which has led to recommendations for women to consume 400 \( \mu g \) (0.4 mg) of folic acid daily to reduce their risk of having an NTD-affected pregnancy (1). However, currently the Essential Medicines List only includes folic acid with iron and high dose folic acid supplements [1000 \( \mu g \) (1 mg) and 5000 \( \mu g \) (5 mg)], the higher dose which has been shown effective in the prevention of recurrence of NTDs. For women who have difficulties in taking iron supplements or who choose not to, it is important they have the option to consume folic acid alone in the recommended dose for the prevention of occurrent NTDs.

This EML application presents evidence from a Cochrane review, literature review and a WHO guideline on the safety and efficacy of 400 \( \mu g \) (0.4 mg) of folic acid daily supplementation in preventing the occurrence NTDs among women of reproductive age.

II. Background on NTD’s

Congenital anomalies, also known as birth defects, can be defined as structural or functional abnormalities, including metabolic disorders, which are present from birth and can be caused by single gene defects, chromosomal disorders, multifactorial inheritance, environmental teratogens or micronutrient deficiencies. (2) NTDs originate from a failure in the development of the embryonic nervous system at very early stages of gestation (3). Moreover, NTDs affect the brain and spinal cord and anencephaly, spina bifida and encephalocele are the most frequent phenotypes of NTDs (4).

Approximately half of all congenital anomalies cannot be linked to a specific cause. However there are some known causes or risk factors for congenital anomalies, including
socioeconomic factors, genetic factors, infections, maternal nutritional status and environmental factors (5). For NTDs specifically, folate insufficiency is considered the most important nutritional risk factor for NTDs and supplementation with folic acid has been associated with a reduced risk of NTDs. (6)(7)

Because the neural tube closes by day 28 of pregnancy, a period when pregnancy may not have been detected, it is important that folic acid be consumed in sufficient amounts prior to pregnancy and in the early stages of pregnancy. Folic acid supplementation after the first month of pregnancy will not prevent neural tube defects, but will contribute to other aspects of maternal and fetal health (8).

In malaria-endemic areas, the use of high-dose folic acid supplementation presents a unique challenge where anti-folate anti-malaria drugs, such as sulfadoxine-pyrimethamine, are used. It is estimated that over 125 million pregnant women are at risk of malaria each year, and the disease has serious implications in pregnancy for both mothers and infants (9). Indeed, placental malaria can lead to placental insufficiency, causing fetal growth restriction and leading to maternal, fetal and infant morbidity and mortality. It is estimated that more than 100 000 infant deaths occur each year as a result of malaria in pregnancy (9).

Starting as early as possible in the second trimester of pregnancy, intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP) is recommended for all pregnant women at each scheduled antenatal care visit until the time of delivery, provided that the doses are given at least one month apart. There are concerns that the use of 5000 µg (5 mg) doses of folic acid may counteract the efficacy of sulfadoxine-pyrimethamine as an antimalarial agent in these areas (9).

1. Public health relevance

Congenital anomalies (also referred as birth defects) affect an estimated 1 in 33 infants and result in approximately 3.2 million birth defect-related disabilities every year (5). The NTD burden was recently assessed in 18 countries in 6 WHO regions (5). The overall burden calculated using the median was 1.67/1000 live births for total NTD burden, 1.13/1000 for spina bifida, 0.25/1000 for anencephaly and 0.15/1000 for encephalocele. It further estimated that about 190 000 neonates were born each year with a NTD in low- and middle-income countries (5). In 2010, an estimated 270 000 deaths globally were attributable to congenital anomalies during the first 28 days of life, with NTDs being one of the most serious and most common of these anomalies (5). In 2005, the rates of spina bifida and anencephaly in the United States were 17.96 per 100 000 live births and 11.11 per 100 000 live births, respectively. (10)

Minimum effective dose of folic acid for the prevention of NTDs

Folic acid supplementation before conception and during the first trimester of pregnancy is one of the few public health interventions effective in reducing the risk of NTD (1). The 400 µg (0.4 mg) dose of folic acid alone (without iron or as part of a multiple micronutrient
supplement) is missing in several National Essential Medicines List (NEML), especially in low and middle income countries who use the WHO EML/EMLc to build their respective national formularies (11). This lack of clarity on the dose of folic acid supplementation and the availability of several doses in the market leads to different approaches among countries where folic acid intake recommendations can range from 400 μg (0.4 mg) or lower to 5000 μg (5 mg) or higher (11). It is also important to recommend the effective minimum dose, since the effect on health of high folic acid doses it is still not clear for certain population groups. (12)(13)(14)(15)

A reliable biomarker, such as red blood cell folate is useful for evaluating the potential public health impact of prevention programmes for neural tube defects and efforts have recently been made to estimate the red blood cell folate concentration associated with the greatest reduction in NTDs (16). Existing definitions of clinical folate deficiency (<340 nmol/L red blood cell folate) were not based on folate sufficiency for the prevention of neural tube defects and this current cut-off value was not associated with the prevention of neural tube defects (17) but with the prevention of megaloblastic anaemias. Therefore, the new recommendation on optimal blood folate concentrations for the prevention of neural tube defects in women of reproductive age to provide a population cut-off for this purpose (18). At the population level, red blood cell folate concentrations above 906 nmol/L (400 ng/mL) in women of reproductive age have been associated with the greatest reduction of NTD.

**Concomitant use of anti-folate anti-malaria drugs and folic acid**

In areas where malaria remains the leading cause of mortality and morbidity in children and pregnant women, there are challenges with the use of folic acid supplementation for the prevention of NTDs and antifolate antimalarial drugs, such as sulfadoxine-pyrimethamine (19). When high doses of folic acid, such as 5000 μg (5 mg), are used concomitantly with antifolate antimalarial drugs there may be a decrease in the malaria treatment efficacy, exposing pregnant women to malaria. Thus, there is concern that this strategy may not be appropriate and that the dose of folic acid should be as low as possible to not interfere with the antifolate antimalarial drugs (20). Therefore, a coherent public health approach is needed in order to promote a minimal effective dose of folic acid that would avoid a risk of interfering with antimalaria drugs in malaria endemic areas.

### 2. Current public health interventions

In an effort to address the emerging importance of birth defect morbidity and mortality, the Sixty-third World Health Assembly adopted on 21 May 2010 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 (21).
In support of public health efforts to prevent NTDs, increased folic acid intake has been promoted through daily or intermittent supplementation among women of childbearing age as well as the fortification of staple foods with folic acid before conception (8) (22) (23).

In malaria-endemic regions, mainly in sub-Saharan Africa, WHO recommends the implementation of an intermittent preventive treatment during pregnancy (IPTp), as part as a focused antenatal care package, using the antifolate drug sulfadoxine-pyrimethamine with a lower 400 µg (0.4 mg) folic acid concomitant intake (9).

3. Proposed public health intervention

WHO recommends all women, from the moment they being trying to conceive until 12 weeks of gestation, take a daily supplement of 400 µg (0.4 mg) folic acid to prevent NTDs (occurring NTDs) and women who have previously had a fetus diagnosed as affected by a NTD, or have given birth to a baby with a NTD, should take a daily supplement of 5000 µg (5 mg) folic acid to prevent recurrent NTDs (1). Currently the Essential Medicine’s List includes folic acid with iron (400 µg folic acid plus 60 mg elemental iron) and high dose folic acid of 1000 µg (1 mg) and 5000 µg (5 mg), but does not include the 400 µg (0.4 mg) folic acid dose without iron. For women who have difficulties in taking iron supplements or who choose not to, it is important they have the option to consume folic acid alone in the recommended dose for the prevention of NTDs. Additionally, the availability of the 400 µg (0.4 mg) dose of folic acid is needed in malaria-endemic areas for women of childbearing age in the general population, in place of the higher doses of supplemental folic acid as to not, affect the preventive and therapeutic activity of the antimalarial drug sulfadoxine-pyrimethamine (9).

III. Methods

1. Methods for assessment of dosing, efficacy and safety

Cochrane review: Effects and safety of periconceptional folate supplementation for preventing birth defects

A Cochrane review (De-Regil 2010) was commissioned to update and extend the evidence on the efficacy and safety of periconceptional folate supplementation for preventing birth defects (24). The review assessed both randomized and quasi-randomized trials including cluster-randomized trials if they were otherwise eligible. The participants were women who became pregnant or were 12 or less weeks pregnant at the time of the intervention (24). The interventions assessed were daily folic acid supplementation (with or without other micronutrients) compared with a placebo, no intervention or other micronutrients without folic acid (24). Studies that were included offered supplementation in the periconceptional period and during early pregnancy. Only interventions related with the supplementation of
folic acid alone compared versus no treatment or placebo are presented in this document. Two studies were found that met these inclusion criteria.

**Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects**
The first study on the *Minimum effective dose of folic acid for food fortification to prevent neural-tube defects* was a double-blind, randomised, controlled trial of several folic acid doses (100 µg, 200 µg, or 400 µg) provided in a tablet compared to placebo (25). The relation between RBC folate and the occurrence of NTD had been established in previous studies and was used to find the lowest folic acid dose needed to reach the protective concentration of RBC folate (26). Women (n=95) with RBC folate values between 150 µg/L and 400µg/L were eligible to take part in the trial and completed the six months study. Participants were randomly assigned tablets containing no folic acid, 100 µg, 200 µg, or 400 µg folic acid, based on a daily supplementation (25).

**Study 2: Prevention of neural–tube defects with folic acid in China**
The second study on the *Prevention of neural–tube defects with folic acid in China* was a cohort study done as part as a public health campaign conducted from 1993 to 1995 in one northern province (with high rates of NTDs) and two southern provinces (with low rates of NTDs) of China (27). Pregnant women and women who were preparing for marriage (n=285,536) registered with a pregnancy-monitoring system that serves as the principal record of prenatal care and delivery in these three provinces. In total 130,142 women took folic acid at any time before or during pregnancy and 117,689 women did not take folic acid (27). This study evaluated the outcomes of NTD prevalence among women who were asked to take 400 µg of folic acid alone daily from the time of their premarital examination until the end of their first trimester of pregnancy. They were compared with women who did not take folic acid (27).

**Study 3: Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model**
An article on *Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model*, was recently published in July 2014 (17). This study was designed to determine the optimal population RBC folate concentration for the prevention of NTDSs by WHO using information in the published literature and individual level data from two studies conducted in China; a community intervention project that included 275 women treated with folic acid 400 µg (0.4 mg) daily (from previous study 2) and a randomized clinical trial of 400 µg (0.4 mg) folic acid supplementation in a subset of 371 Chinese women of reproductive age. The goal of the analysis was to estimate the association between a mother’s RBC folate concentration at the time of her fetus’s neural tube closure and the risk of a NTD in women consuming 400 µg folic acid supplementation daily (17).

---

3 Physical examination and laboratory test before marriage
Literature review: Antifolate antimalaric drugs: Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate

A literature review found seven studies that assessed the impact of folic acid supplementation on the efficacy of sulfadoxine-pyrimethamine, in women and children, of which three studies were related specifically to pregnant women (19). These three studies are summarized in Table 5 of Appendix A.

The review reports a study “A randomized controlled trial of folate supplementation when treating malaria in pregnancy with sulfadoxine-pyrimethamine” completed in 2006 that showed the effect of folate on sulfadoxine/pyrimethamine treatment failure in pregnant women (n=488) with a gestational age between 17 and 34 weeks and presenting with uncomplicated malaria (28). They were treated with sulfadoxine-pyrimethamine and iron supplementation, and were randomized into three arms: 5 mg folic acid (n=161), 400 µg folic acid (n=165) or placebo (n=162). After 14 days, all women received 5 mg folic acid daily and were followed up to day 28 (28).

Using the same study sample, a second study “Plasma folate level and high-dose folate supplementation predict sulfadoxine-pyrimethamine treatment failure in pregnant women in Western Kenya who have uncomplicated malaria” assessed the association between folate blood concentrations and sulfadoxine-pyrimethamine efficacy in 467 pregnant women. Plasma folate levels were determined at enrolment and after seven days (29).

A third study “Lack of inhibition of the antimalarial action of sulfadoxine-pyrimethamine by folic acid supplementation when used for intermittent preventive treatment in Gambian primigravidae” was reported on 1035 Gambian primigravidae who were randomized to receive either folic acid (500–1,500 µg/day) together with oral iron (n=522) or oral iron alone (n=513) for 14 days at the same time as they received IPTp with sulfadoxine-pyrimethamine (30).

2. Methods for the assessment of costs
For tablets containing 400 µg (0.4 mg) folic acid, the 2013 edition of the International Drug Price Indicator Guide, the UNICEF Supply Catalogue website as well as the NEMLs of several countries were searched (31) (32).

3. Methods for the assessment of current availability amongst Member States
A survey of NEMLs of 20 low, middle and high income countries was undertaken to determine availability of folic acid supplements (11).

4. Assessment of the evidence
Strength and quality of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (33). A table showing the characteristics of the studies with an evaluation of their quality is presented in Annex B. The studies presented in this document were all the studies found on the assessment of
the efficacy of 400 µg (0.4 mg) folic acid in the prevention of NTDs. Regarding the concomitant use of folic acid and sulfadoxine-pyrimethamine in pregnant women, no additional studies were found.

IV. Regulatory information on folic acid supplementation

Supplements of folic acid alone in doses of 1000 µg (1 mg) and 5000 µg (5 mg) and folic acid 400 µg in combination with 60 mg elemental iron are currently on the WHO EML for adults. Folic acid supplements are not reviewed for safety or efficacy and are not approved for sale as medications by the Stringent Regulatory Authorities (SRAs) in United States (Food and Drug Administration, FDA), Australia (Therapeutic Goods Administration, TGA) and the United Kingdom (Medicines and Healthcare products Regulatory Agency, MHRA) (34) (35) (36). Rather supplements are registered as food supplements and are held to good manufacturing practices for purities only (36). Therefore, no additional specific analysis of regulatory status of folic acid supplements was warranted. However, manufacturers of supplements must be registered entities and certified to adhere to good manufacturing practices (37).

V. Analysis of costs

Folic acid supplements are currently available in 400 µg (0.4 mg) doses in either high income countries (HIC) or middle and low income countries (MLIC) for this intervention. Supplements containing 400 µg (0.4 mg) folic acid by itself (without other micronutrients) have been found in Chile, Colombia, Costa Rica, Guatemala, France, Mexico, Spain, Switzerland (Table 2), the United Kingdom of Great Britain and Northern Ireland and Uruguay, and is likely commercialized in other countries (11) (38) (39) (40). Furthermore, supplements containing 500 µg (0.5 mg) folic acid have been found in Australia and Peru (11) (35). The most recent International Drug Price Indicator Guide 2013 and the UNICEF Supply Catalogue were reviewed supplements containing 400 µg (0.4 mg) folic acid alone (without other micronutrients) were not found (32). Only single nutrient formulations containing higher amounts of folic acid and the combination of 60 mg elemental iron plus 400 µg (0.4 mg) folic acid tablets were found (Table 1) (10).

<table>
<thead>
<tr>
<th>Source</th>
<th>Compound</th>
<th>Form</th>
<th>Cost per tablet (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH 2011</td>
<td>Folic Acid</td>
<td>5mg tab</td>
<td>0.0023 (median)</td>
</tr>
<tr>
<td>UNICEF</td>
<td>Folic acid</td>
<td>5 mg tabs</td>
<td>0.00429</td>
</tr>
<tr>
<td></td>
<td>Folic Acid</td>
<td>1 mg tab</td>
<td>0.0277 (median)</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------</td>
<td>------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>MSH 2011</td>
<td>Ferrous salt + folic acid</td>
<td>60 mg elemental iron + 0.4 mg folic acid tab</td>
<td>0.0029 (median)</td>
</tr>
<tr>
<td>UNICEF</td>
<td>Ferrous fumarate + folic acid</td>
<td>60 mg elemental iron + 0.4 mg folic acid tab</td>
<td>0.00507</td>
</tr>
</tbody>
</table>

Table 2: Price of supplements containing 400 µg folic acid alone in Switzerland

<table>
<thead>
<tr>
<th>Price (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 30 tablets: 11.48</td>
</tr>
<tr>
<td>• 90 tablets: 29.77</td>
</tr>
<tr>
<td>• 28 tablets: 10.53</td>
</tr>
<tr>
<td>• 98 tablets: 27.31</td>
</tr>
</tbody>
</table>

Average per tablet/capsule: 0.337

**VI. Current NEML availability evaluation**

NEMLs of 20 LMICs were reviewed to determine the current availability of folic acid supplements containing 400 µg per tablet/capsule (11).

The table below shows that the majority of national NEMLs contain the 400 µg (0.4 mg) dose of folic acid only in combination with iron the 5000 µg (5 mg) dose of folic acid (without other micronutrients). None of the NEMLs surveyed contained 400 µg (0.4 mg) folic acid alone (without other micronutrients). This was expected since this formulation is not currently on the EML or EMLc, and most LMICs use the model WHO EML/EMLc to build their respective national formularies (11). However, supplements containing 400 µg (0.4 mg) folic acid alone was found in the NEML of Switzerland, France, Mexico and it is probably present in other countries (11) (39) (40). Peru has registered a 500 µg (0.5 mg) dose of folic acid in its NEML (11).

---

Supplements containing 400 µg folic acid are in the Swiss NEML and are reimbursed by Swiss health insurance.
<table>
<thead>
<tr>
<th>#</th>
<th>country</th>
<th>folic acid 400 µg</th>
<th>folic acid 1 mg</th>
<th>folic acid 5 mg</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Angola</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>250 µg folic acid was found in combination with 200 mg ferrous sulfate.</td>
</tr>
<tr>
<td>2</td>
<td>Bangladesh</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>Folic acid tablet found, unknown strength.</td>
</tr>
<tr>
<td>3</td>
<td>Bhutan</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>400 µg folic acid was found in combination with 60 mg ferrous sulfate.</td>
</tr>
<tr>
<td>4</td>
<td>Central African Republic</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>Folic acid tablet found, unknown strength.</td>
</tr>
<tr>
<td>5</td>
<td>China</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>Folic acid tablet found, unknown strength.</td>
</tr>
<tr>
<td>6</td>
<td>Democratic Republic of Congo</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ecuador</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>Folic acid – the NEML listed tablet strength range from 500 µg to 5 mg.</td>
</tr>
<tr>
<td>8</td>
<td>Fiji</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ghana</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>India</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>100 µg folic acid found in combination with ferrous salt liquid and tablet.</td>
</tr>
<tr>
<td>11</td>
<td>Honduras</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Lesotho</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Malaysia</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Namibia</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Oman</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Pakistan</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>400 µg folic acid was found in combination with 60 mg iron.</td>
</tr>
<tr>
<td>17</td>
<td>Rwanda</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>400 µg folic acid was found in combination with 60 mg iron.</td>
</tr>
<tr>
<td>18</td>
<td>Senegal</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Thailand</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Vanuatu</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
VII. Evidence on dosing, efficacy and safety of folic acid supplementation

Cochrane review outcomes: Effects and safety of periconceptional folate supplementation for preventing birth defects
The Cochrane group undertook several comparisons of evidence but only the comparison between supplementation with folic acid alone and no treatment/placebo was analyzed (24). Only the results for the effect of supplementation with folic acid alone (compared to no treatment/placebo) on the prevalence of NTDs and RBC folate concentrations are presented here.

Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects outcomes:
The effect of supplementation in women with 100 µg, 200 µg or 400 µg folic acid provided in tablet form on RBC folate concentrations and the prevalence of NTDs was assessed in this study (25). The results of this study are summarized in Table 1 of Appendix A.

Study 2: Prevention of neural–tube defects with folic acid in China outcomes:
The comparison undertaken in this trial was based on NTDs prevalence among the 130,142 women who had taken the daily 0.4mg folic acid versus 117,689 women who had not taken folic acid (27). The results of this study are summarized in Table 2 of Appendix A.

Study 3: Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model outcomes:
A Bayesian model was developed to statistically estimate the association between RBC folate concentrations at the time of neural tube closure (embryologic day 28) and risk of NTDs using existing data sources (17). The authors analysed data from two studies in non-fortified populations from two regions in China: a community intervention study testing folic acid supplementation to prevent neural tube defects that included 275 cases in 247,831 women treated with folic acid 400 µg daily; and a population based randomised trial to evaluate the effects of folic acid supplementation in a subset of 371 women of reproductive age who provided RBC folate concentrations after treatment with 400 µg daily. The authors also compared the results of this analysis with another study in the Irish population, the only study in which the association between measured RBC folate concentrations in pregnancy and the risk of NTDs was directly assessed (26) (41). The results of this study are summarized in Table 3 and 4 of Appendix A.
A literature review found seven studies that assessed the impact of folic acid supplementation on the sulfadoxine-pyrimethamine treatment failure in women and children, of which three studies were related specifically to pregnant women (19). These three studies are summarized below and in Table 4 of Appendix A.

The first study was a randomized, double-blind, placebo-controlled trial of pregnant women (n=488) with a gestational age between 17 and 34 weeks presenting with uncomplicated malaria parasitemia (28). All women received sulfadoxine-pyrimethamine and iron supplementation and were randomized to 400 µg folic acid, 5 mg folic acid or placebo daily for 14 days (28).

The second study used samples from study 2 to assess plasma folate concentrations and sulfadoxine-pyrimethamine drug efficacy at baseline and day 7 after treatment (29). Total plasma folate levels were comparable in all groups at enrolment.

In the third study, primigravida women (n=1035) with a gestational age of more than 15 weeks were randomized to receive either folic acid (500-1500 µg per day) together with oral iron (n=522) or iron alone (n=513) for 14 days at the same time as they received sulfadoxine-pyrimethamine (30). This study assessed the effect of folic acid supplementation on the sulfadoxine-pyrimethamine drug efficacy.
1. Quality of the evidence

**Cochrane review: Effects and safety of periconceptional folate supplementation for preventing birth defects**

The trials reporting outcomes for NTD prevalence and RBC folate concentrations in this review were rated moderate to high quality in terms of allocation concealment and blinding. Pooling results in a meta-analysis resulted in fairly low levels of between-study heterogeneity (24). Note that the GRADE Summary of Findings table for the outcomes of NTD prevalence and RBC folate concentrations in Appendix C does not include the two studies in the Cochrane review that provided 3600 µg (3.6 mg) and 4000 µg (4 mg) of folic acid due to the specific evaluation of the effect of 400 µg (0.4 mg) folic acid versus placebo.

**Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects**

This study assessed the effects of folic acid supplementation on RBC folate concentrations. The quality of the evidence for this outcome was considered very low due to their being only one study with few participants (n=45), leading to the risk of imprecise results; inadequate loss of follow up and others study limitations (the primary analysis was by intention to treat and there was no control on participant’s risk of additional folic acid intake). As there is only one trial it was not possible to assess the consistency of the findings, however, consistency is supported by indirect evidence modelling (Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model). See table of quality assessment of evidence for more details in Appendix B. This study reported on the additional outcome of NTD prevalence, but only as an “estimated NTD risk per 1000 births” and not as a direct outcome of the study, therefore the results were not included in the GRADE Summary of Findings table for NTD prevalence. However, the GRADE Summary of Findings table on RBC concentration is presented in Appendix C.

**Study 2: Prevention of neural–tube defects with folic acid in China**

This study assessed the effects of folic acid supplementation on the prevalence of NTDs. The quality of evidence was considered low given that there is only one study reporting this outcome, and although this was a large study, it was a cohort study and not a randomized controlled trial. As there is only one trial it was not possible to assess the consistency of the findings, however, consistency is supported by indirect evidence modelling (Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model). The GRADE Summary of Findings table on the prevalence of NTDs is presented in Appendix C. See table of quality assessment of evidence for more details in Appendix B.
Study 3: Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model
This was a statistical analysis of existing data and not a trial itself and was therefore not included in a GRADE Summary of Findings table. However, these findings are important indirect that helps understand the potential relationship between RBC folate concentrations and NTD prevalence.

Literature review: Antifolate antimalaric drugs: Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate
A literature review found seven studies that assessed the impact of folic acid supplementation on the outcome of sulfadoxine-pyrimethamine treatment failure in women and children, of which three studies were related specifically to pregnant women (19). Of these three studies, two studies contributed to data on the outcome of sulfadoxine-pyrimethamine treatment failure: 400 µg vs 5mg of folic acid supplementation and 0.5 mg-1.5 mg vs no placebo. The overall quality of evidence for this outcome was considered moderate due to indirectness in the study population and the fact that it was a single study (see table of quality assessment of evidence for more details in Appendix B). The GRADE Summary of Findings table is presented in Appendix C.

2. Summary of the evidence

Cochrane review: Effects and safety of periconceptional folate supplementation for preventing birth defects
This systematic review included two trials with 299 women. The prevalence of NTDs was lower in the women who received folic acid alone compared with women who received no treatment or placebo (RR 0.32, 95% CI 0.08 to 1.34) (24). The dose of folic acid was 360 µg (0.36 mg) in one trial and 4000 µg (4 mg) of folic acid in the other. The intervention was provided daily and initiated before pregnancy, continuing throughout the first trimester. In the trial providing 360 µg (0.36 mg)folic acid, the difference between the placebo group was not significant for the prevalence of NTDs (RR 0.12, 95% CI 0.01 to 2.29) (24). However, in both trials women had a history of previous pregnancy affected by a NTD for whom WHO recommends 5000 µg (5 mg) folic acid daily for the prevention of recurrent NTDs (1).

Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects
The effect of supplementation in women with 100 µg, 200 µg or 400 µg folic acid provided in tablet form on RBC folate concentrations and the prevalence of NTDs was assessed in this
study (25). All groups receiving folic acid showed significant increases in RBC folate while the placebo group showed no significant change compared to baseline. Supplementation with 400 µg folic acid daily lead to a higher RBC folate concentration compared to the placebo group (MD 260 µg/L; 95% CI 103.81 to 416.19). The median incremental changes and median post-treatment RBC concentrations are shown below. See Table 1 in Appendix A for more details.

- 100 µg folic acid/day: 67 µg/L (95% CI 43–120) and 375 µg/L (95% CI 354–444);
- 200 µg folic acid/day: 130 µg/L (95% CI 108–184) and 475 µg/L (95% CI 432–503);
- 400 µg folic acid/day: 200 µg/L (95% CI 125–312) and 571 µg/L (95% CI 481–654);

Previous work showed that women with RBC folate values below 150 µg/L had a risk of a NTD of 6.6 per 1000 births, whereas when RBC folate was higher than 400 µg/L the risk of an NTD was only 0.8 per 1000 births with an overall population risk of 1.9 per 1000 births (41). Based on this estimation of the risk of NTD, the above increases in RBC folate of the current Study 1, would therefore result in a 22% reduction in NTD risk in the 100 µg/day group, a 41% reduction in the 200 µg/day group, and a 47% reduction in the 400 µg/day group (25).

Study 2: Prevention of neural–tube defects with folic acid in China
This was a cohort study conducted in China that evaluated the prevalence of NTDs in foetuses and infants born to women taking either 400 µg (0.4 mg) folic acid (n=130,142) or who received no treatment (n=117,689) at any time before or during pregnancy. Supplementation with 400 µg (0.4 mg) folic acid daily lead to a 79% reduction in the risk of having a fetus or infant with a NTD in the northern region of China where the high baseline risk of NTDs was high (RR 0.21, 95% CI 0.10 to 0.43) and a 41% reduction in risk in the southern region of China where the baseline risk of NTDs was low (RR 0.59, 95% CI 0.36 to 0.97). Globally, this would translate to a 50% reduction in risk (RR 0.5, 95% CI 0.39 to 0.64) (27) (see Table 2 in Appendix A for more details). A greater reduction in risk of NTDs was observed in a subgroup of women who took folic acid with a good compliance (>80% of the time) compared to women who did not take folic acid. In this subgroup of women, there was a 85% reduction in the risk of NTDs in the northern region of China (RR 0.15, 95% CI 0.06 to 0.38) and a 40% reduction in the risk of NTDs in the southern region of China (RR 0.60, 95% CI 0.36 to 1.02) (27).

Study 3: Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model
This is a report of results from a Bayesian model developed to statistically estimate the association between RBC folate concentrations at the time of neural tube closure (embryologic day 28) and risk of NTDs using existing data sources (17). The studies included participants in a community intervention project to prevent NTDs with 400 µg (0.4
mg) folic acid daily (included 275 cases in 247,831 women) and participants in a population based randomized trial to evaluate the effect of supplementation with 400 µg folic acid on RBC folate concentrations among 371 women of reproductive age (17). The risk of NTDs was found to be high at the lowest estimated RBC folate concentrations (e.g. 25.4 NTDs per 10,000 births at 500 nmol/L) and decreased as the estimated RBC folate concentration increased. NTD risk was markedly reduced at an estimated RBC folate concentration of ~1000 nmol/L (e.g. 6 NTDs per 10,000 births at 1180 nmol/L). These results indicate that a RBC folate concentration about 1000-1300 nmol/L might achieve optimal prevention of folate sensitive NTDs, with a resulting overall risk of neural tube defects of about 6 per 10,000. These results are in agreement with results of a previous study in the Irish population, which included 84 NTD cases, reporting a RBC folate threshold of 906 nmol/L (41). It was noted that these estimated risks of NTDs at various estimated RBC folate concentrations were consistent with the prevalence of NTDs in the United States of America before and after folic acid fortification of food. From this model, it was concluded that a population threshold for preventing NTDs could be defined as a population RBC folate concentration of ~1000 nmol/L. See Table 3 in Appendix A for more details.

In this study, the amount of folic acid intake necessary to achieve the effective RBC concentrations was not determinate. However if we compare it with the “Minimum effective dose of folic acid for food fortification to prevent neural-tube defects” (Study 1), we observe an association between folic acid intake, RBC folate concentrations and risk of NTDs. In Study 1, the median post-treatment RBC concentration after a daily 400 µg folic acid supplementation was 571 µg/L (equivalent to 1293.61 nmol/L). This RBC concentration is considered to be optimal for prevention of NTDs (about 1000-1300 nmol/L) in the current study (Study 3) (17). These two studies are also concordant with the results of a previous study in the Irish population where the optimal RBC concentration was found to be 906 nmol/L (41).

The fact that these three distinct studies produced comparable estimates of the associations between the dose of folic acid intake and the preventive red blood cell folate concentration provides evidence that 400 µg is likely to be the minimum effective dose for the prevention of NTDs (17) (16) (41).

**Literature review: Antifolate antimalaric drugs: Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate**

This review of the literature found 7 studies that assessed the impact of folic acid supplementation on the efficacy of sulfadoxine-pyrimethamine in women and children, of which three studies were related specifically to pregnant women (19). These three studies are summarized below and in Table 4 of Appendix A.

The first study was a randomized, double-blind, placebo-controlled trial of pregnant women (n=488) with a gestational age between 17 and 34 weeks presenting with uncomplicated
malaria parasitemia (28). All women received sulfadoxine-pyrimethamine and iron supplementation and were randomized to 400 µg folic acid (n=165), 5 mg folic acid (n=161) or placebo (n=162) daily for 14 days. The proportion of treatment failure at day 14 was 13.9% (19/137) in the placebo group, 14.5% (20/138) in the 400 µg (0.4 mg) folic acid group (adjusted hazard ratio [AHR], 1.07; 98.7% CI, 0.48 to 2.37; p = 0.8), and 27.1% (38/140) in the 5 mg folic acid group (AHR, 2.19; 98.7% CI, 1.09 to 4.40; p = 0.005) (28). The rate treatment failure in the placebo group and 400 µg (0.4 mg) folic acid groups were similar (13.9 and 14.5%, respectively, P<0.05), while the failure rate in the 5 mg folic acid group was significantly higher than in the placebo group (27.1% versus 13.9%, respectively, P=0.005) (28). Authors concluded that concomitant use of 5 mg folic acid supplementation compromises the efficacy of sulfadoxine-pyrimethamine for the treatment of uncomplicated malaria in pregnant women (28). See Table 4 in Appendix A for more details.

The second study used samples from study 1 to assess plasma folate concentrations at baseline and day 7 after treatment (29). Total plasma folate levels were comparable in all groups at enrolment. After seven days of folic acid supplementation, the 5 mg folic acid group had a significantly higher median increase in plasma folate level (9.2 ng/mL increase) than the 400 µg folic acid group increase (2.7 ng/mL) (29). At this point, 100 women (2 in the placebo group, 23 in the 400 µg (0.4 mg) folic acid group and 75 in the 5 mg folic acid group) had folate levels exceeding 15.4 ng/mL, and 48 of these (38 in the 5 mg folic acid group and 10 in the 400 µg (0.4 mg) folic acid group) failed the sulfadoxine-pyrimethamine treatment (29). These two studies suggested that 400 µg (0.4 mg) of folic acid lead to a lower rate of sulfadoxine-pyrimethamine treatment failure than high doses (5mg) of folic acid (RR 0.63, 95% CI 0.44 to 0.88) (29).

In the third study, primigravida women (n=1035) with a gestational age of more than 15 weeks were randomized to receive either folic acid (500-1500 µg per day) together with oral iron (n=522) or iron alone (n=513) for 14 days at the same time as they received sulfadoxine-pyrimethamine (30). Prevalences of Plasmodium falciparum parasitemia on day 14 after treatment were similar in both groups: 5.7% (26 of 458) in the iron plus folic acid group and 4.9% (22 of 446) in the iron alone group (risk difference 0.74%, 95% CI −2.2% to 3.7%) (30). Parasitologic cure was observed in 116 (91%) of 128 of women who were parasitemic on presentation and who received iron and folic acid and in 122 (92%) of 133 women who received iron alone (difference 1.1%, 95% CI −5.6% to 8.0%) (30). The rate of treatment failure was similar between the groups treated with 500-1500 µg folic acid and the group treated only with iron (5.7% versus 4.9%, P>.0.05) (30). The results of this study suggested that in an area of low sulfadoxine-pyrimethamine resistance, administration of folic acid to pregnant women in a dose of 500–1,500 µg/day will not interfere with the protective effect of sulfadoxine-pyrimethamine (30).
VIII. Recommendations from the studies and comments

Cochrane Review: Effects and safety of periconceptional folate supplementation for preventing birth defects
This systematic review acknowledges that there is a lack of certainty on the minimum dose of supplemental folic acid that is effective for reducing the occurrence of NTDs. The authors concluded that this wide response to supplementation may be determined by the baseline blood folate concentrations in each population (24).

Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects
The study estimates that delivery of 400 µg (0.4 mg) folic acid daily would yield reductions in NTD incidence of 47%. Overall, between 50% and 70% of NTD is thought to be preventable by folate; this reduction is therefore substantial. If the average value of RBC folate in the population could be increased such that all pregnant women had values above 400 µg/L, the risk of NTD could be reduced by almost 60% (25).

Study 2: Prevention of neural–tube defects with folic acid in China
The conclusion of this study was that daily ingestion of 400 µg folic acid alone during the periconceptional period reduces a woman’s risk of having a fetus or infant with a NTD in areas of both high and low frequency (27). The preventive effect in the southern region of China, in which the base-line rate is similar to that in the United States and elsewhere, suggests that 400 µg folic acid taken daily may help reduce the rate of the first occurrence of NTDs in many parts of the world (27).

Study 3: Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model
This analysis indicated that a RBC folate concentration about 1000-1300 nmol/L might achieve optimal prevention of folate sensitive NTDs, with a resulting overall risk of neural tube defects of about 6 per 10000. The exact amount of folic acid intake for any person or population necessary to achieve the effective RBC folate concentrations was not determined but the analysis suggested that a folic acid dosage of less than 400 µg/day (but greater than 100 µg/day) supplementation, starting more than six months before pregnancy, could be sufficient to prevent most folate sensitive NTDs in many populations of women (17).

Literature review: Antifolate antimalaric drugs: Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate
This review indicates that supplementation with folic acid can make ineffective the efficacy of sulfadoxine-pyrimethamine as part of normal treatment for malaria. However, this
negation is dependent upon the dose of folic acid. Supplementation with 5000 µg (5 mg) folic acid daily was found to significantly decrease the efficacy of sulfadoxine-pyrimethamine (19). On the other hand, doses from 500 µg (0.5 mg) to 1500 µg (1.5 mg) of folic acid did not affect sulfadoxine-pyrimethamine efficacy. Folic acid at doses of 400 µg (0.4 mg) is the lower and more suitable dose for NTD prevention in the context of malaria prevention/treatment (19).

X. Summary and recommendations

WHO currently recommends all women, from the moment they being trying to conceive until 12 weeks of gestation, take a daily supplement of 400 µg (0.4 mg) folic acid to prevent NTDs (occurrent NTDs) and women who have previously had a fetus diagnosed as affected by a NTD, or have given birth to a baby with a NTD, should take a daily supplement of 5mg folic acid to prevent recurrent NTDs (1). Currently the Essential Medicine’s List includes folic acid with iron (400 µg folic acid plus 60 mg iron) and high dose folic acid (1 mg and 5 mg), but does not include the 400 µg folic acid dose without iron. For women who have difficulties in taking iron supplements, those who choose not to, or where iron is not recommended for other reasons it is important they have the option to consume folic acid alone in the recommended dose for the prevention of occurrent NTDs.

The recommendations for changes to the EML Section 27 – Vitamins and minerals, are as follows:

2. Add 400 µg (0.4 mg) folic acid tablet/capsule formulation for the prevention of NTD’s during periconceptional period.
   a. Dose
      i. 400 µg (0.4 mg) folic acid.
   b. Frequency and duration of the supplementation
      iv. One tablet per day
      v. Start two months before the planned pregnancy and continue 12 weeks after the pregnancy
      vi. This dose does not concern pregnant women who have previously had a baby with NTD or who have diabetes or who are under anticonvulsant treatment. A higher dose as 5000 µg (5 mg) is recommended for these cases.
I. References


18. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. 2012.


20. The Roll Back Malaria Partnership (RBM); Malaria in Pregnancy Working Group Consensus Statement. Ensuring pregnant women get enough Folic acid and effective treatment for malaria. Geneva: pending.


### Appendix A: Results of the studies

**Table 1**: Pretreatment and post-treatment red-cell folate (RCF) and estimated NTD risk per 1000 births from *Minimum effective dose of folic acid for food fortification to prevent neural-tube defects* study.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>N</th>
<th>Median (95% CI) RBC (µg/L)</th>
<th>Median (95% CI) incremental change in RBC (µg/L)</th>
<th>Estimated NTD risk per 1000 births</th>
<th>Estimated Reduction in Risk of NTD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>After Trial</td>
<td>Initial</td>
<td>After Trial</td>
</tr>
<tr>
<td>Placebo</td>
<td>19</td>
<td>335 (306-388)</td>
<td>311 (171-343)</td>
<td>-12 (-83 to 4)</td>
<td>1.6</td>
</tr>
<tr>
<td>100 µg/day</td>
<td>22</td>
<td>309 (291-371)</td>
<td>375 (354-444)</td>
<td>67 (43-120)</td>
<td>1.8</td>
</tr>
<tr>
<td>200 µg/day</td>
<td>28</td>
<td>311 (291-337)</td>
<td>475 (432-503)</td>
<td>130 (108-184)</td>
<td>1.7</td>
</tr>
<tr>
<td>400 µg/day</td>
<td>26</td>
<td>350 (319-399)</td>
<td>571 (481-654)</td>
<td>200 (125-312)</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 2: Rates and risks of NTD according to the use of folic acid pills from Prevention of neural-tube defects with folic acid in China study.

<table>
<thead>
<tr>
<th></th>
<th>Northern region</th>
<th></th>
<th>Southern region</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Rate of NTD/1000</td>
<td>Risk Ratio</td>
<td>Reduction in Risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnancies</td>
<td>(95% CI)</td>
<td>(95%)</td>
</tr>
<tr>
<td>Control group</td>
<td>13 369</td>
<td>6.5</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Intervention group</td>
<td>18 591</td>
<td>1.3</td>
<td>0.21 (0.13-0.32)</td>
<td>79 (67-87)</td>
</tr>
</tbody>
</table>

Table 3: Estimated red blood cell folate concentrations associated with specified levels of risk of NTDs among participants in Community Intervention Project from Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model study.

<table>
<thead>
<tr>
<th>Neural tube defect risk per 10 000 births</th>
<th>Red blood cell folate concentration (nmol/L)</th>
<th>95% uncertainty interval†</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>360</td>
<td>420 to 449</td>
</tr>
<tr>
<td>20</td>
<td>560</td>
<td>510 to 640</td>
</tr>
<tr>
<td>10</td>
<td>870</td>
<td>700 to 960</td>
</tr>
<tr>
<td>5</td>
<td>1180</td>
<td>1150 to 1340</td>
</tr>
<tr>
<td>5</td>
<td>1310</td>
<td>1250 to 1520</td>
</tr>
</tbody>
</table>

Estimates were derived using estimated parameters of neural tube defect risk model; details on model, underlying assumptions, and methods used to develop estimates are provided in supplementary material.

*Median (rounded to nearest 10) of posterior distribution for possible values of red blood cell folate concentration associated with specified level of neural tube defect risk.

†Lower value of this interval is 1.5th centile of posterior distribution for possible values of red blood cell folate concentration associated with specified level of neural tube defect risk; upper value is 97.5th centile of distribution.
Table 4: Estimated risk of neural tube defect per 10 000 births for specified red blood cell folate concentrations among participants in Community Intervention Project from Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model study.

<table>
<thead>
<tr>
<th>Red blood cell folate concentration (nmol/L)</th>
<th>Neural tube defect risk per 10 000 births*</th>
<th>95% uncertainty interval†</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>48.8</td>
<td>3.2 to 65.6</td>
</tr>
<tr>
<td>500</td>
<td>25.4</td>
<td>21.8 to 31.2</td>
</tr>
<tr>
<td>700</td>
<td>14.4</td>
<td>11.2 to 16.8</td>
</tr>
<tr>
<td>800</td>
<td>11.8</td>
<td>6.8 to 12.4</td>
</tr>
<tr>
<td>900</td>
<td>9.4</td>
<td>7.9 to 11.0</td>
</tr>
<tr>
<td>1000</td>
<td>7.9</td>
<td>5.5 to 9.3</td>
</tr>
<tr>
<td>1100</td>
<td>6.7</td>
<td>5.5 tol.1</td>
</tr>
<tr>
<td>1200</td>
<td>5.8</td>
<td>4.6 to 7.1</td>
</tr>
<tr>
<td>1300</td>
<td>5.1</td>
<td>4.0 to 6.3</td>
</tr>
<tr>
<td>1400</td>
<td>4.6</td>
<td>3.4 to 6.6</td>
</tr>
</tbody>
</table>

Estimates were derived using estimated parameters of neural tube defect risk model; details on model, underlying assumptions, and methods used to develop estimates are provided in supplementary material. *Median of posterior distribution of possible values for neural tube defect risk associated with specified red blood cell folate concentration. †Lower value of interval is 2.5th centile of posterior distribution for possible values of neural tube defect risk associated with specified red blood cell folate concentration; upper value is 9.5th centile of distribution.

Table 5: Summary of studies carried out in Africa in which the efficacy of antimalarials (SP) was tested in the context of FA supplementation in pregnant women suffering from malaria from Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate article.

<table>
<thead>
<tr>
<th>Country</th>
<th>Study population and length of the intervention</th>
<th>Folic acid supplementation with the number of participants</th>
<th>Rates or events of failure of SP efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gambia (2002-03)</td>
<td>Primigravidae at gestational age &gt;15 weeks (n=1035) 14 days of treatment</td>
<td>0.5mg -1.5mg of FA (n=458) Iron (n=446)</td>
<td>N= 26 5.7% (P&gt;0.05) N= 22 4.9 % (P&gt;0.05)</td>
</tr>
<tr>
<td>Kenya (2003-05)</td>
<td>Pregnant women at gestational age 17–34 weeks (n=488) 14 days of treatment</td>
<td>5mg of FA (n=161) 0.4mg of FA (n=165) Placebo (n=162)</td>
<td>N=44 27.1% (P=0.05) N=24 14.5% (P&lt;0.05) N=23 13.9% (P&lt;0.05)</td>
</tr>
</tbody>
</table>
Appendix B: Characteristics of the studies and quality assessment of the evidence

Study 1: Minimum effective dose of folic acid for food fortification to prevent neural-tube defects(25)

| Methods | • Double-blind, randomised, controlled trial.  
|         | • Randomization: random number assignments with rank ordered red-cell-folate values to ensure a similar distribution of screening values among the four groups.  
|         | • Allocation concealment: participants were randomly assigned tablets containing no folic acid, or 100 µg, 200 µg, or 400 µg folic acid.  
|         | • Blinding: Colour-coded blue, green, yellow, and red tablet dispensers were situated in the hospital canteen. Each participant was asked to take one tablet from the assigned dispenser every day. All the tablets appeared identical and were taken with food. Samples were analysed by investigators unaware of supplement group.  
|         | • Loss to follow-up: 26 women out of 121 were excluded or withdrawn of the trial (21.5 %). They withdrew from the study women who stopped taking the tablets, decided to become pregnant, took non-study vitamin tablets, increased their dietary folate during the study period, or took medications that would interfere with folate status.  

| Participants | • Number: 121 women were randomly assigned supplements.  
|             | • Inclusion/ Exclusion criteria: Women with values between 150 µg/L and 400µg/L were eligible and were asked to take part in the trial. Women were excluded if they were currently pregnant, planning a pregnancy, or believed themselves to be at risk of pregnancy. Other exclusion criteria were current use of folic acid or vitamin B12 supplements, having had a child with an NTD, two or more consecutive miscarriages, previous gastrointestinal surgery, and current hepatic or gastrointestinal disease. There was a 7-day placebo run-in period before the trial began. Women who did not comply were excluded.  

| Interventions | • 121 women were randomly assigned placebo or 100 µg, 200 µg, or 400 µg daily folic acid.  

| Outcomes | • Pretreatment and post-treatment red blood cell folate (RBC) concentrations and estimated NTD risk per 1000 births.  

| Notes | • Compliance was reported as good |
The primary analysis was by intention to treat.
The groups (400 µg and placebo) assessed for RBC concentration in the Summary of Findings (Grade) included only 45 women.

<table>
<thead>
<tr>
<th>Risk of bias</th>
<th>Authors’ judgement</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>Yes</td>
<td>Randomisation was by random number assignments with rank ordered red-cell-folate values to ensure a similar distribution of screening values among the four groups.</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>Yes</td>
<td>Adequate</td>
</tr>
<tr>
<td>Blinding?</td>
<td>Yes</td>
<td>Adequate. Participant and provider blinded.</td>
</tr>
<tr>
<td>Incomplete outcome data addressed?</td>
<td>Yes</td>
<td>Inadequate: 21.5 %</td>
</tr>
</tbody>
</table>

**Factors lowering the quality of evidence**

**Study limitations**
The primary analysis was by intention to treat. No control on the feeding (risk of additional folic acid intake). The study withdrew 21.5 % of women who stopped taking the tablets, decided to become pregnant, took non-study vitamin tablets, increased their dietary folate during the study period, or took medications that would interfere with folate status.

→ Minor limitations

**Consistency**
Only one study: consistency is not applicable as a criterion.

**Directness**
Directness on RBC. Indirectness on the NTD risk because it is an estimation but not included in the Summary of Findings (GRADE).

**Imprecision**
The groups (400 µg and placebo) assessed for RBC concentration in the Summary of Findings (GRADE) included only 45 women.

→ Imprecision

**Reporting bias**
The only study found on the 400 µg folic acid supplementation effect on RBC concentration.

---

**Study 2: Prevention of neural–tube defects with folic acid in China (27)**

**Methods**

- **Observational cohort study**

- **Randomization:** All women who completed the premarital examination were asked to purchase pills containing 400 µg of folic acid and to take one of these pills every day through the end of the first trimester of pregnancy. Each bottle contained 31 pills and the women were asked to take one bottle of pills each month. The dates that the women started and stopped taking folic acid were recorded. At the end of each month, pills remaining in each bottle and the dates of all menstrual periods were recorded.
- **Blinding:** Single blind: three paediatricians, who were unaware of the women’s pill-taking status, independently reviewed the reports and photographs and assigned diagnostic codes, and a clinical geneticist validated the diagnoses.

- **Loss to follow-up:** Among the 285,536 women who registered, 277,287 were pregnant. After the exclusion of pregnant women who were lost to follow-up or those for whom the status of the fetus or infant with respect to a neural-tube defect was unknown, there were 247,831 pregnant women. In total, 29,456 women were lost to follow-up (10.6%).

**Participants**

- **Number:** 130,142 women who took folic acid at any time before or during pregnancy (intervention) and 117,689 women who had not taken folic acid (no intervention). Total: 247,831.

- **Inclusion/Exclusion criteria:** The cohort included all women whose fetuses or infants could be confirmed as either having or not having a neural-tube defect from both high and low rates of neural-tube defects regions.

- The birth defect surveillance system collected detailed data about infants and fetuses with external structural birth defects. Live-born infants with birth defects were included in the surveillance system if they had a gestational age of at least 20 weeks and had a birth defect that was diagnosed by 6 weeks of age.

**Interventions**

- All women who completed a premarital examination were asked to purchase pills containing 400 μg of folic acid and to take one of these pills every day through the end of the first trimester of pregnancy.

**Outcomes**

- The study identified infants with neural-tube defects through a birth defects surveillance system. The prevalence of NTDs was assessed.

**Notes**

- For each woman, the study computed compliance as the percentage of folic acid pills that were taken as compared with the number that could have been taken.

**Risk of bias**

<table>
<thead>
<tr>
<th>Description</th>
<th>Authors’ judgement</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blinding? All outcomes</td>
<td>Yes</td>
<td>Assessor blinded: single blind. Three paediatricians, who were unaware of the women’s pill-taking status, independently reviewed the reports and photographs and assigned diagnostic codes, and a clinical geneticist validated the diagnose.</td>
</tr>
<tr>
<td>Incomplete outcome data addressed? All outcomes</td>
<td>Yes</td>
<td>Adequate: 10.6%</td>
</tr>
</tbody>
</table>

**Factors lowering the quality of evidence**

Study limitations | There is a risk of bias because the selection of women who took folic acid
is not clear. There is only one study.

→ Minor limitations

Consistency

The results were similar across two different populations: an area of China with high rates of neural-tube defects (the northern region) and one with low rates (the southern region). It is not clear if the population who took folic acid was homogeneous: there is a lack of consistency in the selection of women who took folic acid.

→ Inconsistency

Directness

The study identified infants with neural-tube defects through a birth defects surveillance system.

Imprecision

130,142 women who took folic acid at any time before or during pregnancy (intervention) and 117,689 women who had not taken folic acid (no intervention). Total: 247 831.

Reporting bias

Only study found on the direct assessment of NTDs prevalence with 400 μg of folic acid supplementation.

**Literature review: Antifolate antimalaric drugs: Impact of folate supplementation on the efficacy of sulfadoxine/pyrimethamine in preventing malaria in pregnancy: the potential of 5-methyl-tetrahydrofolate**

1. **A randomized Controlled Trial of Folate Supplementation When Treating Malaria in Pregnancy with Sulfadoxine-Pyrimethamine.**(28)

**Methods**

- **Randomized, placebo-controlled, double-blind trial.**

- **Randomization:** One of the investigators generated a randomization list with a block size of 12 using the statistical program SAS (SAS system for Windows version 8; SAS, Cary, North Carolina, United States).

- **Allocation concealment:** All folic acid treatment and placebo tablets were prepared off site and were identical in appearance and taste (Laboratory and Allied). Medicine envelopes with 14 tablets of a treatment arm were prepacked and labelled with the arm by staffs who were not involved in randomization. The medicine envelopes were put in sealed, opaque envelopes with consecutive numbers according to the randomization list by an investigator.

- **Blinding:** A trained clinical officer or nurse randomized eligible women by assigning them the next envelope in order of enrolment. The envelope was opened by the participant, and the study arm was allocated by the study staff according to the arm indicated on the medicine envelope. All folic acid treatment and protocol tablets were prepared off site and were identical in appearance and taste (Laboratory and Allied). All study staff participants were blind to the treatment in each arm. The investigators remained blind until data cleaning, analysis, and quality control of the blood smears were completed.
**Loss to follow-up:** 73 women out of 488 were lost to follow-up (15%).

**Participants**
- The participants were 488 pregnant women presenting at their first antenatal visit with uncomplicated malaria parasitaemia (density of 500 parasites/l), a haemoglobin level higher than 7 g/dl, a gestational age between 17 and 34 weeks, and no history of antimalarial or FA use, or sulfa allergy. A total of 415 women completed the study.

**Interventions**
- All participants received SP and iron supplementation. They were randomized to the following arms: Folic acid 5 mg, Folic acid 0.4 mg, or placebo. After 14 days, all participants continued with FA 5 mg daily as per national guidelines. Participants were followed at days 2, 3, 7, 14, 21, and 28 or until treatment failure.

**Outcomes**
- The outcomes were SP failure rate and change in haemoglobin (note included in the Summary of Findings) at day 14.

**Notes**
- NA

<table>
<thead>
<tr>
<th>Risk of bias</th>
<th>Authors’ judgement</th>
<th>Description</th>
</tr>
</thead>
</table>
| Adequate sequence generation? | Yes | • Adequate. Randomization list with a statistical program.  
• The randomization procedures were appropriate and procedures were used to blind participants and researchers to the different interventions, therefore reducing the risk of bias. Since the trial had a placebo arm, it was possible to conclude that the lower dose of folate (0.4 mg) did not significantly affect efficacy of sulfadoxine-pyrimethamine as compared with placebo. |
| Allocation concealment? | Yes | Adequate. Identical in appearance and taste tablets and envelopes were used. |
| Blinding? All outcomes | Yes | Adequate. Participants and provider blinded. |
| Incomplete outcome data addressed? All outcomes | Yes | Adequate: 15% |

**Factors lowering the quality of evidence**

A limitation of the study is that the length of the intervention was short, since all women reverted to standard 5 mg folate after 14 days. It is therefore not clear whether a longer trial would have shown additional risks or benefits of the different doses of folate. Finally, PCR genotyping was not done on the parasites infecting women in the trial; this procedure could have distinguished between true treatment failures and new infections (but which would have been unlikely within 14 days). The
study analysed the data on an intention-to-treat basis using a pre-established analysis plan. Short duration of the study (only 14 days).

Minor Limitations

Consistency The results of this study are consistent with the results of the two other studies on SP/Folic acid concomitant use.

Directness The study measured the SP failure through assessment of malaria infection (parasiteamia). Indirectness of the population because women in the study were at gestational age between 17 and 34 weeks whereas the recommendation is for women who are planning a pregnancy until 12 weeks after conception.

Indirectness

Imprecision The participants were 488 pregnant women.

Reporting bias One of the 3 studies on folic acid supplementation and concomitant use of SP.

2. Lack of inhibition of the antimalarial action of sulfadoxine-pyrimethamine by folic acid supplementation when used for intermittent preventive treatment in Gambian primigravidae (30)

Methods

- **Randomized, placebo-controlled**

- **Randomization**: Provided that the Hb concentration was > 7 g/dL, the woman was given a study number and SP was administered under supervision. The woman was then given the randomization envelope bearing the same study number. This envelope contained iron and folic acid tablets or iron tablets alone.

- **Allocation concealment**: The woman was then given the randomization envelope bearing the same study number. This envelope contained iron and folic acid tablets or iron tablets alone. Envelopes were pre-packaged by a person who had no further direct involvement in the trial.

- **Blinding**: Not clear

- **Loss to follow-up**: 126 women out of 1035 were lost to follow-up (12%): 62 (12%) in the intervention group (n= 522) and 64 (12%) in the placebo group (n=513) were lost to follow-up, mainly because of their travel outside the study area.

Participants

- **Number**: 1035 Gambian primigravidae

- Eligibility criteria were pregnancy greater than 15 weeks, haemoglobin (Hb) concentration > 7g/dL, absence of any serious underlying disease, absence of a history of an adverse response to sulfonamides, and residence in the study area and willingness to be visited at home.

Interventions

- One group of women (immediate treatment group) was given iron and folic acid tablets (500µg of folic acid and 47 mg of ferrous
sulfate per tablet) to be taken at home once per day for 14 days. Women with an Hb concentration of 7–9 g/dL were asked to take three tablets a day, those with an Hb concentration of 9–11 g/dL were asked to take two tablets a day, and those with an Hb concentration ≥ 11 g/dL were asked to take one tablet a day. Thus, women in this group received 500 µg to 1,500 µg of folic acid per day.

- Another group of women (delayed treatment group) received oral iron tablets (60 mg of ferrous sulfate per tablet) to be taken at home once per day for 14 days, 1–3 tablets according to Hb concentration as described above. At the end of this period, women in this group were given a supply of iron and folic acid tablets to be taken as described above for women in the immediate treatment group.

- All women received directly observed treatment with three tablets of SP (25 mg of pyrimethamine and 500 mg of sulfadoxine)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Risk of parasitologic failure in asymptomatic primigravidae given SP.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notes</td>
<td>Any iron or folic acid tablets that had not been used were collected to measure compliance</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk of bias</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate sequence generation?</td>
<td>Unclear. Based on study number.</td>
</tr>
<tr>
<td>Allocation concealment?</td>
<td>Unclear. No information on the appearance and taste of the tablets. Envelopes were used.</td>
</tr>
<tr>
<td>Blinding? All outcomes</td>
<td>Envelopes were pre-packaged by a person who had no further direct involvement in the trial.</td>
</tr>
<tr>
<td>Incomplete outcome data addressed? All outcomes</td>
<td>Adequate: 12%</td>
</tr>
</tbody>
</table>

Factors lowering the quality of evidence

- Minor Limitations
  - The concealment of allocation to treatment group and the sequence generation are unclear.

- Indirectness
  - The study measured the SP failure through assessment of malaria infection (direct measure of parasitemia). Indirectness of the population because women in the study were at gestational age greater than 15 weeks whereas the recommendation is for women who are planning a pregnancy until 12 weeks after conception.

- Imprecision
  - The participants were 1035 pregnant women (first pregnancy)

- Reporting bias
  - One of the 3 studies on folic acid supplementation and concomitant use of SP.
## Appendix C: Summary of Findings (GRADE) Tables

### Annex 1. GRADE “Summary of findings” tables

#### 400 µg of folic acid versus placebo or no intervention

**Patient or population:** Women in reproductive age  
**Settings:** All settings  
**Intervention:** 400 µg doses of folic acid  
**Comparison:** placebo or no intervention

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect or mean difference (95% CI)</th>
<th>Number of participants (studies)</th>
<th>Quality of the evidence (GRADE)*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of Neural Tube Defects (NTDs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North China</td>
<td>RR 0.21 (0.13–0.32)</td>
<td>(1 study)</td>
<td>low¹</td>
<td>Area with high Prevalence of NTD (5 to 6 per 1000 births)</td>
</tr>
<tr>
<td>South China</td>
<td>RR 0.84 (0.62–1.14)</td>
<td>24,783</td>
<td></td>
<td>Area with low Prevalence of NTD (1 per 1000 births)</td>
</tr>
</tbody>
</table>

| Red blood cell folate (RBC) concentration (µG /L) | MD 260 (103.81–416.19) | (1 study) | very low² |  |

CI, confidence interval; RR, risk ratio; MD, mean difference.  
*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 Single observational cohort study with inconsistency among the population studied.  
² Single study with minor limitations: primary analysis was by intention to treat and there was no control on the feeding (risk of additional folic acid intake). The study withdrew 21.5 % of women (inadequate loss of follow up). This study has a high risk of imprecision given the low numbers of participants. As there is only one trial it was not possible to assess the consistency of the findings, however, consistency is supported by indirect evidence modelling (Crider 2014)(17)
**Annex 2. GRADE “Summary of findings” tables**

**400 µg of folic acid versus 5mg of folic acid**

**Patient or population:** Women in reproductive age  
**Settings:** All settings  
**Intervention:** 5mg of folic acid  
**Comparison:** 400µg doses of folic acid

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Relative effect or mean difference (95% CI)</th>
<th>Number of participants (studies)</th>
<th>Quality of the evidence (GRADE)*</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Failure of antimalarial (SP) drug treatment | RR 1.87  
(1.15–3.05) | (1 study)  
488 | Moderate  
1 |  
1 There is indirectness in the results because women in the study were at gestational age between 17 and 34 weeks whereas the recommendation is for women who are planning a pregnancy until 12 weeks after conception. As there is only one trial it was not possible to assess the consistency of the findings. |

CI, confidence interval; RR, risk ratio; MD, mean difference.  
*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.