

Evidence Synthesis

Number 145

Folic Acid Supplementation: An Evidence Review for the U.S. Preventive Services Task Force

Prepared for:

Agency for Healthcare Research and Quality
U.S. Department of Health and Human Services
5600 Fishers Lane
Rockville, MD 20857
www.ahrq.gov

Contract No. HHSA-290-2012-00015-I, Task Order No. 4

Prepared by:

RTI International–University of North Carolina at Chapel Hill Evidence-based Practice Center
Research Triangle Park, NC

Investigators:

Meera Viswanathan, PhD
Katherine A. Treiman, PhD
Julia Kish Doto, PhD
Jennifer C. Middleton, PhD
Emmanuel J.L. Coker-Schwimmer, MPH
Wanda K. Nicholson, MD, MPH

**AHRQ Publication No. 14-05214-EF-1
January 2017**

This report is based on research conducted by the RTI International–University of North Carolina at Chapel Hill (UNC) Evidence-based Practice Center (EPC) under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract No. HHS-290-2012-00015-I, Task Order No. 4). The findings and conclusions in this document are those of the authors, who are responsible for its contents, and do not necessarily represent the views of AHRQ. Therefore, no statement in this report should be construed as an official position of AHRQ or of the U.S. Department of Health and Human Services.

The information in this report is intended to help health care decisionmakers—patients and clinicians, health system leaders, and policymakers, among others—make well-informed decisions and thereby improve the quality of health care services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information (i.e., in the context of available resources and circumstances presented by individual patients).

This report may be used, in whole or in part, as the basis for development of clinical practice guidelines and other quality enhancement tools, or as a basis for reimbursement and coverage policies. AHRQ or U.S. Department of Health and Human Services endorsement of such derivative products may not be stated or implied.

None of the investigators have any affiliations or financial involvement that conflicts with the material presented in this report.

Acknowledgments

The authors acknowledge the following individuals for their contributions to this project: Iris Mabry-Hernandez, MD, and Tracy Wolff, MD, MPH, of AHRQ; current and former members of the U.S. Preventive Services Task Force who contributed to topic deliberations; expert reviewers (Nancy Chescheir, MD, University of North Carolina at Chapel Hill; Anna Maria Siega-Riz, PhD, University of North Carolina at Chapel Hill; Kimberly D. Gregory, MD, MPH, Cedars-Sinai; Joe Leigh Simpson, MD, March of Dimes Foundation; and Lt. Col. Catherine Witkop, MD, MPH, Uniformed Services University); federal partner reviewers from the National Institute of Environmental Health Sciences, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Center on Birth Defects and Developmental Disabilities, and U.S. Department of Veterans Affairs; Evelyn Whitlock, MD, MPH, formerly of the Kaiser Permanente Research Affiliates EPC; and RTI–UNC EPC staff (Christiane Voisin, MSLS, librarian; Sharon Barrell, MA, editor; and Loraine Monroe, publications specialist).

Suggested Citation

Viswanathan M, Treiman KA, Kish Doto J, Middleton JS, Coker-Schwimmer EJ, Nicholson WS. Folic Acid Supplementation: An Evidence Review for the U.S. Preventive Services Task Force. Evidence Synthesis No. 145. Rockville, MD: Agency for Healthcare Research and Quality; 2017.

Structured Abstract

Purpose: To assess the benefits and harms of folic acid supplementation in reproductive-age women for the prevention of neural tube defects (NTDs).

Data Sources: Systematic review of studies from MEDLINE, the Cochrane Library, EMBASE unpublished literature, and trial registries through January 28, 2016; bibliographies from retrieved articles, outside experts, and reviewers.

Study Selection: Two investigators independently selected studies using a priori inclusion and exclusion criteria. We included studies that focused on the use of folic acid supplementation (by itself or in multivitamin or prenatal supplement form) for the prevention of NTD-affected pregnancies in women of childbearing age. We limited the evaluation of benefits to NTDs.

We excluded poor-quality studies and studies of prepubertal girls, men or women without the potential for childbearing, and NTD recurrence prevention, and studies in developing countries.

Data Extraction: One investigator extracted data and a second checked accuracy. Two reviewers independently rated the quality for all included studies, using predefined criteria.

Data Synthesis: We included 24 studies: 12 on the effect of folic acid supplementation on NTDs, three on variation in the effect on NTDs by race/ethnicity, and eight on variation by dosage or timing. For harms, we focused on two recent systematic reviews on respiratory outcomes, which reported on several included studies. One systematic review also provided data on variation in harms by timing and dose. We paid particular attention in the analysis to variation in effect by study design and over time, particularly before and after food fortification in the United States (1998).

For the question of benefits of folic acid supplementation on NTDs, we found a single randomized, controlled trial (RCT), initiated in 1984 in Hungary, reporting a Peto odds ratio (OR) for NTDs of 0.131 (95% confidence interval [CI], 0.026 to 0.648; $p=0.013$). Two older cohort studies provided an OR of 0.11 (95% CI, 0.011 to 0.91) and 0.27 (95% CI, 0.11 to 0.63). Older case-control studies were also generally consistent with the evidence from the older RCT and cohort studies; odds ranged from 0.6 to 0.7 in three of four case-control studies. This evidence led to food fortification in the United States in 1998, after which no new trials or prospective cohort studies have been conducted. All newer evidence arises from case-control studies only. These newer studies, with inherently weaker designs, are consistent in not demonstrating a protective effect of folic acid supplements on NTDs, with odds ranging from 0.9 to 1.4 and CIs spanning the null.

Regarding variations in benefits by race/ethnicity, we found three eligible studies. One found no effect by race/ethnicity, a second found a higher but nonsignificant risk of NTDs with folic acid supplementation among Hispanic women (adjusted OR for consistent users compared with nonusers, 2.20 [95% CI, 0.98 to 4.92]), and a third found that the risk reduction was of smaller magnitude for Hispanic women compared with white or black women. These inconsistent results could have occurred by chance. Regarding variation in benefits, eight studies provided

information. Of these, four studies provided information on dose, none on duration, and five on timing. We found no indication of a dose-response relationship in three of four studies. One study showed lower odds for daily versus less than daily use (OR, 0.57 [95% CI, 0.35 to 0.93]). Regarding timing, two older studies consistently showed no effect.

Two newer studies found no effect of timing for spina bifida, while one showed a protective effect with supplement use before pregnancy for anencephaly.

Regarding harms, one trial and one cohort study did not find evidence of statistically significant increased risk of twinning in women. The cohort study found that any increased risk of twinning was attenuated when the confounding effects of in vitro fertilization were accounted for. Three systematic reviews evaluated childhood asthma, wheezing, or allergy and found no consistent evidence of harm. One trial evaluated the risks of adverse events in women and found a higher risk of some events (weight gain, diarrhea, constipation) and not others (increased appetite, lack of appetite, exanthema, heartburn, and vertigo), but the event rate was very low and could have occurred by chance or as consequence of pregnancy. One systematic review did not find consistent evidence of an effect of folic acid supplementation on childhood asthma, wheezing, and allergy by timing or dose of intake.

Limitations: We restricted interventions to folic acid supplementation and did not include interventions such as food fortification, counseling to increase dietary intake, or screening for NTDs. We found very limited information on differences in benefits and risks of folic acid supplementation by race/ethnicity, dose, and timing and no information on duration.

Regarding the overall quality of evidence, ethical considerations limit the use of RCTs to answer questions of efficacy. Observational studies carry limitations of case ascertainment and recall bias, and these two sources of bias can serve to reduce the observed effect of NTDs.

Conclusions: Studies conducted before food fortification in the United States in 1998, with fewer design flaws, show that folic acid supplementation provides protection against NTDs. Newer studies, conducted after food fortification with folic acid, do not demonstrate this protective effect. These studies, however, have the potential for misclassification and recall bias, both of which can serve to attenuate the effect of folic acid supplementation on NTDs. Although mandatory food fortification in the United States has been accompanied by a decline in NTD prevalence, variations in intake continue to leave nearly a quarter of the U.S. population with suboptimal red blood cell folate concentrations, suggesting continued importance of folic acid supplement use.

Evidence of variations in effectiveness by race/ethnicity is inconsistent and could have occurred due to chance. We found no evidence of a dose-response effect, but studies had small numbers of cases for subanalyses. We did not find consistent evidence on timing of folic acid for benefits. We also did not find consistent evidence of harms, specifically twinning, respiratory outcomes, and other harms.

Table of Contents

Chapter 1. Introduction	1
Scope and Purpose	1
Condition Background	1
Definition	1
Prevalence and Burden of Illness	1
Etiology and Natural History	2
Risk Factors	2
Prevention	3
Rationale for Intervention	3
Intervention Strategies	3
Dietary Measures and Biomarkers of Folic Acid Intake	4
Sources of Folate and Folic Acid	4
Consumption of Folate and RBC Folate Concentration	5
RBC Folate Concentration and NTDs	6
Folic Acid Consumption and Pregnancy Intention	6
Promoting Folic Acid Consumption	7
Current Clinical Practice	7
Previous USPSTF Recommendation	8
Chapter 2. Methods	9
Key Questions and Analytic Framework	9
Search Strategies	9
Study Selection	10
Population	10
Interventions	10
Comparators	10
Outcomes and Timing	10
Settings	11
Study Designs	11
Data Abstraction and Quality Rating	11
Data Synthesis and Analysis	11
Expert Review and Public Comment	12
USPSTF Involvement	12
Chapter 3. Results	13
Literature Search	13
Results	13
KQ 1a. Effect of Folic Acid Supplementation on NTDs in Women of Childbearing Age	13
KQ 1b. Variation in Effect of Folic Acid Supplementation by Race/Ethnicity	20
KQ 1c. Variation in Effect of Folic Acid Supplementation by Dosage, Timing, and Duration of Therapy	21
KQ 2a. Harms of Folic Acid Supplementation in Women of Childbearing Age	23
KQ 2b. Variation in Harms of Folic Acid Supplementation by Dose, Timing, and Duration of Therapy	27

Chapter 4. Discussion	28
Summary of Evidence.....	28
Evidence for Benefits of Folic Acid Supplementation	28
Evidence for Harms of Folic Acid Supplementation.....	29
Applicability of Evidence	30
Sufficiency of Intake.....	30
Variation of Intake From Diet and Other Sources.....	31
Differences by Age	31
Differences by Race/Ethnicity	31
Differences by Education.....	32
Differences by Income	33
Differences by Insurance Status.....	33
Differences by Employment Status	33
Differences by Marital Status	33
Differences by Pregnancy Intention.....	34
Differences by U.S. Versus Foreign Born and Years in the United States.....	34
Differences by Parity	34
Differences by Health Behaviors/Health Status	34
Effect of Folic Acid Supplementation Outside the Periconceptual Period on NTDs.....	35
Variation in Effect of Folic Acid Supplementation by Medical Risk Factors.....	35
Diabetes.....	35
Maternal Obesity.....	36
Folic Acid Antagonists and Seizure Medications.....	37
Prior NTDs.....	37
Other Fetal, Neonatal, or Maternal Benefits of Folic Acid Supplementation	38
Limitations of the Review.....	38
Future Research Needs	39
Conclusion	40
References.....	41

Figures

Figure 1. Analytic Framework

Figure 2. Preferred Reporting of Systematic Review and Meta-Analysis (PRISMA) Tree

Figure 3. Folic Acid Supplementation and Adverse Events: Forest Plot

Figure 4. Folic Acid Supplementation and Neural Tube Defects by Earliest Year of Recruitment: Forest Plot

Figure 5. Folic Acid Supplementation and Neural Tube Defects by Study Design: Forest Plot

Tables

Table 1. Measures and Definitions

Table 2. Current Guidelines for Folic Acid Supplementation

Table 3. Study Characteristics of Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Table 4. Results of Prospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Table 6. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Race/Ethnicity

Table 7. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Dosage

Table 8. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

Table 9. Harms of Folic Acid Supplementation: Study Characteristics of Included Twinning Studies

Table 10. Harms of Folic Acid Supplementation: Study Characteristics of Included Asthma/Wheezing Studies

Table 11. Results of Prospective Studies on the Associations Between Folic Acid Supplementation and Twinning

Table 12. Results of Meta-Analyses on the Associations Between Folic Acid Supplementation and Asthma/Respiratory Illness

Table 13. Variation in Harms of Folic Acid Supplementation by Timing

Table 14. Variation in Harms of Folic Acid Supplementation by Dose

Table 15. Summary of Evidence for Folic Acid Supplementation

Table 16. Estimates of Folate Sufficiency

Appendices

Appendix A. Search Strategy and Detailed Methods

Appendix B. Inclusion/Exclusion Criteria

Appendix C. Excluded Studies

Appendix D. Risk of Bias Assessments for all Included Studies

Appendix E. Inclusion/Exclusion Status of Studies Included in 2009 Report

Appendix F. Overview of Study Characteristics

Appendix G. Ongoing Trials

Chapter 1. Introduction

Scope and Purpose

The U.S. Preventive Services Task Force (USPSTF) will use this systematic review to update its 2009 recommendation on folic acid supplementation in women of childbearing age for the prevention of neural tube defects (NTDs) in their offspring. This report summarizes the evidence on the benefits and harms of folic acid supplementation and identifies key gaps in the scientific literature. Evidence on folate fortification, counseling to increase dietary intake of folate (folic acid or natural food folate), or screening for NTDs is outside the scope of this report.

Condition Background

Definition

NTDs are abnormalities that can occur in the brain, spine, or spinal canal of a developing embryo and are present at birth.¹ During an embryo's development, specific cells form a neural tube that later becomes the spinal cord, the brain, and the nearby structures (e.g., spinal column) that protect them. The top of the neural tube becomes the brain and the remainder becomes the spinal cord. When the neural tube does not close completely, a hole in the spinal column is left or another type of defect develops.¹ NTDs cover numerous conditions, including spina bifida, encephalocele, and anencephaly. Spina bifida occurs when the neural tube in the cranial region or along the spine does not close by the 28th day of gestation.² Spina bifida can range from mild (no noticeable disability) to severe (limitations in physical movement and function, paralysis, and cognitive deficits). Anencephaly is a more severe NTD that results in the fetus having little to no brain matter; the fetus could also lack part of its skull.^{3,4} All infants with anencephaly are stillborn or die soon after birth.¹ NTDs may be isolated, one of multiple congenital abnormalities, or a component of a syndrome that is caused by a single-gene disorder, chromosomal abnormality, or teratogenic exposure.⁵ Although exact proportions are difficult to estimate, studies suggest that a higher proportion of spina bifida and anencephaly cases can be classified as isolated compared with other forms of NTDs, such as encephalocele or iniencephaly.⁶

Prevalence and Burden of Illness

The Centers for Disease Control and Prevention estimate that the average annual prevalence of anencephaly and spina bifida was 6.5 cases per 10,000 live births for the period from 2009 to 2011.⁷ Estimates of total burden of illness require relying on indirect calculations because fetal deaths and elective terminations attributable to NTDs are underreported. The total number of annual cases from health systems without prenatal ascertainment of NTDs for this period is 2,203. Health systems with prenatal ascertainment yield a higher estimate of annual cases for the same period (2,604). The estimated average number of annual live births for the same period is 4,027,880. Anencephaly accounts for 43 percent of NTDs and is incompatible with life.⁷ Infants

born with spina bifida can survive with treatment but can have a broad range and degree of disabilities depending on the severity of the defect. Disabilities from spina bifida are based on the level of the lesion and the consequent motor and sensory deficits that occur. Lower lesions are associated with a better prognosis. Paralysis, urinary and fecal incontinence, and ventriculomegaly with placement of a ventricular-peritoneal shunt are common complications.

Etiology and Natural History

The neural plate appears during the third week of fetal development and gives rise to the neural folds that fuse in the midline to form the neural tube. Therefore, defects can occur when one or both sites fail to meet and close. Neural tube closure is usually complete by the end of the 4th week after conception.⁸

The etiology of NTDs is believed to be due to a combination of genetic predisposition and environmental influences.⁹ The specific environmental influences are still under investigation. NTDs occur more frequently in certain families.⁸ Parents with one child with an NTD are at increased risk for having another child with a similar defect (2% to 5%).¹⁰ However, the majority of NTD cases occur in families with no prior history of NTDs.⁹ It may be that certain persons with a genetic predisposition have not yet been exposed to the environmental factors necessary to produce an abnormality in their offspring.¹¹ One of the key environmental influences is intake of folic acid. Low concentrations of folate may limit the number of methyl groups available for DNA replication and methylation. Evidence suggests that parents with a pregnancy complicated by an NTD are more likely to carry a variant in the gene (677C→T mutation) encoding the methylenetetrahydrofolate reductase (MTHFR) enzyme. MTHFR¹² is an enzyme that regulates folate and homocysteine levels. Persons who are homozygous for this gene mutation have lower concentrations of folate,¹³ which can decrease the conversion of homocysteine to methionine and may increase the risk of NTDs.¹² A meta-analysis drawn from Dutch and other international sources suggests that the prevalence of this gene mutation is 9.3 percent (95% confidence interval [CI], 8.1 to 10.4).¹² Estimates for subpopulations in the United States range from 1.2 percent for blacks to 20.7 percent for Hispanics in California, indicating a high degree of variation based on subpopulation.¹⁴ Folic acid may help mitigate the effects of this gene mutation, thereby promoting methionine utilization.¹⁵

Risk Factors

Nonmodifiable risk factors for NTDs include race/ethnicity, female sex of the neonate, and family history of NTDs in a first- or second-degree relative.^{9,16} Evidence indicates that certain racial/ethnic groups appear to be at higher risk for NTDs. Both before and after food fortification laws, birth prevalence rates were highest among Hispanics, followed by non-Hispanic whites and non-Hispanic blacks.^{17,18} The prevalence of genetic mutations in certain enzymes may differ among these population groups.^{19,20} Whether differences between racial/ethnic groups are attributable to genetic or environmental traits is unknown.²¹ Folic acid intake from diet is known to vary by race/ethnicity. For example, Hispanics may be at increased risk for having a baby with NTDs, likely because of lower levels of folic acid in their foods, such as unfortified corn masa products, rather than fortified cereals and pasta.²²⁻²⁴

Racial/ethnic differences persist through childhood. In a study of the prevalence of spina bifida among children and adolescents in 10 regions of the United States, the estimated prevalence in 2002 was 3.1 cases per 10,000 (95% CI, 3.0 to 3.3) among non-Hispanic white children and adolescents, 1.9 (95% CI, 1.7 to 2.2) among non-Hispanic black children and adolescents, 3.6 (95% CI, 3.4 to 3.8) among Hispanic children and adolescents, and 1.8 (95% CI, 1.5 to 2.2) among all other children and adolescents.¹⁶ Possible reasons for these differences include differential birth prevalence and survival probability.

Among other risk factors, high maternal body mass index (BMI) is an independent risk factor for lower serum folate²⁵ and NTDs.²⁶ Because as many as 56 percent of women in the United States ages 20 to 39 years are overweight or obese, this risk factor is widely relevant to primary care practice.²⁷ Undiagnosed and pregestational (type 1 or 2) diabetes are also risk factors for NTDs.^{28,29} In addition, women with epilepsy who take certain antiepileptic medications, such as valproic acid or carbamazepine, are at increased risk of spina bifida (1% to 2% and 0.5%, respectively).³⁰ Another medication associated with NTDs is warfarin.³¹ Malabsorption of micronutrients, including dietary folate (e.g., due to bariatric surgery),³² or maternal heat exposure (e.g., from a sauna, hot tub, fever, or electric blanket)³³ also elevate the risk of NTDs.

A study of the proportion of NTDs attributable to known risk factors, modifiable or otherwise, found that the factors responsible for the greatest proportion of cases included maternal Hispanic ethnicity, obesity, low dietary folate intake, female sex of the neonate, and lack of folic acid supplementation.⁹

Prevention

Rationale for Intervention

NTDs are among the most common congenital major anomalies in the United States.³⁴ NTDs occur very early in pregnancy, with no or only limited chance for complete recovery. Prevention is the only medical solution. Periconceptional folic acid supplementation is a primary prevention intervention that can be implemented in primary care settings.

Although often used interchangeably, the term “folate” refers to the water-soluble B vitamin (B₉) that occurs in many chemical forms, including naturally in foods, while “folic acid” is the term applied to the synthetic form of folate that is found in supplements and added to fortified foods.³⁵ Folic acid supplementation is usually provided as a single vitamin or part of a multivitamin.

Intervention Strategies

The main approaches in the United States to achieving adequate folate concentrations in women who are capable of becoming pregnant are ensuring a healthy diet that includes foods fortified with folic acid, providing folic acid supplements, and providing a combination of supplements and a folic acid–rich diet.³⁶ Although other risk factors for NTDs exist, such as diabetes, obesity, and family history, prevention measures have focused primarily on promoting folic acid consumption through diet and supplements.

In 1998, the U.S. Food and Drug Administration required the addition of folic acid to all enriched cereal grain products sold in the United States.³⁷ The rate of NTDs has dropped since food fortification laws were implemented.^{7,18,38,39}

Dietary Measures and Biomarkers of Folic Acid Intake

Several measures are used to assess the adequacy of dietary folic acid consumption: recommended daily allowance (RDA), dietary folic equivalent (DFE), and estimated average requirement (**Table 1**).

Another approach is to use red blood cell (RBC) or serum folate concentration as a biomarker for folic consumption. Plasma or serum concentration of folate reflects transient levels of folate found in circulation. RBC concentration is thought to be a more accurate measure because it reflects body stores of folate. There is no stated threshold value for plasma or serum concentration to determine deficiency as it relates to the risk of NTD. The World Health Organization recommends an RBC folate concentration greater than 400 ng/mL (906 nmol/L) in women of reproductive age to achieve the greatest reduction of NTDs.⁴⁰ Although folate concentrations have traditionally been assessed using microbiological and protein-binding assays, newer assays are being developed but are not yet standardized.⁴¹ Consequently, current assays do not produce comparable results and may lead to inaccurate assessments of folate status. Folate status reflects both dietary intake and absorption. The question of how much natural food folate or folic acid intake is necessary to achieve adequate RBC folate concentration has not yet been resolved.^{40,42}

Sources of Folate and Folic Acid

Women can consume folate by eating foods rich in folate, such as dark green leafy vegetables, oranges, orange juice, and legumes. Women can consume folic acid, the synthetic derivative of folate, by eating food fortified with folic acid, such as cereals, grains, and pasta products, or by taking a dietary supplement or multivitamin containing folic acid of varying doses.

Manufacturers are mandated in the United States to fortify cereal grain products (e.g., grains and pastas) that are labeled as “enriched”—a mandatory addition of folic acid at 0.14 mg per 100 g of grain product.⁴³ Other cereals and related products such as ready-to-eat cereals may be voluntarily fortified, but their folic acid content can change because the level is not mandated.

Naturally occurring food folate is 1.7 times less bioavailable than folic acid.³⁵ Since the 1998 U.S. Food and Drug Administration requirement to fortify enriched cereal grain products, the national incidence of babies born with NTDs has decreased.^{7,18,38,39} However, it remains challenging for most women to consume the recommended daily intake of 0.4 mg of folic acid from diet alone. In the United States, women age 19 years or older have a median daily intake of 0.117 mg of folic acid per day from mandatorily fortified food.⁴⁴ Data from the 2007 to 2012 National Health and Nutrition Examination Survey (NHANES) suggest that 48.4 percent of U.S. women of childbearing age (95% CI, 46.3 to 50.6) reported consuming folic acid from mandatorily fortified foods only.⁴⁵ In another study of pregnant women in North Carolina (n=2,247), only 60 percent met folic acid recommendations from diet alone.⁴⁶ Additionally,

populations that do not consume mandatorily fortified foods (e.g., those on gluten-free or Atkins diets) are not protected by mandatory food fortification.

Folic acid intake from diet varies by race/ethnicity. Some investigators reported on decreased dietary folic acid intake among Mexican American women, who may be at increased risk because of lower levels of consumption of fortified foods, such as cereals and pastas, as a result of their corn masa-based diets.²²⁻²⁴ Dietary supplements, including multivitamins, contain large amounts of folic acid, and U.S. adults commonly use supplements.⁴⁷ Supplements containing folic acid in the United States generally contain 400 to 800 µg of folic acid. However, doses up to 1,000 µg are permitted without a prescription.

More than 28 percent of 2007 to 2012 NHANES participants reported using a dietary supplement containing folic acid (>71% not taking folic acid daily).⁴⁵ Of those who took a supplement containing folic acid, about half (14.6 % of all women) took one that contained less than the daily recommended dose of 400 µg.⁴⁵ The Pregnancy Risk Assessment Monitoring System (PRAMS) data for 2009 report that only 30 percent of women reported taking a multivitamin, prenatal vitamin, or folic acid supplement daily 1 month before conception (70% were not taking folic acid daily).⁴⁸

Blood folate data from NHANES have documented improvements in the folate status of the U.S. population after food fortification was implemented.⁴⁹ The prevalence of low serum folate (<10 nmol/L) among U.S. women of childbearing age (ages 15 to 44 years) declined from 32.2 percent in the prefortification period (1988 to 1994) to 5.5 percent in the postfortification period (1999 to 2010). These changes have been accompanied by a decline in the prevalence of NTDs from 10.7 cases per 10,000 live births before fortification (1995 to 1996) to 7.0 cases after fortification (1999 to 2011).⁷ Other countries such as Canada, South Africa, Costa Rica, Chile, Argentina, and Brazil have also reported reductions in the rate of NTDs following the introduction of food fortification.⁵⁰

Consumption of Folate and RBC Folate Concentration

A systematic review and Bayesian meta-analysis explored the extent to which consumption of natural folate translates to an increase in RBC and serum folate concentrations. The review excluded studies in settings with folic acid consumption through supplements or fortification that did not include an adequate washout period.⁵¹ Six studies of nonpregnant, nonlactating females ages 12 to 49 years with RBC concentrations assessed with microbiological assay contributed to the analysis. The authors found that a 10 percent increase in natural food folate intake can increase RBC folate concentration by approximately 6 percent (95% credible interval [CrI], 4 to 9). Using the model, the authors estimated that in a population with a mean natural food folate intake of 450 µg DFE/day, the mean RBC folate concentration would be approximately 1,070 nmol/L (95% CrI, 770 to 1,440). For every 10 percent increase in natural food folate intake, the authors reported that serum/plasma folate concentration could increase by approximately 7 percent (95% CrI, 1 to 12).

RBC Folate Concentration and NTDs

Findings from two large epidemiological studies support RBC folate concentration as a key prevention strategy for NTDs. Daly and colleagues reassessed the findings of a large case-control study of pregnant women in Ireland presenting for antenatal care in three clinics in Dublin between 1986 and 1990.⁵² The investigators analyzed blood samples from 86 cases and 266 controls (normal live births) in a 1:3 ratio for RBC and plasma folate concentrations. Cases and controls were not matched for maternal or gestational age, but both characteristics were similar between the two groups. Median gestational age at the time the samples were collected was 15 weeks. Using an overall NTD rate of 1.9 cases/1,000 births, Daly estimated the risk of NTDs with RBC and plasma folate levels as continuous variables and at different threshold levels. After adjustment for maternal covariates in logistic regression models, dose-response effects were determined for both plasma and RBC folate levels. In assessing RBC folate levels, an eight-fold difference in the risk of NTDs was found among women with RBC concentrations less than 340 nmol/L (150 ng/mL) compared with those with levels of 906 nmol/L (400 ng/mL) or higher ($p < 0.001$). Notably, the study was conducted in a relatively homogeneous population in Ireland. Blood specimens were obtained at a median of 15 gestational weeks, which is well beyond the time of neural tube closure. Thus, the dose-response effects as summarized may not fully reflect the periconceptual relationship between RBC folate concentration and the risk of NTDs.

In an effort to determine the optimal RBC folate concentration to reduce NTDs, Crider and associates⁴² reviewed data from two population-based Chinese cohorts prior to food fortification programs: more than 240,000 participants (1993 to 1995) in a community-based study of folate supplementation⁵³ (400 $\mu\text{g/day}$) and 1,194 participants (2003 to 2005) in the population-based Folic Acid Dosing Trial.^{54,55} Nonpregnant women without plans to conceive (intrauterine device in place) were randomized to one of four dosage regimens: 100 $\mu\text{g/day}$, 400 $\mu\text{g/day}$, 4,000 $\mu\text{g/day}$, or 4,000 $\mu\text{g/week}$. Measurement of RBC folate concentration and *MTHFR* genotyping were performed at baseline and at 1, 3, and 6 months after supplementation. Crider and colleagues initially analyzed data from the Folic Acid Dosing Trial to assess the association between the length of time that women consumed folic acid supplementation and RBC folate concentration, adjusted for the presence of the *MTHFR* genotype. Findings from this initial model were then used to estimate RBC folate concentrations in the community-based cohort based on participants' report of daily folate supplementation (i.e., pill consumption) to determine the association between RBC folate concentration, folate supplementation, and risk of NTDs. Subsequently, the model was applied to U.S. women using published estimates from NHANES. Estimates of the association between RBC folate concentration and risk of NTDs were consistent with those published in the earlier work by Daly et al. A substantially lower risk of NTDs was seen with RBC concentrations of 1,180 nmol/L (95% uncertainty interval, 1,050 to 1,340 nmol/L). Also, similar to Daly, RBC folate concentration near 1,200 nmol/L was associated with a substantial reduction in NTDs (5.8/10,000 live births). The relative consistency with the earlier estimates by Daly support the potential use of RBC folate concentration in assessing the risk of NTDs and could inform future health policies.

Folic Acid Consumption and Pregnancy Intention

Planning or intention to have a baby influences folic acid consumption. Women who plan to get

pregnant are more likely to take folic acid prior to becoming pregnant.⁵⁶⁻⁵⁹ However, half of all pregnancies in the United States are unplanned,^{60,61} which presents a challenge in terms of promoting folic acid consumption for women who are not planning to but may become pregnant. Interventions to increase use in this group require messaging to encourage women to take folic acid “just in case” they get pregnant. Alternatively, public health campaigns can promote folic acid use as a method of maintaining good health overall, but these campaigns do not appear to have lowered the prevalence of NTDs.²¹ Few studies have assessed which messaging approach is more effective for women who are not intending to but could become pregnant.⁶²⁻⁶⁵ However, formative studies^{64,65} suggest targeting messages for planners (women who are planning to become pregnant in the next 2 years) and nonplanners (women who do not plan to become pregnant in the next 2 years). For the latter, messages should be focused on promoting a woman’s overall health and well-being and how to have a healthy lifestyle (e.g., taking a multivitamin).

Promoting Folic Acid Consumption

Public health campaigns have been effective in increasing awareness, knowledge, and use of folic acid.⁶⁶ Although knowledge about the benefits and sources of folic acid increased postcampaign, campaigns have had less effect on women’s understanding of the correct timing for taking folic acid supplements.⁶⁶ In addition to increasing public folic acid awareness through campaigns, women are increasingly learning about folic acid in the clinical setting. From 1995 to 2008, the proportion of women who reported learning about folic acid from their health care providers increased from 13 to 33 percent, according to U.S. national surveys conducted by the March of Dimes.⁶⁷ Of women surveyed in 2008, 32 percent reported that their health provider discussed the benefits of folic acid.⁶⁷ However, only 12 percent of women reported that their health care provider advised them that folic acid needs to be taken before pregnancy.⁶⁷ Although preconception guidelines from the American College of Obstetricians and Gynecologists exist,⁶⁸ preconception care has yet to become standard practice among health care providers. Providers need to promote and initiate the idea of preconceptional health to help ensure women are as healthy as they can be before they become pregnant.⁶⁹

Current Clinical Practice

Several organizations offer consistent guidance supporting a minimum daily intake of 400 µg per day for women capable of becoming pregnant (**Table 2**).⁷⁰⁻⁷⁴ Additionally, some organizations offer an upper level for general populations (800 µg⁷³ to 1 mg⁷²) or recommendations for high-risk populations.^{35,70,72,75} High-risk populations include women of Hispanic ethnicity, women expecting a neonate of female sex, women who have a family history of NTDs in a first- or second-degree relative, women with a high BMI or undiagnosed pregestational diabetes, women with epilepsy who take certain antiepileptic medications, and women who have low dietary folate intake or currently lack folic acid supplementation.⁹

Previous USPSTF Recommendation

In 2009, the USPSTF recommended that all women planning a pregnancy or capable of pregnancy take a daily supplement containing 0.4 to 0.8 mg (400 to 800 μ g) of folic acid (A recommendation). This recommendation was based on convincing evidence from trial and observational evidence in settings without or before food fortification suggesting a reduction in NTDs with doses from 0.4 to 0.8 mg and adequate evidence that folic acid supplementation at usual doses is not associated with harms. The USPSTF also noted that most women in the United States are not ingesting fortified foods at a level thought to provide optimal benefit.

Chapter 2. Methods

Key Questions and Analytic Framework

The investigators, USPSTF members, and Agency for Healthcare Research and Quality (AHRQ) Medical Officers developed the scope, Key Questions (KQs), and analytic framework (**Figure 1**) that guided our literature search and review. Specifically, our KQs are:

- 1a. To what extent does folic acid supplementation reduce the risk for NTDs (first occurrence) in women of childbearing age?
- 1b. Does the effect of folic acid supplementation on NTDs (first occurrence) differ by race/ethnicity?
- 1c. Do the benefits of folic acid supplementation differ by dosage, timing, or duration of therapy?
- 2a. Are there harms associated with folic acid supplementation to the mother, fetus, neonate, or child?
- 2b. Do the harms of folic acid supplementation differ by dosage, timing, or duration of therapy?

The USPSTF also requested five contextual questions to help inform the report:

1. What is the current intake of folic acid from diet and other sources in women of childbearing age?
2. Does intake of folic acid from diet and other sources differ by pregnancy intention, age, race/ethnicity, or access to foods?
3. Does folic acid supplementation outside of the periconceptional period reduce the risk of NTDs?
4. Does the effect of folic acid supplementation on NTDs in the periconceptional period differ by medical risk factors (obesity, poorly controlled diabetes, seizure medications, methotrexate or other folate-antagonist therapies, or previous pregnancy with an NTD)?
5. Are there other potential fetal, neonatal, or maternal benefits from folic acid supplementation in the periconceptional period?

Search Strategies

We searched PubMed/MEDLINE®, the Cochrane Library, and EMBASE for English-language articles published from database inception through January 28, 2016. We used Medical Subject Headings as search terms when available and keywords when appropriate, focusing on terms to describe relevant populations, interventions, outcomes, and study designs. **Appendix A** describes the complete search strategies. We conducted targeted searches for unpublished literature by searching ClinicalTrials.gov, HSRProj, the World Health Organization's International Clinical Trials Registry Platform, and NIH Reporter. To supplement electronic searches, we reviewed the reference lists of pertinent review articles and studies meeting our inclusion criteria and added all previously unidentified relevant articles. We also manually reviewed all literature suggested by

peer reviewers or public comment respondents and, if appropriate, incorporated it into the final review.

Study Selection

We selected studies on the basis of inclusion and exclusion criteria developed for each KQ based on the PICOTS (population, intervention, comparator, outcome, timing, setting) approach for identifying populations, interventions, comparators, outcomes, timing, settings, and study designs (**Appendix B**). **Appendix C** lists excluded studies. We imported all citations identified through searches and other sources into EndNote v.5 (Thomson Reuters, New York, NY). Two investigators independently reviewed titles and abstracts. We dually and independently reviewed the full text of abstracts marked for potential inclusion by either reviewer. Two experienced team members resolved disagreements. We did not apply date limits to our searches and therefore captured all the studies included in the 2009 review.³⁶ We resolved disagreements by discussion and consensus; if necessary, we sought adjudication of conflicts from other experienced team members.

Population

We included studies that focused on the use of folic acid supplementation for the prevention of NTD-affected pregnancies in women of childbearing age. We did not include studies of prepubertal girls or men or women without the potential for childbearing (e.g., postmenopausal women, women with genetic uterine or ovarian abnormalities).

Interventions

We searched for studies that examined the use of folic acid supplementation with or without food fortification or naturally occurring folate for the prevention of NTDs. We also searched for studies that examined supplementation with micronutrients (e.g., multivitamin, iron) in combination with folic acid for the prevention of NTDs.

Comparators

For KQs 1a, 1b, and 2a, we included studies that compared interventions with placebo, no treatment, dietary supplementation only, supplementation with prenatal vitamins not containing folic acid, or iron supplements not containing folic acid. For KQs 1b, 1c, and 2b, we included studies that compared interventions with lower or higher doses of folic acid supplementation.

Outcomes and Timing

For KQs 1a and 1b, we searched for studies that reported on the benefits of folic acid supplementation initiated before the index pregnancy or in the first trimester to prevent NTDs. For KQs 1c, 2a, and 2b, we searched for studies that reported on the harms of folic acid

supplementation initiated before the index pregnancy and during the first, second, and third trimesters of pregnancy.

Settings

For all KQs, we searched for studies conducted in the United States or in countries with a very high Human Development Index.

Study Designs

For KQs 1a, 1b, and 1c, we included randomized, controlled trials (RCTs), controlled trials, cohort studies, case-control studies, and systematic reviews. For KQs 2a and 2b, we included RCTs, controlled trials, cohort studies, case-control studies, systematic reviews, and registry data.

Data Abstraction and Quality Rating

We abstracted pertinent information from each included study; details included methods and patient populations, interventions, comparators, outcomes, timing, settings, and study designs. A second investigator checked all data abstractions for completeness and accuracy. Using predefined criteria developed by the USPSTF and others⁷⁶⁻⁷⁸ for additional criteria for diagnostic accuracy studies, two investigators independently assessed the quality of each study as good, fair, or poor (**Appendix D**).⁷⁹ Disagreements were resolved by discussion and consensus. Studies with “fatal flaws” were rated as having high risk of bias (i.e., poor quality). Particular considerations for this topic include the risk of misclassification bias (from retrospective recall of level and timing of exposure), the risk of selection bias (from not identifying all cases of the outcome, including fetal deaths), and the risk of confounding (from not appropriately accounting for factors such as infertility that might influence both exposure to folic acid supplementation and the outcome of twinning). We rated studies with one or more of these features as poor quality. Other fatal flaws that resulted in poor-quality ratings included initially assembled groups not close to being comparable or maintained throughout the study (including overall attrition of at least 20% or differential attrition of at least 15% between groups); use of unreliable or invalid measurement instruments or unequal application among groups (including not masking outcome assessment); and, for RCTs, the lack of intention-to-treat analysis.

Two reviewers dually reviewed the quality of all studies included in the 2009 report that met the inclusion criteria for the current review and resolved disagreement by discussion and consensus.

Data Synthesis and Analysis

We qualitatively synthesized findings for each KQ by summarizing the characteristics and results of included studies in tabular or narrative format. To determine whether meta-analyses were appropriate, we assessed the clinical and methodological heterogeneity of the studies following

established guidance.⁸⁰ We qualitatively assessed the populations, similarities and differences in screening tests or treatments used, and similarities in outcomes and timing of outcomes assessed. We produced forest plots without summary estimates to illustrate patterns of effect size across studies.

Expert Review and Public Comment

A draft report was reviewed by content experts, representatives of federal partners, USPSTF members, and AHRQ Medical Officers and was revised based on comments, as appropriate. It was also posted for public comment.

USPSTF Involvement

This review was funded by AHRQ. AHRQ staff and USPSTF members participated in developing the scope of the work and reviewed draft manuscripts, but the authors are solely responsible for the content.

Chapter 3. Results

Literature Search

We identified 5,786 unique records and assessed 757 full-text articles for eligibility (**Figure 2**). We excluded 691 studies for various reasons detailed in **Appendix C** and included 66 published articles (54 studies) of good or fair quality in our main analyses (**Appendix D**). All five studies⁸¹⁻⁸⁵ that were included in the previous review^{36,86} were considered for the current review (**Appendix E**). Of these five studies, three⁸¹⁻⁸³ were included in our main analyses, one⁸⁵ was excluded due to wrong intervention, and one⁸⁴ was excluded for high risk of bias. Of the 49 included studies, 20 studies^{9,11,22,36,81,82,86-99} addressed KQ 1a, three studies^{11,82,87} addressed KQ 1b, eight studies^{11,22,82,87,96-99} addressed KQ 1c, 20 articles^{36,83,86,88-94,100-109} addressed KQ 2a, and six studies^{100-102,107,109,110} addressed KQ 2b. Although we intended to limit studies of harms to folic acid supplementation only, the available evidence was mixed, so we elected to include studies of multivitamins as well. Details of the quality assessment of included studies and studies excluded based on poor quality are provided in **Appendix D**. **Appendix F** provides additional details on study characteristics.

Results

KQ 1a. Effect of Folic Acid Supplementation on NTDs in Women of Childbearing Age

Overview

We found a total of 20 publications on the benefits of folic acid supplementation. Seven publications present results of the only eligible RCT.⁸⁸⁻⁹⁴ The trial, conducted in Hungary, is an RCT that was initiated in 1984 and terminated in 1992, with information collected through 1993. Three publications relate to two cohort studies; one was a Hungarian cohort study of women recruited between 1993 and 1996,⁸¹ and the second was a cohort drawn from women who underwent alpha-fetoprotein screening or amniocentesis between 1984 and 1987.^{97,98} All other studies were case-control studies and compared NTD cases with nonmalformed infants^{9,11,22,82,87,96} or infants with non-NTD malformations.^{95,99} Additionally, we drew on information from two publications in the previous update.^{36,86}

We present information from the RCT first, followed by the cohort studies and the case-control studies. Although the RCT and the cohort studies potentially offer greater control for potential sources of bias, they predate mandatory food fortification. The case-control studies span a period ranging from 1976 through 2008, including several relying exclusively on data collected after food fortification. Because these eight publications of case-control data draw from related, or in some cases, subsets of the same data, we present them by the broadest data source first (national or multistate followed by two-state or single-state studies) and recency of data collection within each section. **Table 3** provides supporting descriptions of each study. **Tables 4** and **5** provide

results. Because of the heterogeneity across studies and the differences in food fortification over time, we did not pool the results.

Study Characteristics of the Included RCT

One RCT, described in seven publications,⁸⁸⁻⁹⁴ randomized women to a vitamin supplement containing folic acid (0.8 mg folic acid and 12 vitamins, four minerals, and three trace elements) or a trace-element supplement (copper, manganese, zinc, and a low dose of vitamin C). Women started the supplement at least 28 days before conception and continued at least until the date of the second missed menstrual period.⁹¹ The trial, as part of the Hungarian Optimal Family Planning Programme, excluded women with delayed conception and infertility or with ongoing pregnancies. For the first 4 years, the program also excluded women older than age 35 years or with a prior wanted pregnancy.⁹⁰ The trial involved repeated contact with women at regular intervals. Women were asked to visit the clinic immediately after the first missed menstrual period. The staff administered a sensitive serum pregnancy test, followed within 2 weeks by an ultrasound.⁹³ As a result, the authors note that they had “nearly total ascertainment of unsuccessful pregnancy outcomes, including fetal deaths and malformations.”⁹³ The trial included only “informative” cases in the analysis; that is, live births, terminations in the second trimester, and stillbirths (late fetal deaths). It did not account for loss to followup, which constituted 0.9 percent of each arm (26 cases of 2,819 confirmed pregnancies in the multivitamin arm and 23 cases of 2,863 confirmed pregnancies in the trace-element supplement arm), or loss due to first-trimester losses, chemical pregnancy, ectopic pregnancy, or miscarriage (395 cases [14%] in the multivitamin arm and 504 cases [17.6%] in the trace-element supplement arm).

The trialists ascertained compliance with the supplement by 1) asking women; 2) checking women’s record of supplement use, recorded daily with basal body temperatures; and 3) checking boxes of supplements for unused tablets.⁹⁰ Women who became pregnant before starting the supplement or during the first month were considered unsupplemented and were referred to prenatal care immediately. Women who became pregnant after a period of supplementation were referred at 12 weeks to prenatal care.

Results of the Included RCT

The trial reported no cases of NTDs in the experimental arm and 6 cases in the control arm. Based on a denominator of “informative” cases only (live births, stillbirths, and second-trimester terminations only; counting each of twin and triplet births separately), the p-value for the Fisher exact test was 0.014. We calculated the Peto odds ratio (OR) as 0.131 (95% CI, 0.0263 to 0.648; p=0.013).

Study Characteristics of Included Cohort Studies

At the conclusion of the RCT described above, no additional RCT was considered ethically possible. The authors continued their investigation using the same intervention (multivitamin supplement containing 0.8 mg of folic acid) in women drawn from the Hungarian Periconceptional Service (1993 to 1996), with supplementation provided before conception.⁸¹ The comparison group comprised unsupplemented pregnant women at their first visit in the

regional antenatal care clinic between the 8th and 12th week of gestation; women who were determined (on a one-page, personally administered questionnaire) to have taken multivitamins or folic acid supplements during the periconceptional period were excluded. Unexposed women were matched to exposed women for age, socioeconomic status, employment status, and residence during the first year of pregnancy. Informative offspring included malformed fetuses, antenatally diagnosed and terminated in the second or third trimester; stillborn fetuses (late fetal death after the 28th week of gestation and/or weighing >1,000 g); and live-born infants. Informative offspring were ascertained in three ways: 1) by antenatal diagnoses of terminated fetuses, supported by a description of pathology; 2) through records at birth; and 3) by examination by a blinded pediatrician at 1 year of age or from pediatricians' records. NTDs included anencephaly and spina bifida. The study had a potential risk of selection bias because women in the supplemented cohort had a higher rate of comorbid conditions and unsuccessful pregnancies. The latter, in particular, likely prompted them to seek entry into the Hungarian Periconceptional Service. Supplemented women, however, were likely to have planned their pregnancies, had healthier behaviors in the periconceptional period, and received better prenatal care than unsupplemented women.

We also identified an eligible cohort study based on 23,491 women undergoing alpha-fetoprotein screening or amniocentesis between 15 and 20 weeks of gestation (1984 to 1987).^{97,98} Most of these women lived in Boston (33%), elsewhere in Massachusetts (48%), elsewhere in New England (5%), and outside New England (14%). Most of the samples were analyzed at the Boston University School of Medicine facilities. Nurses contacted women at the time that their tests were received by the laboratory; 93 percent did not know the results of their tests at the time of the interview. Nurses then asked women to recall their use of multivitamins in the first 3 months before pregnancy and the first 3 months of pregnancy. Exposure was defined as the use of at least one multivitamin containing folic acid per week between weeks 1 and 6 following conception. The maximum period of recall was 8 months (3 months prepregnancy and 5 months of pregnancy).

Results of Included Cohort Studies

The Hungarian cohort study reported 1 case of an NTD in 3,056 supplemented women and 9 cases in 3,056 unsupplemented women.⁸¹ The selection bias arising from higher rates of unsuccessful pregnancies in the supplemented cohort likely biased the results toward the null, while the selection bias from intentional pregnancies likely biased the results toward an effect of the intervention on NTDs. The authors adjusted the OR for birth order, chronic maternal disorder, and history of previous unsuccessful pregnancies. The study reported an adjusted OR (aOR) of 0.11 (95% CI, 0.011 to 0.91; p-value not reported).

The New England study reported 10 cases of NTDs among 10,713 women who took multivitamins containing folic acid in weeks 1 through 6 compared with 11 cases of NTDs among 3,157 women who did not take any supplements (OR, 0.27 [95% CI, 0.11 to 0.63]). By contrast, use of multivitamins containing folic acid from week 7 onward had no statistically significant effect on NTDs (25 cases in 7,883 supplemented women vs. 11 cases in 3,157 unsupplemented women; OR, 91 [95% CI, 0.45 to 1.80]) when compared with nonuse.⁹⁷

Study Characteristics of Case-Control Studies

Data From Multiple States: National Birth Defects Prevention Study

Two included publications used the National Birth Defects Prevention Study.^{9,87} The National Birth Defects Prevention Study was established in 1997 and includes 10 population-based birth defects surveillance systems in Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah. Eight of 10 surveillance sites include live births, fetal deaths, and elective pregnancy terminations, thus mitigating, but not entirely eliminating, the risk of selection bias.⁹ Cases were women with a pregnancy affected by anencephaly or spina bifida that did not result from a single-gene or chromosomal abnormality. Diagnosis from medical records of fetuses or infants were confirmed following review of clinical descriptions and surgery or autopsy reports. A random sample of women from each site who delivered live-born infants without structural birth defects served as controls. Interviews targeted for completion within 6 months of the expected delivery date collected information on dietary and supplement intake but could take place no earlier than 6 weeks and no later than 24 months following the expected date of delivery.⁹ Women were asked to recall use of multivitamins or supplements from 3 months before pregnancy through the last month of pregnancy, resulting in a maximum recall period of 3 years (24 months postpartum, 9 months of pregnancy, 3 months pre-pregnancy).

The two publications presented adjusted ORs for the risk of NTDs^{9,87} but did not report on the same time period. The more recent of these two publications, from 2013, focused on births from 1997 to 2007 and defined exposure as any use of folic acid, multivitamin, or prenatal vitamin supplement during the month before pregnancy and the first month of pregnancy. The earlier publication, from 2008, focused on births from 1998 to 2003. The authors compared the outcomes of consistent use (taking supplements at least half the number of days, ≥ 60 days, or from 3 months before pregnancy to the first month of pregnancy) with nonuse. The 2008 publication also provided data on outcomes associated with timing of folic acid supplementation (consistent use from 3 months before pregnancy to the first month after pregnancy vs. initiating supplement use in the first month of pregnancy).⁸⁷

To avoid double-counting cases and to use the largest potential study, we focus primarily on the 2013 study,⁹ with the longer time span, and discuss the 2008 study in the results.⁸⁷

Data From Multiple States: Slone Birth Defects Study

Three included studies drew on the Slone Birth Defects Study and were published in 2011,¹¹ 2001,⁹⁵ and 1993,⁹⁹ respectively. The Slone Birth Defects Study began in 1976. It identifies cases, largely from hospital discharge records; randomly selects controls; and identifies exposure to folic acid supplements through an interview conducted within 6 months of delivery going back to 6 months before pregnancy. Over the course of several decades, the list of included sites and sources has shifted.

The most recent Slone Birth Defects Study, published in 2011, identifies cases as arising from discharge records of participating hospitals serving areas surrounding Boston, Philadelphia, San

Diego, and Toronto (Canada).¹¹ Additionally, the study included some cases identified through birth defect registries in Massachusetts and parts of New York. Nonmalformed controls were selected randomly each month from discharge lists from the same hospitals or from statewide birth records. Not all data sources in the study included fetal deaths and elective terminations of pregnancy.¹¹ This publication focused on births in the postfortification era, from June 1998 to 2008, and defined nonusers as those who use supplements less than 1 day a week or only 2 lunar months before the last menstrual period. It compares the effect of supplementation on spina bifida for consistent users (≥ 4 days per week during at least 2 of the 3 periconceptional months, 1 or 2 lunar months before the last menstrual period, or 1 month after the last menstrual period), early pregnancy initiators (≥ 4 days per week beginning 1 or 2 months after the last menstrual period), and inconsistent users (all other use patterns) with nonuse. The maximum period of recall for this study is 17 months (6 months postpartum, 9 months of pregnancy, 2 months prepregnancy).

The 2001 Slone Birth Defects study includes births from 1976 to 1998 in the greater metropolitan areas of Boston, Philadelphia, and Toronto and, between 1983 and 1985, part of Iowa.⁹⁵ The data sources for this study included fetal deaths and elective terminations from 1988 onward. The study defined folic acid supplementation as never, occasional, or daily use in the 2 months after the last menstrual period, but the primary focus of the study was on the effect of exposure to folic acid antagonists in the same 2-month postconceptional period. It compared cases of NTDs (anencephaly, spina bifida, encephalocele, and others) with controls that had non-NTD malformations (i.e., hypertrophic pyloric stenosis, indeterminate sex or pseudohermaphroditism, musculoskeletal anomalies of the skull or face, feet deformities, anomalies of the diaphragm, gastroschisis/omphalocele, esophageal stenosis, stenosis of the large intestine or anus, congenital dislocation of the hip, hypospadias, and others). The maximum period of recall for this study was 15 months (6 months postpartum, 9 months of pregnancy).

The 1993 study from the same center evaluated births from 1988 to 1991 in the greater metropolitan areas of Boston, Philadelphia, and Toronto.⁹⁹ It compared cases (anencephaly, spina bifida, or encephalocele) with controls (other major malformations such as chromosomal abnormalities, ventricular septal defects, renal defects, transposition of great vessels, hypospadias, limb reduction defects, and craniosynostosis) for the effect of multivitamins. Exposure was defined as daily use of a vitamin supplement containing folic acid in the period spanning from 28 days before the last menstrual period to 28 days after, less than daily use in the same period, use of a multivitamin with unknown folic acid status any time in the periconceptional period, and use of a multivitamin with unknown folic acid status. The maximum period of recall for this study was 16 months (6 months postpartum, 9 months of pregnancy, 1 month prepregnancy).

We focus on the 2011 study spanning 1998 to 2008¹¹ in the analysis because it is consistent with other studies in comparing NTD cases with nonmalformed controls. We also discuss and compare the results of the 2001 study⁹⁵ and the 1993 study in the results.⁹⁹

Single-State or Two-State Data Sources

The most recent study, from a more limited data source, collected data from January 1995 to

February 1999 from 148 Mexican American women living along the Texas-Mexico border with NTD-affected pregnancies (including live births, stillbirths, spontaneous abortions, and elective terminations) and 158 control women with normal live births.²² NTDs included anencephaly, spina bifida, and encephalocele. Cases were ascertained using the Texas Department of Health's Neural Tube Defect Project, which relied on prospective case finding through hospitals, birthing centers, ultrasound centers, abortion centers, and midwives. Control women were randomly selected residents of the study area with normal births during the same time period. Exposure was defined as daily use in every month in the preconception period (≤ 3 months before conception), any reported use, or postconception use (≤ 3 months before conception). Interviews were conducted approximately 1 month postpartum. The average period of recall for this study was 13 months (1 month postpartum, 9 months of pregnancy, 1 month prepregnancy).

A case-control study drew on cases from the California Birth Defects Monitoring Program. These cases included singleton fetuses and live-birth infants diagnosed with an NTD (anencephaly, spina bifida, and other [combined anencephaly and spina bifida, craniorhachischisis, and iniencephaly]) between June 1989 and May 1991 and electively-terminated fetuses with an NTD from February 1989 to January 1991. The study identified cases randomly from area hospitals, drawing from each in proportion to the hospital's contribution to the total population of infants born alive.⁸² The study was able to reach 549 (88.0%) of 624 eligible case mothers and 540 (88.2%) of 612 eligible control mothers. Exposure was defined as any use in the 3 months before or after conception. Additionally, the study estimated, based on self-reports of composition and frequency of use, the average daily folic acid supplement intake from all supplements: less than 0.4 mg, 0.4 to 0.9 mg, and 1.0 mg or greater. Interviews were conducted on average 5 months after birth, leading to an average recall period of 17 months (5 months postpartum, 9 months of pregnancy, 3 months prepregnancy).

An older case-control study drew from cases identified in California and Illinois between 1985 and 1987 (the National Institute of Child Health and Human Development [NICHD] Neural Tube Defects Study).⁹⁶ The authors included anencephaly, meningocele, myelomeningocele, encephalocele, rachischisis, iniencephaly, and lipomeningocele in their definition of cases. In California, cases were identified through a state-mandated reporting systems for NTDs or through contracts with ultrasound centers, amniocentesis centers, disability services, and parents' support groups. In Illinois, cases were identified through ultrasound and genetic units, perinatal networks, hospital neurosurgery services, and vital records. Controls were matched for race/ethnicity, gestational age at diagnosis, date of diagnosis, and geographic area. In California, cases were matched by zip code, and in Illinois, they were matched by county. Exposure was defined as the RDA or more (i.e., women took supplements containing the RDA of at least four vitamins or a higher dose at least 6 days per week), less than the RDA, and none. The authors calculated the amount of folate received based on direct reports of use of brand-name supplements. Interviews were conducted no more than 3 months after birth, leading to a maximum recall period of 13 months (1 month postpartum, 9 months of pregnancy, 3 months prepregnancy).

Results of Included Case-Control Studies

Despite differences in definition of exposure, comparison, and timing across the two National

Birth Defects Prevention Study publications, both are consistent in demonstrating a lack of effect of folic acid supplementation on benefits (aOR for anencephaly and spina bifida, 0.93 [95% CI, 0.82 to 1.06] and aOR for anencephaly, 1.2 [95% CI, 0.8 to 1.9], respectively).^{9,87} A potential explanation for the findings from this surveillance-based database is that in the postfortification era, the majority of cases of NTDs arising from folate deficiency have been averted, and the remainder of the cases represent other potential etiologies. A second explanation is that these findings could have arisen from bias. The National Birth Defects Prevention Study is a surveillance-based database in which eight of 10 sites recorded fetal deaths and elective pregnancy terminations in addition to live births. This case ascertainment approach mitigates the risk of bias from selection that otherwise occurs in studies focusing on live births only, where potentially eligible cases (i.e., fetal deaths and elective pregnancy terminations attributable to NTDs) are lost to analysis. If folic acid supplementation is protective, a sample that is selectively missing women who do not use folic acid supplements and have NTD-affected pregnancies that end in terminations or stillbirths will have higher odds of NTDs with folic acid supplementation than a sample without selection bias. The risk of recall bias, however, is a concern with all retrospective studies. An additional risk of differential recall bias may occur if study participants are generally aware of the potential benefits of folic acid and case mothers systematically overreport its use. A “yes/no” categorization of folic acid supplementation further risks misclassifying exposure.⁹ One publication attempted to address recall bias by focusing on consistent use,⁸⁷ but the risks stemming from recall over the course of up to 3 years persist.

The 2011 Slone Birth Defects Study found no effect of folic acid supplementation on the risk of spina bifida, regardless of the level of supplementation. Consistent users, when compared with nonusers, had an adjusted odds of 1.11 (95% CI, 0.74 to 1.65). Early pregnancy initiators had an adjusted odds of 0.79 (95% CI, 0.54 to 1.16). Inconsistent users had an odds of 2.20 (95% CI, 0.64 to 7.62). These results could be explained by the ceiling effect—all cases of NTDs preventable through supplementation were averted by food fortification, and the remainder constitute a population with a different etiology. Alternatively, these results could arise from bias. Specifically, the sources of bias include 1) differential recall of supplementation, particularly in an era with more widespread knowledge of the support and claims for the use of folic acid supplementation in pregnancy, and 2) selection bias from incomplete case ascertainment, because the study did not consistently include terminated spina bifida cases, which were available consistently from only one site.

The two prefortification Slone Birth Defect Studies with overlapping time periods consistently demonstrate that daily use of supplements reduces the risk of NTDs compared with nonuse (aOR, 0.7 [95% CI, 0.5 to 0.8] in the 2001 study;⁹⁵ adjusted relative risk [RR], 0.6 [95% CI, 0.4 to 0.8] in the 1993 study⁹⁹). The Slone Birth Defect Study, particularly in the early years, had a potential risk of selection bias by not including stillbirths and elective terminations. Later rounds of analysis included a more complete case ascertainment process. The risks of recall bias were somewhat mitigated by having a shorter recall period and a calendar aid highlighting the woman’s last menstrual period. Additionally, these two studies attempted to correct for the issue of differential recall of periconceptional exposure in cases and controls^{95,99} by comparing cases of NTDs with controls of other malformed infants. The 1993 study found a much higher rate of knowledge of the folic acid hypothesis among NTD case mothers than among control mothers of other malformed infants (74/432 [17%] vs. 65/2,561 [2.5%]), suggesting that the knowledge of

the hypothesis could skew recollections of folic acid supplement intake.⁹⁹ However, all cases in this study belong in the prefortification era and do not address the effect of folic acid supplementation in the current environment.

Notably, the 2001 study also offered, through the analysis of the effect of folic acid antagonists, a perspective on what would occur when women are folate deficient because of a folate antagonist. The study found that the adjusted odds of NTDs among women exposed to folic acid antagonists was 2.8 (95% CI, 1.7 to 4.6).

The study of Mexican Americans, spanning the pre- and postfortification era, found a nonsignificant reduction in the odds of NTDs associated with daily consumption of multivitamins containing folic acid (0.77 [95% CI, 0.19 to 3.22]); when adjusted for maternal age, education, obesity, and previous stillbirth or miscarriage, the direction of effect altered (aOR, 1.12 [95% CI, 0.22 to 5.78]; p-value not reported).²² Of note are the extremely low levels of folic acid supplement use in both arms (3 cases of daily use in the 3-month preconceptional period vs. 66 cases of no use in the 6-month periconceptional period among cases; 4 cases of daily use in the 3-month preconceptional period vs. 68 cases of no use in the 6-month periconceptional period among controls).

Two other studies were conducted in the prefortification era. Both studies drew on data from the California Birth Defects Monitoring Program, using cases from 1989 to 1991⁸² and 1985 to 1987. The Shaw et al study found an OR of 0.65 (95% CI, 0.45 to 0.94) for any use in the 3 months before conception. A larger analysis comprising women reporting supplement use in the 3 months before and after conception found an OR of 0.6 (95% CI, 0.46 to 0.79). The NICHD Neural Tube Defects Study, using a combination of slightly older California data (1985 to 1987) and Illinois data (also from 1985 to 1987), reported no effect of supplements on NTDs (calculated OR, 1.00 [95% CI, 0.73 to 1.40]; p=0.97).⁹⁶ The Shaw et al study was able to ascertain the status of approximately 88 percent of eligible cases and controls.⁸² By contrast, the case ascertainment of the Mills et al study was estimated, based on a re-evaluation of the likely prevalence, to be as low as 43 percent.⁸²

KQ 1b. Variation in Effect of Folic Acid Supplementation by Race/Ethnicity

Study Characteristics

Three case-control studies provide limited information about the effects of folic acid supplementation by racial/ethnic and other maternal characteristics.^{11,82,87} **Table 3** presents study characteristics and **Table 6** provides results. The Slone Birth Defects Study provides the most recent data (1998 to 2008).¹¹ In this study, mothers of infants with and without birth defects were interviewed within 6 months of delivery about pregnancy exposures, including details of diet and vitamin intake. Periconceptional folic acid supplementation and dietary folate consumption were compared between 205 mothers of spina bifida cases and 6,357 mothers of nonmalformed controls. Women who reported folic acid supplement use of at least 4 days per week during at least 2 of the 3 periconceptional months (2 months before to 2 months after last menstrual period) were considered to be “consistent users.” A second case-control study analyzed the data

from 1998 to 2003 from the National Birth Defects Prevention Study.⁸⁷ It used logistic regression to compute crude and aORs between cases and controls assessing maternal periconceptional use of folic acid supplements and intake of dietary folic acid. The third case-control study used data from the California Birth Defects Monitoring Program (1989 to 1991). Mothers of 549 cases and 540 controls were interviewed about vitamin supplements used in the 3 months before or after conception.⁸²

Results

The Slone Birth Defects Study found that in the setting of folic acid fortification, folic acid supplementation does not appear to offer further benefit for reducing spina bifida risk.¹¹ Women who reported taking folic acid supplements at least 4 days per week during the months before neural tube closure did not have decreased risk of spina bifida compared with women who reported no supplementation. The lack of protective relationship was observed for white women. The study found a possible increased risk of spina bifida among consistent supplement users of Hispanic ethnicity compared with nonusers (aOR, 2.20 [95% CI, 0.98 to 4.92]); however, the authors note this finding may be due to chance.

The National Birth Defects Prevention Study found that periconceptional supplement use did not reduce the risk of having a pregnancy affected by an NTD, and there were no differences in the effects of folic acid supplementation by race/ethnicity.⁸⁷ Supplement use-race interactions were not significant for anencephaly ($p=0.57$) or spina bifida ($p=0.08$). However, the authors note that the number of cases among non-Hispanic black and Hispanic populations were relatively small, so findings should be interpreted with caution.

The California Birth Defects Monitoring Program found that women who used any folic acid-containing vitamin in the 3 months before conception had a lower risk of having an NTD-affected pregnancy.⁸² Reduction in risk for Hispanics was of smaller magnitude (OR, 0.96 [95% CI, 0.44 to 2.10]) than that observed for non-Hispanic whites (OR, 0.62 [95% CI, 0.35 to 1.10]) and blacks (OR, 0.54 [95% CI, 0.09 to 3.20]), but these results were not statistically significant and could have occurred due to chance.

Although a study focusing on Mexican Americans²² does not provide information about differences by race/ethnicity, it provides an estimate of effect among Hispanic women, albeit in a limited geographical context. When adjusted for maternal age, education, obesity, and previous stillbirth or miscarriage, the OR was 1.12 (95% CI, 0.22 to 5.78; p -value not reported).

KQ 1c. Variation in Effect of Folic Acid Supplementation by Dosage, Timing, and Duration of Therapy

Study Characteristics

One cohort study, set in New England (1984 to 1987) and described in two publications,^{97,98} and six case-control studies^{11,22,82,87,96,99} provided information on the effect of dosage and timing of folic acid supplementation on NTDs. Of these, the most recent case-control studies drew from the Slone Birth Defects Study (1998 to 2008)¹¹ and the National Birth Defects Prevention Study

(1998 to 2003).⁸⁷ A third, focusing on Mexican Americans along the Texas-Mexico border, was conducted between 1995 and 1999.²² Two older case-control studies drew from the California Birth Defects Monitoring Program (1989 to 1991)⁸² and the Slone Birth Defects Study (1988 to 1991),⁹⁹ respectively. The oldest case-control study, the NICHD Neural Tube Defects Study, drew from both California and Illinois (1985 to 1987).⁹⁶ Four studies (one cohort⁹⁸ and three case-control studies^{82,96,99}) reported on dose of folic acid supplementation. Five studies (one cohort⁹⁷ and four case-control studies^{11,22,82,87}) reported on timing of folic acid supplementation.

Table 3 provides further details on study characteristics. We report the cohort study first and then report on the case-control studies in order of recency.

Results

Folic Acid Supplementation Variation by Dosage

All included studies on dose predate the food fortification era (**Table 7**). The New England cohort study (1984 to 1987)⁹⁸ found no statistically significant differences by dose (1 to 399 DFEs, 400–799 DFEs, and ≥ 800 DFEs vs. none). Although authors infer that the study provides no evidence of a dose-response relationship, the number of NTDs for each dose category was low.

The case-control study using data from the California Birth Defects Monitoring Program compared the effect of three levels of dosage (<0.4 mg, 0.4 to 0.9 mg, and ≥ 1.0 mg) with no folic acid supplementation in the 3 months before or after conception.⁸² The study found no differences by dose for women reporting use in the 3 months before conception. In a larger sample of women reporting use in the 3 months after conception (including those who started before conception and continued), the effect of doses below 0.4 mg or above 0.9 mg was not statistically significant compared with no use (OR for 0.4 mg, 0.99 [95% CI, 0.56 to 1.80]; OR for ≥ 1.0 mg, 0.92 [95% CI, 0.54 to 1.60]); only the use of 0.4 to 0.9 mg had a statistically significant effect on NTDs compared with nonuse (OR, 0.54 [95% CI, 0.41 to 0.72]). Of note, however, is the small sample size for the higher and lower doses (54 cases and controls took <0.4 mg and 75 cases and controls took ≥ 1.0 mg).

Data from the Slone Birth Defects Study (1988 to 1991)⁹⁹ suggest lower odds of NTDs for daily use versus less than daily use (calculated OR, 0.57 [95% CI, 0.35 to 0.93]). A supplemental analysis in the same study of differences by dosage among women who did not know the hypothesis between folic acid supplementation and NTDs failed to find a dose-response effect. An older case-control study, the NICHD Neural Tube Defects Study, drawing from cases identified in California and Illinois between 1985 and 1987, reported on the number of NTDs in women receiving the RDA from supplements compared with those receiving less than the RDA.⁹⁶ The study reported no statistically significant differences between different levels of exposure (calculated OR, 1.84 [95% CI, 0.92 to 3.71]). Of note, this study likely had problems with case ascertainment.

Folic Acid Supplementation Variation by Timing

The single cohort study (drawing on cases from 1984 to 1987 and set in New England⁹⁷) reported that using multivitamins in weeks 1 through 6 resulted in a lower odds of NTDs than using multivitamins in weeks 7 and later (10/10,731 vs. 25/7,795; calculated OR, 0.29 [95% CI, 0.14 to 0.60]) (**Table 8**).

Of the four case-control studies, two were set in the postfortification era,^{11,87} one spanned the pre- and postfortification era,²² and one predated the food fortification era.⁸² The most recent case-control study¹¹ reported the risk of consistent use (defined as ≥ 4 days of use per week in 2 of 3 periconceptional months) versus initiating use in the first month of pregnancy (≥ 4 days per week starting in the first or second month postconception). Consistent users had a higher but statistically nonsignificant risk of spina bifida (calculated OR, 1.23 [95% CI, 0.88 to 1.73]). The second postfortification case-control study⁸⁷ reported the risk of anencephaly and spina bifida separately. Women who started folic acid supplementation use before pregnancy had a lower risk of anencephaly compared with women who started during the first month of pregnancy (calculated OR, 0.61 [95% CI, 0.40 to 0.93]). No difference was found for the spina bifida cases (calculated OR, 0.95 [95% CI, 0.71 to 1.28]).

A study of Mexican American women along the Texas-Mexico border, drawing from cases from 1995 to 1999, did not find any statistically significant differences in the odds of NTDs by preconceptional versus postconceptional use (calculated OR, 1.84 [95% CI, 0.58 to 5.86]).²²

The case-control study using data from the California Birth Defects Monitoring Program (1989 to 1991) found lower odds but wide CIs for the use of folic acid supplements in the 3 months before conception compared with no use. It also found no statistically significant effect of NTDs compared with any use of folic acid supplements in the 3 months after conception (88 cases and 98 controls 3 months before vs. 322 cases and 384 controls 3 months after conception; calculated OR, 1.07 [95% CI, 0.77 to 1.48]).⁸²

KQ 2a. Harms of Folic Acid Supplementation in Women of Childbearing Age

Study Characteristics

We included one RCT comparing folic acid supplementation with a multivitamin versus trace elements described in seven publications⁸⁸⁻⁹⁴ and one cohort study (**Table 9**).⁸³ Additionally, the previous review also reported on twinning.^{36,86,88-90,93,94} The trial characteristics are described under KQ 1a.¹⁰¹ As noted previously, the trial included only “informative” cases in the analysis (i.e., live births and stillbirths [late fetal deaths]). The authors noted that it was generally not possible to recognize multiple gestations in miscarriages or ectopic pregnancies.

In a retrospective, population-based cohort study in Norway (N=176,042) of births from December 1998 through the end of 2001,⁸³ the use of folic acid supplements and multivitamins was ascertained using a birth notification form submitted through the Norway Birth Registry. For multiple gestations, the registry received one form for each birth. Separate notification was made

for pregnancies conceived through in vitro fertilization.

Three meta-analyses^{101,108,109} met our initial inclusion criteria and evaluated the effects of periconceptional folic acid supplementation on childhood respiratory illness. One meta-analysis¹⁰¹ with a low risk of bias evaluated the association of folic acid supplementation during the specified time frame of 1 month prior to pregnancy or the first 12 weeks of pregnancy with childhood asthma or wheezing and allergy-related outcomes. However, because of heterogeneity in the type of folic acid supplementation (e.g., folate, combination of folate and dietary folate) and measure of exposure during the periconceptional period, authors limited the pooled estimate to five studies (three cohort, two nested case-control)¹⁰²⁻¹⁰⁶ that assessed folic acid in the periconceptional period (from the month prior to pregnancy) or first trimester. Folic acid supplementation was operationalized as “yes/no.” In the three studies that reported the dose of folic acid, the range was 400 to 600 µg/day.¹⁰³⁻¹⁰⁵ In one study, the average dose was not reported but the investigators suggested it was 400 µg/day.¹⁰² Asthma or wheezing was assessed through a structured parental interview or parental completion of a medical questionnaire. Two studies reported on asthma,^{105,106} two studies reported on wheezing,^{102,103} and one study reported on wheezing and asthma.¹⁰⁴

A second meta-analysis¹⁰⁸ with a medium risk of bias included five published studies and data from a review of a longitudinal cohort study of folic acid supplementation and asthma. We describe the two meta-analyses noted above in **Table 10**. Additionally, a third eligible meta-analysis¹⁰⁹ used a subset of the evidence in the other two meta-analyses; we focus on concordance of these results with other meta-analyses.

Results

Twinning in Women

In an analysis of informative pregnancies in the trial,⁹¹ the proportion of twin pregnancies and twin births (live and stillbirths) was not statistically significantly different between the multivitamin and trace-element arms (**Table 11**). Out of the total pregnancies in the multivitamin group, 1.9 percent (46/2,421) were determined to be twin gestations compared with 1.36 percent (32/2,346) of pregnancies in the trace element group ($\chi^2=2.13$; $p=0.15$). The RR (1.4 [95% CI, 0.87 to 2.26]) was not statistically significantly different between the two groups. The proportion of twin births (as opposed to pregnancies) was higher in the multivitamin group (93/2,468 [3.77%]) than in the trace element group (64/2,378 [2.69%]; RR, 1.42 [95% CI, 1.01 to 1.98]).

In a further analysis for the same trial, women who were not supplemented were excluded (i.e., women who became pregnant before or during the first month of supplementation). The analysis continued to demonstrate a lack of significant difference in the risk of twin pregnancies (calculated RR, 1.5 [95% CI, 0.94 to 2.39]). The study found an increased risk of twin births in the multivitamin group compared with the trace element group (RR, 1.53 [95% CI, 1.08 to 2.16]). The increased risk in the multivitamin group may be due to several factors, including differences in maternal characteristics and pregnancy-specific or delivery-related complications. Limited information is available on differences in maternal characteristics and essentially no data are available on pregnancy-related factors. The findings should be interpreted with caution

because births include live births and stillbirths, and there are no data to discern the proportion of live or stillbirths in each treatment arm.

Twinning and Ovarian Stimulation in Women

The Hungarian trial did not find evidence⁹¹ for an increased risk of twinning among women receiving fertility treatments and randomized to a multivitamin or trace element. The proportion of women in the multivitamin and trace element groups who received fertility treatment was similar at 6.4 and 6.6 percent, respectively. Among the 2,198 women who received multivitamin supplementation, 141 received clomiphene citrate, with or without other infertility drugs, and 19/141 (13.5%) resulted in a multiple gestation. Of the 2,057 women in the multivitamin group who did not receive fertility drugs, 25/2,057 (1.2%) resulted in a multiple gestation. Among the 2,170 women who received trace element supplementation, 143 underwent ovarian stimulation and 12/143 (8.4%) resulted in a multiple gestation. Of the remaining 2,027 pregnancies conceived without ovarian stimulation, 17/2,027 (0.8%) twin pregnancies were identified. Among pregnancies that were conceived without ovarian stimulation, the study found no statistically significantly increased risk of twinning among women who received multivitamin compared with trace element supplementation (OR, 1.46 [95% CI, 0.78 to 2.70]). Among women who underwent ovarian stimulation, the odds of twinning in those receiving multivitamins was calculated to be 1.70 [95% CI, 0.79 to 3.65]. The point estimates and wide CIs are largely due to the relatively small total number of twin gestations in this subgroup analysis (n=73) and the similarly small number (proportion) of twin gestations in the multivitamin and trace element groups.

The initial analysis of the cohort study⁸³ found an increased odds (baseline adjustment for maternal age and parity) of twinning among pregnancies with folic acid supplementation use compared with those with no folic acid supplementation use. With further adjustment for in vitro fertilization, the OR was attenuated and no longer statistically significant (1.04 [95% CI, 0.91 to 1.18]).

In analyses stratified by method of conception (in vitro fertilization or natural conception), the odds of twinning with folic acid supplement use in natural conception (OR, 1.13 [95% CI, 0.97 to 1.33]) were slightly higher than with in vitro fertilization (OR, 0.90 [95% CI, 0.73 to 1.11]). In an analysis stratified by parity, women with no prior pregnancies had slightly higher odds of twinning with folic acid supplement use (OR, 1.31 [95% CI, 1.05 to 1.62]). In a subsequent modeling analysis, the authors assumed that 12.7 percent of pregnancies conceived by true in vitro fertilization were misclassified as natural conception and that 45 percent of women were misclassified as folic acid supplement users. Authors found an attenuated effect of folic acid supplementation and multivitamin use before pregnancy after adjusting for age, parity, and potential misclassification; neither was statistically significant (OR for folic acid supplements before pregnancy, 1.02 [95% CI, 0.85 to 1.24]; OR for multivitamins before pregnancy, 0.98 [95% CI, 0.83 to 1.17]). The authors found elevated risks of twinning with folic acid supplementation (OR, 1.14 [95% CI, 1.00 to 1.23]) or multivitamin use (OR, 1.30 [95% CI, 1.14 to 1.49]) during pregnancy after adjusting for age and parity. The authors note, however, that this effect could be explained by confounding by indication; that is, an increased use of folic acid or multivitamin supplements once the multiple gestation is recognized.

Childhood Asthma or Wheezing and Allergen-Related Outcomes

We identified eight eligible articles,^{100,102-107,110} which were synthesized in three systematic reviews.^{101,108,109} All included primary studies were observational, with attendant risks of misclassification and recall bias. We discuss pooled estimates from the meta-analyses below (**Table 12**). With regard to asthma, the pooled estimate from one meta-analysis¹⁰¹ focusing on the prepregnancy period through the first trimester (N not reported) found no evidence from three studies¹⁰⁴⁻¹⁰⁶ of an association between maternal folic acid supplementation compared with no use and childhood asthma (pooled RR, 1.01 [95% CI, 0.78 to 1.30]; $I^2=0.00$; $p=0.95$ and 0.73 , respectively). For the combined outcomes of wheezing in infants and toddlers and asthma in children, the pooled estimate from five studies¹⁰²⁻¹⁰⁶ resulted in a slightly elevated risk with the use of folic acid supplements before pregnancy or during the first trimester (RR, 1.05 [95% CI, 1.02 to 1.09]; $I^2=0.00$; $p=0.01$ and 0.68 , respectively).

A second meta-analysis evaluating any exposure from the periconceptual period through pregnancy ($n=14,438$)¹⁰⁸ included five studies in the pooled estimate.^{100,104-107} The meta-analysis found no association between folic acid supplementation during the periconceptual period or pregnancy and the development of child asthma (OR, 1.06 [95% CI, 0.99 to 1.14]), but the authors reported wide variations in the dose of folic acid supplementation across included studies. Other allergen-related outcomes included a combination of atopy, eczema, and atopic dermatitis. One meta-analysis¹⁰¹ evaluated these outcomes for periconceptual and first trimester exposure from four studies^{102-104,107} and found two reports of elevated risk from one study¹⁰² among 13 reported associations of lower respiratory tract infections (adjusted RR, 1.09 [95% CI, 1.01 to 1.15]) and hospitalizations from lower respiratory tract infections (adjusted RR, 1.24 [95% CI, 1.09 to 1.41]) among infants ages 0 to 18 months.

A third meta-analysis, using a smaller subset of studies,^{100,103-105} also found no statistically significant differences in the incidence of asthma, wheezing, atopic dermatitis, eczema, or sensitization.¹⁰⁹

Other Reported Harms in Women

The Hungarian trial also reported on other harms.⁹³ The presence or absence of these harms represents potential side effects of folic acid supplementation, many of which are common pregnancy symptoms, and provides reassurance of the safety of folic acid supplementation in the preconceptional period. The Hungarian trial⁹³ reported on differences between weight gain, body weight, gastrointestinal symptoms (hunger or increased appetite, lack of appetite, heartburn and indigestion, constipation, diarrhea, irregular and/or colic defecation [urge to defecate after a meal]), and exanthema (a skin disorder characterized by a rash and skin eruptions) after periconceptual multivitamin and trace element supplementation (**Figure 3**). The study found no statistically significant differences in the report of most of these symptoms between the two groups from before pregnancy through pregnancy confirmation. Women who continued supplementation through the first 12 weeks of pregnancy had an increased risk of weight gain (calculated RR, 1.78 [95% CI, 1.23 to 2.57]), diarrhea (calculated RR, 7.09 [95% CI, 2.72 to 18.47]), and constipation (calculated RR, 1.67 [95% CI, 1.06 to 2.63]) compared with the trace element group. They also had a lower risk of irregular and/or colic defecation compared with the

trace element group (RR, 0.33 [95% CI, 0.16 to 0.68]). The study found no difference in the risk of exanthema, although two participants in the multivitamin group and one in the trace element group withdrew from the study because of this disorder.

KQ 2b. Variation in Harms of Folic Acid Supplementation by Dose, Timing, and Duration of Therapy

Study Characteristics

One meta-analysis¹⁰¹ with a low risk of bias evaluated the association of folic acid supplementation with childhood asthma or wheezing and allergy-related outcomes. Studies were grouped by the timing of exposure: early (preconceptional, periconceptional, first trimester) versus late (second and third trimesters).^{100,102,107} A second meta-analysis also examined timing of supplementation (prepregnancy, early pregnancy, other period in pregnancy).¹⁰⁹ One meta-analysis found one study reporting on dose (<200 µg/day, 200 to 499 µg/day, and >500 µg/day).¹¹⁰

Results

As noted in KQ 2, the meta-analysis that analyzed folic acid supplement use as a dichotomous variable and reported its association with asthma in childhood showed a pooled RR of 1.01 (95% CI, 0.78 to 1.30; $I^2=0.00$; $p=0.95$ and 0.73 , respectively) (**Table 13**).¹⁰¹ Two of the cohort studies included in the meta-analysis examined the association between prenatal use of a supplement containing folic acid (compared with no use) in the second or third trimester and asthma or wheezing in childhood.^{100,102}

Of the 15 associations across two studies, only one association was significantly elevated. Specifically, one study showed that maternal use of folic acid supplements in the third trimester was associated with increased risk of maternal report of wheezing at age 1 year (adjusted prevalence ratio, 1.20 [95% CI, 1.04 to 1.39]).¹⁰⁰ Regarding other outcomes, three cohort studies examined the use of supplements containing folic acid during the second or third trimester and risk of other allergy outcomes.^{100,102,107} The meta-analysis reported no significant findings in 38 reported associations across these three studies.

A meta-analysis examined the incidence of asthma and wheezing by timing of supplementation (prepregnancy, early pregnancy, other period in pregnancy).¹⁰⁹ Four of five reported associations showed no statistically significant effect of folic acid supplementation on asthma or wheezing in childhood. The one statistically significant effect on wheezing in childhood was associated with exposure in early pregnancy (RR, 1.06 [95% CI, 1.02 to 1.09]).¹⁰²⁻¹⁰⁴ One study separated the study population into tertiles (<0.2 mg/day, 0.2–0.499 mg/day, and >0.5 mg/day) and compared the second and third tertiles with the first for the incidence of any allergic disease, sensitization, recurrent wheezing, eczema, food reactions, immunoglobulin E–mediated food allergy, and sensitization to food allergens (**Table 14**). In all cases, the number of events was small, ranging from 16 to 69. All results had wide CIs spanning or overlapping the line of no difference.¹¹⁰

Chapter 4. Discussion

Summary of Evidence

Table 15 provides a summary of findings in this evidence review. This table is organized by KQ and provides a summary of outcomes along with a description of precision, risk of bias, and applicability.

Evidence for Benefits of Folic Acid Supplementation

One RCT, two cohort studies, and eight case-control studies met eligibility criteria, in addition to two publications from the prior review.^{36,86} These 20 publications, comprising 11 primary studies and one systematic review, drew from eight data sources (Hungarian trial,⁸⁸⁻⁹⁴ Hungarian cohort,⁸¹ New England study,^{97,98} National Birth Defects Prevention Study,^{9,87} Slone Birth Defects Study,^{11,95,99} Texas Department of Health's Neural Tube Defect Project,²² California Birth Defects Monitoring Program,⁸² and NICHD Neural Tube Defects Study⁹⁶). Together they span births occurring over three decades, from 1976 through 2007.

After the publication of the Hungarian trial and other trials in women with recurrent NTDs, the clear evidence of benefit pointed to the need for large-scale public health interventions, and the United States added folate to grain products in 1998. The clear evidence of benefit also made the conduct of additional trials unethical. As a consequence, all subsequent studies relied on observational data.

Although all included studies in this review avoid fatal flaws, their designs contain inherent and unavoidable sources of bias. Prospective studies may not be able to ascertain all cases. Retrospective studies have a risk of recall bias. In the case-control studies included in this review, women were asked to recall frequency and dose of supplements over a relatively short period of exposure occurring between 13 months and 3 years prior to the interview. Compounding the risk of recall bias is the relatively widespread knowledge of the protective effect of folic acid supplementation on NTDs. Studies that compare cases with NTD malformations with controls with non-NTD malformations may have a lower risk of differential recall bias. Two such studies showed a clear and consistent protective effect of folic acid supplementation but were based on data collected in the prefortification era.^{95,99}

Both of the risks of bias described above (case ascertainment and recall) will reduce the differences between study groups. A further issue with the included study designs is the relatively rarity of the outcome and the difficulty of adequately powering studies to determine benefits.

Figures 4 and 5 present the results in a forest plot of the largest or more representative study from each data source. **Figure 4** demonstrates that the older studies (with one exception) show a protective effect of folic acid supplementation on NTDs, and the newer studies, all conducted after the introduction of food fortification, do not show a protective effect. The same studies,

when sorted by study design (**Figure 5**), show greater consistency in direction of effect in the cohort studies and greater variation in direction of effect in the case-control studies.

Although the incidence of NTDs has declined in conjunction with food fortification, 23 percent of U.S. women have suboptimal RBC folate concentrations.⁴⁵ Among women who consume supplements, the proportion is less than 10 percent; among women who do not consume supplements, it is 28 percent. Nearly 33 percent of women who consume mandatorily fortified foods alone (i.e., they do not consume folic acid supplements or voluntarily fortified foods) have suboptimal RBC folate concentrations. These statistics suggest a continued and important role for supplements.

Three case-control studies provide information about the effects of folic acid supplementation by racial/ethnic and other maternal characteristics.^{11,82,87} One study suggested that folic acid supplementation may be less protective for Hispanic women, a second showed a higher risk of NTDs among Hispanic women, and the third did not show an effect. None of the studies are conclusive because of small numbers that could have resulted in chance findings.

One cohort study, set in New England (1984 to 1987) and described in two publications,^{97,98} and six case-control studies^{11,22,82,87,96,99} provided evidence on dose and timing. All four studies on dose (one cohort⁹⁸ and three case-control studies^{82,96,99}) predate food fortification; none show a dose-response effect. Notably, the number of cases for varying levels of dosage was small. The five studies (one cohort⁹⁷ and four case-control studies^{11,22,82,87}) reporting on timing of folic acid supplementation did not consistently compare the same timing of exposure. The two older studies did not find statistically significant effects of folic acid supplementation by timing of supplementation.^{22,82} One newer study, conducted entirely in the postfortification era, found more protective effects for women who started before pregnancy compared with during the first month of pregnancy for anencephaly; the protective effect of early timing of exposure did not appear to hold for spina bifida.⁸⁷ The other new study, focusing on spina bifida only, did not find a statistically significant effect of timing of folic acid supplementation on the odds of spina bifida.¹¹

Evidence for Harms of Folic Acid Supplementation

We included one RCT comparing folic acid supplementation with a multivitamin versus trace elements described in seven publications⁸⁸⁻⁹⁴ that evaluated the harms of folic acid supplementation in the periconceptional period to prevent NTDs. We also included one fair-quality cohort study⁸³ of women with and without folic acid supplementation use that met our inclusion criteria. Although we could not rule out risk of an increase in higher-level multiple gestations (triplets or greater) in the RCT^{88-90,93,94} due to a limited number of events, analyses focused on twinning among women with or without treatment with fertility drugs (clomiphene citrate) were reassuring, with point estimates and CIs that did not indicate a risk for multiple gestations. Among a general population of women in the RCT, there was no evidence of a higher risk of twin pregnancies or twin births (combination of live births and stillbirths). When the analyses were limited to only women who completed full or partial folic acid supplementation, there was no evidence of a higher risk of twin pregnancies. Suggestion of a higher likelihood of twin births (live births and stillbirths) among women who completed full or partial folic acid

supplementation compared with those who completed full or partial trace element supplementation should be interpreted cautiously, given the similarity in point estimates and overlap in the calculated 95% CIs. Findings from the observational study⁸³ support the findings from the RCT. After adjustment for in vitro fertilization, the authors found no association between periconceptional folic acid supplement use and twinning.

Comparison of maternal symptoms between the multivitamin with folic acid and trace elements groups in the RCT^{88-90,93,94} suggested a potential higher risk of maternal weight gain, diarrhea, and constipation at 12 weeks of gestation. These symptoms are common in pregnancy and, thus, limit our ability to draw definitive conclusions about the association of folic acid supplementation and these symptomatic outcomes.

With regard to childhood respiratory illness, three meta-analyses^{101,108,109} provided no evidence of a higher risk of child asthma or other allergen-related illness (e.g., atopy, eczema, and atopic dermatitis) and no consistent variation in these outcomes by timing or dose of exposure.¹⁰¹

Applicability of Evidence

Most of the studies included in this review are applicable to primary care. One cohort study of women undergoing alpha-fetoprotein screening or amniocentesis is representative of older pregnant women but not all women.⁹⁷ The modal age for the cohort study ranged from 30 to 39 years; in contrast, the average age of women in other studies was in the 20s. A majority of studies reported that they excluded NTD cases with multiple malformations or known syndromal causes of NTDs.

Sufficiency of Intake

Estimates of folate sufficiency of intake vary widely by measure (**Table 16**). When the highest threshold, the recommended usual intake for preventing NTDs, is used, NHANES data from 2003 to 2006 suggest that 75 percent of nonpregnant women ages 15 to 44 years did not consume the recommended daily intake. Among all women, the median intake of folic acid overall was 245 µg, which is less than the recommended amount of 400 µg.¹¹¹ Although the proportion of intake varies by race/ethnicity, the proportion of women not consuming the recommended usual intake varies from 70 to 91 percent.

Another approach is to set the threshold for insufficiency based on RBC folate concentration. A threshold of 400 ng/mL or more (906 nmol/L) is based on an association with an NTD prevalence of more than 9 cases per 10,000 live births. This threshold yields an estimate suggesting a greater level of sufficiency, on average, with 22.8 percent of nonpregnant women ages 12 to 49 years having suboptimal RBC folate concentrations for NTD prevention. Levels vary by use of dietary supplements containing folic acid, consumption of mandatorily fortified enriched cereal grain products as the only source of folic acid, non-Hispanic black or Hispanic race/ethnicity, or current smoking status. Among women who consume any supplements containing folic acid, 9.7 percent are associated with insufficiency; 28 percent of women who do not consume supplements have insufficient RBC folate concentrations.

The estimated average requirement sets the lowest threshold for folate sufficiency based on the prevention of megaloblastic anemia (not NTD risk reduction).³⁵ The estimated average requirement for individuals ages 14 to 18 years is 330 DFE; for individuals age 19 years or older, it is 320 DFE. NHANES data (2003 to 2006) suggest a much lower level of insufficiency; on average, approximately 15 to 19 percent of childbearing-age women (levels vary by age) did not meet the estimated average requirement for folate.

Very few women exceed the upper level for folic acid consumption. According to the 2015 Dietary Guideline Advisory Committee report, which uses NHANES 2007 to 2010 dietary intake data, less than 3 percent of women ages 14 to 50 years get more than 1,000 µg/day from food, beverages, and dietary supplements.¹¹²

Variation of Intake From Diet and Other Sources

The National Birth Defects Prevention Study¹¹³ and PRAMS^{48,114,115} provide data on folic acid intake (diet and supplemental) prior to pregnancy among women of childbearing age. NHANES,¹¹¹ the March of Dimes surveys,^{116,117} and other studies provide data on folic acid intake among women of reproductive age overall but do not focus on consumption prior to pregnancy specifically. Across these studies, we found differences in consumption of supplemental folic acid by age, race/ethnicity, and other characteristics. We also found differences in total folic acid intake—dietary and supplemental.

Differences by Age

Studies consistently found that among women of reproductive age, supplemental folic acid intake increases with age. PRAMS 2009 found that rates of folic acid consumption (multivitamins, prenatal vitamins, or a folic acid supplement every day of the month before pregnancy) increased as follows: 42.4 percent among women ages 35 to 55 years ; 34.5 percent among women ages 25 to 34 years; and 16.1 percent among women ages 18 to 24 years ($p < 0.05$).⁴⁸ The National Birth Defects Prevention Study (1997 to 2005) found rates of compliant folic acid use (defined as ≥ 5 times per week during the 3 months before conception) as follows: 7.23 percent among women age 19 years or younger, 16.18 percent among women ages 20 to 24 years, 39.30 percent among women ages 25 to 34 years, and 51.96 percent among women age 35 years and older.¹¹³

The March of Dimes surveys and NHANES also provide data on folic acid consumption among women of reproductive age but do not focus on consumption prior to pregnancy. These studies also found that use of supplemental folic acid increases with age.^{111,116,117}

Differences by Race/Ethnicity

Significant improvements in RBC folate status have occurred among all racial/ethnic groups; among non-Hispanic black women, the prevalence of low RBC folate declined from 59.6 percent between 1998 and 1994 to 12.1 percent between 1999 and 2000. During the same time period,

the prevalence of low RBC folate declined from 34.5 to 4.5 percent among non-Hispanic white women and from 38.7 to 1.6 percent among Mexican American women.⁴⁹

Although all women of childbearing age increased their median total folate intake by at least 100 µg/day since fortification, increases were larger for whites than for blacks and Mexican Americans.¹¹⁸ White women were also more likely to have reached the 400 µg/day threshold both pre- and postfortification (30% and 39%, respectively) than black women (20% and 26%, respectively) and Mexican American women (17% and 28%, respectively).¹¹⁸

Consumption of supplements containing folic acid also varies by race/ethnicity and other characteristics. According to the 2007 March of Dimes survey, women who were nonwhite, ages 18 to 24 years, had less than a high school education, or had a household income less than \$25,000 were the least likely to report daily consumption of a supplement containing folic acid.¹¹⁹ Studies consistently found that among women of reproductive age, supplemental folic acid intake is higher among whites than blacks and among non-Hispanics than Hispanics. The National Birth Defects Prevention Study found higher rates of compliant folic acid supplement use among white women (43.73%) compared with black (16.89%), Hispanic (10.87%), or other women (27.98%).¹¹³ PRAMS 2009 found that folic acid consumption was highest among white women (34.2%), followed by other race/ethnicity (33.0%), Hispanic (22.5%), and black women (19.5%) ($p < 0.05$).⁴⁸

The March of Dimes 2008 survey, NHANES,¹¹¹ and the California Women's Health Survey¹²⁰ found similar patterns by race/ethnicity. NHANES 2003 to 2006 data found non-Hispanic whites were most likely to take supplemental folic acid (37.2%), followed by other race/multiracial (24.7%), Mexican Americans (20%), and non-Hispanic blacks (20%).¹¹¹ The California Women's Health Survey 2006 found more white women reported daily use of folic acid containing supplements (50.6%) compared with blacks (39.5%) and Asians (40%). Non-Hispanic women were more likely to report folic acid supplement use (46.9%) than Hispanic women (30.2%).¹²⁰

The March of Dimes conducted a survey of Spanish-language–dominant Hispanic women in 2008. Overall, 21 percent of women took folic acid daily (multivitamins, folic acid supplements, or prenatal vitamins). Rates of daily folic acid supplement use varied by ancestry: Mexican (19%), Central American (22%), South American (35%), and Caribbean/other (25%).¹¹⁷

Differences by Education

Studies consistently found that the use of supplemental folic acid increases with education. The National Birth Defects Prevention Study found rates of compliant folic acid supplement use as follows: 9.04 percent among women with less than a high school education; 17.36 percent among women with a high school education; 30.98 percent among women with 1 to 3 years of college education; and 58.20 percent among women with 4 or more years of college education.¹¹³ State-level PRAMS data from Rhode Island¹¹⁵ and Texas¹¹⁴ found similar patterns. In Rhode Island (PRAMS 2004 to 2008), daily multivitamin use was as follows: 25.1 percent among women with less than a high school education; 23.1 percent among women with a high school education; and 44.5 percent among women with more than a high school education ($p < 0.0001$).

In Texas (PRAMS 2002 to 2010), no daily multivitamin use was highest among women with less than a high school education (80.3%) and high school graduates (82.2%) compared with those with more than a high school education (66.6%). Differences were statistically significant in multivariate models that included age, race/ethnicity, education, insurance status, Medicaid coverage, and pregnancy intention. March of Dimes surveys^{111,116,117} and the California Health Information Survey¹²⁰ also found that folic acid supplement consumption increases with education.

Differences by Income

Studies consistently found that supplemental folic acid use increases with income. The National Birth Defects Prevention Study found rates of compliant folic acid supplement use as follows: 10.07 percent for households making less than \$10,000; 26.05 percent for households making \$10,000 to \$50,000; and 55.21 percent for households making more than \$50,000.¹¹³ Rhode Island PRAMS data (2004 to 2008) found a similar pattern: multivitamin use prior to pregnancy was 21.1 percent for households making less than \$25,000; 30.2 percent for households making \$25,000 to less than \$50,000; and 50.5 percent for households making \$50,000 or more (p<0.0001).¹¹⁵ March of Dimes surveys,^{116,117} NHANES,¹¹¹ and other surveys¹²⁰ also found that folic acid supplement consumption increases with household income.

Differences by Insurance Status

State-level analyses of PRAMS data found differences in supplemental folic acid use by insurance status. Rhode Island PRAMS data (2004 to 2008) found significant differences in folic acid supplement use prior to pregnancy as follows: 17.3 percent among women with no insurance; 25.5 percent among women with public insurance; and 44 percent among women with private insurance (p<0.0001).¹¹⁵ Texas PRAMS data (2002 to 2010) found no daily multivitamin use was higher among women without health care coverage before pregnancy (83.2%) compared with those with coverage (67.9%) and among women on Medicaid (82.7%) compared with those not on Medicaid (64.7%). Differences were statistically significant in multivariate models that included age, race/ethnicity, education, insurance status, Medicaid coverage, and pregnancy intention.¹¹⁴

Differences by Employment Status

The National Birth Defects Prevention Study found higher rates of compliant folic acid supplement use among women who were employed (35.47%) versus those who were unemployed (24.93%).¹¹³

Differences by Marital Status

Rhode Island PRAMS data (2004 to 2008) found higher supplemental folic acid use prior to pregnancy among married women (44%) compared with nonmarried women (21.2%) (p<0.0001).¹¹⁵

Differences by Pregnancy Intention

State-level analyses of PRAMS data found that women intending pregnancy have higher rates of supplemental folic acid use than women not intending pregnancy. Rhode Island PRAMS data (2004 to 2008) found significant differences in folic acid supplement use prior to pregnancy by pregnancy intention (44.5% among women intending pregnancy, 19.4% among women not intending pregnancy; $p < 0.001$).¹¹⁵ Texas PRAMS data (2002 to 2010) found that no daily multivitamin use was more common among women not intending pregnancy (85.6%) than among those intending pregnancy (66.5%). Differences were statistically significant in multivariate models that included age, race/ethnicity, education, insurance status, Medicaid coverage, and pregnancy intention.¹¹⁴

The National Birth Defects Prevention Study found higher rates of compliant folic acid supplement use among women not using birth control (33.51%)—who may be intending pregnancy—than among women using birth control (29.48%).¹¹³

Differences by U.S. Versus Foreign Born and Years in the United States

The National Birth Defects Prevention Study found higher rates of compliant folic acid supplement use among women born in the United States (35.94%) than among foreign-born women (18.5%). The 2008 March of Dimes survey of Spanish-language–dominant Hispanic women found rates of daily folic acid supplement use varied by number of years in the United States (<5 years, 10%; 5 to 10 years, 19%; ≥ 10 years, 24%; born in the United States, 18%).¹¹⁷

Differences by Parity

Rhode Island PRAMS data (2004 to 2008) found differences in folic acid supplement use prior to pregnancy by parity: 37.1 percent among women having their first birth and 32.6 percent among women having their second or higher birth ($p = 0.0012$). NHANES 2003 to 2006 reports folic acid supplement use by parity: 32.4 percent for women with no live births, 30.3 percent for women with one live birth, and 31.3 percent for women with two or more live births.¹¹¹ The 2008 March of Dimes survey of Spanish-language–dominant Hispanic women found that daily folic acid supplement use was lowest among women who had never been pregnant (12%) compared with women who had been pregnant in the past 2 years or who were currently pregnant (20%), last pregnant 3 to 4 years ago (26%), or last pregnant 5 or more years ago (23%).¹¹⁷

Differences by Health Behaviors/Health Status

The National Birth Defects Prevention Study found higher rates of compliant folic acid supplement use among nonsmokers (35.13%) versus smokers (21.93%) and among women who had any alcohol intake in the 3 months prior to conception (38.01%) compared with no alcohol intake (27.65%).¹¹³ NHANES 2003 to 2006 found use of folic acid supplements was lower

among women who have diabetes (17%) than among women who do not have diabetes (32.1%).¹¹¹

Effect of Folic Acid Supplementation Outside the Periconceptual Period on NTDs

We did not find studies examining the effect of folic acid supplementation outside the periconceptual period on NTDs.

Variation in Effect of Folic Acid Supplementation by Medical Risk Factors

To date, the effect of folic acid supplementation on NTDs in pregnancies complicated by medical risk factors, including obesity, diabetes, seizure disorders, and therapy with folic acid antagonists (e.g., methotrexate), has been incompletely investigated, leaving physicians and their patients with limited guidance on dosage or interval of dosing in important subgroups of women. We sought to determine the available evidence on the effect of periconceptual folic acid supplementation in certain high-risk yet moderately prevalent subgroups of pregnant women. Published studies do not provide direct evidence for developing clinical guidelines but do provide insight on the current state of knowledge of the effects of folic acid supplement in women with medical risk factors and identify important gaps and research needs. Although the findings from several large population-based case-control studies are available for review, there are relatively few cases of NTDs. Also, there is potential recall bias because folic acid supplementation is assessed primarily by maternal interviews or questionnaires completed 6 months or more after infant delivery.

Diabetes

Offspring of women with pregestational diabetes have a two- to fourfold increased risk of a wide range of birth defects. Prior studies in humans and animal models show that glucose control is an important prevention method. Additionally, studies suggest that micronutrient levels, including folic acid, may play a key role in the pathogenesis of NTDs and other birth defects. Larger epidemiologic studies in this area have focused primarily on the joint effects of diabetes and obesity and folic acid intake to better determine the association of folic acid supplementation across key groups: no diabetes or obesity and folic acid use (reference group), no diabetes or obesity and no folic acid intake, diabetes and/or obesity and folic acid use, diabetes and/or obesity and no folic acid use (highest risk group). One of the largest population-based studies, the Birth Defects Prevention Study (1997 to 2004),^{28,121} identified 14,721 cases (infants with cardiac or noncardiac birth defects, including spina bifida and anencephaly) and 5,437 controls and assessed the joint effects of maternal diabetes and folic acid consumption on birth defect development. Periconceptual folic acid supplementation was defined as intake in the month before conception or during the first 3 months of pregnancy. Women with pregestational diabetes and no use of folic acid supplementation had an increased odds of birth defects overall, but the

findings for specific birth defects must be interpreted with caution because they are limited by a small number of cases. Regarding spina bifida, there was a nonsignificant twofold increase (OR, 2.37 [95% CI, 0.21 to 26.65]) in the risk of spina bifida among women with diabetes and no folic acid supplementation compared with women without diabetes who were taking folic acid supplements (reference group). There was a higher odds of anencephaly in this same group (OR, 31.56 [95% CI, 4.98 to 199.94]).

The Slone Epidemiology Birth Defects Study¹²² assessed the effect of periconceptional folic acid supplementation on NTDs within the setting of diabetes and obesity. Folic acid intake was ascertained through maternal interviews conducted within 6 months of delivery and was operationalized for analysis as 400 µg/day or more versus less than 400 µg/day. Spina bifida was more likely to occur in women who had diabetes (0.7% vs. 0.4%) or obesity (19% vs. 10.8%) than in those without either condition. In analyses stratified by folic acid use, pregnancies with diabetes and less than 400 µg/day of folic acid had a statistically significant odds of NTDs (aOR, 3.95 [95% CI, 1.56 to 10.00]). Among pregnancies with appropriate folic acid supplementation and diabetes, there was a lower but statistically significant odds of NTDs (aOR, 1.31 [95% CI, 0.7 to 10]).

A smaller study¹²³ among pregnant women with diabetes (n=31) and without diabetes (n=54) found no difference in dietary, serum, or RBC folate concentrations between the two groups, after adjusting for the extent of folic acid supplementation. These findings suggest that there is no difference in folic acid metabolism among women with and without diabetes, but the findings will need to be confirmed in a larger study. Additionally, while the level of glycemic control in early pregnancy is hypothesized to influence development of cardiac defects, the role of glycemic control on the conversion of homocysteine to methionine, the pathway thought to be most important to the development of NTDs, is not known, and there was no adjustment for glucose levels or the degree of glucose control in the analysis. Finally, it may be that the NTDs among women with diabetes may occur through multiple pathways.

Maternal Obesity

The epidemic of maternal obesity in the United States has drawn attention to whether the recommended daily dose of periconceptional folic acid supplementations is sufficient for women with an elevated BMI. It is unclear, however, whether lower concentrations of folate in overweight/obese pregnant women are associated with a higher risk of NTDs and, therefore, whether the effect of folic acid supplementation varies with obesity. Although there are no recommendations specifically for overweight or obese women in the United States, some countries have recommended higher dosages up to 5 mg daily for overweight women. Prior studies have reported an inverse association between BMI and concentrations of micronutrients, including folic acid. Lower folic acid levels have been documented with increasing BMI, although a direct effect on the incidence of NTDs has not been observed. In one study,¹²⁴ serum folate concentrations and dietary intake of folate were assessed across BMI quartiles at mid- and late pregnancy among 802 and 660 women, respectively. A statistically significant association between BMI and folate concentration was reported at mid-pregnancy (p=0.001 for trend). While these data suggest a decrease in folic acid levels during pregnancy, the time frame is mid- to late pregnancy, which is well beyond the first 28 days of gestation, in which maternal folate

concentrations prevent NTDs.

Folic Acid Antagonists and Seizure Medications

We did not find conclusive evidence that the effect of folic acid supplementation on prevention of NTDs varies with the use of folic acid antagonists or seizure medications. Jentink and colleagues¹²⁵ conducted a registry-based case-control study of infants with spina bifida (cases) compared with infants with other congenital malformations unrelated to folic acid metabolism (controls) to determine the effect of periconceptional folic acid supplementation (4 weeks prior to conception through the first 8 weeks of pregnancy) on the risk of NTDs among women with epilepsy and treatment with valproic acid or carbamazepine. Over the study time period, 11,864 pregnancies with congenital malformations (197 pregnancies with spina bifida) were identified. Sixty-six women were exposed to antiepileptic drugs. There was no statistically significant effect of folic acid supplementation on pregnancy outcomes in women exposed to valproic acid or carbamazepine (OR, 0.9 [95% CI, 0.2 to 4.3]). In a subsequent analysis, Jentink¹²⁶ examined the effect of folic acid supplementation on NTDs in pregnancies exposed and unexposed to valproic acid alone. Among pregnancies unexposed to valproic acid, there was a 50 percent reduction in NTDs with folic acid supplementation (OR, 0.5 [95% CI, 0.3 to 0.7]). Among pregnancies exposed to valproic acid, there was no effect of folic acid supplementation on the incidence of NTDs (OR, 1.0 [95% CI, 0.1 to 7.6]). There is little evidence to suggest that folic acid has a diminished protective effect on NTDs in women taking antiepileptic drugs and essentially no data on the effect of various dosages of folic acid supplementation on NTDs. First trimester exposure to valproic acid is associated with a 1 to 2 percent risk of NTDs, but the mechanism by which NTDs occur in the setting of valproic acid exposure is unclear, and further investigations are needed to better understand the biological pathways in which valproic acid may affect neural tube closure.

Several folic acid antagonists were evaluated in a large case-control study by Hernandez-Diaz.⁹⁵ Reported findings indicate a higher likelihood of NTDs (OR, 2.8 [95% CI, 1.7 to 4.6]) with exposure to any one of several folic acid antagonists (including carbamazepine, phenobarbital, phenytoin, primidone, sulfasalazine, triamterene, and trimethoprim) in the first or second month of pregnancy compared with no exposure, even after adjusting for maternal characteristics, reproductive history, and folic acid supplementation. Subgroup analyses show a higher adjusted odds of NTDs with exposure to carbamazepine (aOR, 6.9 [95% CI, 1.9 to 25.7]) or trimethoprim (aOR, 4.8 [95% CI, 1.5 to 16.1]). These findings should be interpreted with caution because the number of cases identified, particularly in subgroup analyses, was small. No studies were identified that evaluated the impact of folic acid supplementation in the setting of methotrexate exposure, a folic acid antagonist used in the treatment of ectopic (i.e., tubal) pregnancy.

Prior NTDs

Meta-analysis findings¹²⁷ indicated that periconceptional folic acid supplementation was effective in reducing the risk of recurrent NTDs in the included studies of women with prior pregnancies complicated by NTDs. Evidence from three studies¹²⁸⁻¹³⁰ (n=1,650 total pregnancies) of folic acid (doses ranging from 360 µg/day^{102,128} to 4 mg/day^{129,130}) with a

multivitamin (10/817 cases) compared with multivitamin alone or iron and calcium alone (32/833 cases) indicated a 67 percent reduction in NTDs (RR, 0.33 [95% CI, 0.17 to 0.66]); folic acid of 400 µg/day combined with a multivitamin (10/817 cases) compared with multivitamin alone (32/833 cases) indicated a 67 percent reduction in NTDs (RR, 0.33 [95% CI, 0.17 to 0.66]). Heterogeneity of the pooled analysis was low ($\chi^2=1.25$; $I^2=0.0\%$; $p=0.74$). The pooled RR was largely driven by the Medical Research Council Study, which had a total of 27/1,195 cases. Individual RR estimates, however, were consistent and statistically significantly associated with a reduction in the recurrence of NTDs across all three studies (range, 0.29 to 0.42).

Other Fetal, Neonatal, or Maternal Benefits of Folic Acid Supplementation

Two Cochrane reviews evaluated the effect of folic acid supplementation on birth defects¹³¹ and on maternal health and pregnancy outcomes.¹³² Neither found other benefits of folic acid supplementation.

The Cochrane review on birth defects searched for evidence through July 2010. The review found no statistically significant effect of folic acid supplementation during the perinatal period compared with no treatment, other micronutrients, or placebo on the prevention of congenital cardiovascular defects (3 studies; N=2,869; RR, 0.55 [95% CI, 0.27 to 1.14]), cleft palate (3 studies; N=2,869; RR, 0.66 [95% CI, 0.11 to 3.92]), cleft lip (3 studies; N=2,869; RR, 1.00 [95% CI, 0.27 to 3.74]), and other birth defects, excluding NTDs, cleft lip, cleft palate, and cardiovascular defects (average RR, 0.81 [95% CI, 0.38 to 1.77]). The same review found no effect on miscarriage (5 studies; N=7,618; RR, 1.10 [95% CI, 0.97 to 1.26]), stillbirth (4 studies; N=5,994; RR, 0.96 [95% CI, 0.51 to 1.83]), or low birth weight (1 study; N=186; RR, 0.80 [95% CI, 0.39 to 1.64]).¹³¹ In other analyses (restricted to placebo-controlled studies, expanded to include folic acid plus other micronutrients), the lack of effect persisted.

The Cochrane review on maternal health and pregnancy outcomes searched for evidence through December 2012.¹³² The reviews found no effect of folic acid compared with no folic acid on preterm birth (3 studies; N=2,959; RR, 1.01 [95% CI, 0.73 to 1.38]), stillbirths or neonatal deaths (3 studies; N=3,110; RR, 1.33 [95% CI, 0.96 to 1.85]), low birth weight of less than 2,500 g (3 studies; N=3,089; RR, 0.80 [95% CI, 0.63 to 1.02]), or predelivery anemia (8 studies; N=4,149; RR, 0.62 [95% CI, 0.35 to 1.10]).¹³²

Limitations of the Review

The limitations of this review arise from scoping decisions and the limitations of the evidence. As with the previous review, we restricted interventions to folic acid supplementation and did not consider food fortification, counseling to increase dietary intake, or screening for NTDs. The review did not systematically examine the benefits of folic acid supplementation on benefits other than averted NTDs, although we considered this issue as a contextual question. Finally, we did not systematically evaluate the effect of folic acid supplementation among high-risk populations such as women with previous pregnancies with NTDs; we considered this issue as a

contextual question.

Limitations of the evidence relate to insufficient data and the quality of evidence as a whole. We found very limited information on differences in benefits and risks of folic acid supplementation by race/ethnicity, dose, and timing and no information on duration. Regarding the overall quality of evidence, ethical considerations limit the use of RCTs for this question. Observational studies carry limitations of case ascertainment and recall bias, and these two sources of bias serve to reduce the observed effect of folic acid supplements on NTDs.

Future Research Needs

The results of more recent studies (case-control studies) showing lack of benefit from folic acid supplementation run counter to the relatively consistent results from older studies (trials, cohort studies, and case-control studies) showing benefit. This difference can potentially be attributed to a real attenuation of the effect of folic acid supplementation in the context of food fortification, or it can be attributed to study design issues. Future research attempting to separate design effects from the real effects of the intervention need to consider important study design constraints.

Given ethical considerations about withholding folic acid from pregnant women, no trials of folic acid supplementation versus placebo are likely in the future. Additional case-control studies will likely encounter very similar issues of recall bias. Prospective studies could potentially shed light on the real effect of the intervention, provided they collect complete dietary and supplemental intake using consistent measurement tools and ensure good case ascertainment. Medication event monitoring systems (such as pill bottles fitted with a tracking device that records the date and times the container is opened and closed) could potentially improve the validity of adherence. Prospective studies that have access to state-based registries of birth defects may be able to ensure a higher rate of case ascertainment. Prospective studies by definition, however, will not be entirely representative of the primary care population because they will recruit women intending to become pregnant. Studies have shown that over half of the births in the United States were unplanned and that the rate of unintended pregnancy is higher among younger women than older women of childbearing age.¹³³

Future research may have to rely on the intermediate links between folic acid intake, RBC folate concentration, and outcomes in place of studies evaluating the direct link between intake and outcomes. As discussed earlier in this report, current studies of the effects of folic acid supplementation are limited by the inability to fully measure a woman's total consumption of folate (natural intake and supplementation). Studies that prospectively assess and quantify dietary intake, including the intake of foods fortified with folic acid and folic acid supplementation, and RBC folate concentration can provide further evidence on the first part of this intermediate evidence chain. Retrospective or prospective studies that link RBC folate concentration and outcomes can provide further evidence on the second part of the intermediate evidence chain.

These findings can provide additional data to inform public health messaging about folic acid

supplementation regimens that, with compliance, can achieve optimal concentrations of folate in the periconception period. As assays are standardized, future studies may be able to measure folate status more efficiently through plasma, serum, or RBC concentrations. RBC folate concentration may offer a sufficiently consistent and precise measure of maternal folate levels and ensure maximal benefit in the reduction of NTDs. Further studies are needed to evaluate the effect of measuring this biomarker on NTD reduction and the ability to integrate RBC folate concentration into population-based health policies.

Another potential research direction is to use simulation studies to discern the magnitude of effect of food fortification versus folic acid supplementation using older studies with fewer design flaws. Two ongoing trials (**Appendix G**) of high- versus low-dose folic acid (4 vs. 0.4 mg per day) in the Netherlands and Italy, expected to be completed in 2016, can provide additional relevant information.

Future updates of this systematic review should evaluate the intermediate links between folic acid supplementation and other sources of folate intake, folate status, and outcomes.

In addition to unanswered questions of overall effectiveness, future research should evaluate differences in subpopulations. We found limited information on the extent to which folic acid supplementation had a protective effect among Hispanic women and possible reasons for differences in subgroups.

Conclusion

Older studies with fewer design flaws, conducted before food fortification, show that folic acid supplementation provides protection against NTDs. Newer studies, conducted after food fortification with folate in the United States, do not demonstrate this protective effect. These studies, however, have the potential for misclassification and recall bias, both of which can serve to attenuate the effect of folic acid supplementation on NTDs. Although mandatory food fortification in the United States has been accompanied by a decline in NTD prevalence, variations in intake continue to leave nearly a quarter of the U.S. population with suboptimal RBC folate concentrations, suggesting a continued important role for folic acid supplement use.

Inconsistent results of effectiveness of folic acid on the risk of NTDs among Hispanic women compared with white or black women could be due to chance. We found no evidence of a dose-response effect but studies had small numbers of cases for subanalyses. We did not find consistent evidence on timing of folic acid supplementation for benefits. We did not find consistent evidence on harms, specifically twinning, respiratory outcomes, and other harms (e.g., gastrointestinal symptoms, weight gain or loss). Limited evidence suggests that the association of folic acid supplementation with twinning is attenuated after adjustment for covariates.

References

1. National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development. Neural Tube Defects (NTDs): Condition Information. Bethesda, MD: USA.gov; 2012 November 30
<http://www.nichd.nih.gov/health/topics/ntds/conditioninfo/Pages/default.aspx>. Accessed May 2, 2014.
2. Centers for Disease Control and Prevention (CDC). CDC Grand Rounds: additional opportunities to prevent neural tube defects with folic acid fortification. *MMWR Morb Mortal Wkly Rep*. 2010 Aug 13;59(31):980-4. PMID: 20703205.
3. National Institute of Neurological Disorders and Stroke. NINDS Anencephaly Information Page. Bethesda, MD: NINDS; 2014 April 16.
<http://www.ninds.nih.gov/disorders/anencephaly/anencephaly.htm>. Accessed July 23, 2012.
4. National Institute of Neurological Disorders and Stroke. Cephalic disorders fact sheet. Bethesda, MD: NINDS; 2012 April 16, 2014.
http://www.ninds.nih.gov/disorders/cephalic_disorders/detail_cephalic_disorders.htm. Accessed July 23, 2012.
5. Rasmussen SA, Olney RS, Holmes LB, et al. Guidelines for case classification for the National Birth Defects Prevention Study. *Birth Defects Res A Clin Mol Teratol*. 2003 Mar;67(3):193-201. PMID: 12797461.
6. Rasmussen SR, Fraus JL. Genetics of syndromic neural tube defects. In: Wyszynski DF, ed. *Neural Tube Defects: From Origin to Treatment*. New York, NY: Oxford University Press; 2006.
7. Williams J, Mai CT, Mulinare J, et al. Updated estimates of neural tube defects prevented by mandatory folic acid fortification - United States, 1995-2011. *MMWR Morb Mortal Wkly Rep*. 2015 Jan 16;64(1):1-5. PMID: 25590678.
8. American College of Obstetricians and Gynecologists. Neural tube defects. *ACOG Practice Bulletin No. 44*. *Obstet Gynecol*. 2003;102:203-13.
9. Agopian AJ, Tinker SC, Lupo PJ, et al. Proportion of neural tube defects attributable to known risk factors. *Birth Defects Res A Clin Mol Teratol*. 2013 Jan;97(1):42-6. PMID: 23427344.
10. Elwood JM, Little J, Elwood JH. *Epidemiology and control of neural tube defects*. Oxford, UK: Oxford University Press; 1992.
11. Ahrens K, Yazdy MM, Mitchell AA, et al. Folic acid intake and spina bifida in the era of dietary folic acid fortification. *Epidemiology*. 2011 Sep;22(5):731-7. PMID: 21659881.
12. van der Put NM, Eskes TK, Blom HJ. Is the common 677C->T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *QJM*. 1997 Feb;90(2):111-5. PMID: 9068801.
13. Tsang BL, Devine OJ, Cordero AM, 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 Jun;101(6):1286-94. PMID: 25788000.

14. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol*. 2000 May 1;151(9):862-77. PMID: 10791559.
15. van der Put NM, Steegers-Theunissen RP, Frosst P, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet*. 1995 Oct 21;346(8982):1070-1. PMID: 7564788.
16. Shin M, Besser LM, Siffel C, et al. Prevalence of spina bifida among children and adolescents in 10 regions in the United States. *Pediatrics*. 2010 Aug;126(2):274-9. PMID: 20624803.
17. Goldman P, Peppercorn MA. Drug therapy: sulfasalazine. *N Engl J Med*. 1975 Jul 3;293(1):20-3. PMID: 236516.
18. Williams LJ, Rasmussen SA, Flores A, et al. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995-2002. *Pediatrics*. 2005 Sep;116(3):580-6. PMID: 16140696.
19. Aneji CN, Northrup H, Au KS. Deep sequencing study of the MTHFR gene to identify variants associated with myelomeningocele. *Birth Defects Res A Clin Mol Teratol*. 2012 Feb;94(2):84-90. PMID: 22241680.
20. Centers for Disease Control and Prevention (CDC). Racial/ethnic differences in the birth prevalence of spina bifida - United States, 1995-2005. *MMWR Morb Mortal Wkly Rep*. 2009 Jan 9;57(53):1409-13. PMID: 19129744.
21. Mitchell LE. Epidemiology of neural tube defects. *Am J Med Genet C Semin Med Genet*. 2005 May 15;135C(1):88-94. PMID: 15800877.
22. Suarez L, Hendricks KA, Cooper SP, et al. Neural tube defects among Mexican Americans living on the US-Mexico border: effects of folic acid and dietary folate. *Am J Epidemiol*. 2000 Dec 1;152(11):1017-23. PMID: 11117610.
23. Suarez L, Felkner M, Brender JD, et al. Neural tube defects on the Texas-Mexico border: what we've learned in the 20 years since the Brownsville cluster. *Birth Defects Res A Clin Mol Teratol*. 2012 Nov;94(11):882-92. PMID: 22945287.
24. Hamner HC, Mulinare J, Cogswell ME, et al. Predicted contribution of folic acid fortification of corn masa flour to the usual folic acid intake for the US population: National Health and Nutrition Examination Survey 2001-2004. *Am J Clin Nutr*. 2009 Jan;89(1):305-15. PMID: 19056605.
25. da Silva VR, Hausman DB, Kauwell GP, et al. Obesity affects short-term folate pharmacokinetics in women of childbearing age. *Int J Obes (Lond)*. 2013 Dec;37(12):1608-10. PMID: 23567925.
26. McMahon DM, Liu J, Zhang H, et al. Maternal obesity, folate intake, and neural tube defects in offspring. *Birth Defects Res A Clin Mol Teratol*. 2013 Feb;97(2):115-22. PMID: 23404872.
27. Flegal KM, Carroll MD, Kit BK, et al. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012 Feb 1;307(5):491-7. PMID: 22253363.
28. Correa A, Gilboa SM, Botto LD, et al. Lack of periconceptional vitamins or supplements that contain folic acid and diabetes mellitus-associated birth defects. *Am J Obstet Gynecol*. 2012 Mar;206(3):218.e1-13. PMID: 22284962.

29. Dheen ST, Tay SS, Boran J, et al. Recent studies on neural tube defects in embryos of diabetic pregnancy: an overview. *Curr Med Chem*. 2009;16(18):2345-54. PMID: 19519395.
30. Yerby MS. Management issues for women with epilepsy: neural tube defects and folic acid supplementation. *Neurology*. 2003 Sep 1;61(6 Suppl 2):S23-6. PMID: 14504306.
31. Shaer CM, Chescheir N, Schulkin J. Myelomeningocele: a review of the epidemiology, genetics, risk factors for conception, prenatal diagnosis, and prognosis for affected individuals. *Obstet Gynecol Surv*. 2007 Jul;62(7):471-9. PMID: 17572919.
32. Chen CP. Syndromes, disorders and maternal risk factors associated with neural tube defects (VII). *Taiwan J Obstet Gynecol*. 2008 Sep;47(3):276-82. PMID: 18935989.
33. Milunsky A, Ulcickas M, Rothman KJ, et al. Maternal heat exposure and neural tube defects. *JAMA*. 1992 Aug 19;268(7):882-5. PMID: 1640616.
34. Parker SE, Mai CT, Canfield MA, et al. Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004-2006. *Birth Defects Res A Clin Mol Teratol*. 2010 Dec;88(12):1008-16. PMID: 20878909.
35. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academies Press; 1998.
36. Wolff T, Witkop CT, Miller T, et al. Folic Acid Supplementation for the Prevention of Neural Tube Defects: An Update of the Evidence for the U.S. Preventive Services Task Force. Evidence Synthesis No. 70 AHRQ Publication No. 09-051132-EF-1. Rockville MD: Agency for Healthcare Research and Quality; May 2009. PMID: 20722169.
37. Food and Drug Administration (FDA). Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Final Rule. *Fed Regist*. 1996;61(44):8781-97.
38. Honein MA, Paulozzi LJ, Mathews TJ, et al. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA*. 2001 Jun 20;285(23):2981-6. PMID: 11410096.
39. National Birth Defects Prevention Network. Neural tube defect ascertainment project 2010. 2004 http://www.nbdpn.org/current/resources/ntd_fa_info.html. Accessed 29 May 2015.
40. World Health Organization. *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.
41. Shane B. Folate status assessment history: implications for measurement of biomarkers in NHANES. *Am J Clin Nutr*. 2011 Jul;94(1):337S-42S. PMID: 21593497.
42. Crider KS, Devine O, Hao L, et al. Population red blood cell folate concentrations for prevention of neural tube defects: bayesian model. *BMJ*. 2014;349:g4554. PMID: 25073783.
43. U.S. Department of Health and Human Services. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. *Fed Regist*. 1996 March 5;61(44):8781-97.

44. Yang Q, Cogswell ME, Hamner HC, et al. Folic acid source, usual intake, and folate and vitamin B-12 status in US adults: National Health and Nutrition Examination Survey (NHANES) 2003-2006. *Am J Clin Nutr*. 2010 Jan;91(1):64-72. PMID: 19828716.
45. Tinker SC, Hamner HC, Qi YP, et al. U.S. women of childbearing age who are at possible increased risk of a neural tube defect-affected pregnancy due to suboptimal red blood cell folate concentrations, National Health and Nutrition Examination Survey 2007 to 2012. *Birth Defects Res A Clin Mol Teratol*. 2015 Jun;103(6):517-26. PMID: 25884850.
46. Siega-Riz AM, Bodnar LM, Savitz DA. What are pregnant women eating? Nutrient and food group differences by race. *Am J Obstet Gynecol*. 2002 Mar;186(3):480-6. PMID: 11904611.
47. Gahche J, Bailey R, Burt V, et al. Dietary supplement use among U.S. adults has increased since NHANES III (1988-1994). *NCHS Data Brief*. 2011 Apr(61):1-8. PMID: 21592424.
48. Robbins CL, Zapata LB, Farr SL, et al. Core state preconception health indicators - pregnancy risk assessment monitoring system and behavioral risk factor surveillance system, 2009. *MMWR Surveill Summ*. 2014 Apr 25;63(3):1-62. PMID: 24759729.
49. McDowell MA, Lacher DA, Pfeiffer CM, et al. Blood folate levels: the latest NHANES results. *NCHS Data Brief*. 2008 May(6):1-8. PMID: 19389320.
50. Crider KS, Bailey LB, Berry RJ. Folic acid food fortification-its history, effect, concerns, and future directions. *Nutrients*. 2011 Mar;3(3):370-84. PMID: 22254102.
51. Marchetta CM, Devine OJ, Crider KS, 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 Apr;7(4):2663-86. PMID: 25867949.
52. Daly LE, Kirke PN, Molloy A, et al. Folate levels and neural tube defects. Implications for prevention. *JAMA*. 1995 Dec 6;274(21):1698-702. PMID: 7474275.
53. Berry RJ, Li Z, Erickson JD, 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 Nov 11;341(20):1485-90. PMID: 10559448.
54. Hao L, Yang QH, Li Z, et al. Folate status and homocysteine response to folic acid doses and withdrawal among young Chinese women in a large-scale randomized double-blind trial. *Am J Clin Nutr*. 2008 Aug;88(2):448-57. PMID: 18689382.
55. Crider KS, Zhu JH, Hao L, 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 Jun;93(6):1365-72. PMID: 21508090.
56. Chuang CH, Hillemeier MM, Dyer AM, et al. The relationship between pregnancy intention and preconception health behaviors. *Prev Med*. 2011 Jul-Aug;53(1-2):85-8. PMID: 21539855.
57. Dott M, Rasmussen SA, Hogue CJ, et al. Association between pregnancy intention and reproductive-health related behaviors before and after pregnancy recognition, National Birth Defects Prevention Study, 1997-2002. *Matern Child Health J*. 2010 May;14(3):373-81. PMID: 19252975.
58. Goldberg BB, Alvarado S, Chavez C, et al. Prevalence of periconceptual folic acid use and perceived barriers to the postgestation continuance of supplemental folic acid: survey

- results from a Teratogen Information Service. *Birth Defects Res A Clin Mol Teratol*. 2006 Mar;76(3):193-9. PMID: 16511885.
59. Rosenberg KD, Gelow JM, Sandoval AP. Pregnancy intendedness and the use of periconceptional folic acid. *Pediatrics*. 2003 May;111(5 Pt 2):1142-5. PMID: 12728127.
 60. Finer LB, Zolna MR. Unintended pregnancy in the United States: incidence and disparities, 2006. *Contraception*. 2011 Nov;84(5):478-85. PMID: 22018121.
 61. Robbins CL, Zapata LB, Farr SL, et al. Core state preconception health indicators - pregnancy risk assessment monitoring system and behavioral risk factor surveillance system, 2009. *MMWR Surveill Summ*. 2014 Apr 25;63 Suppl 3:1-62. PMID: 24759729.
 62. Centers for Disease Control and Prevention. Preconception Care and Health Care. Atlanta, GA: Centers for Disease Control and Prevention; 2013 February 1. <http://www.cdc.gov/preconception/showyourlove/>. Accessed 31 July 2015.
 63. Lynch MM, Squiers LB, Lewis MA, et al. Understanding women's preconception health goals: audience segmentation strategies for a preconception health campaign. *Soc Mar Q*. 2014;20:148-64.
 64. Squiers L, Mitchell EW, Levis DM, et al. Consumers' perceptions of preconception health. *Am J Health Promot*. 2013 Jan-Feb;27(3 Suppl):S10-9. PMID: 23286658.
 65. Lewis MA, Mitchell EW, Levis DM, et al. Couples' notions about preconception health: implications for framing social marketing plans. *Am J Health Promot*. 2013 Jan-Feb;27(3):S20-S7. PMID: WOS:000323479400009.
 66. Rofail D, Colligs A, Abetz L, et al. Factors contributing to the success of folic acid public health campaigns. *J Public Health (Oxf)*. 2012 Mar;34(1):90-9. PMID: 21727078.
 67. March of Dimes. Improving Preconception Health: Women's Knowledge and Use of Folic Acid. March of Dimes Foundation Item # 50-2392-08. White Plains, NY: December 2008. <http://www.marchofdimes.com/peristats/pdfdocs/2008FolicAcidSurveyReport.pdf>
 68. American College of Obstetricians and Gynecologists. The importance of preconception care in the continuum of women's health care. Committee Opinion Number 313. Washington, DC: American College of Obstetricians and Gynecologists; September 2005.
 69. Callegari LS, Ma EW, Schwarz EB. Preconception care and reproductive planning in primary care. *Med Clin North Am*. 2015 May;99(3):663-82. PMID: 25841606.
 70. American Congress of Obstetricians and Gynecologists. ACOG practice bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 44, July 2003. (Replaces Committee Opinion Number 252, March 2001). *Obstet Gynecol*. 2003 Jul;102(1):203-13. PMID: 12850637.
 71. American Academy of Pediatrics. Committee on Genetics. Folic acid for the prevention of neural tube defects. *Pediatrics*. 1999 Aug;104(2 Pt 1):325-7. PMID: 10429019.
 72. Centers for Disease Control and Prevention. From the Centers for Disease Control and Prevention. Recommendations for use of folic acid to reduce number of spina bifida cases and other neural tube defects. *JAMA*. 1993 Mar 10;269(10):1233, 6-8. PMID: 8437292.
 73. American Academy of Family Physicians. Summary of Recommendations for Clinical and Preventive Services. Neural Tube Defects, Prevention, Folic Acid Supplementation, Women. Lakewood, KS: AAFP; 2009 www.aafp.org/patient-care/clinical-recommendations/all/neural-tube-defects.html. Accessed 2 May 2014.

74. National Institute for Health and Care Excellence. NICE public health guidance: PH11 Maternal and child nutrition. London: NICE; 2008
<http://publications.nice.org.uk/maternal-and-child-nutrition-ph11/recommendations#folic-acid-2on>. Accessed 2 May 2014.
75. Quality Standards Subcommittee of the American Academy of Neurology. Practice parameter: management issues for women with epilepsy (summary statement). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Epilepsia*. 1998 Nov;39(11):1226-31. PMID: 9821989.
76. Sterne JAC, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions. *BMJ (Online)*. in press.
77. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343(d5928):d5928. PMID: 22008217.
78. Whiting P, Savovic J, Higgins JP, et al. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. *J Clin Epidemiol*. 2016 Jan;69:225-34. PMID: 26092286.
79. Harris RP, Helfand M, Woolf SH, et al. Current methods of the US Preventive Services Task Force: a review of the process. *Am J Prev Med*. 2001 Apr;20(3 Suppl):21-35. PMID: 11306229.
80. West SL, Gartlehner G, Mansfield AJ, et al. Comparative Effectiveness Review Methods: Clinical Heterogeneity. Methods Research Report AHRQ Publication No. 10-EHC070-EF. Rockville, MD: Agency for Healthcare Research and Quality; 2010.
<http://effectivehealthcare.ahrq.gov/>
81. Czeizel AE, Dobo M, Vargha P. Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol*. 2004 Nov;70(11):853-61. PMID: 15523663.
82. Shaw GM, Schaffer D, Velie EM, et al. Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology*. 1995 May;6(3):219-26. PMID: 7619926.
83. Vollset SE, Gjessing HK, Tandberg A, et al. Folate supplementation and twin pregnancies. *Epidemiology*. 2005 Mar;16(2):201-5. PMID: 15703534.
84. Goh YI, Bollano E, Einarson TR, et al. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis. *J Obstet Gynaecol Can*. 2006 Aug;28(8):680-9. PMID: 17022907.
85. Thompson SJ, Torres ME, Stevenson RE, et al. Periconceptional multivitamin folic acid use, dietary folate, total folate and risk of neural tube defects in South Carolina. *Ann Epidemiol*. 2003 Jul;13(6):412-8. PMID: 12875798.
86. Wolff T, Witkop CT, Miller T, et al. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*. 2009 May 5;150(9):632-9. PMID: 19414843.
87. Mosley BS, Cleves MA, Siega-Riz AM, et al. Neural tube defects and maternal folate intake among pregnancies conceived after folic acid fortification in the United States. *Am J Epidemiol*. 2009 Jan 1;169(1):9-17. PMID: 18953063.
88. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. 1992 Dec 24;327(26):1832-5. PMID: 1307234.

89. Czeizel AE. Prevention of congenital abnormalities by periconceptional multivitamin supplementation. *BMJ*. 1993 Jun 19;306(6893):1645-8. PMID: 8324432.
90. Czeizel AE, Dudas I, Metneki J. Pregnancy outcomes in a randomised controlled trial of periconceptional multivitamin supplementation. Final report. *Arch Gynecol Obstet*. 1994;255(3):131-9. PMID: 7979565.
91. Czeizel AE, Metneki J, Dudas I. The higher rate of multiple births after periconceptional multivitamin supplementation: an analysis of causes. *Acta Genet Med Gemellol (Roma)*. 1994;43(3-4):175-84. PMID: 8588492.
92. Czeizel AE. Controlled studies of multivitamin supplementation on pregnancy outcomes. *Ann N Y Acad Sci*. 1993 Mar 15;678:266-75. PMID: 8257482.
93. Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. *Eur J Obstet Gynecol Reprod Biol*. 1998 Jun;78(2):151-61. PMID: 9622312.
94. Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. *Am J Med Genet*. 1996 Mar 15;62(2):179-83. PMID: 8882400.
95. Hernandez-Diaz S, Werler MM, Walker AM, et al. Neural tube defects in relation to use of folic acid antagonists during pregnancy. *Am J Epidemiol*. 2001 May 15;153(10):961-8. PMID: 11384952.
96. Mills JL, Rhoads GG, Simpson JL, et al. The absence of a relation between the periconceptional use of vitamins and neural-tube defects. National Institute of Child Health and Human Development Neural Tube Defects Study Group. *N Engl J Med*. 1989 Aug 17;321(7):430-5. PMID: 2761577.
97. Milunsky A, Jick H, Jick SS, et al. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA*. 1989 Nov 24;262(20):2847-52. PMID: 2478730.
98. Moore LL, Bradlee ML, Singer MR, et al. Folate intake and the risk of neural tube defects: an estimation of dose-response. *Epidemiology*. 2003 Mar;14(2):200-5. PMID: 12606886.
99. Werler MM, Shapiro S, Mitchell AA. Periconceptional folic acid exposure and risk of occurrent neural tube defects. *JAMA*. 1993 Mar 10;269(10):1257-61. PMID: 8437302.
100. Bekkers MB, Elstgeest LE, Scholtens S, et al. Maternal use of folic acid supplements during pregnancy, and childhood respiratory health and atopy. *Eur Respir J*. 2012 Jun;39(6):1468-74. PMID: 22034647.
101. Crider KS, Cordero AM, Qi YP, et al. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. *Am J Clin Nutr*. 2013 Nov;98(5):1272-81. PMID: 24004895.
102. Haberg SE, London SJ, Stigum H, et al. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child*. 2009 Mar;94(3):180-4. PMID: 19052032.
103. Kieft-de Jong JC, Timmermans S, Jaddoe VW, et al. High circulating folate and vitamin B-12 concentrations in women during pregnancy are associated with increased prevalence of atopic dermatitis in their offspring. *J Nutr*. 2012 Apr;142(4):731-8. PMID: 22399526.
104. Magdelijns FJ, Mommers M, Penders J, et al. Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics*. 2011 Jul;128(1):e135-44. PMID: 21690114.

105. Martinussen MP, Risnes KR, Jacobsen GW, et al. Folic acid supplementation in early pregnancy and asthma in children aged 6 years. *Am J Obstet Gynecol.* 2012 Jan;206(1):72.e1-7. PMID: 21982024.
106. Whitrow MJ, Moore VM, Rumbold AR, et al. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. *Am J Epidemiol.* 2009 Dec 15;170(12):1486-93. PMID: 19880541.
107. Granell R, Heron J, Lewis S, et al. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. *Clin Exp Allergy.* 2008 Feb;38(2):320-8. PMID: 18070159.
108. Yang L, Jiang L, Bi M, et al. High dose of maternal folic acid supplementation is associated to infant asthma. *Food Chem Toxicol.* 2015 Jan;75:88-93. PMID: 25449200.
109. Wang T, Zhang HP, Zhang X, et al. Is folate status a risk factor for asthma or other allergic diseases? *Allergy Asthma Immunol Res.* 2015 Nov;7(6):538-46. PMID: 26333700.
110. Dunstan JA, West C, McCarthy S, et al. The relationship between maternal folate status in pregnancy, cord blood folate levels, and allergic outcomes in early childhood. *Allergy.* 2012 Jan;67(1):50-7. PMID: 21923665.
111. Tinker SC, Cogswell ME, Devine O, et al. Folic acid intake among U.S. women aged 15-44 years, National Health and Nutrition Examination Survey, 2003-2006. *Am J Prev Med.* 2010 May;38(5):534-42. PMID: 20347553.
112. Dietary Guidelines Advisory Committee. Scientific Report of the 2015 Dietary Guidelines Advisory Committee: Advisory Report to the Secretary of Health and Human Services. Rockville, MD: U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA); 2015. <http://health.gov/dietaryguidelines/2015-scientific-report/PDFs/Scientific-Report-of-the-2015-Dietary-Guidelines-Advisory-Committee.pdf>.
113. Khodr ZG, Lupo PJ, Agopian AJ, et al. Preconceptional folic acid-containing supplement use in the national birth defects prevention study. *Birth Defects Res A Clin Mol Teratol.* 2014 Apr 7. PMID: 24706436.
114. Centers for Disease Control and Prevention. Preconception health indicators among women--Texas, 2002-2010. *MMWR Morb Mortal Wkly Rep.* 2012 Jul 27;61(29):550-5. PMID: 22832936.
115. Kim H, Cain R, Viner-Brown S, et al. Multivitamin use prior to pregnancy in Rhode Island. *Med Health R I.* 2011 Sep;94(9):276-8. PMID: 22010325.
116. March of Dimes. Improving preconception health: women's knowledge and use of folic acid. 2008.
117. March of Dimes. Improving preconception health: knowledge and use of vitamins and folic acid among Spanish-language-dominant Hispanic women. 2009.
118. Bentley TG, Willett WC, Weinstein MC, et al. Population-level changes in folate intake by age, gender, and race/ethnicity after folic acid fortification. *Am J Public Health.* 2006 Nov;96(11):2040-7. PMID: 17018833.
119. Centers for Disease Control and Prevention (CDC). Use of supplements containing folic acid among women of childbearing age--United States, 2007. *MMWR Morb Mortal Wkly Rep.* 2008 Jan 11;57(1):5-8. PMID: 18185493.
120. Centers for Disease Control and Prevention. Trends in folic acid supplement intake among women of reproductive age--California, 2002-2006. *MMWR Morb Mortal Wkly Rep.* 2007 Oct 26;56(42):1106-9. PMID: 17962802.

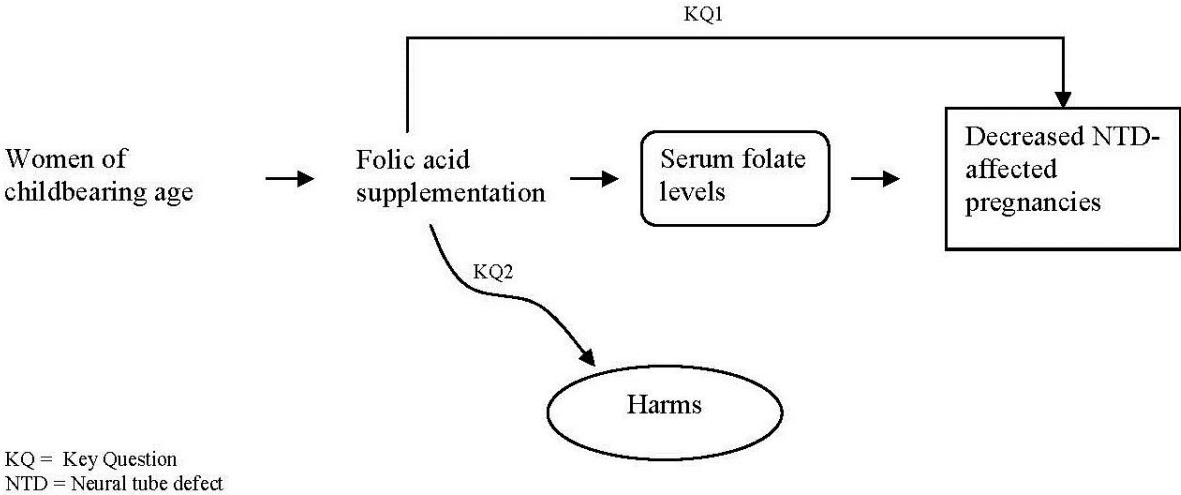
121. Correa A, Stolley A, Liu Y. Prenatal tea consumption and risks of anencephaly and spina bifida. *Ann Epidemiol.* 2000 Oct 1;10(7):476-7. PMID: 11018418.
122. Parker SE, Yazdy MM, Tinker SC, et al. The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. *Am J Obstet Gynecol.* 2013 Sep;209(3):239.e1-8. PMID: 23711668.
123. Kaplan JS, Iqbal S, England BG, et al. Is pregnancy in diabetic women associated with folate deficiency? *Diabetes Care.* 1999 Jul;22(7):1017-21. PMID: 10388960.
124. Kim H, Lee YA, Hwang JY, et al. Relationship between body-mass index and serum folate concentrations in pregnant women: Mothers and Children's Environmental Health (MOCEH). *FASEB J.* 2011;25.
125. Jentink J, Bakker MK, De Jong-van Den Berg LTW. Effect of correct folic acid use on spina bifida in pregnancies exposed to valproic acid or carbamazepine in the Netherlands. *Pharmacoepidemiol Drug Saf.* 2009;18(S1):S13.
126. Jentink J, Bakker MK, Nijenhuis CM, et al. Does folic acid use decrease the risk for spina bifida after in utero exposure to valproic acid? *Pharmacoepidemiol Drug Saf.* 2010 Aug;19(8):803-7. PMID: 20680999.
127. Van Allen MI, Boyle E, Thiessen P, et al. The impact of prenatal diagnosis on neural tube defect (NTD) pregnancy versus birth incidence in British Columbia. *J Appl Genet.* 2006;47(2):151-8. PMID: 16682757.
128. Kirke PN, Daly LE, Elwood JH. A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child.* 1992 Dec;67(12):1442-6. PMID: 1489222.
129. Central Technical Co-Ordinating Unit. Multicentric study of efficacy of periconceptional folic acid containing vitamin supplementation in prevention of open neural tube defects from India. *Indian J Med Res.* 2000;112:206-11. PMID: CN-00328155.
130. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet.* 1991 Jul 20;338(8760):131-7. PMID: 1677062.
131. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, et al. Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database Syst Rev.* 2010(10):Cd007950. PMID: 20927767.
132. Lassi Zohra S, Salam Rehana A, Haider Batool A, et al. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. *Cochrane Database of Syst Rev.* 2013(3). PMID: CD006896.
133. Finer LB, Zolna MR. Shifts in intended and unintended pregnancies in the United States, 2001-2008. *Am J Public Health.* 2014 Feb;104 Suppl 1:S43-8. PMID: 24354819.
134. Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* Washington DC: National Academy Press; 2001.
135. O'Keefe CA, Bailey LB, Thomas EA, et al. Controlled dietary folate affects folate status in nonpregnant women. *J Nutr.* 1995 Oct;125(10):2717-25. PMID: 7562109.
136. Herbert V. Experimental nutritional folate deficiency in man. *Trans Assoc Am Physicians.* 1962;75:307-20.

137. Eichner ER, Pierce HI, Hillman RS. Folate balance in dietary-induced megaloblastic anemia. *N Engl J Med.* 1971 Apr 29;284(17):933-8. PMID: 5551802.
138. Hoffbrand AV, Newcombe BF, Mollin DL. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. *J Clin Pathol.* 1966;19:17-28.
139. Varadi S, Abbott D, Elwis A. Correlation of peripheral white cell and bone marrow changes with folate levels in pregnancy and their clinical significance. *J Clin Pathol.* 1966;19(1):33-&. PMID: WOS:A19667336800005.
140. Yoon PW, Rasmussen SA, Lynberg MC, et al. The National Birth Defects Prevention Study. *Public Health Rep.* 2001;116 Suppl 1:32-40. PMID: 11889273.
141. Bailey RL, Dodd KW, Gahche JJ, et al. Total folate and folic acid intake from foods and dietary supplements in the United States: 2003-2006. *Am J Clin Nutr.* 2010 Jan;91(1):231-7. PMID: 19923379.
142. U.S. Department of Agriculture. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Appendix E-2: Supplementary Documentation to the 2015 DGAC Report. Washington, DC: U.S. Department of Agriculture; February 2015.
143. Brown SB, Reeves KW, Bertone-Johnson ER. Maternal folate exposure in pregnancy and childhood asthma and allergy: a systematic review. *Nutr Rev.* 2014 Jan;72(1):55-64. PMID: 24551950.
144. Abe M. Folic acid supplementation during pregnancy affects food allergy risk in offspring depending on parental allergies. *Allergy.* 2014;69:152.
145. Abe M. The effect of maternal folic acid intake during pregnancy on incidence of child food allergy. *Allergy.* 2013;68:559-60.
146. Abe M. The effect of folic acid supplementation before conception on the onset of food allergies in infants. *Allergy.* 2015;70:266.
147. Berry RJ, Kihlberg R, Devine O. Impact of misclassification of in vitro fertilisation in studies of folic acid and twinning: modelling using population based Swedish vital records. *BMJ.* 2005 Apr 9;330(7495):815. PMID: 15722370.
148. Botto LD, Erickson JD, Mulinare J, et al. Maternal fever, multivitamin use, and selected birth defects: evidence of interaction? *Epidemiology.* 2002 Jul;13(4):485-8. PMID: 12094106.
149. Bower C, Stanley FJ. Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. *Med J Aust.* 1989 Jun 5;150(11):613-9. PMID: 2725375.
150. Bower C, Stanley FJ. Periconceptional vitamin supplementation and neural tube defects; evidence from a case-control study in Western Australia and a review of recent publications. *J Epidemiol Community Health.* 1992 Apr;46(2):157-61. PMID: 1583432.
151. Brescianini S, Cotichini R, Nistico L, et al. Folic acid supplementation before conception and chance of a twin pregnancy: Preliminary results from an Italian case-control study. *Twin Res Hum Genet.* 2012;15(2):177.
152. Carmichael SL, Yang W, Shaw GM. Periconceptional nutrient intakes and risks of neural tube defects in California. *Birth Defects Res A Clin Mol Teratol.* 2010 Aug;88(8):670-8. PMID: 20740594.
153. Chandler AL, Hobbs CA, Mosley BS, et al. Neural tube defects and maternal intake of micronutrients related to one-carbon metabolism or antioxidant activity. *Birth Defects Res A Clin Mol Teratol.* 2012 Nov;94(11):864-74. PMID: 22933447.

154. Charles D, Ness AR, Campbell D, et al. Taking folate in pregnancy and risk of maternal breast cancer. *BMJ*. 2004 Dec 11;329(7479):1375-6. PMID: 15591563.
155. Charles DH, Ness AR, Campbell D, et al. Folic acid supplements in pregnancy and birth outcome: re-analysis of a large randomised controlled trial and update of Cochrane review. *Paediatr Perinat Epidemiol*. 2005 Mar;19(2):112-24. PMID: 15787886.
156. Taylor CM, Atkinson C, Penfold C, et al. Folic acid in pregnancy and mortality from cancer and cardiovascular disease: further follow-up of the Aberdeen folic acid supplementation trial. *J Epidemiol Community Health*. 2015 Aug;69(8):789-94. PMID: 25855124.
157. Czeizel AE, Vargha P. Periconceptional folic acid/multivitamin supplementation and twin pregnancy. *Am J Obstet Gynecol*. 2004 Sep;191(3):790-4. PMID: 15467542.
158. Czeizel AE, Toth M, Rockenbauer M. Population-based case control study of folic acid supplementation during pregnancy. *Teratology*. 1996 Jun;53(6):345-51. PMID: 8910980.
159. De Marco P, Merello E, Calevo MG, et al. Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: an Italian case-control study. *Childs Nerv Syst*. 2011 Jul;27(7):1073-81. PMID: 21207040.
160. Desoto MC, Hitlan RT. Synthetic folic acid supplementation during pregnancy may increase the risk of developing autism. *J Pediatr Biochem*. 2012;2(4):251-61.
161. Ericson A, Kallen B, Aberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res*. 2001 Apr;4(2):63-6. PMID: 11665336.
162. Gildestad T, Nordtveit TI, Nilsen RM, et al. Maternal folic acid and multivitamin supplementation and risk of neural tube defects: a population-based registry study. *Eur J Epidemiol*. 2013;28(1):S242-S3.
163. Haberg SE. Folic acid supplements in pregnancy and respiratory health in early childhood. *J Allergy Clin Immunol*. 2009;123(2):S18.
164. Kallen B. Use of folic acid supplementation and risk for dizygotic twinning. *Early Hum Dev*. 2004 Nov;80(2):143-51. PMID: 15500994.
165. Kallen B. Congenital malformations in infants whose mothers reported the use of folic acid in early pregnancy in Sweden. A prospective population study. *Congenit Anom (Kyoto)*. 2007 Dec;47(4):119-24. PMID: 17988253.
166. Kondo A, Morota N, Date H, et al. Awareness of folic acid use increases its consumption, and reduces the risk of spina bifida. *Br J Nutr*. 2015 Jul 14;114(1):84-90. PMID: 25999131.
167. Medveczky E, Puho E. Parental employment status and neural-tube defects and folic acid/multivitamin supplementation in Hungary. *Eur J Obstet Gynecol Reprod Biol*. 2004 Aug 10;115(2):178-84. PMID: 15262352.
168. Mulinare J, Cordero JF, Erickson JD, et al. Periconceptional use of multivitamins and the occurrence of neural tube defects. *JAMA*. 1988 Dec 2;260(21):3141-5. PMID: 3184392.
169. Ohya Y, Yonemoto J, Ogata T, et al. Influence of environmental chemicals and drugs taken before and during pregnancy on onset of childhood asthma and eczema. *Allergy*. 2011;66:554.
170. Shaw GM, Nelson V, Carmichael SL, et al. Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? *Epidemiology*. 2002 Nov;13(6):625-30. PMID: 12410002.

171. Veeranki SP, Gebretsadik T, Dorris SL, et al. Association of folic acid supplementation during pregnancy and infant bronchiolitis. *Am J Epidemiol.* 2014 Apr 15;179(8):938-46. PMID: 24671071.
172. Veeranki SP, Gebretsadik T, Mitchel EF, et al. Timing of prenatal folic acid supplementation and risk of allergic rhinitis in early childhood. *Am J Respir Crit Care Med.* 2014;189.
173. Di Renzo GC, Spano F, Giardina I, et al. Iron deficiency anemia in pregnancy. *Womens Health (Lond Engl).* 2015 Oct 16. PMID: 26472066.
174. Locksmith GJ, Duff P. Preventing neural tube defects: the importance of periconceptual folic acid supplements. *Obstet Gynecol.* 1998 Jun;91(6):1027-34. PMID: 9611019.
175. Kallen BA, Olausson PO. Use of folic acid and delivery outcome: a prospective registry study. *Reprod Toxicol.* 2002 Jul-Aug;16(4):327-32. PMID: 12220592.
176. Lumley J, Watson L, Watson M, et al. Periconceptual supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane Database Syst Rev.* 2001(3):Cd001056. PMID: 11686974.
177. Shaw GM, Rozen R, Finnell RH, et al. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. *Am J Epidemiol.* 1998 Jul 1;148(1):30-7. PMID: 9663401.
178. Shaw GM, Todoroff K, Carmichael SL, et al. Lowered weight gain during pregnancy and risk of neural tube defects among offspring. *Int J Epidemiol.* 2001 Feb;30(1):60-5. PMID: 11171858.
179. Shaw GM, Velie EM, Schaffer D. Risk of neural tube defect-affected pregnancies among obese women. *JAMA.* 1996 Apr 10;275(14):1093-6. PMID: 8601928.

Figure 1. Analytic Framework



Abbreviations: KQ=Key Question; NTD=neural tube defect.

Figure 2. Preferred Reporting of Systematic Review and Meta-Analysis (PRISMA) Tree

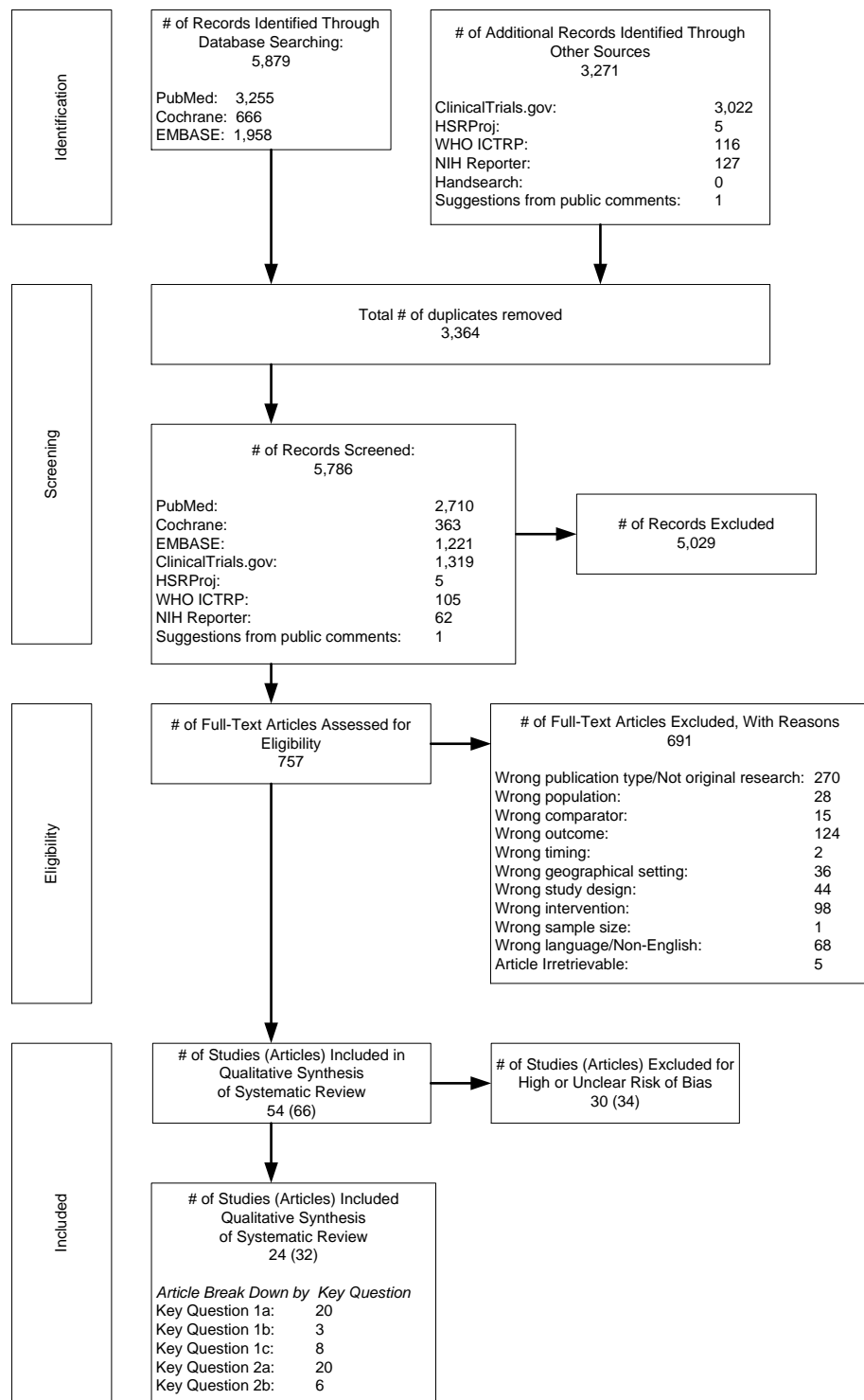


Figure 4. Folic Acid Supplementation and Neural Tube Defects by Earliest Year of Recruitment: Forest Plot

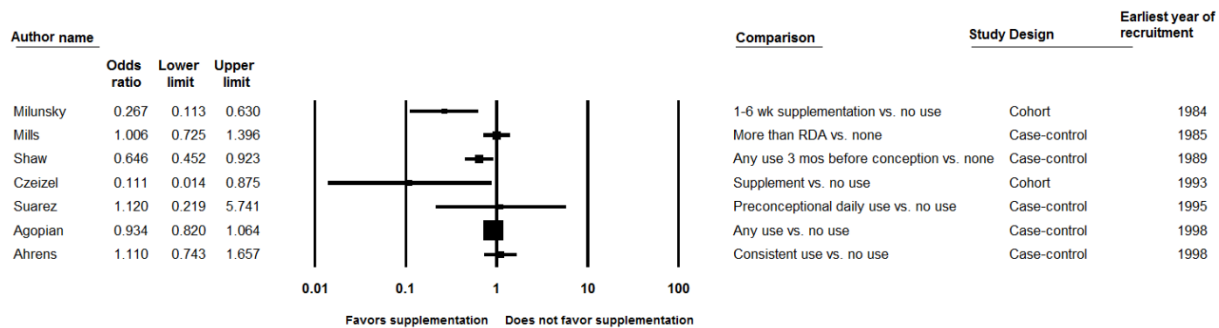


Figure 5. Folic Acid Supplementation and Neural Tube Defects by Study Design: Forest Plot

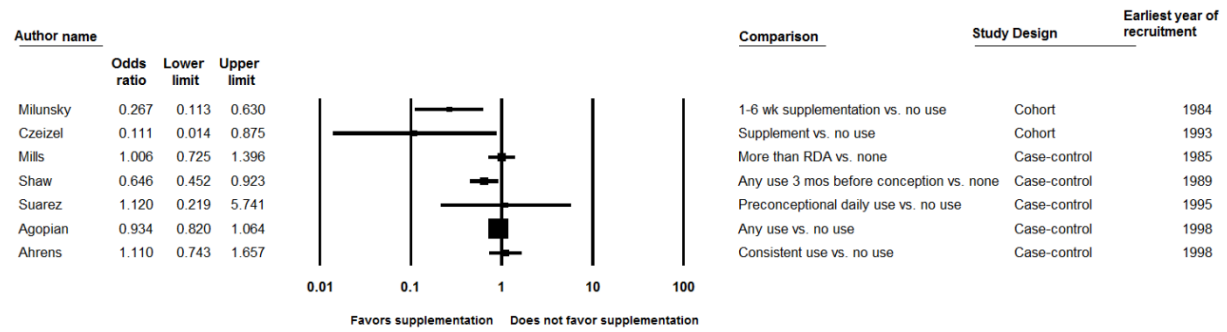


Table 1. Measures and Definitions

Measure	Definition
Recommended Daily Allowance (RDA) ¹³⁴	<ul style="list-style-type: none"> The RDA is the average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group. If the standard deviation is available and the data are normally distributed, the RDA = estimated average requirement (EAR) + 2 SD of EAR. If data about variability in requirements are insufficient to calculate an SD, a coefficient of variation for the EAR of 10% is assumed. The resulting equation for the RDA is then RDA = 1.2 × EAR. The RDA for folate is set by assuming a coefficient of variation of 10% because information is not available on the standard deviation of the requirement for folate; the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97 to 98% of the individuals in the group. For folate the RDA is 120% of the EAR. The RDA for both men and women is 400 µg/day of dietary folate equivalents.
Dietary Folic Equivalent (DFE)	<ul style="list-style-type: none"> 1 DFE: 0.6 µg of folic acid from fortified food or as a supplement consumed with food = 0.5 µg of a supplement taken on an empty stomach³⁵
Estimated Average Requirement (EAR)	<ul style="list-style-type: none"> The EAR is the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group. EAR for females 15–50: 320 DFE³⁵ The 320 DFE is based on 1 study of 5 patients who were fed a diet of 319 DFE.¹³⁵ Of these women, 3 had RBC folate <305 nmol/L, suggesting that with 320 DFE half would have RBC folate over 305 nmol/L. The threshold of 305 nmol/L (140 ng/mL) of folate was chosen as the cutoff point for adequate folate status based on evidence that lower levels were associated with the appearance of hypersegmented neutrophils (1 case¹³⁶; 2 cases¹³⁷ and its association with megaloblastic anemia (40 patients with megaloblastic anemia also had RBC folate <305 nmol/L¹³⁸; 238 pregnant women with RBC <327 nmol/L had megoblastic marrow¹³⁹ or chromosomal damage (8 patients with RBC folate <305 nmol/L had a threefold higher frequency of cellular micronuclei (suggesting DNA and chromosomal damage) than 14 control patients
Plasma/serum folate concentration	<ul style="list-style-type: none"> Reflects current concentrations of folate in the circulation based on intake of folate that occurs naturally in food, fortified foods with folic acid and folic acid supplementation. Because plasma/serum levels represent recent folate intake, it is not possible to differentiate between whether the result indicates a transient decline in folate intake or chronic deficiency. No threshold value for plasma/serum folate concentration to prevent NTDs. Further research is necessary to correlate plasma/serum levels with RBC folate concentrations.
Red Blood Cell (RBC) Folate Concentrations	<ul style="list-style-type: none"> Reflects body stores of folate; therefore, considered to be more accurate of folate status than plasma or serum folate concentrations. RBC folate levels can be assessed with microbiological assays or commercial protein-binding assays on automated clinical analyzers. At the population level, RBC folate concentrations should be above 400 ng/mL (906 nmol/L).⁴⁰ RBC is preferred over blood serum concentrations because there is less variation. It is unknown how much natural food folate or folic acid intake is necessary to achieve adequate RBC folate concentrations.^{40,42}

Abbreviations: CV=coefficient of variation; DNA=deoxyribonucleic acid; NTD=neural tube defect; SD=standard deviation.

Table 2. Current Guidelines for Folic Acid Supplementation

Organization	Definition of Treatment Population	Guideline
American College of Obstetrics and Gynecology ⁷⁰	General population: Women capable of becoming pregnant	Folic acid supplementation of 400 µg per day is recommended during the periconceptional period to reduce the occurrence and recurrence of NTDs in low-risk women.
	High-risk population: women at high risk of NTDs or with a previous pregnancy with an NTD	Folic acid supplementation of 4 mg per day is recommended for women at high risk of NTDs.
American Academy of Pediatrics ⁷¹	General population: Women with no history of a previous pregnancy affected by an NTD	All women of childbearing age, capable of becoming pregnant, and having no history of a previous pregnancy affected by an NTD should consume 400 µg (0.4 mg) of folic acid.
	High-risk population: Women with a previous pregnancy affected by an NTD, having a close relative with an NTD, having diabetes, receiving treatment of valproic acid or carbamazepine for a seizure disorder, and having an NTD, or having a partner with an NTD	Women with a previous pregnancy affected by an NTD should consume 4,000 µg (4 mg) of folic acid per day starting 1 month before the time they plan to become pregnant and the first 3 months of pregnancy, unless contraindicated. Women should be advised not to attempt to achieve the 4,000 µg daily dosage of folic acid by taking over-the-counter or prescription multivitamins containing folic acid because of the possibility of ingesting harmful levels of other vitamins. Women of other high-risk groups who are planning a pregnancy should discuss with their physician the advantages and disadvantages of increasing their daily periconceptional folic acid intake to 4,000 µg.
Public Health Service ⁷²	General population: Women of childbearing age in the United States	Women of childbearing age in the United States who are capable of becoming pregnant should consume 0.4 mg of folic acid per day to reduce the risk of having a pregnancy affected with spina bifida or other NTDs. Because the effects of high intakes are not well known but include complicating the diagnosis of vitamin B12 deficiency, care should be taken to keep total folate consumption at less than 1 mg per day, except under the supervision of a physician.
	High-risk population: Women who have had a previous pregnancy affected by an NTD	Women with a previous pregnancy affected by an NTD should consult their physicians for advice when planning to become pregnant.
American Academy of Family Physicians ⁷³	Women planning or capable of pregnancy	Daily supplement containing 0.43 to 0.8 mg (400 to 800 µg) of folic acid is recommended for women planning a pregnancy or capable of pregnancy.
American Academy of Neurology ⁷⁵	Women with epilepsy	Folic acid supplementation should be instituted in women with epilepsy: no less than 0.4 mg/day and continued throughout pregnancy.
Institute of Medicine ³⁵	Women capable of becoming pregnant	400 µg of folic acid daily from fortified foods, supplements, or both in addition to consuming food folate from a varied diet
National Institute for Health and Care Excellence ⁷⁴	General population: Women who may become pregnant and women in early pregnancy	A daily dose of 400 µg of folic acid before pregnancy and throughout the first 12 weeks is recommended.
	High-risk population: <ul style="list-style-type: none"> • Women or their partners have an NTD • Women who have had a previous baby with an NTD • Women or their partners who have a family history of NTDs • Women who have diabetes 	A daily dose of 5 mg of folic acid is recommended for women at high risk who are planning a pregnancy or are in the early stages of pregnancy.

Abbreviation: NTD=neural tube defect.

Table 3. Study Characteristics of Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Study Name Design Risk of Bias	Population	Intervention	Timing Setting
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1993 ⁹² Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³ Hungarian RCT RCT Medium (fair quality)	Women planning a pregnancy without any delayed conception or infertility and not currently pregnant	G1: Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) ⁹³ (n=2,793) G2: Trace-element supplement (copper, manganese, zinc, low dose of vitamin C) ⁹⁴ (n=2,660)	28 days before conception and at least until the date of the second missed menstrual period. ⁹¹ HPS began 3 months before a pregnancy is planned and continues for the first 3 months after conception. HPS provided information and counseling, examinations, and interventions during all trimesters by qualified nurses.
Czeizel et al, 2004 ⁸¹ Hungarian Cohort Cohort Medium (fair quality)	Women planning a pregnancy without any delayed conception or infertility and not currently pregnant	G1: Women supplemented with multivitamin (n=3,056) G2: Nonsupplemented women (n=3,056)	Before conception and at least until first missed menstrual period. Supplemented cohort was recruited from the HPS. HPS provides information and counseling, examinations, and interventions during all trimesters by qualified nurses. Unsupplemented cohort was recruited during their first visit at an antenatal care clinic.
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort Medium (fair quality)	Women undergoing MSAFP screen or an amniocentesis	G1: Use of multivitamins containing folic acid G2: No use of multivitamins containing folic acid (or use less than 1 a week) Total: (n=22,715, multivitamin use information available)	3 months prior to pregnancy through 1st 3 months of pregnancy. Women were identified and recruited when they had a MSAFP screen or an amniocentesis at 16 weeks of pregnancy between October 1984 and June 1987. Women were receiving prenatal care and routine MSAFP screening in the practices of over 100 participating obstetricians.
Agopian et al, 2013 ⁹ National Birth Defects Prevention Study Case-control Medium (fair quality)	Mothers with and without pregnancies affected by birth defects	G1: Spina bifida or anencephaly live births, fetal deaths, and elective pregnancy terminations (n=1,239) G2: Live-born controls without major birth defects (n=8,494)	Folic acid supplementation before pregnancy through 1st month of pregnancy. Population-based surveillance systems in 10 states. Data collected from medical records, birth certificate data, or hospital birth logs.
Mosley et al, 2009 ⁸⁷ National Birth Defects Prevention Study Case-control Medium (fair quality)	Mothers with and without pregnancies affected by birth defects	G1: Spina bifida or anencephaly live births, fetal deaths, and elective pregnancy terminations (n=565) G2: Live-born controls without major birth defects (n=3,691)	Folic acid supplementation before pregnancy through 1st month of pregnancy. Population-based surveillance systems in 10 states. Data collected from medical records, birth certificate data, or hospital birth logs.

Table 3. Study Characteristics of Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Study Name Design Risk of Bias	Population	Intervention	Timing Setting
<p>Ahrens et al, 2011¹¹</p> <p>Slone Birth Defects Study</p> <p>Case-control</p> <p>Medium (fair quality)</p>	<p>Mothers with and without pregnancies affected by birth defects</p>	<p>G1: Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation (n=205)</p> <p>G2: Live-born nonmalformed infants (n=6,357)</p>	<p>Folic acid supplementation 2 months before the last menstrual period and 1 month after last menstrual period.</p> <p>Cases identified from discharge records of participating hospitals serving the areas surrounding Boston, MA; Philadelphia, PA; San Diego, CA; and Toronto, Canada and through birth defect registries in Massachusetts and New York State. Nonmalformed controls selected each month from study hospitals' discharge lists or from statewide birth records.</p>
<p>Hernandez-Diaz et al, 2001⁹⁵</p> <p>Slone Birth Defects Study</p> <p>Case-control</p> <p>Medium (fair quality)</p>	<p>Mothers of malformed children</p>	<p>G1: Cases with NTDs (spina bifida, anencephaly, and encephalocele, or other NTDs) (n=1,242)</p> <p>G2: Infants with malformations not related to vitamin supplementation (n=6,660)</p>	<p>Any time during the 2 months after the last menstrual period.</p> <p>Participants of the Slone Epidemiology Unit Birth Defects Study. Study interviewed mothers of malformed children born in the greater metropolitan areas of Boston, MA; Philadelphia, PA; Toronto, Canada; and between 1983 and 1985, part of the state of Iowa. Subjects identified through review of admissions and discharges at major referral hospitals and clinics and through regular contact with newborn nurseries in community hospitals.</p> <p>A random sample of nonmalformed infants was identified at the birth hospitals as potential controls (only after 1993).</p>
<p>Werler et al, 1993⁹⁹</p> <p>Case-control</p> <p>Slone Birth Defects Study</p> <p>Medium (fair quality)</p>	<p>Mothers with NTD-affected pregnancies and mothers with pregnancies affected by other major malformations</p>	<p>G1: Live-born, stillborn infants, and therapeutic abortions with anencephaly, spina bifida, or encephalocele (n=436)</p> <p>G2: Live-born, stillborn infants, and therapeutic abortions with other major malformations (n=2,615)</p>	<p>Periconceptional period (interval from 28 days before the LMP through the 28 days after the LMP) (the first lunar month).</p> <p>Study subjects recruited from tertiary and birth hospitals in greater metropolitan Boston, MA; Philadelphia, PA; and Toronto, Ontario. Primary physicians of potential subjects were asked for permission to contact mothers.</p>

Table 3. Study Characteristics of Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Study Name Design Risk of Bias	Population	Intervention	Timing Setting
<p>Suarez et al, 2000²²</p> <p>Texas Department of Health's Neural Tube Defect Project</p> <p>Medium (fair quality)</p>	<p>Mothers with and without NTD-affected pregnancies</p>	<p>G1: Infants or fetuses who had anencephaly (including craniorachischisis and iniencephaly), spina bifida, or encephalocele identified at birth or prenatally (n=148) G2: Normal live births (n=158)</p>	<p>3 months before conception to 3 months after conception.</p> <p>Texas Department of Health's Neural Tube Defect Project, occurrence of NTDs in 14 Texas counties along the U.S.-Mexico border identified at birth or prenatally between January 1995 and February 1999. Surveillance included hospitals, birthing centers, genetics clinics, ultrasound centers, licensed abortion centers, and approximately 150 midwives in the region.</p>
<p>Shaw et al, 1995⁸²</p> <p>California Birth Defects Monitoring Program</p> <p>Case-control</p> <p>Medium (fair quality)</p>	<p>Mothers with and without singleton pregnancies affected by reportable birth defects</p>	<p>G1: Singleton live-born infants and electively terminated fetuses with an NTD (anencephaly, spina bifida cystic, craniorachischisis, and iniencephaly) (n=538) G2: Singleton live births without a reportable birth defect (n=539)</p>	<p>3 months before pregnancy and/or first 3 months after conception.</p> <p>CBDMP, birth years between June 1, 1989 and May 31, 1991. Cases were women who had live-born and stillborn infants with NTDs and those who had NTD-affected pregnancies that were terminated after prenatal diagnosis (February 1, 1989–January 31, 1991). Controls were an equal number of singleton live births randomly selected in proportion to hospital's contributions to total population of infants born alive in CA.</p>
<p>Mills et al, 1989⁹⁶</p> <p>National Institute of Child Health and Human Development Neural Tube Defects Study (data from California and Illinois)</p> <p>Case-control</p> <p>Medium (fair quality)</p>	<p>Mothers with and without pregnancies affected by birth defects</p>	<p>G1: Cases, mothers of an infant or fetus with an NTD (n= 571) G2: Controls, mothers of normal infants (n=573) G3: Controls, mothers of an abnormal or stillborn infant or fetus (n=546)</p>	<p>Vitamin use 30 days before the first day of LMP and ending approximately 45 days thereafter.</p> <p>Study based in CA and IL. Cases included mothers of an infant or fetus with an NTD diagnosis prenatally or postnatally between June 15, 1985, and April 30, 1987, in IL or between August 1, 1985, and April 30, 1987, in CA.</p>

Abbreviations: CBDMP=California Birth Defects Monitoring Program; G=group; HPS=Hungarian Preconceptional Service; LMP=last menstrual period; MSAFP=maternal serum alpha-fetoprotein; n=number; NTD=neural tube defect; RCT=randomized, controlled trial.

Table 4. Results of Prospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

First Author, Year Design Risk of Bias	Interventions	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1993 ⁹² Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³ RCT Medium (fair quality)	Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) Trace element supplement (copper, manganese, zinc, low dose of vitamin C)	Prospective, confirmed by (1) asking women; (2) checking women's record of supplement use, recorded daily with basal body temperatures; and (3) checking boxes of supplements for unused tablets	Live births, termination in the second trimester following prenatal diagnosis, and stillbirths with NTD	Live births, terminations in the second trimester following prenatal diagnosis, and stillbirths without NTD	1984–1992	(Peto) 0.131 (0.026–0.648)	Cases in exposed arm: 0 Cases in control arm: 6 N in exposed arm: 2,471 N in control arm: 2,391	None
Czeizel et al, 2004 ⁸¹ Cohort Medium (fair quality)	Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) No supplement	Prospective, confirmed by (1) asking women; (2) checking women's record of supplement use, recorded daily with basal body temperatures; and (3) checking boxes of supplements for unused tablets	Live births, terminations in the second or third trimester following prenatal diagnosis, and stillbirths (late fetal death after 28th week of gestation and/or weighing >1,000 g) with NTD	Live births, terminations in the second or third trimester following prenatal diagnosis, and stillbirths (late fetal death after 28th week of gestation and/or weighing >1,000 g) without NTD	1993–1996	0.11 (0.01–0.91)	Cases in exposed arm: 1 Cases in control arm: 9 N in exposed arm: 3,056 N in control arm: 3,056	Birth order (first or second and more), chronic maternal disorder, and history of previous unsuccessful pregnancies including fetal death or congenital abnormalities in fetuses or newborn infants

Table 4. Results of Prospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

First Author, Year Design Risk of Bias	Interventions	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort Medium (fair quality)	Multivitamins containing folic acid weeks 1-6 No multivitamins or multivitamins not containing folic acid weeks 7 and onward	Interviewed at the time that the laboratory received the alpha-fetoprotein or amniocentesis test (15–20 weeks of pregnancy)	NTD, defined as spina bifida, anencephaly, or encephalocele alone or in combination with other defects from pregnancy outcome data ascertained through questionnaires to delivering physician or mothers (for nonresponsive physician)	No NTD	1984–1987	0.27 (0.11–0.63)	Cases in exposed arm: 10 Cases in control arm: 11 N in exposed arm: 10,713 N in control arm: 3,157	None
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort Medium (fair quality)	Multivitamins containing folic acid weeks 7 and beyond No multivitamins or multivitamins not containing folic acid weeks 7 and onward	Interviewed at the time that the laboratory received the alpha-fetoprotein or amniocentesis test (15–20 weeks of pregnancy)	NTD, defined as spina bifida, anencephaly, or encephalocele alone or in combination with other defects from pregnancy outcome data ascertained through questionnaires to delivering physician or mothers (for nonresponsive physician)	No NTD	1984–1987	0.92 (0.45–1.87)	Cases in exposed arm: 25 Cases in control arm: 11 N in exposed arm: 7,795 N in control arm: 3,157	None

Abbreviations: CI=confidence interval; N=number; NTD=neural tube defect.

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Agopian et al, 2013 ⁹ Case-control Medium (fair quality)	Any folic acid supplement (folic acid, multivitamin, or prenatal supplement) during the month before pregnancy and the first month of pregnancy No supplements during the month before pregnancy and the first month of pregnancy	Interviews are targeted for completion within 6 months of the EDD but must be completed no earlier than 6 weeks and no later than 24 months after the EDD.	Spina bifida or anencephaly live births, fetal deaths, and elective pregnancy terminations	Live-born controls without major birth defects	1997–2007	0.93 (0.82–1.06)	Cases: 1,239 N exposed: 617 N not exposed: 619 Controls: 8,494 N exposed: 4,293 N not exposed: 4,167	Body mass index ≥30.0, low dietary folate intake, anticonvulsant medication use, female infant sex, family history of NTDs in a first- or second-degree relative, maternal Hispanic ethnicity
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair quality)	Consistent use (taking supplements at least half the number of days, ≥60 days, from 3 months before pregnancy to the first month of pregnancy) No supplements during the month before pregnancy and the first month of pregnancy	Interviews are targeted for completion within 6 months of the EDD but must be completed no earlier than 6 weeks and no later than 24 months after the EDD.	Anencephaly live births, fetal deaths, and elective pregnancy terminations	Live-born controls without major birth defects	1998–2003	1.2 (0.8–1.9)	Cases: 180 N exposed: 38 N not exposed: 81 Controls: 3,691 N exposed: 965 N not exposed: 1,778	Maternal race and education

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair quality)	Consistent use (taking supplements at least half the number of days, ≥60 days, from 3 months before pregnancy to the first month of pregnancy) No supplements during the month before pregnancy and the first month of pregnancy	Interviews are targeted for completion within 6 months of the EDD but must be completed no earlier than 6 weeks and no later than 24 months of the EDD.	Spina bifida live births, fetal deaths, and elective pregnancy terminations	Live-born controls without major birth defects	1998– 2003	1.4 (1.0– 1.8)	Cases: 385 N exposed: 97 N not exposed: 188 Controls: 3,691 N exposed: 965 N not exposed: 1,778	Maternal race, BMI, and pregnancy
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair quality)	Initiating supplement use in the first month of pregnancy No supplement use during the month before pregnancy and the first month of pregnancy	Interviews are targeted for completion within 6 months of the EDD but must be completed no earlier than 6 weeks and no later than 24 months of the EDD.	Anencephaly live births, fetal deaths, and elective pregnancy terminations	Live-born controls without major birth defects	1998– 2003	1.7 (1.2– 2.4)	Cases: 180 N exposed: 61 N not exposed: 81 Controls: 3,691 N exposed: 948 N not exposed: 1,778	Maternal race and education
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair quality)	Initiating supplement use in the first month of pregnancy No supplementation during the month before pregnancy and the first month of pregnancy	Interviews are targeted for completion within 6 months of the EDD but must be completed no earlier than 6 weeks and no later than 24 months of the EDD.	Spina bifida live births, fetal deaths, and elective pregnancy terminations	Live-born controls without major birth defects	1998– 2003	1.1 (0.9– 1.5)	Cases: 385 N exposed: 100 N not exposed: 188 Controls: 3,691 N exposed: 948 N not exposed: 1,778	Maternal race, BMI, and pregnancy
Ahrens et al, 2011 ¹¹ Case-control Medium (fair quality)	Consistent users of prenatal vitamins, multi- vitamins, and folic acid supplements (≥4 days per week at least 2 of 3 periconceptional months) No folic acid supplement use (<1 day per month, or use only during LM-2)	Interviews conducted within 6 months of delivery	Malformed live- born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation	Live-born nonmalformed infants	1998– 2008	1.11 (0.74– 1.65)	Cases: 205 N exposed: 83 N not exposed: 59 Controls: 6,357 N exposed: 2,573 N not exposed: 1,438	Race, BMI, pregnancy intent, and study center

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Ahrens et al, 2011 ¹¹ Case-control Medium (fair quality)	Early pregnancy initiators of prenatal vitamins, multivitamins, and folic acid supplements (≥4 days per week beginning in first or second postconceptional months) No folic acid supplement use (<1 day per month, or use only in LM-2)	Interviews conducted within 6 months of delivery	Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation	Live-born nonmalformed infants	1998–2008	0.79 (0.54–1.16)	Cases: 205 N in exposed: 60 N not exposed: 59 Controls: 6,357 N exposed: 2,293 N not exposed: 1,438	Race, BMI, pregnancy intent, and study center
Ahrens et al, 2011 ¹¹ Case-control Medium (fair quality)	Inconsistent users of prenatal vitamins, multivitamins, and folic acid supplements (use patterns not defined as consistent), early pregnancy or nonuse No folic acid supplement use (<1 day per month, or use only during LM-2)	Interviews conducted within 6 months of delivery	Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation	Live-born nonmalformed infants	1998–2008	2.20 (0.64–7.62)	Cases: 205 N in exposed: 3 N not exposed: 59 Controls: 6,357 N exposed: 53 N not exposed: 1,438	Race, BMI, pregnancy intent, and study center
Hernandez-Diaz et al, 2001 ⁹⁵ Case-control Medium (fair quality)	Folic acid during the 2 months after LMP No folic acid use	Interviews conducted within 6 months of delivery	Live-born and stillborn infants and therapeutic abortions with NTD (anencephaly, spina bifida, encephalocele, or other NTD); stillbirths and therapeutic abortions included from 1988 onward	Malformations other than NTDs	1976–1998	0.7 (0.5–0.8)	Cases: 1,242 N exposed: 140 N not exposed: 715 Controls: 6,660 N exposed: 939 N not exposed: 3,695	Interview year, region, maternal age, education, weight before pregnancy, and urinary tract infections or other infections early in pregnancy

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Werler et al, 1993 ⁹⁹ Case-control Medium (fair quality)	Daily use in the periconceptional period (28 days before LMP through 28 days after LMP) No folic acid use	Interviews conducted within 6 months of delivery	Live-born and stillborn infants and therapeutic abortions with NTD (anencephaly, spina bifida, or encephalocele)	Other major malformations	1988– 1991	0.6 (0.4– 0.8)	Cases: 436 N exposed: 34 N not exposed: 250 Controls: 2,615 N exposed: 339 N not exposed: 1,253	Maternal age, maternal education, annual family income, birth status
Suarez et al, 2000 ²² Case-control Medium (fair quality)	Daily use in every month in the preconception period (≤3 months before conception) No folic acid use	Interviews were conducted approximately 1 month postpartum	Infants or fetuses who had anencephaly, spina bifida, or encephalocele identified at birth or prenatally	Control, normal live births	1995– 1999	0.77 (0.17– 3.59)	Cases: 148 N exposed: 3 N not exposed: 66 Controls: 158 N exposed: 4 N not exposed: 68	None
Suarez et al, 2000 ²² Case-control Medium (fair quality)	Daily use in every month in the preconception period (≤3 months before conception) No folic acid use	Interviews were conducted approximately 1 month postpartum	Infants or fetuses who had anencephaly, spina bifida, or encephalocele identified at birth or prenatally	Control, normal live births	1995– 1999	1.12 (0.22– 5.78)	Cases: 148 N exposed: 3 N not exposed: 66 Controls: 158 N exposed: 4 N not exposed: 68	Maternal age, education, obesity, and previous stillbirth or miscarriage
Suarez et al, 2000 ²² Case-control Medium (fair quality)	Any use in every month in the preconception period (≤3 months before conception) No folic acid use	Interviews were conducted approximately 1 month postpartum	Infants or fetuses who had anencephaly, spina bifida, or encephalocele identified at birth or prenatally	Control, normal live births	1995– 1999	1.65 (0.51– 5.30)	Cases: 148 N exposed: 8 N not exposed: 66 Controls: 158 N exposed: 5 N not exposed: 68	None

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Suarez et al, 2000 ²² Case-control Medium (fair quality)	Post conception period (≤3 months after conception) No folic acid use	Interviews were conducted approximately 1 month postpartum	Infants or fetuses who had anencephaly, spina bifida, or encephalocele identified at birth or prenatally	Control, normal live births	1995–1999	0.90 (0.57–1.42)	Cases: 148 N exposed: 74 N not exposed: 66 Controls: 158 N exposed: 85 N not exposed: 68	None
Shaw et al, 1995 ⁸² Case-control Medium (fair quality)	Vitamin supplements containing folic acid in the 3 months before conception No folic acid use	Interviews conducted an average of 5 months after delivery	Cases: Singleton live-born infants and electively terminated fetuses with an NTD (anencephaly, spina bifida cystic, cranio-rachischisis, and iniencephaly)	Singleton live births without a reportable birth defect	1989–1991	0.65 (0.45–0.94)	Cases: 538 N exposed: 88 N not exposed: 207 Controls: 539 N exposed: 98 N not exposed: 149	None
Shaw et al, 1995 ⁸² Case-control Medium (fair quality)	Vitamin supplements containing folic acid in the 3 months after conception (assuming women who started in period before conception continued) No folic acid use	Interviews conducted an average of 5 months after delivery	Cases: Singleton live-born infants and electively terminated fetuses with an NTD (anencephaly, spina bifida cystic, cranio-rachischisis, and iniencephaly)	Singleton live births without a reportable birth defect	1989–1991	0.60 (0.46–0.79)	Cases: 538 N exposed: 322 N not exposed: 207 Controls: 539 N exposed: 384 N not exposed: 149	None

Table 5. Results of Retrospective Studies on the Effect of Folic Acid Supplementation on Neural Tube Defects

Author Design Risk of Bias	Intervention Comparison	Timing of Measurement of Exposure	Outcome	Comparison	Period of Exposure	Odds Ratio (95% CI)	N	Adjustments
Mills et al, 1989 ⁹⁶ Case-control Medium (fair quality)	Vitamin supplements containing folic acid (exposure defined as taking supplements containing the RDA of at least 4 vitamins or a higher dose ≥6 days per week) None	Interviews were conducted no more than 3 months after delivery	NTDs including anencephaly, meningocele, myelomengo- cele, encephalocele, rachischisis, iniencephaly, and lipomeningo- cele	Controls, mothers of normal infants	1985– 1987	1.00 (0.73– 1.40)	Cases: 565 N exposed: 86 N not exposed: 464 Controls: 567 N exposed: 84 N not exposed: 456	None

Abbreviations: BMI=body mass index; CI=confidence interval; EDD=expected date of delivery; LM-2=2 months before last menstrual period; LMP=last menstrual period; N=number; NTD=neural tube defect.

Table 6. Variations in the Effect of Folic Acid Supplementation on Neural Tube Defects by Race/Ethnicity

Author Design Risk of Bias	Subgroup	N	Results
Ahrens et al, 2011 ¹¹ Case-control Medium (fair quality)	White, non-Hispanic black, non-Hispanic Hispanic	White, non-Hispanic G1: 128 G2: 4535 Black, non-Hispanic G1: 22 G2: 459 Hispanic G1: 39 G2: 892	White, non-Hispanic Crude OR (95% CI) Consistent users: 0.78 (0.49–1.25) Early pregnancy initiators: 0.63 (0.38–1.06) Adjusted OR (95% CI) Consistent users: 0.93 (0.56–1.54) Early pregnancy initiators: 0.68 (0.40–1.16) Black, non-Hispanic Crude OR (95% CI) Consistent users: 1.11 (0.34–3.61) Early pregnancy initiators: 0.77(0.29–2.02) Adjusted OR (95% CI) Consistent users: NC Early pregnancy initiators: 0.86 (0.32–2.30) Hispanic Crude OR (95% CI) Consistent users: 1.81 (0.85–3.84) Early pregnancy initiators: 0.61 (0.27–1.38) Adjusted OR (95% CI) Consistent users: 2.20 (0.98–4.92) Early pregnancy initiators: 0.74 (0.32–1.70)
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair-quality)	White, black, Hispanic	Anencephaly White, non-Hispanic: 83 Black, non-Hispanic: 18 Hispanic: 67 Spina bifida White, non-Hispanic: 191 Black, non-Hispanic: 42 Hispanic: 134 Controls White, non-Hispanic: 2,173 Black, non-Hispanic: 431 Hispanic: 865	Anencephaly White, non-Hispanic Crude OR (95% CI) 3 months before pregnancy: 1.2 (0.7–2.1) First month of pregnancy: 1.5 (0.9–2.6) Black, non-Hispanic Crude OR (95% CI) 3 months before pregnancy: 2.8 (0.8–10.4) 1st month of pregnancy: 3.9 (1.3–11.5) Hispanic Crude OR (95% CI) 3 months before pregnancy: 0.7 (0.2–2.2) 1st month of pregnancy: 1.4 (0.8–2.5) Spina bifida White, non-Hispanic Crude OR (95% CI) 3 months before pregnancy: 1.3 (0.9–1.9) 1st month of pregnancy: 1.1 (0.8–1.7) Black, non-Hispanic Crude OR (95% CI) 3 months before pregnancy: 1.2 (0.5–2.8) 1st month of pregnancy: 0.6 (0.3–1.6) Hispanic Crude OR (95% CI) 3 months before pregnancy: 0.4 (0.2–1.2) 1st month of pregnancy: 1.3 (0.9–2.0)

Table 6. Variations in the Effect of Folic Acid Supplementation on Neural Tube Defects by Race/Ethnicity

Author Design Risk of Bias	Subgroup	N	Results
Shaw et al, 1995 ⁸² Case-control Medium (fair-quality)	Hispanic, non-Hispanic white, black, other	Hispanic G1: 265 G2: 196	OR for NTD from maternal use of a folic acid-containing vitamin in 3 months before conception OR (95% CI) Hispanic: 0.96 (0.44–2.10)
		Non-Hispanic white G1: 217 G2: 272	Non-Hispanic: 0.62 (0.3 to -1.10) African American: 0.54 (0.09–3.20) Other: 4.3 (0.23–145)
		Black G1: 27 G2: 31	OR for NTD from maternal use of a folic acid-containing vitamin in first 3 months postconception OR (95% CI) Hispanic: 0.73 (0.49–1.10)
		Other G1: 28 G2: 39	Non-Hispanic: 0.58 (0.36–0.94) African American: 0.29 (0.08–1.10) Other: 1.9 (0.57–6.30)

Abbreviations: CI=confidence interval; N=number; NTD=neural tube defect; OR=odds ratio.

Table 7. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Dosage

Author Design Risk of Bias	Subgroup	N	Results
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort Medium (fair quality)	0, 1–399, 400–799, ≥800	0: 13,431 1–399: 2,489 400–799: 1,812 ≥800: 5,494	Relative risk of NTD (95% CI) dietary folate from supplements dietary folate equivalent/day (weeks 1–5) 1–399: 0.29 (0.07–1.2) 400–799: 0.41 (0.10–1.7) ≥800: 0.56 (0.24–1.3)
Shaw et al, 1995 ⁸² Case-control Medium (fair-quality)	Any, <0.4, 0.4–0.9, ≥1.0	Use in 3 months before conception <0.4 G1: 53 G2: 56 0.4–0.9 G1: 29 G2: 32 ≥1.0 G1: 5 G2: 6 Use in 3 months after conception <0.4 G1: 37 G2: 27 0.4–0.9 G1: 243 G2: 322 ≥1.0 G1: 42 G2: 33 None G1: 207 G2: 149 Unknown G1: 4 G2: 2	OR for NTDs with maternal use of a folic acid–containing vitamin supplement in the 3 months before conception OR (95%) <0.4: 0.68 (0.43–1.10) 0.4–0.9: 0.65 (0.37–1.20) ≥1.0: 0.60 (0.16–2.30) OR for NTDs with maternal use of a folic acid–containing vitamin supplement 3 months after conception OR (95%) <0.4: 0.99 (0.56–1.80) 0.4–0.9: 0.54 (0.41–0.72) ≥1.0: 0.92 (0.54–1.60)
Werler et al, 1993 ⁹⁹ Case-control Medium (fair quality)	≥1 mg, 0.5–0.9 mg, 0.4 mg, <0.4 mg	Daily dose G1: 34 G2: 339 Less than daily dose G1: 41 G2: 234 RR according to daily folic acid dose in 18 case mothers (G1) and 322 control mothers (G2) who received periconceptional supplements and did not report knowledge of the hypothesis ≥1 mg G1: 3 G2: 52	Calculated OR for daily vs. less than daily dose: 0.57 (95% CI, 0.35–0.93) RR by dose among women who did not know hypothesis ≥1 mg folic acid dose in supplement RR (95% CI) (unclear if crude or multivariate): 0.4 (0.1–1.3) 0.5–0.9 mg folic acid dose in supplement RR (95% CI) (unclear if crude or multivariate): 0.9 (0.2–4.2) 0.4 mg folic acid dose in supplement RR (95% CI) (unclear if crude or multivariate): 0.3 (0.1–0.6)

Table 7. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Dosage

Author Design Risk of Bias	Subgroup	N	Results
		0.5–0.9 mg G1: 2 G2: 15 0.4 mg G1: 8 G2: 185 <0.4 mg G1: 3 G2: 50	<0.4 mg folic acid dose in supplement RR (95% CI) (unclear if crude or multivariate): 0.5 (0.2–1.5)
Mills et al, 1989 ⁹⁶ Case-control Medium (fair quality)	RDA or more vs. none Any amount vs. none	RDA or more G1: 86 G2: 70 G3: 84 Less than RDA G1: 15 G2: 17 G3: 27 None G1: 464 G2: 451 G3: 456	Calculated OR of RDA or more vs. less than RDA: 1.84 (95% CI, 0.92– 3.71)

Abbreviations: CI=confidence interval; G=group; N=number; NTD=neural tube defect; RDA=recommended daily allowance; OR=odds ratio; RR=relative risk.

Table 8. Variations in Effect of Folic Acid Supplementation on Neural Tube Defects by Timing

Author Design Risk of Bias	Subgroup	N	Results
Ahrens et al, 2011 ¹¹ Case-control Medium (fair quality)	Consistent users (4 or more days per week) 2 of 3 periconceptional months vs. initiating in the first month (4 or more days per week)	Spina bifida Consistent users G1: 83 G2: 2,573 Initiating in the first month G1: 60 G2: 2,293	Calculated OR, 1.23 (95% CI, 0.88–1.73)
Mosley et al, 2009 ⁸⁷ Case-control Medium (fair quality)	Consistent users 3 months before pregnancy through first month of pregnancy vs. initiating in the first month	Anencephaly Consistent users G1: 38 G2: 61 Initiating in the first month G1: 965 G2: 948	Calculated OR, 0.61 (95% CI, 0.40–0.93)
		Spina bifida Consistent users G1: 97 G2: 100 Initiating in the first month G1: 965 G2: 948	Calculated OR, 0.95 (95% CI, 0.71–1.28)
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort Medium (fair quality)	Women who did not use multivitamins after conception vs. women who used multivitamins in the first 6 weeks of pregnancy and women who started multivitamin use only after week 6	Use in weeks 1-6 G1: 10 G2: 10,731 Use in weeks 7 and later G1: 25 G2: 7,795	Calculated OR, 0.29 (95% CI, 0.14–0.60)
Suarez et al, 2000 ²² Case-control Medium (fair quality)	Preconceptional use vs. postconceptional use	Preconceptional use G1: 8 G2: 5 Postconceptional use G1: 74 G2: 85	Calculated OR, 1.84 (95% CI, 0.58–5.86)
Shaw et al, 1995 ⁸² Case-control Medium (fair quality)	Use in 3 months before conception vs. use in 3 months before conception	Use in 3 months before conception Any G1: 88 G2: 98 Use in 3 months after conception Any G1: 322 G2: 384	Calculated OR, 1.07 (95% CI, 0.77–1.48)

Abbreviations: CI=confidence interval; G=group; N=number; OR=odds ratio.

Table 9. Harms of Folic Acid Supplementation: Study Characteristics of Included Twinning Studies

Author Study Name Design Risk of Bias	Population	Intervention	Timing and Setting
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1993 ⁹² Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³ Hungarian RCT RCT Medium (fair quality)	Women planning a pregnancy without any delayed conception or infertility and not currently pregnant	G1: Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) ⁹³ (n=2,793) G2: Trace-element supplement (copper, manganese, zinc, low dose of vitamin C) ⁹⁴ (n=2,660)	28 days before conception and at least until the date of the second missed menstrual period ⁹¹ HPS began 3 months before a pregnancy is planned and continues for the first 3 months after conception. HPS provided information and counseling, examinations, and interventions during all trimesters by qualified nurses.
Vollset et al, 2005 ⁸³ Medical Birth Registry of Norway Cohort Medium (fair quality)	Women with singleton and twin pregnancies	G1: Preconceptional use of folate (n=11,077) G2: No preconceptional use of folate (n=164,965)	Preconception Medical Birth Registry of Norway, women who gave birth from December 1998 through the end of 2001. Information on IVF pregnancies obtained by contacting fertility clinics in Denmark and Sweden.

Abbreviations: G=group; HPS=Hungarian Preconceptional Service; IVF=in vitro fertilization; n=number; RCT=randomized, controlled trials.

Table 10. Harms of Folic Acid Supplementation: Study Characteristics of Included Asthma/Wheezing Studies

Author Study Name Design Risk of Bias	Population	Intervention	Timing and Setting
Yang, 2014 ¹⁰⁸ Meta-analysis Medium (fair quality)	Studies that examined folic acid exposure during the periconceptional period or during pregnancy and provided results on at least one allergic or respiratory outcome or outcome of interest	Folic acid supplementation	Supplementation during preconception and during pregnancy All available settings
Crider, 2013 ¹⁰¹ Meta-analysis Low (high quality)	Studies that examined maternal folic acid supplementation during pregnancy on infant asthma	Folic acid supplementation	Supplementation during preconception and during pregnancy All available settings

Table 11. Results of Prospective Studies on the Associations Between Folic Acid Supplementation and Twinning

First Author, Design Risk of Bias	Intervention Groups (n)	Timing of Measurement Exposure	Outcomes	Relative Risk or Odds Ratio (95% CI)	N	Adjustments
Czeizel, 1994 ^{91,94} RCT Medium (fair quality)	G1: Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) ⁹³ (n=2,793) G2: Trace-element supplement (copper, manganese, zinc, low dose of vitamin C) ⁹⁴ (n=2,660)	28 days before conception and at least until the date of the second missed menstrual period ⁹¹	Twinning	Calculated OR: 1.40 (0.89–2.21) Calculated OR: 1.70 (0.79–3.65)	Multiple pregnancy cases in exposed arm: 46 N informative pregnancies in exposed arm: 2,421 Multiple pregnancy cases in control arm: 32 N informative pregnancies in control arm: 2,346 Clomiphene (infertility treatment) subgroup: Multiple pregnancy cases in exposed arm: 19 N in exposed arm: 141 Multiple pregnancy cases in control arm: 12 N in control arm: 143	None
Vollset et al, 2005 ⁸³ Cohort Medium (fair quality)	All pregnancies: G1: Preconceptional use of folate (N=11,077) G2: No preconceptional use of folate (N=164,965) Natural conception: G1: 10,457 G2: 164,965 IVF: G1: 620 G2: 2,000	Preconception	Twinning	OR of twin pregnancies with adjustments for maternal age and parity: 1.59 (1.41–1.78) OR of twin pregnancies with adjustments for maternal age, parity, and IVF: 1.04 (0.91–1.18)	Multiple pregnancy cases in exposed arm for all women: 329 N in exposed arm: 11,077 Multiple pregnancy cases in comparison arm: 2,825 N in comparison arm: 164,965 Multiple pregnancy cases in exposed arm: 154 N in exposed arm: 620 Multiple pregnancy cases in comparison arm: 543 N in comparison arm: 2,000	Maternal age and parity Maternal age, parity, and IVF

Abbreviations: CI=confidence interval; G=group; IVF=in vitro fertilization; n=number; N=number; OR=odds ratio; RR=relative risk.

Table 12. Results of Meta-Analyses on the Associations Between Folic Acid Supplementation and Asthma/Respiratory Illness

First Author, Design Quality	Intervention Groups (n)	Timing of Measurement Exposure	Outcomes	Relative Risk or Odds Ratio (95% CI)
Yang, 2014 ¹⁰⁸ Meta-analysis Fair quality	G1: Periconceptual use of folic acid (NR) G2: No use (NR)	Periconceptual period through pregnancy	Child asthma	OR: 1.06 (0.99–1.14)
Crider, 2013 ¹⁰¹ Meta-analysis Fair quality	G1: Periconceptual use of folic acid (NR) G2: No use (NR)	Periconceptual or first trimester	Asthma or wheezing Other outcomes: atopy, eczema, and atopic dermatitis (includes LRTI, URTI, food reaction, sensitization)	Asthma: RR: 1.01 (0.78–1.30) Wheeze in infants/toddler; asthma in children: RR: 1.05 (1.02–1.09) Atopy, eczema, and atopic dermatitis (includes LRTI, URTI, food reaction, sensitization): no statistically significant differences

Abbreviations: CI=confidence interval; G=group; LRTI=lower respiratory tract infection; n=number; N=number; NR=not reported; OR=odds ratio; RR=relative risk; URTI=upper respiratory tract infection.

Table 13. Variation in Harms of Folic Acid Supplementation by Timing

First Author, Design	Intervention Groups (n)	Timing of Measurement Exposure	Outcomes	Relative Risk or Odds Ratio (95% CI)
Crider, 2013 ¹⁰¹ Systematic review	G1: Second or third trimester use of folic acid (NR) G2: No use (NR)	Second or third trimester	Asthma or wheezing Other allergic outcomes	Wheeze in infants/toddler RR: 1.20 (1.04–1.39) in 1 study ¹⁰⁰ 14 other associations for asthma or wheezing not statistically significantly different ^{100,102} 38 associations for other allergic outcomes not statistically significant

Abbreviations: CI=confidence interval; G=group; NR=not reported; RR=relative risk.

Table 14. Variation in Harms of Folic Acid Supplementation by Dose

First Author, Design	Intervention Groups (n)	Timing of Measurement Exposure	Outcomes	Relative Risk or Odds Ratio (95% CI)
Crider, 2013 ¹⁰¹ (citing Dunstan, 2012 ¹¹⁰) Systematic review	G1: >0.5 mg/day G2: 0.2–0.499 mg/day G3: <0.2 mg/day	Third trimester	Any allergic disease, sensitization, recurrent wheeze, eczema, food reactions, IgE-mediated food allergy, and sensitization to food allergens	12 associations overlap line of no difference; 2 span line of no difference for G3 vs. G1 (OR, 1.5 [1.0–2.5]) and G2 vs. G1 (OR, 1.7 [1.0–2.8]) for eczema

Abbreviations: CI=confidence interval; G=group; IgE=immunoglobulin E; RR=relative risk.

Table 15. Summary of Evidence for Folic Acid Supplementation

Key Question	Number of Studies (Study Designs); N	Summary of Findings	Consistency/Precision	Reporting Bias	Overall Quality	Body of Evidence Limitations	EPC Assessment of Strength of Evidence for Key Question	Applicability
KQ 1a: Extent to which folic acid supplements reduce the risk for NTDs	12 (1 RCT, 2 cohort studies, 8 case-control studies, 1 previous review); N>41,802	<p>RCT (prefortification): Peto OR for NTD, 0.131 (95% CI, 0.026–0.648); p=0.013</p> <p>Cohort studies (prefortification): aOR for NTD, 0.11 (95% CI, 0.01–0.91); OR, 0.27 (95% CI, 0.11–0.63)</p> <p>Case-control studies (prefortification): aOR for NTD, 0.7 (95% CI, 0.5–0.8); RR for NTD, 0.6 (95% CI, 0.4–0.8); OR for NTD, 0.65 (95% CI, 0.45–0.94); OR, 1.00 (95% CI, 0.73–1.40); p=0.97</p> <p>Case control studies (spanning pre- and postfortification): aOR for NTD, 1.12 (95% CI, 0.22–5.78)</p> <p>Case control studies (postfortification): OR for NTD, 1.11 (95% CI, 0.74–1.65) for consistent users; aOR for NTD (anencephaly+spina bifida), 0.93 (95% CI, 0.82–1.06); aOR (anencephaly), 1.2 (95% CI, 0.8–1.9); aOR (spina bifida), 1.4 (95% CI, 1.0–1.8)</p>	<p>Consistency: Generally consistent within the prefortification and postfortification eras, inconsistent over time</p> <p>Precision: Wide confidence intervals but clear indication of benefit in the prefortification era, narrower confidence intervals with confidence intervals spanning the null in postfortification era</p>	Undetected	Fair	No new trials can be conducted on this topic. New studies must rely on observational data with inherent risks of case ascertainment bias (prospective cohort studies) or recall bias (retrospective studies)	High for prefortification data; low for postfortification data	Generally applicable to primary care

Table 15. Summary of Evidence for Folic Acid Supplementation

Key Question	Number of Studies (Study Designs); N	Summary of Findings	Consistency/Precision	Reporting Bias	Overall Quality	Body of Evidence Limitations	EPC Assessment of Strength of Evidence for Key Question	Applicability
KQ 1b: Differences in effect of folic acid supplements on NTDs by race/ethnicity	3 (3 case-control studies); N=11,154	No effect in first study; higher risk in second (aOR for Hispanic women, 2.20 [95% CI, 0.98–4.92]); less protective effect in third (OR for Hispanic women, 0.96 [95% CI, 0.44–2.10]) vs. 0.62 [95% CI, 0.35–1.10]) for non-Hispanic whites vs. 0.54 [95% CI, 0.09–3.20] for blacks)	Inconsistent Imprecise	Undetected	Fair	Small numbers in each comparison, effects possibly due to chance	Low	Generally applicable to primary care
KQ 1c: Differences in effect of folic acid supplements on NTDs by dosage, duration, and timing	Dosage: 4 (1 cohort study, 3 case-control studies); N=26,791 Duration: 0 Timing: 5 (1 cohort study, 4 case-control studies); N=26,808	No indication of dose response in 3 of 4 studies. One study shows lower odds for daily use vs. less than daily use (OR, 0.57 [95% CI, 0.35–0.93]) Duration: none Timing: Calculated OR from cohort study for use weeks 1-6 vs. weeks 7 and later: 0.29 [95% CI, 0.14–0.60]. Older studies consistently show no effect of timing; 1 new study (postfortification) shows a protective effect of use before pregnancy on anencephaly but not spina bifida. The other new study did not find a protective effect for spina bifida.	Inconsistent Imprecise	Undetected	Fair	Small numbers in each comparison, effects possibly due to chance, studies use different measures of dose and timing	Low	Generally applicable to primary care

Table 15. Summary of Evidence for Folic Acid Supplementation

Key Question	Number of Studies (Study Designs); N	Summary of Findings	Consistency/Precision	Reporting Bias	Overall Quality	Body of Evidence Limitations	EPC Assessment of Strength of Evidence for Key Question	Applicability
KQ 2a: Harms associated with folic acid supplements	Twinning in women: 2 (1 trial, 1 cohort); N=7,387	Trial found no statistically significant differences in twin pregnancy rate (RR, 1.4 [95% CI, 0.87–2.26]). Cohort found higher risk of twin birth for folate use (OR, 1.59 [95% CI, 1.41–1.78]) was attenuated once potential misclassification was accounted for (OR, 1.04 [95% CI, 0.91 to 1.18])	Consistent Imprecise	Undetected	Fair	Low event rate, wide confidence intervals	Moderate for no effect	Generally applicable to primary care
	Childhood asthma, wheezing, allergy (3 SRs, 8 observational studies); N>14,438	No effect for a large majority of comparisons and outcomes	Consistent Precise			Variable measures of outcomes and exposure, all observation studies with risks of bias from case ascertainment and recall	Moderate for no effect	
	Other adverse events in women (1 RCT); N=4,862	Increased risk for weight gain, diarrhea, constipation; reduced risk for irregular defecation; no difference for increased appetite, lack of appetite, exanthema, heartburn, and vertigo	Consistency unknown, single study, imprecise			Low event rate, wide confidence intervals	Low for no effect	

Table 15. Summary of Evidence for Folic Acid Supplementation

Key Question	Number of Studies (Study Designs); N	Summary of Findings	Consistency/Precision	Reporting Bias	Overall Quality	Body of Evidence Limitations	EPC Assessment of Strength of Evidence for Key Question	Applicability
KQ 2b: Differences in harms associated with folic acid supplements by dosage, timing, and duration	Dosage: 1 SR, 1 observational study; N=484 Duration: 0 Timing of asthma, wheezing, allergy (2 SRs, 3 observational studies); N varies by outcome	Dosage: no consistent increase in the risk of childhood asthma, wheeze, or allergies by dosage Duration: none Timing: no consistent increase in the risk of childhood asthma, wheeze, or allergies by timing	Consistent Precise	Undetected		Variable measures of outcomes and exposure, all observation studies with risks of bias from case ascertainment and recall	Low for no effect	Generally applicable to primary care

Abbreviations: CI=confidence interval; EPC=Evidence-based Practice Center; KQ=key question; N=number; NTD=neural tube defect; OR=odds ratio; RCT=randomized, controlled trial; RR=relative risk; SR=systematic review.

Table 16. Estimates of Folate Sufficiency

Data Source	Population	What Was Measured	Measure	Results
NHANES 2003-2006 ¹¹¹	Nonpregnant women ages 15-44	<ul style="list-style-type: none"> Two 24-hour dietary recalls; accounts for within individual variation FA values assigned to foods using USDA National Nutrient Database for Standard Reference Dietary supplement use in past 30 days, no. of days taken; recorded info on dosage from bottles; calculated average daily intake 	Recommended daily allowance (≥ 400 $\mu\text{g/day}$)	23.8% consuming recommended amount (≥ 400 $\mu\text{g/day}$) (76.2% of women <u>not</u> consuming recommended amount)
NHANES 1999-2000 ¹¹⁸	Women ages 15-44	<ul style="list-style-type: none"> Food and supplements One 24-hour dietary recall Included only subjects who had complete data for food folate and supplemental FA Adjusted for measurement error using a subsample of NHANES III subjects who had provided 2 separate 24-hour recalls Supplement intake over 1 month 	% women ages 15-44 years consuming >400 $\mu\text{g/day}$	Non-Hispanic white: 39% Non-Hispanic black: 26% Mexican American: 28% (61%-74% <u>not</u> consuming recommended amount)
NHANES 2007-2012 ⁴⁵	Nonpregnant women ages 12-49	<ul style="list-style-type: none"> Two 24-hour dietary recalls RBC folate concentrations from analysis of blood samples using microbiologic assay method from 2007-2012 Optimal RBC folate concentrations established by WHO to be >906 nmol/L (400 ng/L). For purposes of this analysis, optimal RBD folate concentration considered to be 748 nmol/L NHANES assay, the concentrations associated with an NTD risk of <9 NTDs per 10,000 live births based on the Daly et al^{52*} and Crider et al^{42†} studies 	% women with RBC folate concentration associated with an NTD prevalence of ≥ 9 per 10,000 live births	22.8% of women of have suboptimal RBC folate concentrations for NTD prevention
NHANES 2003-2006 ¹⁴¹	Women age ≥ 14 (pregnant and lactating women excluded)	<ul style="list-style-type: none"> Dietary folate (measured by two 24-hour recalls) and supplements (collected with 30-day frequency questionnaire) Bias-corrected best power method to adjust for within-person variability Dietary and total nutrient intakes estimated in 2 ways: 1) dietary and total folate in DFE and 2) dietary and total folic acid in micrograms Estimated average requirement (EAR) is for folate DFE EAR for those ages 14-18 years is 330 DFE and 320 DFE for those age ≥ 19 years 	% of women below the EAR	Ages 14-18: 19% 19-30: 16.9% 31-50: 14.6%
NHANES 2007-2010 ¹⁴²	Women ages 14-50	<ul style="list-style-type: none"> Total usual intake from food, beverages, and dietary supplements 1-day dietary recall and 30-day supplement questionnaire Folate measured in DFE 	% of women below EAR	Ages 14-18: 20% 19-30: 12% 31-50: 173%

*Daly et al reported that with RBC folate >906 nmol/L, the risk of NTD was 0.8 (95% CI, 0.43 to 1.5); with RBC folate ≤ 339 nmol/L, the risk was more than 8 times higher at 6.6 (95% CI, 3.3 to 11.7).

Table 16. Estimates of Folate Sufficiency

[†]Crider et al estimated a risk of 25.4 NTDs per 10,000 births at the lowest estimated RBC folate concentration of 500 nmol/L and 6 NTDs per 10,000 births at 1180 nmol/L, which they note likely corresponds to the lowest feasible prevalence of NTDs that can be obtained with folic acid–based interventions.

Abbreviations: FA=folic acid; NHANES=National Health and Nutrition Examination Survey; NTD=neural tube defect; RBC=red blood cell; USDA=U.S. Department of Agriculture; WHO=World Health Organization.

Appendix A. Search Strategy and Detailed Methods

6/23/14 PubMed Benefits Search

Search	Query	Items found
#1	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	786032
#2	Search (“folic acid”[MeSH] OR “vitamin b9”[tw] OR “vitamin m”[tw] or “Pteroylglutamic Acid”[tw] OR “folvite”[tw] OR “folacin”[tw] OR “folate”[tw] OR “folic acid”[tw])	46413
#3	Search (multivitamin[all fields] OR “prenatal vitamin”[all fields] OR multivitamins[all fields] OR “prenatal vitamins”[all fields] OR “vitamin supplement”[all fields] OR “vitamin supplements”[all fields])	4154
#4	Search (“neural tube defects”[MeSH Terms] OR “spina bifida”[All Fields] OR “neural tube damage”[All Fields] OR “neural tube defect”[All Fields] OR “neural tube defects”[All Fields] OR “neural tube disorders”[All Fields] OR “Neural tube defect, folate-sensitive”[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR “Tethered Cord Syndrome”[tw] OR “Tethered Cord Syndromes”[tw] OR “Occult Spinal Dysraphism Sequence”[tw] OR “Tethered Spinal Cord Syndrome”[tw] OR “Occult Spinal Dysraphism”[tw] OR “Occult Spinal Dysraphisms”[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR “Neurenteric Cyst”[tw] OR “Neurenteric Cysts”[tw] OR “Neuroenteric Cyst”[tw] OR “Neuroenteric Cysts”[tw] OR “Spinal Cord Myelodysplasia”[tw] OR “Spinal Cord Myelodysplasias”[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw])	27529
#5	Search (#1 AND (#2 OR #3) AND #4)	1794
#6	Search (#1 AND (#2 OR #3) AND #4) Filters: Humans	1623
#7	Search (#1 AND (#2 OR #3) AND #4) Filters: Other Animals	201
#8	Search (#7 NOT #6)	96
#9	Search (#5 NOT #8)	1698

9/4/14 PubMed Benefits Addendum Search

Added: “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate”

Search	Query	Items found
#1	Search (5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR 5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR 5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	2015
#2	Search (“folic acid”[MeSH] OR “vitamin b9”[tw] OR “vitamin m”[tw] or “Pteroylglutamic Acid”[tw] OR “folvite”[tw] OR “folacin”[tw] OR “folate”[tw] OR “folic acid”[tw] OR multivitamin[all fields] OR “prenatal vitamin”[all fields] OR multivitamins[all fields] OR “prenatal vitamins”[all fields] OR “vitamin supplement”[all fields] OR “vitamin supplements”[all fields])	50052
#3	Search (#1 NOT #2)	534
#4	Search (“neural tube defects”[MeSH Terms] OR “spina bifida”[All Fields] OR “neural tube damage”[All Fields] OR “neural tube defect”[All Fields] OR “neural tube defects”[All Fields] OR “neural tube disorders”[All Fields] OR “Neural tube defect, folate-sensitive”[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR “Tethered Cord Syndrome”[tw] OR “Tethered Cord Syndromes”[tw] OR “Occult Spinal Dysraphism Sequence”[tw] OR “Tethered Spinal Cord Syndrome”[tw] OR “Occult Spinal Dysraphism”[tw] OR “Occult Spinal Dysraphisms”[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR “Neurenteric Cyst”[tw] OR “Neurenteric Cysts”[tw] OR “Neuroenteric Cyst”[tw] OR “Neuroenteric Cysts”[tw] OR “Spinal Cord Myelodysplasia”[tw] OR “Spinal Cord Myelodysplasias”[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw])	27715
#5	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	792595
#6	Search (#3 and #4 and #5)	5
#7	Search (#3 and #4 and #5) Filters: Humans	4
#8	Search (#3 and #4 and #5) Filters: Other Animals	3
#9	Search (#8 NOT #7)	1
#10	Search (#6 NOT #9)	4

Appendix A. Search Strategy and Detailed Methods

11/10/14 PubMed Benefits Search

Search	Query	Items found
#1	Search ((Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR "pregnant women"[MeSH]))	797377
#2	Search ("folic acid"[MeSH] OR "vitamin b9"[tw] OR "vitamin m"[tw] OR "Pteroylglutamic Acid"[tw] OR "folvite"[tw] OR "folacin"[tw] OR "folate"[tw] OR "folic acid"[tw] OR "5-Me-THF" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate"[Supplementary Concept] OR "5-methyltetrahydrofolate triglutamate"[Supplementary Concept])	47728
#3	Search ((multivitamin[all fields] OR "prenatal vitamin"[all fields] OR multivitamins[all fields] OR "prenatal vitamins"[all fields] OR "vitamin supplement"[all fields] OR "vitamin supplements"[all fields]))	4245
#4	Search (("neural tube defects"[MeSH Terms] OR "spina bifida"[All Fields] OR "neural tube damage"[All Fields] OR "neural tube defect"[All Fields] OR "neural tube defects"[All Fields] OR "neural tube disorders"[All Fields] OR "Neural tube defect, folate-sensitive"[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR "Tethered Cord Syndrome"[tw] OR "Tethered Cord Syndromes"[tw] OR "Occult Spinal Dysraphism Sequence"[tw] OR "Tethered Spinal Cord Syndrome"[tw] OR "Occult Spinal Dysraphism"[tw] OR "Occult Spinal Dysraphisms"[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR "Neurenteric Cyst"[tw] OR "Neurenteric Cysts"[tw] OR "Neuroenteric Cyst"[tw] OR "Neuroenteric Cysts"[tw] OR "Spinal Cord Myelodysplasia"[tw] OR "Spinal Cord Myelodysplasias"[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw]))	27869
#5	Search ((#1 AND (#2 OR #3) AND #4))	1821
#6	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Humans	1647
#7	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Other Animals	207
#8	Search ((#7 NOT #6))	97
#9	Search (#5 NOT #8)	1724
#10	Search ("retraction"[All Fields] OR "Retracted Publication"[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	28946
#11	Search (#9 and #10)	1
#13	Search (#5 NOT #8) Filters: Publication date from 2013/06/23	88

3/24/15 PubMed Benefits Search

Search	Query	Items found
#1	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR "pregnant women"[MeSH])	807095
#2	Search ("folic acid"[MeSH] OR "vitamin b9"[tw] OR "vitamin m"[tw] OR "Pteroylglutamic Acid"[tw] OR "folvite"[tw] OR "folacin"[tw] OR "folate"[tw] OR "folic acid"[tw] OR "5-Me-THF" OR "5-Me-H4F" OR "5-methyltetrahydrofolate" OR "5-methyltetrahydropteroylpentaglutamate"[Supplementary Concept] OR "5-methyltetrahydrofolate triglutamate"[Supplementary Concept])	48429
#3	Search (multivitamin[all fields] OR "prenatal vitamin"[all fields] OR multivitamins[all fields] OR "prenatal vitamins"[all fields] OR "vitamin supplement"[all fields] OR "vitamin supplements"[all fields])	4335
#4	Search (("neural tube defects"[MeSH Terms] OR "spina bifida"[All Fields] OR "neural tube damage"[All Fields] OR "neural tube defect"[All Fields] OR "neural tube defects"[All Fields] OR "neural tube disorders"[All Fields] OR "Neural tube defect, folate-sensitive"[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR "Tethered Cord Syndrome"[tw] OR "Tethered Cord Syndromes"[tw] OR "Occult Spinal Dysraphism Sequence"[tw] OR "Tethered Spinal Cord Syndrome"[tw] OR "Occult Spinal Dysraphism"[tw] OR "Occult Spinal Dysraphisms"[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR "Neurenteric Cyst"[tw] OR "Neurenteric Cysts"[tw] OR "Neuroenteric Cyst"[tw] OR "Neuroenteric Cysts"[tw] OR "Spinal Cord Myelodysplasia"[tw] OR "Spinal Cord Myelodysplasias"[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw]))	28158
#5	Search ((#1 AND (#2 OR #3) AND #4))	1855
#6	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Humans	1669
#7	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Other Animals	211
#8	Search (#7 not #6)	99
#9	Search (#5 not #8)	1756
#10	Search ("retraction"[All Fields] OR "Retracted Publication"[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	30021
#11	Search (#9 and #10)	1
#12	Search (#5 not #8) Filters: Publication date from 2014/05/10	55

Appendix A. Search Strategy and Detailed Methods

7/27/15 PubMed Benefits Search

Search	Query	Items found
#1	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	817957
#2	Search (“folic acid”[MeSH] OR “vitamin b9”[tw] OR “vitamin m”[tw] or “Pteroylglutamic Acid”[tw] OR “folvite”[tw] OR “folacin”[tw] OR “folate”[tw] OR “folic acid”[tw] OR “5-Me-THF” OR “5-Me-H4F” OR “5-methyltetrahydrofolate” OR “5-methyltetrahydropteroylpentaglutamate”[Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate”[Supplementary Concept])	49245
#3	Search (multivitamin[all fields] OR “prenatal vitamin”[all fields] OR multivitamins[all fields] OR “prenatal vitamins”[all fields] OR “vitamin supplement”[all fields] OR “vitamin supplements”[all fields])	4431
#4	Search (“neural tube defects”[MeSH Terms] OR “spina bifida”[All Fields] OR “neural tube damage”[All Fields] OR “neural tube defect”[All Fields] OR “neural tube defects”[All Fields] OR “neural tube disorders”[All Fields] OR “Neural tube defect, folate-sensitive”[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR “Tethered Cord Syndrome”[tw] OR “Tethered Cord Syndromes”[tw] OR “Occult Spinal Dysraphism Sequence”[tw] OR “Tethered Spinal Cord Syndrome”[tw] OR “Occult Spinal Dysraphism”[tw] OR “Occult Spinal Dysraphisms”[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR “Neurenteric Cyst”[tw] OR “Neurenteric Cysts”[tw] OR “Neuroenteric Cyst”[tw] OR “Neuroenteric Cysts”[tw] OR “Spinal Cord Myelodysplasia”[tw] OR “Spinal Cord Myelodysplasias”[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw])	28482
#5	Search (#1 AND (#2 OR #3) AND #4)	1880
#6	Search (#1 AND (#2 OR #3) AND #4) Filters: Humans	1685
#7	Search (#1 AND (#2 OR #3) AND #4) Filters: Other Animals	213
#8	Search (#7 NOT #6)	100
#9	Search (#5 NOT #8)	1780
#10	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	34641
#11	Search (#9 and #10) Retraction Search	1
#12	Search (#5 NOT #8) Filters: Publication date from 2014/10/24	49

11/18/15 PubMed Benefits Search

Search	Query	Items found
#1	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	826665
#2	Search (“folic acid”[MeSH] OR “vitamin b9”[tw] OR “vitamin m”[tw] or “Pteroylglutamic Acid”[tw] OR “folvite”[tw] OR “folacin”[tw] OR “folate”[tw] OR “folic acid”[tw] OR “5-Me-THF” OR “5-Me-H4F” OR “5-methyltetrahydrofolate” OR “5-methyltetrahydropteroylpentaglutamate”[Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate”[Supplementary Concept])	49896
#3	Search (multivitamin[all fields] OR “prenatal vitamin”[all fields] OR multivitamins[all fields] OR “prenatal vitamins”[all fields] OR “vitamin supplement”[all fields] OR “vitamin supplements”[all fields])	4490
#4	Search (“neural tube defects”[MeSH Terms] OR “spina bifida”[All Fields] OR “neural tube damage”[All Fields] OR “neural tube defect”[All Fields] OR “neural tube defects”[All Fields] OR “neural tube disorders”[All Fields] OR “Neural tube defect, folate-sensitive”[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR “Tethered Cord Syndrome”[tw] OR “Tethered Cord Syndromes”[tw] OR “Occult Spinal Dysraphism Sequence”[tw] OR “Tethered Spinal Cord Syndrome”[tw] OR “Occult Spinal Dysraphism”[tw] OR “Occult Spinal Dysraphisms”[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR “Neurenteric Cyst”[tw] OR “Neurenteric Cysts”[tw] OR “Neuroenteric Cyst”[tw] OR “Neuroenteric Cysts”[tw] OR “Spinal Cord Myelodysplasia”[tw] OR “Spinal Cord Myelodysplasias”[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw])	28752
#5	Search (#1 AND (#2 OR #3) AND #4)	1906
#6	Search (#1 AND (#2 OR #3) AND #4) Filters: Humans	1707
#7	Search (#1 AND (#2 OR #3) AND #4) Filters: Other Animals	213
#8	Search (#7 NOT #6)	100
#9	Search (#5 NOT #8)	1806

Appendix A. Search Strategy and Detailed Methods

#10	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	38379
#11	Search (#9 and #10) Retraction	1
#12	Search (#5 NOT #8) Filters: Publication date from 2015/02/27	48

1/28/16 PubMed Benefits Search

Search	Query	Items found
#1	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	854509
#2	Search (“folic acid”[MeSH] OR “vitamin b9”[tw] OR “vitamin m”[tw] or “Pteroylglutamic Acid”[tw] OR “folvite”[tw] OR “folacin”[tw] OR “folate”[tw] OR “folic acid”[tw] OR “5-Me-THF” OR “5-Me-H4F” OR “5-methyltetrahydrofolate” OR “5-methyltetrahydropteroylpentaglutamate”[Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate”[Supplementary Concept]))	51191
#3	Search ((multivitamin[all fields] OR “prenatal vitamin”[all fields] OR multivitamins[all fields] OR “prenatal vitamins”[all fields] OR “vitamin supplement”[all fields] OR “vitamin supplements”[all fields]))	4536
#4	Search (“neural tube defects”[MeSH Terms] OR “spina bifida”[All Fields] OR “neural tube damage”[All Fields] OR “neural tube defect”[All Fields] OR “neural tube defects”[All Fields] OR “neural tube disorders”[All Fields] OR “Neural tube defect, folate-sensitive”[Supplementary Concept] OR Craniorachischisis[tw] OR Craniorachischises[tw] OR Diastematomyelia[tw] OR Diastematomyelias[tw] OR “Tethered Cord Syndrome”[tw] OR “Tethered Cord Syndromes”[tw] OR “Occult Spinal Dysraphism Sequence”[tw] OR “Tethered Spinal Cord Syndrome”[tw] OR “Occult Spinal Dysraphism”[tw] OR “Occult Spinal Dysraphisms”[tw] OR Iniencephaly[tw] OR Iniencephalies[tw] OR “Neurenteric Cyst”[tw] OR “Neurenteric Cysts”[tw] OR “Neuroenteric Cyst”[tw] OR “Neuroenteric Cysts”[tw] OR “Spinal Cord Myelodysplasia”[tw] OR “Spinal Cord Myelodysplasias”[tw] OR Acrania[tw] OR Acranias[tw] OR Exencephaly[tw] OR Exencephalies[tw]))	29292
#5	Search ((#1 AND (#2 OR #3) AND #4))	1923
#6	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Humans	1715
#7	Search ((#1 AND (#2 OR #3) AND #4)) Filters: Other Animals	216
#8	Search ((#7 NOT #6))	103
#9	Search ((#5 NOT #8))	1820
#10	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	40500
#11	Search ((#9 and #10))	1
#12	Search ((#5 NOT #8)) Filters: Publication date from 2015/10/18	22

9/9/14 PubMed Harms Search

Search	Query	Items found
#1	Search “folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept]	35919
#2	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	792843
#3	Search (#1 and #2)	5060
#4	Search (“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] harm[tiab] OR harms[tiab] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab]))	941528
#5	Search (#3 and #4)	681
#6	Search (#3 and #4) Filters: Humans	586
#7	Search (#3 and #4) Filters: Other Animals	90
#8	Search (#7 not #6)	47
#9	Search (#5 not #8)	634

Appendix A. Search Strategy and Detailed Methods

11/11/14 PubMed Harms Search

Search	Query	Items found
#1	Search (“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	36189
#2	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	797435
#3	Search (#1 and #2)	5102
#4	Search “Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR “Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word]) OR “Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] harm[tiab] OR harms[tiab] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab]	952237
#5	Search ((#3 and #4))	689
#6	Search #5 Filters: Humans	592
#7	Search #5	689
#8	Search #5 Filters: Other Animals	93
#9	Search (#8 NOT #6)	49
#10	Search (#5 NOT #9)	640
#11	Search (#5 NOT #9) Filters: Publication date from 2013/09/09	30
#12	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	28984
#13	Search (#5 and #12)	2

1/6/15 PubMed Harms Addendum Search

Search	Query	Items found
#4	Search “folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept]	36405
#5	Search ((Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH]))	801618
#6	Search (#4 and #5)	5145
#7	Search (“Twins”[mesh] OR “Pregnancy, Twin”[mesh] OR twinning OR twins)	39869
#8	Search (#6 and #7)	57
#9	Search ((“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] harm[tiab] OR harms[tiab] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab]))))	962466
#10	Search (#8 NOT #9)	47
#11	Search (#8 NOT #9) Filters: Humans	46
#12	Search (#8 NOT #9) Filters: Other Animals	4
#13	Search (#12 NOT #11)	0
#14	Search (#10 NOT #13)	47

Appendix A. Search Strategy and Detailed Methods

3/24/15 and 3/27/15 PubMed Harms Search

Search	Query	Items found
#23	Search (“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	36728
#24	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	807095
#25	Search (#23 and #24)	5210
#26	Search (“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR “Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word]) OR “Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] harm[tiab] OR harms[tiab] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab])	821329
#27	Search (#25 and #26)	332
#28	Search (#25 and #26) Filters: Humans	284
#29	Search (#25 and #26) Filters: Other Animals	42
#30	Search (#29 not #28)	21
#31	Search (#27 not #30)	311
#32	Search (#27 not #30) Filters: Publication date from 2014/05/10	11
#33	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	30021
#34	Search (#27 and #33)	0

6/10/15 PubMed Harms Addendum Search

Search	Query	Items found
#1	Search ((Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH]))	813856
#2	Search (“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	37102
#3	Search (#1 and #2)	5261
#4	Search “Asthma”[Mesh]	107710
#5	Search asthma	150552
#6	Search (#4 or #5)	150552
#7	Search “adverse effects” [Subheading]	1765128
#8	Search (#3 and #6)	22
#9	Search (#3 and #7)	779
#10	Search (#8 or #9)	788
#11	Search (#8 or #9) Filters: Humans	671
#12	Search (#8 or #9) Filters: Other Animals	195
#13	Search (#12 not #11)	114
#14	Search (#10 not #13)	674
#15	Search (“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] harm[tiab] OR harms[tiab] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab])	992424
#16	Search (#14 NOT #15)	562

Appendix A. Search Strategy and Detailed Methods

7/27/15 PubMed Harms Search

Search	Query	Items found
#1	Search (“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	37694
#2	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	817957
#3	Search (#1 and #2)	5319
#4	Search ((“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] OR harm[tiab] OR harms[tiab] OR “adverse effects” [Subheading] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab]) OR “Twins”[mesh] OR “Pregnancy, Twin”[mesh] OR twinning OR twins OR asthma[Mesh] OR asthma))	2779068
#5	Search (#3 and #4)	1499
#6	Search (#3 and #4) Filters: Humans	1228
#7	Search (#3 and #4) Filters: Other Animals	271
#8	Search (#7 NOT #6)	158
#9	Search (#5 NOT #8)	1341
#10	Search (#5 NOT #8) Filters: Publication date from 2014/07/06	40
#11	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	34641
#12	Search (#5 and #11) Retractions	2

11/18/15 PubMed Harms Search

Search	Query	Items found
#1	Search (“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept])	38183
#2	Search (Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH])	826665
#3	Search (#1 and #2)	5404
#4	Search ((“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] OR harm[tiab] OR harms[tiab] OR “adverse effects” [Subheading] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab]) OR “Twins”[mesh] OR “Pregnancy, Twin”[mesh] OR twinning OR twins OR asthma[Mesh] OR asthma))	2822126
#5	Search (#3 and #4)	1522
#6	Search (#3 and #4) Filters: Humans	1243
#7	Search (#3 and #4) Filters: Other Animals	275
#8	Search (#7 NOT #6)	161
#9	Search (#5 not #8)	1361
#10	Search (#5 not #8) Filters: Publication date from 2015/02/27	23
#11	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields])	38379
#12	Search (#5 and #11)	2

Appendix A. Search Strategy and Detailed Methods

1/28/16 PubMed Harms Search

Search	Query	Items found
#1	Search ((“folic acid”[mesh] OR “folic acid”[tiab] OR “folvite”[tiab] OR “folacin”[tiab] OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate OR “5-methyltetrahydropteroylpentaglutamate” [Supplementary Concept] OR “5-methyltetrahydrofolate triglutamate” [Supplementary Concept]))	39456
#2	Search ((Pregnancy[MeSH] OR pregnancy[tw] OR pregnant[tw] OR “pregnant women”[MeSH]))	854509
#3	Search ((#1 and #2))	5507
#4	Search (((“Colorectal Neoplasms”[Mesh] OR (“Vitamin B 12 Deficiency”[Mesh] OR (“vitamin b 12”[MeSH Terms] AND deficien*[Text Word])) OR (“Vitamin B 6 Deficiency”[Mesh] OR (“vitamin b 6”[MeSH Terms] AND deficien*[Text Word])) OR (“Drug-Related Side Effects and Adverse Reactions”[Majr] OR “Patient Harm”[Majr] OR harm[tiab] OR harms[tiab] OR “adverse effects” [Subheading] OR “adverse effect”[tiab] OR “adverse effects”[tiab] OR “adverse event”[tiab] OR “adverse events”[tiab] OR complication[tiab] OR complications[tiab] OR “Twins”[mesh] OR “Pregnancy, Twin”[mesh] OR twinning OR twins OR asthma[Mesh] OR asthma)))	2852040
#5	Search ((#3 and #4))	1545
#6	Search ((#3 and #4)) Filters: Humans	1324
#7	Search ((#3 and #4)) Filters: Other Animals	279
#8	Search ((#7 NOT #6))	164
#9	Search ((#5 not #8))	1381
#10	Search ((#5 not #8)) Filters: Publication date from 2015/10/18	10
#11	Search (“retraction”[All Fields] OR “Retracted Publication”[pt] OR Duplicate Publication [PT] OR Erratum[All Fields]))	40500
#12	Search ((#5 and #11))	2

6/23/14 Cochrane Benefits Search

Search	Query	Items found
#1	[mh Pregnancy] OR pregnancy OR pregnant OR [mh “pregnant women”]	27927
#2	[mh “folic acid”] OR “vitamin b9” OR “vitamin m” or “Pteroylglutamic Acid” OR “folvite” OR “folacin” OR “folate” OR “folic acid”	3775
#3	Multivitamin OR “prenatal vitamin” OR multivitamins OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements”	915
#4	[mh “neural tube defects”] OR “spina bifida” OR “neural tube damage” OR “neural tube defect” OR “neural tube defects” OR “neural tube disorders” OR “Neural tube defect, folate-sensitive” OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR “Tethered Cord Syndrome” OR “Tethered Cord Syndromes” OR “Occult Spinal Dysraphism Sequence” OR “Tethered Spinal Cord Syndrome” OR “Occult Spinal Dysraphism” OR “Occult Spinal Dysraphisms” OR Iniencephaly OR Iniencephalies OR “Neurenteric Cyst” OR “Neurenteric Cysts” OR “Neuroenteric Cyst” OR “Neuroenteric Cysts” OR “Spinal Cord Myelodysplasia” OR “Spinal Cord Myelodysplasias” OR Acrania OR Acranias OR Exencephaly OR Exencephalies	374
#5	Search (#1 AND (#2 OR #3) AND #4)	105

Appendix A. Search Strategy and Detailed Methods

9/4/14 Cochrane Benefits Addendum Search

ID	Search	Hits
#1	5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or 5-methyltetrahydropteroylpentaglutamate or "5-methyltetrahydrofolate triglutamate"	96
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	4467
#3	#1 not #2	16
#4	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	28255
#5	#3 and #4	2
#6	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	376
#7	#5 and #6	0

11/11/14 Cochrane Benefits Search

ID	Search	Hits
#1	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	29122
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate	3909
#3	Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	944
#4	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	383
#5	(#1 and (#2 or #3) and #4)	108
#6	(#1 and (#2 or #3) and #4) Publication Year from 2013 to 2014	16

3/24/15 Cochrane Benefits Search

ID	Search	Hits
#1	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	29687
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate	4051
#3	Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	988
#4	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	392
#5	(#1 and (#2 or #3) and #4)	108
#6	(#1 and (#2 or #3) and #4) Publication Year from 2014 to 2015	8

Appendix A. Search Strategy and Detailed Methods

7/27/15 Cochrane Benefits Search

ID	Search	Hits
#1	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	30805
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate 4051	4253
#3	Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	1065
#4	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	408
#5	(#1 and (#2 or #3) and #4)	115
#6	(#1 and (#2 or #3) and #4) Publication Year from 2014 to 2015	13

11/18/15 Cochrane Benefits Search

ID	Search	Hits
#1	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	31487
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate 4051	4353
#3	Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	1098
#4	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	411
#5	(#1 and (#2 or #3) and #4) Publication Year from 2014 to 2015	16

1/28/16 Cochrane Benefits Search

ID	Search	Hits
#1	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	32013
#2	[mh "folic acid"] or "vitamin b9" or "vitamin m" or "Pteroylglutamic Acid" or "folvite" or "folacin" or "folate" or "folic acid" or 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate 4051	4403
#3	Multivitamin or "prenatal vitamin" or multivitamins or "prenatal vitamins" or "vitamin supplement" or "vitamin supplements"	1115
#4	[mh "neural tube defects"] or "spina bifida" or "neural tube damage" or "neural tube defect" or "neural tube defects" or "neural tube disorders" or "Neural tube defect, folate-sensitive" or Craniorachischisis or Craniorachischises or Diastematomyelia or Diastematomyelias or "Tethered Cord Syndrome" or "Tethered Cord Syndromes" or "Occult Spinal Dysraphism Sequence" or "Tethered Spinal Cord Syndrome" or "Occult Spinal Dysraphism" or "Occult Spinal Dysraphisms" or Iniencephaly or Iniencephalies or "Neurenteric Cyst" or "Neurenteric Cysts" or "Neuroenteric Cyst" or "Neuroenteric Cysts" or "Spinal Cord Myelodysplasia" or "Spinal Cord Myelodysplasias" or Acrania or Acranias or Exencephaly or Exencephalies	414
#5	(#1 and (#2 or #3) and #4) Online Publication Date from Oct 2015	5

Appendix A. Search Strategy and Detailed Methods

9/9/14 Cochrane Harms Search

ID	Search Terms	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3438
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	28256
#3	#1 and #2	579
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mj]] or [mh "Patient Harm" [mj]] or harm or harms or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications)	217992
#5	#3 and #4	330

11/11/14 Cochrane Harms Search

ID	Search	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3511
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	29122
#3	#1 and #2	598
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mj]] or [mh "Patient Harm" [mj]] or harm or harms or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications)	221497
#5	#3 and #4	338
#6	#3 and #4 Publication Year from 2013 to 2014	65

3/24/15 Cochrane Harms Search

ID	Search	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3650
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	29687
#3	#1 and #2	627
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mj]] or [mh "Patient Harm" [mj]] or harm or harms or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications)	228315
#5	#3 and #4	338
#6	#3 and #4 Publication Year from 2014 to 2015	41

Appendix A. Search Strategy and Detailed Methods

6/10/15 Cochrane Harms Addendum Search

ID	Search	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3752
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	30578
#3	#1 and #2	649
#4	asthma	24345
#5	#3 and #4	22
#6	[mh /AE]	105460
#7	#3 and #6	73
#8	#5 or #7	84
#9	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mjj] or [mh "Patient Harm" [mjj] or harm or harms or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications)	234745
#10	#8 not #9	0

7/27/15 Cochrane Harms Search

ID	Search	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3867
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	30805
#3	#1 and #2	676
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mjj] or [mh "Patient Harm" [mjj] or harm or harms or [mh /AE] or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications or [mh Asthma] or asthma)	256428
#5	#3 and #4	366
#6	#3 and #4 Publication Year from 2014 to 2015	56

11/18/15 Cochrane Harms Search

ID	Search	Hits
#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	3962
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	31487
#3	#1 and #2	710
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mjj] or [mh "Patient Harm" [mjj] or harm or harms or [mh /AE] or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications or [mh Asthma] or asthma)	261297
#5	#3 and #4 Publication Year from 2015 to 2015	44

Appendix A. Search Strategy and Detailed Methods

1/28/16 Cochrane Harms Search

#1	[mh "folic acid"] or "folic acid" or folvite or folacin 5-Me-THF or 5-Me-H4F or 5-methyltetrahydrofolate or "5-methyltetrahydropteroylpentaglutamate" or "5-methyltetrahydrofolate triglutamate"	4011
#2	[mh Pregnancy] or pregnancy or pregnant or [mh "pregnant women"]	32013
#3	#1 and #2	722
#4	[mh Twins] or [mh "Pregnancy, Twin"] or twinning or twins or [mh "Colorectal Neoplasms"] or ([mh "Vitamin B 12 Deficiency"] or ([mh "vitamin b 12"] and deficien*)) or ([mh "Vitamin B 6 Deficiency"] or ([mh "vitamin b 6"] and deficien*)) or ([mh "Drug-Related Side Effects and Adverse Reactions" [mjj]] or [mh "Patient Harm" [mjj]] or harm or harms or [mh /AE] or "adverse effect" or "adverse effects" or "adverse event" or "adverse events" or complication or complications or [mh Asthma] or asthma)	263974
#5	#3 and #4 Online Publication Date from Oct 2015	16

6/23/14 EMBASE Benefits Search

No.	Query	Results
#11	#9 NOT #10	543
#10	#9 AND [medline]/lim	1,373
#9	#5 NOT #8	1,916
#8	#7 NOT #6 AND [animals]/lim	77
#7	#5 AND [animals]/lim	343
#6	#5 AND [humans]/lim	1,832
#5	#1 AND (#2 OR #3) AND #4	1,993
#4	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly'/exp OR exencephaly OR exencephalies	30,718
#3	'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements'	8,726
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid'	59,344
#1	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	648,650

9/8/14 EMBASE Benefits Addendum Search

No.	Query	Results
#7	#5 AND #6	0
#6	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly'/exp OR exencephaly OR exencephalies	31,029
#5	#3 AND #4	11
#4	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	655,680
#3	#1 NOT #2	379
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR	66,754

Appendix A. Search Strategy and Detailed Methods

	'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements'	
#1	'5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	1,429

3/24/15 EMBASE Benefits Search

No.	Query	Results
#11	#9 NOT #10	70
#10	#9 AND [medline]/lim	41
#9	#8 AND [2014-2015]/py	111
#8	#4 NOT #7	2,394
#7	#6 NOT #5	101
#6	#4 AND [animals]/lim	423
#5	#4 AND [humans]/lim	2,288
#4	#1 AND #2 AND #3	2,495
#3	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly'/exp OR exencephaly OR exencephalies	31,662
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements' OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	69,216
#1	'pregnant women'/exp OR 'pregnant women' OR 'pregnancy'/exp OR 'pregnancy' OR pregnant	784,028

7/27/15 EMBASE Benefits Search

#9	#8 AND [24-10-2014]/sd NOT [27-7-2015]/sd	95
#8	#4 NOT #7	2,456
#7	#6 NOT #5	95
#6	#4 AND [animals]/lim	172
#5	#4 AND [humans]/lim	2,340
#4	#1 AND #2 AND #3	2,551
#3	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly'/exp OR exencephaly OR exencephalies	32,319
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements' OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	71,041
#1	'pregnant women'/exp OR 'pregnant women' OR 'pregnancy'/exp OR 'pregnancy' OR pregnant	802,358

Appendix A. Search Strategy and Detailed Methods

11/18/15 EMBASE Benefits Search

#9	#8 AND [27-2-2015]/sd NOT [18-11-2015]/sd	108
#8	#4 NOT #7	2,500
#7	#6 NOT #5	97
#6	#4 AND [animals]/lim	176
#5	#4 AND [humans]/lim	2,384
#4	#1 AND #2 AND #3	2,597
#3	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia' OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly' OR 'exencephaly'/exp OR exencephaly OR exencephalies	32,708
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements' OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate'/exp OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	72,556
#1	'pregnant women'/exp OR 'pregnant women' OR 'pregnancy'/exp OR 'pregnancy' OR pregnant	816,404

1/28/16 EMBASE Benefits Search

#9	#8 AND [18-10-2015]/sd	23
#8	#4 NOT #7	23
#7	#6 NOT #5	2,512
#6	#4 AND [animals]/lim	98
#5	#4 AND [humans]/lim	177
#4	#1 AND #2 AND #3	2,396
#3	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia' OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly' OR 'exencephaly'/exp OR exencephaly OR exencephalies	2,610
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements' OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate'/exp OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	32,949
#1	'pregnant women'/exp OR 'pregnant women' OR 'pregnancy'/exp OR 'pregnancy' OR pregnant	73,279

Appendix A. Search Strategy and Detailed Methods

9/9/14 EMBASE Harms Search

No.	Query	Results
#11	#9 NOT #10	517
#10	#9 AND [medline]/lim	1,422
#9	#5 NOT #8	1,939
#8	#7 NOT #6	75
#7	#5 AND [animals]/lim	316
#6	#5 AND [humans]/lim	1,777
#5	#3 AND #4	2,014
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications	2,688,524
#3	#1 AND #2	6,768
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	655,686
#1	'folic acid'/exp OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	49,006

11/11/14 EMBASE Harms Search

No.	Query	Results
#11	#9 NOT #10	128
#10	#9 AND [medline]/lim	100
#9	#8 AND [2013-2014]/py	228
#8	#4 NOT #7	2,362
#7	#6 NOT #5	97
#6	#4 AND [animals]/lim	420
#5	#4 AND [humans]/lim	2,257
#4	#1 AND #2 AND #3	2,459
#3	'spinal dysraphism'/exp OR 'spinal dysraphism' OR 'neural tube damage' OR 'neural tube defect'/exp OR 'neural tube defect' OR 'neural tube defects'/exp OR 'neural tube defects' OR 'neural tube disorders' OR 'neural tube defect, folate-sensitive' OR craniorachischisis OR craniorachischises OR 'diastematomyelia'/exp OR diastematomyelia OR diastematomyelias OR 'tethered cord syndrome'/exp OR 'tethered cord syndrome' OR 'tethered cord syndromes' OR 'occult spinal dysraphism sequence' OR 'tethered spinal cord syndrome' OR 'occult spinal dysraphism'/exp OR 'occult spinal dysraphism' OR 'occult spinal dysraphisms' OR 'iniencephaly'/exp OR iniencephaly OR iniencephalies OR 'neurenteric cyst' OR 'neurenteric cysts' OR 'neuroenteric cyst' OR 'neuroenteric cysts' OR 'spinal cord myelodysplasia' OR 'spinal cord myelodysplasias' OR acrania OR acranias OR 'exencephaly'/exp OR exencephaly OR exencephalies	31,173
#2	'vitamin b9' OR 'vitamin m'/exp OR 'vitamin m' OR 'pteroylglutamic acid'/exp OR 'pteroylglutamic acid' OR 'folvite'/exp OR 'folvite' OR 'folacin'/exp OR 'folacin' OR 'folate'/exp OR 'folate' OR 'folic acid'/exp OR 'folic acid' OR 'multivitamin'/exp OR multivitamin OR 'prenatal vitamin' OR 'multivitamins'/exp OR multivitamins OR 'prenatal vitamins' OR 'vitamin supplement' OR 'vitamin supplements' OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	67,655
#1	'pregnant women'/exp OR 'pregnant women' OR 'pregnancy'/exp OR 'pregnancy' OR pregnant	769,699

Appendix A. Search Strategy and Detailed Methods

11/11/14 EMBASE Harms Search

No.	Query	Results
#12	#9 NOT #10 AND [2013-2014]/py	142
#11	#9 NOT #10	522
#10	#9 AND [medline]/lim	1,431
#9	#5 NOT #8	1,953
#8	#7 NOT #6	75
#7	#5 AND [animals]/lim	320
#6	#5 AND [humans]/lim	1,791
#5	#3 AND #4	2,028
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications	2,711,985
#3	#1 AND #2	6,816
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	660,630
#1	'folic acid'/exp OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	49,375

3/24/15 EMBASE Harms Search

No.	Query	Results
#12	#9 NOT #10 AND [2014-2015]/py	77
#11	#9 NOT #10	551
#10	#9 AND [medline]/lim	1,446
#9	#5 NOT #8	1,997
#8	#7 NOT #6	76
#7	#5 AND [animals]/lim	328
#6	#5 AND [humans]/lim	1,835
#5	#3 AND #4	2,073
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications	2,778,112
#3	#1 AND #2	6,984
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	673,619
#1	'folic acid'/exp OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	50,573

6/10/15 EMBASE Harms Addendum Search

No.	Query	Results
#12	#10 NOT #11	164
#11	#8 NOT #9 AND [medline]/lim	379
#10	#8 NOT #9	543
#9	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications	2,826,063
#8	#5 OR #7	1,016
#7	#3 AND #6	949
#6	'adverse drug reaction'/exp OR 'adverse drug reaction'	1,320,972
#5	#3 AND #4	95
#4	'asthma' OR 'asthma'/exp OR asthma	238,789
#3	#1 AND #2	7,124
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	682,899
#1	'folic acid'/exp OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	51,450

Appendix A. Search Strategy and Detailed Methods

7/27/15 EMBASE Harms Search

No.	Query	Results
#12	#11 AND [6-7-2014]/sd NOT [27-7-2015]/sd	116
#11	#7 NOT #10	827
#10	#9 NOT #8	25
#9	#7 AND [animals]/lim	65
#8	#7 AND [humans]/lim	730
#7	#5 NOT #6	852
#6	#5 AND [medline]/lim	2,191
#5	#3 AND #4	3,043
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse drug reaction'/exp OR 'adverse drug reaction' OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications OR 'twin pregnancy'/exp OR 'twins'/exp OR twin OR twins OR twinning OR 'asthma'/exp OR asthma	3,903,874
#3	#1 AND #2	7,683
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	687,384
#1	'folic acid'/exp OR 'folic acid' OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate'/exp OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	52,898

11/18/15 EMBASE Harms Search

No.	Query	Results
#12	#11 AND [27-2-2015]/sd NOT [18-11-2015]/sd	95
#11	#7 NOT #10	853
#10	#9 NOT #8	25
#9	#7 AND [animals]/lim	65
#8	#7 AND [humans]/lim	755
#7	#5 NOT #6	878
#6	#5 AND [medline]/lim	2,210
#5	#3 AND #4	3,088
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse drug reaction'/exp OR 'adverse drug reaction' OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications OR 'twin pregnancy'/exp OR 'twins'/exp OR twin OR twins OR twinning OR 'asthma'/exp OR asthma	3,976,920
#3	#1 AND #2	7,857
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	698,517
#1	'folic acid'/exp OR 'folic acid' OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate'/exp OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	59,181

Appendix A. Search Strategy and Detailed Methods

1/28/16 EMBASE Harms Search

No.	Query	Results
#12	#11 AND [18-10-2015]/sd	27
#11	#7 NOT #10	866
#10	#9 NOT #8	25
#9	#7 AND [animals]/lim	65
#8	#7 AND [humans]/lim	767
#7	#5 NOT #6	891
#6	#5 AND [medline]/lim	2,217
#5	#3 AND #4	3,108
#4	'colorectal tumor'/exp OR 'cyanocobalamin deficiency'/exp OR (b12 AND deficien*) OR 'pyridoxine deficiency'/exp OR (b6 AND deficien*) OR 'adverse drug reaction'/exp/mj OR 'patient harm'/exp/mj OR harm OR harms OR 'adverse drug reaction'/exp OR 'adverse drug reaction' OR 'adverse effect' OR 'adverse effects' OR 'adverse event' OR 'adverse events' OR complication OR complications OR 'twin pregnancy'/exp OR 'twins'/exp OR twin OR twins OR twinning OR 'asthma'/exp OR asthma	4,017,311
#3	#1 AND #2	7,935
#2	'pregnancy'/exp OR 'pregnant women'/exp OR 'pregnancy'/de OR pregnant	705,446
#1	'folic acid'/exp OR 'folic acid' OR 'folic acid':ti OR 'folic acid':ab OR folvite:ti OR folvite:ab OR folacin:ti OR folacin:ab OR '5 me thf' OR '5 me h4f' OR '5 methyltetrahydrofolate'/exp OR '5 methyltetrahydrofolate' OR '5 methyltetrahydropteroylpentaglutamate' OR '5-methyltetrahydrofolate triglutamate'	59,748

7/1/14 Gray Literature Benefits Search

ClinicalTrials.gov – 8

HSRProj (Health Services Research in Progress) – 5

Cochrane Library CENTRAL – no need to search separately, as this was included during the published literature search (there were 59 trials)

WHO ICTRP – searched the neural tube defect terms in the Condition box, and the folate terms in the intervention box, and found 7 trials

NIH RePORTER – used entire quoted search string and retrieved 15 grants

Websites:

NICHD – navigated to publications from the main page, and found and saves 3 pertinent folic acid documents. Also used the search box on the main site to search for “folic acid” and retrieved 45 other links to recent publications.

HRSA Maternal & Child Health Bureau – A search of “folic acid” yielded ~30 websites.

8/6/14 Gray Literature Harms Search

I. SPECIFIC HARMS

(“folic acid” OR folvite OR folacin) AND ((twin OR twin OR twinning) OR “colorectal neoplasms” OR “colorectal cancer” OR “colorectal tumor” OR “vitamin b12 deficiency” OR “vitamin b6 deficiency”) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications)

ClinicalTrials.gov = 421

HSRProj = 0

WHO ICTRP - put folate string in Title search and the other terms in Condition search = 45 records for 36 trials found.

NIH RePORTER - Advanced Search Logic - 2

HRSA Maternal and Child Health Bureau website - 6

NICHD - only small amount of text fits in search box. searched “folic acid” AND (harm* OR adverse*) - 10 results

II. GENERAL HARMS

(“folic acid” OR folvite OR folacin) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications)

ClinicalTrials.gov = 1085

HSRProj = 0

WHO ICTRP = 1, duplicate with the specific search above, not saved.

NIH RePORTER - 30

Appendix A. Search Strategy and Detailed Methods

HRSA Maternal and Child Health Bureau website - 21

3/27/15 Gray Literature Benefits Search

(Pregnancy OR pregnant OR “pregnant women”) AND (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR “folic acid” OR multivitamin OR “prenatal vitamin” OR multivitamins OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate) AND (“neural tube defects” OR “spina bifida” OR “neural tube damage” OR “neural tube defect” OR “neural tube disorders” OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR “Tethered Cord Syndrome” OR “Tethered Cord Syndromes” OR “Tethered Spinal Cord Syndrome” OR “Occult Spinal Dysraphism” OR “Occult Spinal Dysraphisms” OR Iniencephaly OR Iniencephalies OR “Neurenteric Cyst” OR “Neurenteric Cysts” OR “Neuroenteric Cyst” OR “Neuroenteric Cysts” OR “Spinal Cord Myelodysplasia” OR “Spinal Cord Myelodysplasias” OR Acrania OR Acranias OR Exencephaly OR Exencephalies) ClinicalTrials.gov – 7 studies

HSRProj – 0

WHO ICTRP – searched the neural tube defect terms in the Condition box, and the folate terms in the intervention box, and found 2 trials

NIH RePORTER – Award notice date 7-1-14 or greater – 6 results

3/27/15 Gray Literature Harms Search

I. SPECIFIC HARMES

(“folic acid” OR folvite OR folacin) AND ((twin OR twin OR twinning) OR “colorectal neoplasms” OR “colorectal cancer” OR “colorectal tumor” OR “vitamin b12 deficiency” OR “vitamin b6 deficiency”) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications)

ClinicalTrials.gov = 105

HSRProj = 0

WHO ICTRP - put folate string in Title search and the other terms in Condition search = 5 records for 5 trials found.

NIH RePORTER - Advanced Search Logic - 1

II. GENERAL HARMES

(“folic acid” OR folvite OR folacin) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications)

ClinicalTrials.gov = 280

HSRProj = 0

WHO ICTRP = 0

NIH RePORTER - 13

6/11/15 Gray Literature Addendum for Asthma

Previous gray literature searches already covered all needed adverse effects terms.

Search strategy:

(“folic acid” OR folvite OR folacin) AND asthma

-ClinicalTrials.gov = 8

-HSRProj = 0

-WHO ICTRP (<http://apps.who.int/trialsearch/AdvSearch.aspx>) - put folate string in Title search and asthma in Condition search and search Recruitment status ALL = 56,

-NIH RePORTER (<http://projectreporter.nih.gov/reporter.cfm>) - Advanced Search Logic – 3

-HRSA Maternal and Child Health Bureau website (mchb.hrsa.gov) – 22

-**Eunice Kennedy Shriver** National Institute of Child Health and Human Development (NICHD)

(<https://www.nichd.nih.gov/Pages/index.aspx>) - 0 results

7/28/15 Gray Literature Benefits Search

(Pregnancy OR pregnant OR “pregnant women”) AND (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR “folic acid” OR multivitamin OR “prenatal vitamin” OR multivitamins OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate) AND (“neural tube defects” OR “spina bifida” OR “neural tube damage” OR “neural tube defect” OR “neural tube disorders” OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR “Tethered Cord Syndrome” OR “Tethered Cord Syndromes” OR “Tethered Spinal Cord Syndrome” OR “Occult Spinal Dysraphism” OR “Occult Spinal Dysraphisms” OR Iniencephaly OR Iniencephalies OR “Neurenteric Cyst” OR “Neurenteric Cysts” OR “Neuroenteric Cyst” OR “Neuroenteric Cysts” OR “Spinal Cord Myelodysplasia” OR “Spinal Cord Myelodysplasias” OR Acrania OR Acranias OR Exencephaly OR Exencephalies)

Appendix A. Search Strategy and Detailed Methods

ClinicalTrials.gov – 4 studies

HSRProj – 0 added to HSRProj in 2015

WHO ICTRP - searched the neural tube defect terms in the Condition box, and the folate terms in the intervention box, and found 0 trials.

NIH RePORTER – Award notice date 2-1-15 or greater– 10 results.

7/28/15 Gray Literature HARMS Search

I. SPECIFIC HARMS

("folic acid" OR folvite OR folacin) AND ((twin OR twin OR twinning) OR "colorectal neoplasms" OR "colorectal cancer" OR "colorectal tumor" OR "vitamin b12 deficiency" OR "vitamin b6 deficiency") AND ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications OR asthma)

ClinicalTrials.gov = 114

HSRProj = 0

WHO ICTRP - put all terms in title search = 0 records

NIH RePORTER - Advanced Search Logic - 0

II. GENERAL HARMS

(folic acid OR folvite OR folacin) AND ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications)

ClinicalTrials.gov = 312

HSRProj = 0

WHO ICTRP = 0

NIH RePORTER - 19

11/18/15 Gray Literature Benefits Search

Benefits search strategy:

(Pregnancy OR pregnant OR "pregnant women") AND ("folic acid" OR "vitamin b9" OR "vitamin m" OR "Pteroylglutamic Acid" OR folvite OR folacin OR folate OR "folic acid" OR multivitamin OR "prenatal vitamin" OR multivitamins OR "prenatal vitamins" OR "vitamin supplement" OR "vitamin supplements" OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate) AND ("neural tube defects" OR "spina bifida" OR "neural tube damage" OR "neural tube defect" OR "neural tube disorders" OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR "Tethered Cord Syndrome" OR "Tethered Cord Syndromes" OR "Tethered Spinal Cord Syndrome" OR "Occult Spinal Dysraphism" OR "Occult Spinal Dysraphisms" OR Iniencephaly OR Iniencephalies OR "Neurenteric Cyst" OR "Neurenteric Cysts" OR "Neuroenteric Cyst" OR "Neuroenteric Cysts" OR "Spinal Cord Myelodysplasia" OR "Spinal Cord Myelodysplasias" OR Acrania OR Acranias OR Exencephaly OR Exencephalies)

ClinicalTrials.gov – 2 studies

HSRProj – 0

WHO ICTRP – searched the neural tube defect terms in the Condition box, and the folate terms in the intervention box, and found 0 trials.

NIH RePORTER – Advanced Text search logic/ Award notice date 7-1-15 or greater – 11 results.

11/18/15 Gray Literature HARMS Search

I. SPECIFIC HARMS

("folic acid" OR folvite OR folacin) AND ((twin OR twin OR twinning) OR "colorectal neoplasms" OR "colorectal cancer" OR "colorectal tumor" OR "vitamin b12 deficiency" OR "vitamin b6 deficiency") AND ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications OR asthma)

ClinicalTrials.gov = 90

HSRProj = 0

WHO ICTRP - put all terms in title search = 0 records

NIH RePORTER - Advanced Search Logic – 1 record

II. GENERAL HARMS

(folic acid OR folvite OR folacin) AND ("drug-related side effects" OR "adverse reaction" OR harm OR harms OR "adverse effect" OR "adverse effects" OR "adverse event" OR "adverse events" OR Complication OR Complications)

Appendix A. Search Strategy and Detailed Methods

ClinicalTrials.gov = 268
HSRProj = 0
WHO ICTRP = 0
NIH RePORTER - 12

2/1/16 Gray Literature Benefits Search

Benefits search strategy:

(Pregnancy OR pregnant OR “pregnant women”) AND (“folic acid” OR “vitamin b9” OR “vitamin m” OR “Pteroylglutamic Acid” OR folvite OR folacin OR folate OR “folic acid” OR multivitamin OR “prenatal vitamin” OR multivitamins OR “prenatal vitamins” OR “vitamin supplement” OR “vitamin supplements” OR 5-Me-THF OR 5-Me-H4F OR 5-methyltetrahydrofolate) AND (“neural tube defects” OR “spina bifida” OR “neural tube damage” OR “neural tube defect” OR “neural tube disorders” OR Craniorachischisis OR Craniorachischises OR Diastematomyelia OR Diastematomyelias OR “Tethered Cord Syndrome” OR “Tethered Cord Syndromes” OR “Tethered Spinal Cord Syndrome” OR “Occult Spinal Dysraphism” OR “Occult Spinal Dysraphisms” OR Iniencephaly OR Iniencephalies OR “Neurenteric Cyst” OR “Neurenteric Cysts” OR “Neuroenteric Cyst” OR “Neuroenteric Cysts” OR “Spinal Cord Myelodysplasia” OR “Spinal Cord Myelodysplasias” OR Acrania OR Acranias OR Exencephaly OR Exencephalies)

ClinicalTrials.gov – 0 studies
HSRProj – 0
WHO ICTRP - 0
NIH RePORTER – 1
Advanced Text search logic/ Award notice date 10-18-15 or greater – 1 result.

2/1/16 Gray Literature HARMS Search

I. SPECIFIC HARMS

(“folic acid” OR folvite OR folacin) AND ((twin OR twin OR twinning) OR “colorectal neoplasms” OR “colorectal cancer” OR “colorectal tumor” OR “vitamin b12 deficiency” OR “vitamin b6 deficiency”) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications OR asthma)

ClinicalTrials.gov = 73
HSRProj = 0
WHO ICTRP - put all terms in title search = 0 records
NIH RePORTER - Advanced Search Logic – 0 records

II. GENERAL HARMS

(folic acid OR folvite OR folacin) AND (“drug-related side effects” OR “adverse reaction” OR harm OR harms OR “adverse effect” OR “adverse effects” OR “adverse event” OR “adverse events” OR Complication OR Complications)

ClinicalTrials.gov = 245
HSRProj = 0
WHO ICTRP = 0
NIH RePORTER - 3

Appendix B. Inclusion/Exclusion Criteria

	Include	Exclude
Populations	<p>KQ 1: Women of childbearing age (postmenarchal and premenopausal; women with the potential for or planning childbearing)</p> <p>KQ 2: Women of childbearing age (postmenarchal and premenopausal; women with the potential for or planning childbearing); fetus, neonate, or child from index pregnancy</p>	KQ 1: Prepubertal girls; men; women without the potential for childbearing (e.g., women who are postmenopausal or have genetic uterine or ovarian abnormalities)
Interventions	<p>Folic acid supplementation, with or without food fortification or naturally occurring folate, for the prevention of neural tube defects and other birth defects</p> <p>Supplementation with micronutrients (e.g., multivitamins, iron) in combination with folic acid for the prevention of neural tube defects only</p>	<p>Food fortification only</p> <p>Naturally occurring folate only</p> <p>Counseling to improve dietary supplementation</p> <p>Supplementation with micronutrients (e.g., multivitamins, iron) in combination with folic acid for the prevention of harms only</p>
Comparisons	<p>KQs 1a, 1b, 2a: Placebo or no treatment; dietary supplementation only; supplementation with prenatal vitamins without folic acid; iron supplements without folic acid</p> <p>KQs 1b, 1c, 2b: All of the above plus folic acid supplementation of varying dosages</p>	<p>KQs 1a, 1b, 2a: Lower or higher doses of folic acid supplementation; folic acid vs. other active comparators</p> <p>KQs 1c, 2b: Folic acid vs. other active comparators (e.g., multivitamins)</p>
Outcomes	<p>Neonatal outcomes: Neural tube defects</p> <p>Harms from treatment: Twins Colorectal cancer or other reported types of cancer Vitamin B12 deficiency Vitamin B6 deficiency Other reported child, neonatal, fetal, or maternal harms</p>	Benefits not specified in inclusion criteria
Timing	<p>KQs 1a, 1b: Supplementation initiated before index pregnancy or in the first trimester</p> <p>KQs 1c, 2a, 2b: All timing</p>	KQs 1a, 1b: Supplementation initiated after the first trimester of pregnancy
Settings	Developed countries categorized as “Very High” on the Human Development Index (as defined by the United Nations Development Programme)	Countries not categorized as “Very High” on the Human Development Index
Study designs	<p>Efficacy (KQ 1): Randomized, controlled trials; controlled clinical trials; cohort or case-control studies</p> <p>Harms (KQ 2): Randomized, controlled trials; controlled clinical trials; or observational studies (case-control, cohort, registry data)</p>	Commentaries, editorials, case reports
Sample size	More than 50 participants	50 participants or less
Quality	Good and fair quality	Poor quality
Language	English	Non-English studies

Appendix C. Excluded Studies

- X 1 Wrong publication type (Editorials, Letters, Opinions, or Commentaries to the editor with no primary data, Nonsystematic Review articles)
- X 2 Wrong population (Populations were not women of childbearing age [e.g., pre-menarchy, post-menopausal, no potential for childbearing])
- X 3 Wrong or no comparator (Single group design with no comparator; Active comparator [e.g., head-to-head trial])
- X 4 Wrong or no outcome (See Include/Exclude criteria for exceptions)
- X 5 Wrong timing (supplementation initiated after the first trimester for benefits only)
- X 6 Wrong geographical setting (Countries with human development index of low to high)
- X 7 Wrong Study Design (Case reports, case series, cross-sectional designs)
- X 8 Wrong or no intervention (As defined in the Include/Exclude criteria)
- X 9 Study size <50 subjects
- X 10 Wrong language (non-English)
- X 11 Full-text irretrievable
-
1. Folic acid and combined iron and folic acid preparations. *Br Med J*. 1968 Oct 12;4(5623):102-3. PMID: 5696538. Exclusion Code: X 1
 2. Foliates and the fetus. *Lancet*. 1977 Feb 26;1(8009):462. PMID: 65568. Exclusion Code: X 1
 3. Trial of folate treatment to prevent recurrence of neural tube defects. *Br Med J (Clin Res Ed)*. 1981 May 30;282(6278):1793. PMID: 6786625. Exclusion Code: X 1
 4. Vitamin supplements to prevent neural tube defects. *Lancet*. 1982 May 8;1(8280):1075. PMID: 6122879. Exclusion Code: X 1
 5. Vitamins during pregnancy and neural tube defects. *JAMA*. 1990 May 23-30;263(20):2747-9. PMID: 2332916. Exclusion Code: X 1
 6. Periconceptional use of multivitamins and the prevalence of neural-tube defects. *N Engl J Med*. 1990 Apr 12;322(15):1082-4. PMID: 2320071. Exclusion Code: X 1
 7. Vitamins to prevent neural tube defects. *Lancet*. 1991 Oct 5;338(8771):894-5. PMID: 1681257. Exclusion Code: X 1
 8. From the Centers for Disease Control. Use of folic acid for prevention of spina bifida and other neural tube defects--1983-1991. *JAMA*. 1991 Sep 4;266(9):1190-1. PMID: 1870237. Exclusion Code: X 1
 9. Folic acid to prevent neural tube defects. *Lancet*. 1991 Aug 24;338(8765):505-6. PMID: 1678457. Exclusion Code: X 1
 10. Folic acid and neural tube defects. *Lancet*. 1991 Jul 20;338(8760):153-4. PMID: 1677069. Exclusion Code: X 1
 11. Folic acid in the prevention of neural tube defects. *Geneesmiddelenbulletin*. 1991;25(12):56. Exclusion Code: X 1
 12. CDC report: Folic acid and pregnancy. *Am Fam Physician*. 1992;46(6):1842. Exclusion Code: X 1
 13. Periodic health examination, 1994 update: 3. Primary and secondary prevention of neural tube defects. Canadian Task Force on the Periodic Health Examination. *CMAJ*. 1994 Jul 15;151(2):159-66. PMID: 7518734. Exclusion Code: X 1
 14. Folic acid fortification. *Nutr Rev*. 1996 Mar;54(3):94-5. PMID: 8935221. Exclusion Code: X 1
 15. Folic acid. *Can Pharm J*. 1997;130(9):28. Exclusion Code: X 1
 16. Low-dosage folic acid reduces the incidence of neural tube defects. *Drugs and Therapy Perspectives*. 1997;10(2):10-1. Exclusion Code: X 1
 17. Folate delivers head-to-toe health advantages. The B vitamin that benefits unborn babies may also protect the hearts and minds (and colons) of adults. *Health News*. 2002 Dec;8(12):1-2. PMID: 12523265. Exclusion Code: X 1
 18. Folic acid supplementation to prevent neural tube defects. *Med Lett Drugs Ther*. 2004 Mar 1;46(1177):17-8. PMID: 15041889. Exclusion Code: X 1
 19. Folate and vitamin B12 deficiencies: proceedings of a WHO technical consultation held 18-21 October, 2005, in Geneva, Switzerland. Introduction. *Food Nutr Bull*. 2008 Jun;29(2 Suppl):S3-4. PMID: 18709877. Exclusion Code: X 1
 20. Cochrane Pregnancy and Childbirth Group. About The Cochrane Collaboration: John Wiley & Sons, Ltd; 2015. Exclusion Code: X 1
 21. Abramsky L, Noble J. Effects of folic acid. *Lancet*. 2002 Jun 8;359(9322):2039-40. PMID: 12076582. Exclusion Code: X 1

Appendix C. Excluded Studies

22. Al-Mendalawi MD. Towards a national program to combat neural tube defects in Iraq. *Congenital Anomalies*. 2014;54(2):123. Exclusion Code: X 1
23. Althaus F. Folic acid supplementation during early pregnancy appears to lessen risk of neural tube defects. *Fam Plann Perspect*. 1990;22(3):140-1. Exclusion Code: X 1
24. Banhidly F, Czeizel AE. Primary prevention of congenital abnormalities with specified origin. *Clin Chem Lab Med*. 2012;50(2):A65-A6. Exclusion Code: X 1
25. Baxter P. Valproate and folic acid in pregnancy: associations with autism. *Dev Med Child Neurol*. 2014 Jul;56(7):604. PMID: 24924417. Exclusion Code: X 1
26. Bitran JD, Miller JB, Golomb HM. Megaloblastic anemia during pregnancy. *J Reprod Med*. 1977 Oct;19(4):186-92. PMID: 915880. Exclusion Code: X 1
27. Bland JM. Taking folate in pregnancy and risk of maternal breast cancer: what's in a name? *BMJ*. 2005 Mar 12;330(7491):600; author reply -1. PMID: 15761004. Exclusion Code: X 1
28. Bower C. Folate and neural tube defects. *Nutr Rev*. 1995 Sep;53(9 Pt 2):S33-8. PMID: 8577416. Exclusion Code: X 1
29. Bower C, Kurinczuk JJ, Stanley FJ. Spina bifida and folate. *Med J Aust*. 1999 Feb 1;170(3):143-4. PMID: 10065135. Exclusion Code: X 1
30. Bower C, Raymond M, Lumley J, et al. Trends in neural tube defects 1980-1989. *Med J Aust*. 1993 Feb 1;158(3):152-4. PMID: 8450777. Exclusion Code: X 1
31. Bower C, Werler MM. Folate before pregnancy: are we doing enough? *Med J Aust*. 2001 Jun 18;174(12):619-20. PMID: 11480680. Exclusion Code: X 1
32. Breach J. Folic acid - Pharmacy or flour? *Australian Journal of Pharmacy*. 2007;88(1045):52-3. Exclusion Code: X 1
33. Britton T. Women need to take folic acid sooner rather than later. *Nurs Times*. 1998 Mar 11-17;94(10):16. PMID: 9735762. Exclusion Code: X 1
34. Buehler JW, Mulinare J. Preventing neural tube defects. *Pediatr Ann*. 1997 Sep;26(9):535-9. PMID: 9302715. Exclusion Code: X 1
35. Burry J. Preventing neural tube defects with folic acid in pregnancy. *Canadian Pharmacists Journal*. 2008;141(2):90-4. Exclusion Code: X 1
36. Buyse M. Preconceptual folic acid: opportunity for preconceptual counseling offers opportunity to help prevent neural tube defects. *Med Health R I*. 2001 Jan;84(1):21. PMID: 11210288. Exclusion Code: X 1
37. Byran JA, 2nd. Use and abuse of hematinics. *Am Fam Physician*. 1973 Jun;7(6):121-8. PMID: 4708269. Exclusion Code: X 1
38. Campbell RK. The unnecessary epidemic of folic acid-preventable spina bifida and anencephaly. *Pediatrics*. 2001 Oct;108(4):1048-50. PMID: 11589212. Exclusion Code: X 1
39. Capel I, Corcoy R. What dose of folic acid should be used for pregnant diabetic women? *Diabetes Care*. 2007 Jul;30(7):e63. PMID: 17596486. Exclusion Code: X 1
40. Chalmers TC, Sacks H. Vitamin supplements to prevent neural tube defects. *Lancet*. 1982 Mar 27;1(8274):748. PMID: 6122046. Exclusion Code: X 1
41. Chan A, Pickering J, Haan E, et al. "Folate before pregnancy": the impact on women and health professionals of a population-based health promotion campaign in South Australia. *Med J Aust*. 2001 Jun 18;174(12):631-6. PMID: 11480683. Exclusion Code: X 1
42. Chidambaram B. Folate in pregnancy. *J Pediatr Neurosci*. 2012;7(2):81. Exclusion Code: X 1
43. Clarke R, Bennett D. Folate and prevention of neural tube defects. *BMJ*. 2014;349:g4810. PMID: 25073785. Exclusion Code: X 1
44. Crandall BF, Corson VL, Goldberg JD, et al. Folic acid and pregnancy. *Am J Med Genet*. 1995 Jan 2;55(1):134-5. PMID: 7702087. Exclusion Code: X 1
45. Cumming FJ, Bradley S. Spina bifida and folate. *Med J Aust*. 1999 Feb 1;170(3):144. PMID: 10065136. Exclusion Code: X 1
46. Czeizel AE. Folic acid fortification and supplementation. *Am J Med Genet*. 1999 Feb 12;82(4):362. PMID: 10051175. Exclusion Code: X 1
47. Czeizel AE. Folic acid and human malformations: misunderstandings. *Reprod Toxicol*. 2001 Jul-Aug;15(4):441-4. PMID: 11515501. Exclusion Code: X 1
48. Davis JA. Multivitamin prophylaxis against neural-tube defects. *Lancet*. 1980 Jun 14;1(8181):1302. PMID: 6104105. Exclusion Code: X 1

Appendix C. Excluded Studies

49. Davis RE. Folate supplementation. *Med J Aust.* 1992 Sep 21;157(6):425. PMID: 1448000. Exclusion Code: X 1
50. Davis RE. Effects of folic acid. *Lancet.* 2002 Jun 8;359(9322):2038-9. PMID: 12076580. Exclusion Code: X 1
51. Drife J, MacNab G. Prescribing in pregnancy. Mineral and vitamin supplements. *Clin Obstet Gynaecol.* 1986 Jun;13(2):253-67. PMID: 3524949. Exclusion Code: X 1
52. Ebrahim GJ. Role of folate in preventive medicine. *J Trop Pediatr.* 2001 Feb;47(1):2-3. PMID: 11245346. Exclusion Code: X 1
53. Elwood M. Folic acid prevents neural tube defects. *Med J Aust.* 1991 Nov 4;155(9):579-81. PMID: 1943950. Exclusion Code: X 1
54. Emery AE. Foliates and fetal central-nervous-system malformations. *Lancet.* 1977 Mar 26;1(8013):703. PMID: 66501. Exclusion Code: X 1
55. Eskes TK, Steegers-Theunissen RP. Primary prevention of neural-tube defects with folic acid. *Eur J Obstet Gynecol Reprod Biol.* 1994 Feb;53(2):147-52. PMID: 8194653. Exclusion Code: X 1
56. Etzel RA. Foreword: Environmental chemical exposures and autism spectrum disorders: A review of the epidemiological evidence. *Curr Probl Pediatr Adolesc Health Care.* 2014;44(10):275-6. Exclusion Code: X 1
57. Facchinetti F, Dante G. How we might do without vitamins. *Pregnancy Hypertens.* 2011;1(3-4):251. Exclusion Code: X 1
58. Fekete K, Berti C, Cetin I, et al. Perinatal folate supply: relevance in health outcome parameters. *Matern Child Nutr.* 2010 Oct;6 Suppl 2:23-38. PMID: 22296249. Exclusion Code: X 1
59. Fleming A. The role of folate in the prevention of neural tube defects: human and animal studies. *Nutr Rev.* 2001 Aug;59(8 Pt 2):S13-20; discussion S1-3. PMID: 11519664. Exclusion Code: X 1
60. Franzen W. Prevention of neural tube defects. *Lancet.* 1994 Jan 8;343(8889):124. PMID: 7903763. Exclusion Code: X 1
61. Freda MC. "Point four" the future. *MCN Am J Matern Child Nurs.* 1999 Sep-Oct;24(5):223. PMID: 10479803. Exclusion Code: X 1
62. Freeman NV, Rajab A. Neural tube defects. *Trop Doct.* 1999 Oct;29(4):235-7. PMID: 10578641. Exclusion Code: X 1
63. Frenkel EP, Yardley DA. Clinical and laboratory features and sequelae of deficiency of folic acid (folate) and vitamin B12 (cobalamin) in pregnancy and gynecology. *Hematol Oncol Clin North Am.* 2000 Oct;14(5):1079-100, viii. PMID: 11005035. Exclusion Code: X 1
64. Frost J. Folic acid: consider the evidence. *MCN Am J Matern Child Nurs.* 1993 Nov-Dec;18(6):302, 4. PMID: 8295515. Exclusion Code: X 1
65. Gaul GE. Fortifying policy with science--the case of folate. *J Nutr.* 1996 Mar;126(3):749s-50s. PMID: 8598559. Exclusion Code: X 1
66. Gillies A, McLeod S, Carter Y. Periconceptual folate supplementation. *Br J Gen Pract.* 1996 Apr;46(405):254. PMID: 8703532. Exclusion Code: X 1
67. Glanville NT, Cook HW. Folic acid and prevention of neural tube defects. *CMAJ.* 1992 Jan 1;146(1):39. PMID: 1728351. Exclusion Code: X 1
68. Goldenberg RL, Tamura T. Prepregnancy weight and pregnancy outcome. *JAMA.* 1996 Apr 10;275(14):1127-8. PMID: 8601934. Exclusion Code: X 1
69. Goldsmith GA. Vitamin B complex. Thiamine, riboflavin, niacin, folic acid (folacin), vitamin B12, biotin. *Prog Food Nutr Sci.* 1975;1(9):559-609. PMID: 772756. Exclusion Code: X 1
70. Goodman M. Vital ingredient. Interview by Claire Laurent. *Nurs Times.* 1992 Jul 29-Aug 4;88(31):21. PMID: 1502070. Exclusion Code: X 1
71. Gordon N. Folate metabolism and neural tube defects. *Brain Dev.* 1995 Sep-Oct;17(5):307-11. PMID: 8579214. Exclusion Code: X 1
72. Greydanus DE, Van Dyke D. The sacred disease and the clinician's sacred duty. *Int Pediatr.* 2005;20(2):69-71. Exclusion Code: X 1
73. Griffin JP. Vitamins and neural tube defects. *Br Med J (Clin Res Ed).* 1988 Feb 6;296(6619):430. PMID: 3125934. Exclusion Code: X 1
74. Gupta H, Gupta P. Neural tube defects and folic acid. *Indian Pediatr.* 2004 Jun;41(6):577-86. PMID: 15235163. Exclusion Code: X 1
75. Guthrie EW. Bariatric surgery. 2007. p. HS-27-HS-37. Exclusion Code: X 1

Appendix C. Excluded Studies

76. Hacoheh Y, Lerman-Sagie T. Should folic acid fortification be mandatory in Israel? *Isr Med Assoc J*. 2006 Sep;8(9):649-50. PMID: 17058420. Exclusion Code: X 1
77. Haddow JE. Folic acid and neural tube defects. *Genet Med*. 2011 Sep;13(9):849; author reply PMID: 21885925. Exclusion Code: X 1
78. Hall JG. Give the embryo a chance. *Nat Med*. 1997 Jan;3(1):24-5. PMID: 8986732. Exclusion Code: X 1
79. Hall JG. Terathanasia, folic acid, and birth defects. *Lancet*. 1997 Nov 1;350(9087):1322; author reply 3-4. PMID: 9357423. Exclusion Code: X 1
80. Hall JG, Solehdin F. Folate and its various ramifications. *Adv Pediatr*. 1998;45:1-35. PMID: 9742296. Exclusion Code: X 1
81. Hall JG, Solehdin F. Genetics of neural tube defects. *Mental Retardation and Developmental Disabilities Research Reviews*. 1998;4(4):269-81. Exclusion Code: X 1
82. Hansen HA. On the diagnosis of folic acid deficiency. *Acta Obstet Gynecol Scand*. 1967;46(7):Suppl 7:13-37. PMID: 6072972. Exclusion Code: X 1
83. Harris R, Read AP. Spina bifida and vitamins. *Br Med J*. 1982;285(6355):1651. Exclusion Code: X 1
84. Harrison KA. Sick cell disease in pregnancy. *Trop Doct*. 1976 Apr;6(2):74-80. PMID: 1265839. Exclusion Code: X 1
85. Harrison KA. Anaemia, malaria and sickle cell disease. *Clin Obstet Gynaecol*. 1982 Dec;9(3):445-77. PMID: 6756746. Exclusion Code: X 1
86. Hauser WA. Folic acid supplementation: too much of a good thing? *J Neurol Neurosurg Psychiatry*. 2009 May;80(5):468. PMID: 19372285. Exclusion Code: X 1
87. Havens DH, Levin BR. Good news for women and babies: folic acid prevents birth defects. *J Pediatr Health Care*. 1999 Sep-Oct;13(5):255-8. PMID: 10776204. Exclusion Code: X 1
88. Hawkins DF. Antimicrobial drugs in pregnancy and adverse effects on the fetus. *J Obstet Gynaecol*. 1986;6(SUPPL. 1):S11-S3. Exclusion Code: X 1
89. Hayden MR. New use for folic acid. *Mo Med*. 2003 Jan-Feb;100(1):14-5. PMID: 12664703. Exclusion Code: X 1
90. Hendricks K. Fecal folate. *Am J Clin Nutr*. 2001 Mar;73(3):658-9. PMID: 11237951. Exclusion Code: X 1
91. Herbert V. The five possible causes of all nutrient deficiency: illustrated by deficiencies of vitamin B 12. *Am J Clin Nutr*. 1973 Jan;26(1):77-86. PMID: 4577371. Exclusion Code: X 1
92. Herbert V. Anemias. *Curr Concepts Nutr*. 1977;5:79-89. PMID: 322954. Exclusion Code: X 1
93. Herbert V. Megaloblastic anemias. *Lab Invest*. 1985 Jan;52(1):3-19. PMID: 3880840. Exclusion Code: X 1
94. Hesecker H. Folic acid and other potential measures in the prevention of neural tube defects. *Ann Nutr Metab*. 2011;59(1):41-5. PMID: 22123637. Exclusion Code: X 1
95. Hibbard BM. Folates and fetal development. *Br J Obstet Gynaecol*. 1993 Apr;100(4):307-9. PMID: 8110212. Exclusion Code: X 1
96. Hibbard ED. Folate and vitamin B12 metabolism in pregnancy. *Midwife Health Visit*. 1972 Aug;8(8):280-2. PMID: 4489693. Exclusion Code: X 1
97. Hine RJ. What practitioners need to know about folic acid. *J Am Diet Assoc*. 1996 May;96(5):451-2. PMID: 8621868. Exclusion Code: X 1
98. Ho Jacqueline J, Chun Wearn KOH, Chang Alvin SM. Folic acid supplementation for the prevention of anaemia in preterm neonates. *Cochrane Database of Systematic Reviews*: John Wiley & Sons, Ltd; 2011. Exclusion Code: X 1
99. Holmes LB. Prevention of neural tube defects. *J Pediatr*. 1992 Jun;120(6):918-9. PMID: 1593351. Exclusion Code: X 1
100. Hook EB. Neural-tube defects. *N Engl J Med*. 2000 Apr 13;342(15):1136; author reply -7. PMID: 10766584. Exclusion Code: X 1
101. Howell S, Underwood M, Carter Y. Folic acid supplements. *Br J Gen Pract*. 1998 Jan;48(426):922-3. PMID: 9604422. Exclusion Code: X 1
102. Howse JL. Stopping neural tube defects. *AWHONN Lifelines*. 1999 Jun-Jul;3(3):10. PMID: 10689997. Exclusion Code: X 1
103. Hróbjartsson A, Gøtzsche Peter C. Placebo interventions for all clinical conditions. *Cochrane Database of Systematic Reviews*: John Wiley & Sons, Ltd; 2010. Exclusion Code: X 1

Appendix C. Excluded Studies

104. Huang HY, Caballero B, Chang S, et al. The efficacy and safety of multivitamin and mineral supplement use to prevent cancer and chronic disease in adults: a systematic review for a National Institutes of Health state-of-the-science conference. *Ann Intern Med.* 2006 Sep 5;145(5):372-85. PMID: 16880453. Exclusion Code: X 1
105. Hueston WJ. Folic acid for the prevention of neural tube defects. *Am Fam Physician.* 1993 Apr;47(5):1058-60. PMID: 8465700. Exclusion Code: X 1
106. Hughes R. In this issue. *Public Health Nutr.* 2009 May;12(5):591. PMID: 19366464. Exclusion Code: X 1
107. Hume K. Fetal defects and multivitamin therapy. *Med J Aust.* 1980 Dec 27;2(13):731-2. PMID: 7207314. Exclusion Code: X 1
108. Hume K. Neural tube defect. *Med J Aust.* 1981 Oct 3;2(7):315. PMID: 7300770. Exclusion Code: X 1
109. Inman K. Flour power. *Nurs Times.* 1998 Mar 11-17;94(10):16-7. PMID: 9735764. Exclusion Code: X 1
110. Iqbal MM. Birth defects: prevention of neural tube defects by periconceptional use of folic acid and screening. *J Prev Soc Med.* 1999 Jun;18(1):52-65. PMID: 12179656. Exclusion Code: X 1
111. Jacobsen DW. Serum and erythrocyte folates: a matter of life and premature death. *Clin Chem.* 1996 Oct;42(10):1579-81. PMID: 8855138. Exclusion Code: X 1
112. Jacobson B. Folic acid and the prevention of neural tube defects. Chapati flour should be fortified as well. *BMJ.* 1995 Jul 22;311(6999):256. PMID: 7627049. Exclusion Code: X 1
113. Jain R, Acharya AS. Supplemental folic acid in pregnancy and childhood asthma. *Natl Med J India.* 2010 Nov-Dec;23(6):351-2. PMID: 21561047. Exclusion Code: X 1
114. Johnston RB, Jr. Folic acid: new dimensions of an old friendship. *Adv Pediatr.* 1997;44:231-61. PMID: 9265972. Exclusion Code: X 1
115. Jukes TH. Folic acid deficiency. *Nature.* 1983 Jan 20;301(5897):192. PMID: 6337342. Exclusion Code: X 1
116. Kadir RA, Economides DL. Neural tube defects and periconceptional folic acid. *CMAJ.* 2002 Aug 6;167(3):255-6. PMID: 12186171. Exclusion Code: X 1
117. Kalter H. Folic acid and NTD-disputed connection. *Teratology.* 2001 Dec;64(6):318-9. PMID: 11754174. Exclusion Code: X 1
118. Kaneko S. Epilepsy, pregnancy and antiepileptic drugs. *Hirosaki Medical Journal.* 1986;38(3):434-56. Exclusion Code: X 1
119. Kee M. Spina bifida trial. Research council goes public. *Nature.* 1983 Jan 20;301(5897):190. PMID: 6337341. Exclusion Code: X 1
120. Kennedy D, Pastuszak A, Koren G. Taking folic acid during pregnancy. Don't leave it too late. *Can Fam Physician.* 1997 Dec;43:2113-4. PMID: 9426926. Exclusion Code: X 1
121. Kennedy DS. Spina bifida. *Med J Aust.* 1998 Aug 17;169(4):182-3. PMID: 9734573. Exclusion Code: X 1
122. Kirsch SH, Herrmann W, Obeid R. Genetic defects in folate and cobalamin pathways affecting the brain. *Clin Chem Lab Med.* 2013 Jan;51(1):139-55. PMID: 23183749. Exclusion Code: X 1
123. Klein NW. Caution advised before rushing to start folic acid supplements. *Teratology.* 1996 Jun;53(6):331. PMID: 8910976. Exclusion Code: X 1
124. Knight M. Sharper focus on uncomplicated pregnancy. *BMJ (Online).* 2013;347. Exclusion Code: X 1
125. Kokalj TS, Rejc B, Gersak K. Incidence and prevention of neural tube defects in Slovenia. *Eur J Obstet Gynecol Reprod Biol.* 2011 May;156(1):119-20. PMID: 21300428. Exclusion Code: X 1
126. Koren G, Cohn T, Chitayat D, et al. Use of atypical antipsychotics during pregnancy and the risk of neural tube defects in infants. *Am J Psychiatry.* 2002 Jan;159(1):136-7. PMID: 11772703. Exclusion Code: X 1
127. Koren G, Goh I. Increasing folate supplementation for selected groups of Canadian women. *J Obstet Gynaecol Can.* 2007 Dec;29(12):992-6. PMID: 18053385. Exclusion Code: X 1
128. Krone CA, Ely JTA, Harms LC. Nutritional supplements: Friend or foe? *N Z Med J.* 2004;117(1196). Exclusion Code: X 1
129. Kumana CR, Yu YL, Richens A. Antiepileptic treatment during pregnancy. *Journal of the Hong Kong Medical Association.* 1985;37(2):98-9. Exclusion Code: X 1

Appendix C. Excluded Studies

130. Kurzawinska G, Seremak-Mrozikiewicz A, Drews K, et al. [Genetic conditioned changes in activity of 5,10-methylenetetrahydrofolate reductase (MTHFR) and recurrent miscarriages]. *Ginekol Pol.* 2009 Oct;80(10):762-7. PMID: 19943541. Exclusion Code: X 1
131. Laurence KM. Neural tube defects: a two-pronged approach to primary prevention. *Pediatrics.* 1982 Oct;70(4):648-50. PMID: 7122167. Exclusion Code: X 1
132. Laurence KM, Campbell H. Trial of folate treatment to prevent recurrence of neural tube defect. *Br Med J (Clin Res Ed).* 1981 Jun 27;282(6282):2131. PMID: 6788230. Exclusion Code: X 1
133. Leung DT, Ernest JM. Pre-conceptional folic acid supplementation in uninsured pregnant Hispanic women. *South Med J.* 2007 Jan;100(1):85-6. PMID: 17269538. Exclusion Code: X 1
134. Levy T, Blickstein I. Does the use of folic acid increase the risk of twinning? *Int J Fertil Womens Med.* 2006 May-Jun;51(3):130-5. PMID: 17039857. Exclusion Code: X 1
135. Lewis DP, Van Dyke DC, Stumbo PJ, et al. Drug and environmental factors associated with adverse pregnancy outcomes. Part III: Folic acid: pharmacology, therapeutic recommendations, and economics. *Ann Pharmacother.* 1998 Oct;32(10):1087-95. PMID: 9793602. Exclusion Code: X 1
136. Lewis DP, Van Dyke DC, Stumbo PJ, et al. Drug and environmental factors associated with adverse pregnancy outcomes. Part II: Improvement with folic acid. *Ann Pharmacother.* 1998 Sep;32(9):947-61. PMID: 9762383. Exclusion Code: X 1
137. Lewis DP, Van Dyke DC, Stumbo PJ, et al. Drug and environmental factors associated with adverse pregnancy outcomes. Part I: Antiepileptic drugs, contraceptives, smoking, and folate. *Ann Pharmacother.* 1998 Jul-Aug;32(7-8):802-17. PMID: 9681097. Exclusion Code: X 1
138. Leyva M. Folic acid intake and its effects on the prevention of neural tube defects, the masking of vitamin B12 deficiency and the reduction of homocysteine. *J Okla State Med Assoc.* 2002 May;95(5):339-42. PMID: 12043109. Exclusion Code: X 1
139. Lin KW, Birmingham E. Folic Acid for the prevention of neural tube defects. *Am Fam Physician.* 2010 Dec 15;82(12):1533. PMID: 21166375. Exclusion Code: X 1
140. Louis-Ferdinand RT. Myelotoxic, neurotoxic and reproductive adverse effects of nitrous oxide. *Adverse Drug React Toxicol Rev.* 1994 Winter;13(4):193-206. PMID: 7734639. Exclusion Code: X 1
141. Mackerras D, Cunningham J, Hunt A, et al. Re: "effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study". *Am J Epidemiol.* 2010 Mar 15;171(6):746-7; author reply 7. PMID: 20145008. Exclusion Code: X 1
142. Mahomed K. Iron supplementation in pregnancy. *Cochrane Database of Systematic Reviews:* John Wiley & Sons, Ltd; 2006. Exclusion Code: X 1
143. Mahomed K. Iron and folate supplementation in pregnancy. *Cochrane Database of Systematic Reviews:* John Wiley & Sons, Ltd; 2006. Exclusion Code: X 1
144. Makatsariya N, Bitsadze V, Panfilova O, et al. Maternal and paternal hyperhomocysteinemia and prophylaxis of recurrent antenatal fetal death. *J Perinat Med.* 2013;41. Exclusion Code: X 1
145. Mamtani R, Watkins SJ. Trial of folate treatment to prevent recurrence of neural tube defects. *Br Med J (Clin Res Ed).* 1981 Jun 20;282(6281):2056-7. PMID: 6788196. Exclusion Code: X 1
146. Markova V, Nørgaard A, Jørgensen Karsten J, et al. Treatment for women with postpartum iron deficiency anaemia. *Cochrane Database of Systematic Reviews:* John Wiley & Sons, Ltd; 2013. Exclusion Code: X 1
147. Meher S, Duley L. Interventions for preventing pre-eclampsia and its consequences: generic protocol. *Cochrane Database of Systematic Reviews:* John Wiley & Sons, Ltd; 2005. Exclusion Code: X 1
148. Melse-Boonstra A, Kruger S. Potential health risks of folic acid supplementation of 5 mg per day during pregnancy. *South Afr J Clin Nutr.* 2006;19(2):88. Exclusion Code: X 1
149. Metz J. Folate, B12 and neural tube defects. *Med J Aust.* 1995 Sep 4;163(5):231-2. PMID: 7565205. Exclusion Code: X 1
150. Metz J. Haematological implications of folate food fortification. *S Afr Med J.* 2013 Dec;103(12 Suppl 1):978-81. PMID: 24300642. Exclusion Code: X 1

Appendix C. Excluded Studies

151. Michie CA, Narang I, Rogers J, et al. Folate supplementation and neural-tube defects. *Lancet*. 2000 Jan 8;355(9198):147. PMID: 10675195. Exclusion Code: X 1
152. Mills JL, Signore CC. Folic acid and the prevention of neural-tube defects. *N Engl J Med*. 2004 May 20;350(21):2209-11; author reply -11. PMID: 15152070. Exclusion Code: X 1
153. Mills JL, Signore CC. Folic acid and the prevention of neural-tube defects. *N Engl J Med*. 2004 May 20;350(21):2209-11; author reply -11. PMID: 15152070. Exclusion Code: X 1
154. Milunsky A. Folic acid and neural tube defect avoidance. *Prenat Diagn*. 1992 Oct;12(10):856-9. PMID: 1475260. Exclusion Code: X 1
155. Molloy AM. Folate and homocysteine interrelationships including genetics of the relevant enzymes. *Curr Opin Lipidol*. 2004 Feb;15(1):49-57. PMID: 15166809. Exclusion Code: X 1
156. Morrison G. Neural tube defects. *Int Pediatr*. 2001;16(1):3-4. Exclusion Code: X 1
157. Mulinare J. Epidemiologic associations of multivitamin supplementation and occurrence of neural tube defects. *Ann N Y Acad Sci*. 1993 Mar 15;678:130-6. PMID: 8494255. Exclusion Code: X 1
158. Mulinare J, Erickson JD. Prevention of neural tube defects. *Teratology*. 1997 Jul-Aug;56(1-2):17-8. PMID: 9329164. Exclusion Code: X 1
159. Murphy PA. Beneficial effects of folic acid supplementation in the prevention of neural tube defects (NTDs). *J Nurse Midwifery*. 1993 Jan-Feb;38(1):54. PMID: 8380852. Exclusion Code: X 1
160. Murthy EK, Pavlic-Renar I, Metelko Z. Diabetes and pregnancy. *Diabetol Croat*. 2002;31(3):131-46. Exclusion Code: X 1
161. Oakeshott P, Hunt G. Prevention of neural tube defects. *Lancet*. 1994 Jan 8;343(8889):123. PMID: 7903761. Exclusion Code: X 1
162. Oakley GP, Jr. Prevention of neural-tube defects. *N Engl J Med*. 1999 Nov 11;341(20):1546. PMID: 10577108. Exclusion Code: X 1
163. Pearson K. Folic acid to prevent recurrence of neural tube defects. *J Okla State Med Assoc*. 1999 Oct;92(10):513. PMID: 10528476. Exclusion Code: X 1
164. Pendleton HJ. Acute folic acid deficiency of pregnancy associated with oral ulceration and anencephaly. *Proc R Soc Med*. 1969 Aug;62(8):834. PMID: 5810307. Exclusion Code: X 1
165. Pennell PB. Neurology illnesses and pregnancy. *Neurol Clin*. 2004;22(4 SPEC. ISS.):ix-x. Exclusion Code: X 1
166. Quinlivan EP, Gregory JF, 3rd. Folic acid and the prevention of neural-tube defects. *N Engl J Med*. 2004 May 20;350(21):2209-11; author reply -11. PMID: 15156593. Exclusion Code: X 1
167. Rader JI, Schneeman BO. Prevalence of neural tube defects, folate status, and folate fortification of enriched cereal-grain products in the United States. *Pediatrics*. 2006 Apr;117(4):1394-9. PMID: 16585338. Exclusion Code: X 1
168. Rhoads GG, Mills JL. The role of the case-control study in evaluating health interventions. Vitamin supplementation and neural tube defects. *Am J Epidemiol*. 1984 Dec;120(6):803-8. PMID: 6391155. Exclusion Code: X 1
169. Rose NC, Mennuti MT. Periconceptional folate supplementation and neural tube defects. *Clin Obstet Gynecol*. 1994 Sep;37(3):605-20. PMID: 7955648. Exclusion Code: X 1
170. Rush D, Rosenberg IH. Folate supplements and neural tube defects. *Nutr Rev*. 1992 Jan;50(1):25-6. PMID: 1315943. Exclusion Code: X 1
171. Ryan M. Folic acid to prevent neural tube defects: A potential pharmacy initiative with public health implications. *Hosp Pharm*. 2004;39(10):962-9. Exclusion Code: X 1
172. Salvi VS. Folic acid and birth defects. *J Postgrad Med*. 2003 Jul-Sep;49(3):195-6. PMID: 14597778. Exclusion Code: X 1
173. Schiller-Fruhvirth I, Mittermayr T, Wild C. [Neural tube defects in Austria: Assumption and calculations on the prevention potential through folic acid enrichment and supplementation]. *Gesundheitswesen*. 2010 Dec;72(12):880-5. PMID: 20166011. Exclusion Code: X 1
174. Schorah C. Dick Smithells, folic acid, and the prevention of neural tube defects. *Birth Defects Res A Clin Mol Teratol*. 2009 Apr;85(4):254-9. PMID: 19067403. Exclusion Code: X 1

Appendix C. Excluded Studies

175. Schwantzer M, Colvin J, Robertson A. Folic acid supplementation in the periconceptional period [2]. *Australian Journal of Hospital Pharmacy*. 1994;24(5):439-40. Exclusion Code: X 1
176. Scioscia AL. Maternal-fetal medicine. *Curr Opin Obstet Gynecol*. 1995;7(2):83-4. Exclusion Code: X 1
177. Scott J, Weir DG, Kirke PN. Prevention of neural tube defects with folic acid a success but. *QJM*. 1994 Dec;87(12):705-7. PMID: 7859046. Exclusion Code: X 1
178. Sharland E, Montgomery B, Granell R. Folic acid in pregnancy - is there a link with childhood asthma or wheeze? *Aust Fam Physician*. 2011 Jun;40(6):421-4. PMID: 21655492. Exclusion Code: X 1
179. Sharma JB, Newman MR, Smith RJ. Folic acid, pernicious anaemia, and prevention of neural tube defects. *Lancet*. 1994 Apr 9;343(8902):923. PMID: 7908389. Exclusion Code: X 1
180. Shaw MT, Hoffbrand AV. The use and abuse of folic acid. *Practitioner*. 1970 Jun;204(224):795-804. PMID: 5452206. Exclusion Code: X 1
181. Sheikh A. Folic acid supplements and NTDs. *Br J Gen Pract*. 1997 Dec;47(425):836. PMID: 9463994. Exclusion Code: X 1
182. Simister K. Primary prevention of neural tube defects with folic acid. *BMJ*. 1993 Apr 24;306(6885):1123. PMID: 8495164. Exclusion Code: X 1
183. Simpson JL, Mills JL, Rhoads GG, et al. Vitamins, folic acid and neural tube defects: comments on investigations in the United States. *Prenat Diagn*. 1991 Aug;11(8):641-8. PMID: 1766937. Exclusion Code: X 1
184. Smith RB, Davies N, Davies J. Prevention of neural tube defects. *Lancet*. 1994 Jan 8;343(8889):123-4. PMID: 7903762. Exclusion Code: X 1
185. Smithells RW, Sheppard S, Schorah CJ. Folates and the fetus. *Lancet*. 1977 Apr 30;1(8018):955. PMID: 67414. Exclusion Code: X 1
186. Speidel BD. Folic acid deficiency and congenital malformation. *Dev Med Child Neurol*. 1973 Feb;15(1):81-3. PMID: 4729417. Exclusion Code: X 1
187. Stewart CP, Christian P, West KP, et al. Low maternal B12 status is associated with offspring insulin resistance but B12 or folate supplementation does not alter that risk. *FASEB J*. 2011;25. Exclusion Code: X 1
188. Stirrat G. Taking folate in pregnancy and risk of maternal breast cancer: authors and publishers must not disclaim ethical responsibility. *BMJ*. 2005 Mar 12;330(7491):600; author reply -1. PMID: 15761005. Exclusion Code: X 1
189. Super M, Summers EM, Meylan B. Preventing neural tube defects. *Lancet*. 1991 Sep 21;338(8769):755-6. PMID: 1679883. Exclusion Code: X 1
190. Toriello HV. Folic acid and neural tube defects. *Genet Med*. 2005 Apr;7(4):283-4. PMID: 15834247. Exclusion Code: X 1
191. Van Allen MI. "Folate up" for healthy babies. *CMAJ*. 1994 Jul 15;151(2):151-4. PMID: 8039061. Exclusion Code: X 1
192. Wald N. Folic acid and the prevention of neural tube defects. *Ann N Y Acad Sci*. 1993 Mar 15;678:112-29. PMID: 8494254. Exclusion Code: X 1
193. Wald NJ. Folic acid and the prevention of neural-tube defects. *N Engl J Med*. 2004 Jan 8;350(2):101-3. PMID: 14711907. Exclusion Code: X 1
194. Wald NJ, Bower C. Folic acid, pernicious anaemia, and prevention of neural tube defects. *Lancet*. 1994 Feb 5;343(8893):307. PMID: 7905138. Exclusion Code: X 1
195. Wald NJ, Gilbertson MP. Folic acid in prevention of neural tube defects. *Lancet*. 1995 Feb 11;345(8946):389. PMID: 7845138. Exclusion Code: X 1
196. Wald NJ, Hackshaw AK. Folic acid supplementation and neural tube defects. *Am J Med Genet*. 2002 Apr 15;109(1):79. PMID: 11933000. Exclusion Code: X 1
197. Wald NJ, Law MR, Morris JK, et al. Quantifying the effect of folic acid. *Lancet*. 2001 Dec 15;358(9298):2069-73. PMID: 11755633. Exclusion Code: X 1
198. Wani MA. Neural tube defect and folic acid. *JK Pract*. 2000 Jan-Mar;7(1):1-3. PMID: 12295955. Exclusion Code: X 1
199. Watermeyer G. Pregnancy and inflammatory bowel disease. *South African Gastroenterology Review*. 2007;5(1):4-6. Exclusion Code: X 1
200. Welch JP. Folic acid supplementation to prevent neural tube defects. *CMAJ*. 1994 Jul 1;151(1):15-6. PMID: 8004554. Exclusion Code: X 1
201. Werler MM, Mitchell AA. More on folic acid and neural tube defects. *Am J Public Health*. 1995 Feb;85(2):269. PMID: 7856794. Exclusion Code: X 1

Appendix C. Excluded Studies

202. Wild J, Lucock MD, Schorah CJ. Periconceptional folate and neural tube defects. *Am J Clin Nutr*. 1995 Mar;61(3):615-6. PMID: 7872229. Exclusion Code: X 1
203. Wilson RD, Audibert F, Brock JA, et al. Pre-conception Folic Acid and Multivitamin Supplementation for the Primary and Secondary Prevention of Neural Tube Defects and Other Folic Acid-Sensitive Congenital Anomalies. *Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstétrique et gynécologie du Canada : JOGC*. 2015;37(6):534-52. Exclusion Code: X 1
204. Wilson RD, Johnson JA, Wyatt P, et al. Pre-conceptional vitamin/folic acid supplementation 2007: the use of folic acid in combination with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies. *J Obstet Gynaecol Can*. 2007 Dec;29(12):1003-26. PMID: 18053387. Exclusion Code: X 1
205. Yerby MS. Erratum: Clinical Care of Pregnant Women with Epilepsy: Neural Tube Defects and Folic Acid Supplementation (*Epilepsia* (2003) 44: Suppl 3 (33-40)). *Epilepsia*. 2003;44(11):1465. Exclusion Code: X 1
206. Yoder DK. Preventing neural tube defects with folic acid. *Tenn Med*. 1999 May;92(5):177-8. PMID: 10230320. Exclusion Code: X 1
207. Folate supplements prevent recurrence of neural tube defects. *Nutr Rev*. 1992 Jan;50(1):22-4. PMID: 1579267. Exclusion Code: X 1
208. Neural tube defects and folic acid supplements. *J Okla State Med Assoc*. 1994 Dec;87(12):562-3. PMID: 7837011. Exclusion Code: X 1
209. Molloy AM, Kirke PN, Brody LC, et al. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. *Food Nutr Bull*. 2008 Jun;29(2 Suppl):S101-11; discussion S12-5. PMID: 18709885. Exclusion Code: X 1
210. Willett WC. Folic acid and neural tube defect: can't we come to closure? *Am J Public Health*. 1992 May;82(5):666-8. PMID: 1566943. Exclusion Code: X 1
211. Periconceptional use of multivitamins and the occurrence of anencephaly and spina bifida. *MMWR Morb Mortal Wkly Rep*. 1988 Dec 2;37(47):727-30. PMID: 3141772. Exclusion Code: X 1
212. Iron/folate for iron deficiency anaemia in pregnancy. *Australian Journal of Pharmacy*. 2008;89(1056):76. Exclusion Code: X 1
213. Berry RJ, Kihlberg R. Folic acid supplementation is not associated with an increase in dizygotic twinning. *Early Hum Dev*. 2005 May;81(5):465-7; author reply 9-70. PMID: 15935924. Exclusion Code: X 1
214. Bower C. Epilepsy in pregnancy: neural tube defects and folate. *Med J Aust*. 1994 Jan 17;160(2):56-7. PMID: 8309368. Exclusion Code: X 1
215. Butterworth CE, Jr. Interactions of nutrients with oral contraceptives and other drugs. *J Am Diet Assoc*. 1973 May;62(5):510-4. PMID: 4540565. Exclusion Code: X 1
216. Buttriss J. Strategies to increase folate/folic acid intake in women: An overview. *Nutr Bull*. 2004;29(3):234-44. Exclusion Code: X 1
217. Chanarin I. Folate and cobalamin. *Clin Haematol*. 1985 Oct;14(3):629-41. PMID: 3907912. Exclusion Code: X 1
218. Cunningham GC. California's public health policy on preventing neural tube defects by folate supplementation. *West J Med*. 1995 Mar;162(3):265-7. PMID: 7725720. Exclusion Code: X 1
219. Czeizel AE. Terathanasia, folic acid, and birth defects. *Lancet*. 1998 Feb 7;351(9100):450. PMID: 9482337. Exclusion Code: X 1
220. Czeizel AE. Folic acid and the prevention of neural-tube defects. *N Engl J Med*. 2004 May 20;350(21):2209-11; author reply -11. PMID: 15156594. Exclusion Code: X 1
221. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull*. 2008 Jun;29(2 Suppl):S238-44. PMID: 18709899. Exclusion Code: X 1
222. Doyle W. Folic acid in prevention of neural tube defects. *Lancet*. 1995 Feb 11;345(8946):389-90. PMID: 7845140. Exclusion Code: X 1
223. Einarson A, Parshuram C, Koren G. Periconceptional use of folic acid to reduce the rates of neural tube defects: is it working? *Reprod Toxicol*. 2000 Jul-Aug;14(4):291-2. PMID: 10908831. Exclusion Code: X 1

Appendix C. Excluded Studies

224. Evans JA. Pre-conceptional vitamin/folic acid supplementation 2007. *J Obstet Gynaecol Can.* 2008 Aug;30(8):656-7; author reply 8. PMID: 18786285. Exclusion Code: X 1
225. Friel JK, Frecker M, Fraser FC. Nutritional patterns of mothers of children with neural tube defects in Newfoundland. *Am J Med Genet.* 1995 Jan 16;55(2):195-9. PMID: 7717417. Exclusion Code: X 1
226. Halsted CH. Periconceptional use of multivitamins and the prevalence of neural-tube defects (I). *N Engl J Med.* 1990;322(15):1082. Exclusion Code: X 1
227. Hawkins DF. Teratogenesis and other adverse effects on the fetus of antimicrobial drugs used in pregnancy. *Clin Exp Obstet Gynecol.* 1986;13(3-4):113-24. PMID: 3536180. Exclusion Code: X 1
228. Holzgreve W, Pietrzik K, Koletzko B, et al. Adding folate to the contraceptive pill: a new concept for the prevention of neural tube defects. *J Matern Fetal Neonatal Med.* 2012 Sep;25(9):1529-36. PMID: 22272814. Exclusion Code: X 1
229. Koren G. Folic acid and colorectal cancer: unwarranted fears. *Can Fam Physician.* 2011 Aug;57(8):889-90. PMID: 21841107. Exclusion Code: X 1
230. Mason JB. Folate and colon cancer: a fascinating puzzle we have yet to complete. *Clin Nutr.* 1998 Apr;17(2):41-3. PMID: 10205315. Exclusion Code: X 1
231. Mathews F, Murphy M, Wald NJ, et al. Twinning and folic acid use. *Lancet.* 1999 Jan 23;353(9149):291-2. PMID: 9929025. Exclusion Code: X 1
232. Milunsky A, Jick H, Jick SS, et al. Periconceptional use of multivitamins and the prevalence of neural-tube defects (II). *N Engl J Med.* 1990;322(15):1082-3. Exclusion Code: X 1
233. Mulinare J, Cordero JF, Erickson JD, et al. Periconceptional use of multivitamins and the occurrence of neural tube defects. *Obstet Gynecol Surv.* 1989;44(5):362-3. Exclusion Code: X 1
234. Mulinare J, Cordero JF, Erickson JD, et al. Periconceptional use of multivitamins and the prevalence of neural-tube defects (III). *N Engl J Med.* 1990;322(15):1083. Exclusion Code: X 1
235. Obeid R, Koletzko B, Pietrzik K. Critical evaluation of lowering the recommended dietary intake of folate. *Clin Nutr.* 2014 Apr;33(2):252-9. PMID: 24503418. Exclusion Code: X 1
236. O'Connor G, Doherty CP. Folic acid: When to supplement and by how much. *European Neurological Journal.* 2013;4(2). Exclusion Code: X 1
237. Olsen J. Some options in studying side effects of drugs taken during pregnancy. *Eur J Epidemiol.* 2015;30(11):1137-9. Exclusion Code: X 1
238. Ownby DR. Has mandatory folic acid supplementation of foods increased the risk of asthma and allergic disease? *J Allergy Clin Immunol.* 2009 Jun;123(6):1260-1. PMID: 19447481. Exclusion Code: X 1
239. Reynolds EH. Folic acid and the prevention of neural tube defects. Folate has potential to cause harm. *BMJ.* 1995 Jul 22;311(6999):257. PMID: 7503870. Exclusion Code: X 1
240. Scott JM. How does folic acid prevent neural tube defects? *Nat Med.* 1998 Aug;4(8):895-6. PMID: 9701237. Exclusion Code: X 1
241. Strohle A, Bohn T. Folate and Prevention of Neural Tube Defects: New Insights from a Bayesian Model. *Int J Vitam Nutr Res.* 2015 Dec;85(3-4):109-11. PMID: 26780389. Exclusion Code: X 1
242. Sutcliffe M, Schorah CJ, Perry A, et al. Prevention of neural tube defects. *Lancet.* 1993 Nov 6;342(8880):1174. PMID: 7901496. Exclusion Code: X 1
243. Tuerxunjiang M, Wang YP. Folic acid, zinc, iodine, selenium and maternal health. *Journal of Clinical Rehabilitative Tissue Engineering Research.* 2007;11(39):8015-7+20. Exclusion Code: X 1
244. Wald NJ. Folic acid and neural tube defects. *Bibl Nutr Dieta.* 2001(55):22-33. PMID: 11125581. Exclusion Code: X 1
245. Wen SW, Walker M. An exploration of health effects of folic acid in pregnancy beyond reducing neural tube defects. *J Obstet Gynaecol Can.* 2005 Jan;27(1):13-9. PMID: 15937577. Exclusion Code: X 1
246. Wilson RD, Davies G, Desilets V, et al. The use of folic acid for the prevention of neural tube defects and other congenital anomalies. *J Obstet Gynaecol Can.* 2003 Nov;25(11):959-73. PMID: 14608448. Exclusion Code: X 1

Appendix C. Excluded Studies

247. Yu J. Gene-environment interactions should be considered in future studies to understand the association between prenatal folate supplementation and asthma development. *Allergy, Asthma and Immunology Research*. 2015;7(6):523-4. Exclusion Code: X 1
248. Japanese Teratology Society 50th Annual Meeting. *Congenital Anomalies*. 2010;50(4). Exclusion Code: X 1
249. Phull E, Hirst SL. Folic acid in pregnancy. *Br J Gen Pract*. 1995 Dec;45(401):688. PMID: 8745870. Exclusion Code: X 1
250. Daly L, Doyle S. Peri-conceptual folic acid. *Ir Med J*. 2002 Oct;95(9):280. PMID: 12470004. Exclusion Code: X 1
251. Fraser FC. Folic acid and neural tube defects. *CMAJ*. 1995 May 1;152(9):1380-1. PMID: 7728683. Exclusion Code: X 1
252. Laurence KM. Towards the prevention of neural tube defects: discussion paper. *J R Soc Med*. 1982 Sep;75(9):723-8. PMID: 7120258. Exclusion Code: X 1
253. Smithells RW. Neural tube defects: prevention by vitamin supplements. *Pediatrics*. 1982 Apr;69(4):498-9. PMID: 7070901. Exclusion Code: X 1
254. Smithells RW. Multivitamins for the prevention of neural tube defects. How convincing is the evidence? *Drugs*. 1989 Dec;38(6):849-54. PMID: 2693043. Exclusion Code: X 1
255. Weggemans RM, Schaafsma G, Kromhout D. Toward an optimal use of folic acid: an advisory report of the Health Council of the Netherlands. *Eur J Clin Nutr*. 2009 Aug;63(8):1034-6. PMID: 19209186. Exclusion Code: X 1
256. Metson D, Kassianos GS, Kremer MG, et al. Supplementation with folic acid. *BMJ*. 1995 Apr 8;310(6984):942. PMID: 7719213. Exclusion Code: X 1
257. Suarez L, Felkner M, Brender JD, et al. Neural tube defects on the Texas-Mexico border: what we've learned in the 20 years since the Brownsville cluster. *Birth Defects Res A Clin Mol Teratol*. 2012 Nov;94(11):882-92. PMID: 22945287. Exclusion Code: X 1
258. Dean H, Watson K, Sellers EA. Neural tube defects. *CMAJ*. 2005 Jan 18;172(2):158-9; author reply 60. PMID: 15655222. Exclusion Code: X 1
259. Livingstone A. Primary prevention of neural tube defects with folic acid. *BMJ*. 1993 Feb 27;306(6877):584. PMID: 8329023. Exclusion Code: X 1
260. Schorah CJ, Smithells RW. Primary prevention of neural tube defects with folic acid. *BMJ*. 1993 Apr 24;306(6885):1123-4. PMID: 8495165. Exclusion Code: X 1
261. Wang FL, Quan H, Schopflocher D. Neural tube defects. *CMAJ*. 2005 Jan 18;172(2):158; author reply 60. PMID: 15655224. Exclusion Code: X 1
262. Girling JC, Shennan AH. Epilepsy and pregnancy. Emphasise the importance of extra folate. *BMJ*. 1993 Oct 9;307(6909):937. PMID: 8241868. Exclusion Code: X 1
263. Harris AA, Barger MK. Specialized care for women pregnant after bariatric surgery. *J Midwifery Womens Health*. 2010 Nov-Dec;55(6):529-39. PMID: 20974415. Exclusion Code: X 1
264. Hauer P. Neural tube defects in relation to use of folic Acid antagonists during pregnancy. *Pediatr Phys Ther*. 2001 Winter;13(4):215. PMID: 17053639. Exclusion Code: X 1
265. Kaplan JS, Iqbal S, England BG, et al. Is pregnancy in diabetic women associated with folate deficiency? *Diabetes Care*. 1999 Jul;22(7):1017-21. PMID: 10388960. Exclusion Code: X 1
266. Kim H, Lee YA, Hwang JY, et al. Relationship between body-mass index and serum folate concentrations in pregnant women: Mothers and Children's Environmental Health (MOCEH). *FASEB J*. 2011;25. Exclusion Code: X 1
267. Jenkins KJ, Correa A, Feinstein JA, et al. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. *Circulation*. 2007 Jun 12;115(23):2995-3014. PMID: 17519397. Exclusion Code: X 1
268. Shaw GM, Todoroff K, Lammer EJ. NTD phenotypes in infants and fetuses whose mothers used multivitamins containing folic acid in early pregnancy compared to those who did not. *Am J Med Genet*. 1997 Jun 13;70(3):330-1. PMID: 9188676. Exclusion Code: X 1
269. Silver RM. Folic acid: Building bigger babies. *BJOG*. 2014;122(4):490. Exclusion Code: X 1

Appendix C. Excluded Studies

270. Czeizel AE. Periconceptional folic acid and multivitamin supplementation for the prevention of neural tube defects and other congenital abnormalities. *Birth Defects Res A Clin Mol Teratol.* 2009 Apr;85(4):260-8. PMID: 19161162. Exclusion Code: X 1
271. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet.* 1991 Jul 20;338(8760):131-7. PMID: 1677062. Exclusion Code: X 2
272. Akinshina S, Makatsaria A, Bitsadze V, et al. Management principles in pregnancy, delivery and postnatal period in patients with thromboembolism in anamnesis. *Hamostaseologie.* 2013;33(1):A20. Exclusion Code: X 2
273. Brent RL, Oakley GP, Jr., Mattison DR. The unnecessary epidemic of folic acid-preventable spina bifida and anencephaly. *Pediatrics.* 2000 Oct;106(4):825-7. PMID: 11015526. Exclusion Code: X 2
274. Collins JS, Atkinson KK, Dean JH, et al. Long term maintenance of neural tube defects prevention in a high prevalence state. *J Pediatr.* 2011 Jul;159(1):143-9.e2. PMID: 21345450. Exclusion Code: X 2
275. De-Regil LM, Pena-Rosas JP, Fernandez-Gaxiola AC, et al. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database Syst Rev.* 2015;12:Cd007950. PMID: 26662928. Exclusion Code: X 2
276. Harden CL, Meador KJ, Pennell PB, et al. Practice parameter update: management issues for women with epilepsy - focus on pregnancy (an evidence-based review): teratogenesis and perinatal outcomes. Report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society (Structured abstract). *Neurology;* 2009. p. 133-41. Exclusion Code: X 2
277. Heseke HB, Mason JB, Selhub J, et al. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. *Br J Nutr.* 2009 Jul;102(2):173-80. PMID: 19079944. Exclusion Code: X 2
278. Holmes-Siedle M, Dennis J, Lindenbaum RH, et al. Long term effects of periconceptional multivitamin supplements for prevention of neural tube defects: a seven to 10 year follow up. *Arch Dis Child.* 1992 Dec;67(12):1436-41. PMID: 1489221. Exclusion Code: X 2
279. Jeha LE, Morris HH. Optimizing outcomes in pregnant women with epilepsy. *Cleve Clin J Med.* 2005 Oct;72(10):938-40, 42-5. PMID: 16231691. Exclusion Code: X 2
280. Kent A. Research snippets. *BJOG.* 2013;120(12):1445-6. Exclusion Code: X 2
281. Khoury MJ, Shaw GM, Moore CA, et al. Does periconceptional multivitamin use reduce the risk of neural tube defects associated with other birth defects? data from two population-based case-control studies. *Am J Med Genet.* 1996 Jan 2;61(1):30-6. PMID: 8741914. Exclusion Code: X 2
282. Kirke PN, Daly LE, Elwood JH. A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child.* 1992 Dec;67(12):1442-6. PMID: 1489222. Exclusion Code: X 2
283. Laurence KM, James N, Miller MH, et al. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. *Br Med J (Clin Res Ed).* 1981 May 9;282(6275):1509-11. PMID: 6786536. Exclusion Code: X 2
284. Lumley J, Watson L, Watson M, et al. Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane Database Syst Rev.* 2001(3):Cd001056. PMID: 11686974. Exclusion Code: X 2
285. Marik PE, Flemmer M. Do dietary supplements have beneficial health effects in industrialized nations: what is the evidence? (Structured abstract). *Journal of Parenteral and Enteral Nutrition;* 2012. p. 159-68. Exclusion Code: X 2
286. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet.* 1991 Jul 20;338(8760):131-7. PMID: 1677062. Exclusion Code: X 2
287. Muggli EE, Halliday JL. Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006. *Med J Aust.* 2007 Mar 5;186(5):243-8. PMID: 17391087. Exclusion Code: X 2
288. Noventa M, Vitagliano A, Quaranta M, et al. Preventive and Therapeutic Role of Dietary Inositol Supplementation in Periconceptional Period and During Pregnancy: A Summary of Evidences and Future Applications. *Reprod Sci.* 2015 Jul 14 PMID: 26175275. Exclusion Code: X 2

Appendix C. Excluded Studies

289. Schorah CJ, Habibzadeh N, Wild J, et al. Possible abnormalities of folate and vitamin B12 metabolism associated with neural tube defects. *Ann N Y Acad Sci.* 1993 Mar 15;678:81-91. PMID: 8494295. Exclusion Code: X 2
290. Zetstra-van der Woude PA, De Walle HE, Hoek A, et al. Maternal high-dose folic acid during pregnancy and asthma medication in the offspring. *Pharmacoepidemiol Drug Saf.* 2014 Jun 14;PMID: 24930442. Exclusion Code: X 2
291. Smithells RW, Sheppard S, Schorah CJ, et al. Possible prevention of neural-tube defects by periconceptional vitamin supplementation. *Lancet.* 1980 Feb 16;1(8164):339-40. PMID: 6101792. Exclusion Code: X 2
292. Akinshina S, Makatsariya A, Bitsadze V, et al. Pathogenetic prophylaxis of obstetric complications and recurrent thrombosis in pregnant women with history of stroke. *International Journal of Gynecology and Obstetrics.* 2009;107:S101. Exclusion Code: X 2
293. Wald NJ, Hackshaw AD, Stone R, et al. Blood folic acid and vitamin B12 in relation to neural tube defects. *Br J Obstet Gynaecol.* 1996 Apr;103(4):319-24. PMID: 8605127. Exclusion Code: X 2
294. Jentink J, Bakker MK, Nijenhuis CM, et al. Does folic acid use decrease the risk for spina bifida after in utero exposure to valproic acid? *Pharmacoepidemiol Drug Saf.* 2010 Aug;19(8):803-7. PMID: 20680999. Exclusion Code: X 2
295. Parker SE, Yazdy MM, Tinker SC, et al. The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. *Am J Obstet Gynecol.* 2013 Sep;209(3):239.e1-8. PMID: 23711668. Exclusion Code: X 2
296. Harden CL, Pennell PB, Koppel BS, et al. Practice parameter update: management issues for women with epilepsy--focus on pregnancy (an evidence-based review): vitamin K, folic acid, blood levels, and breastfeeding: report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and American Epilepsy Society. *Neurology.* 2009 Jul 14;73(2):142-9. PMID: 19398680. Exclusion Code: X 2
297. Akinshina SV, Makatsaria AD. Prevention of recurrent thrombotic and thromboembolic complications during pregnancy. *J Thromb Haemost.* 2011;9:630. Exclusion Code: X 2
298. Korenbrot CC, Steinberg A, Bender C, et al. Preconception care: a systematic review (Structured abstract). *Maternal and Child Health Journal;* 2002. p. 75-88. Exclusion Code: X 2
299. Prevention program for reducing risk for neural tube defects--South Carolina, 1992-1994. *MMWR Morb Mortal Wkly Rep.* 1995 Mar 3;44(8):141-2. PMID: 7862080. Exclusion Code: X 3
300. Ballas SK, Baxter JK, Riddick G. Folate supplementation and twinning in patients with sickle cell disease. *Am J Hematol.* 2006 Apr;81(4):296-7. PMID: 16550509. Exclusion Code: X 3
301. Ben-Ami I, Vaknin Z, Reish O, et al. Is there an increased rate of anencephaly in twins? *Prenat Diagn.* 2005 Nov;25(11):1007-10. PMID: 16231303. Exclusion Code: X 3
302. Binkley KE, Leaver C, Ray JG. Antenatal risk factors for peanut allergy in children. *Allergy Asthma Clin Immunol.* 2011;7:17. PMID: 21970733. Exclusion Code: X 3
303. Gucciardi E, Pietrusiak MA, Reynolds DL, et al. Incidence of neural tube defects in Ontario, 1986-1999. *CMAJ.* 2002 Aug 6;167(3):237-40. PMID: 12186167. Exclusion Code: X 3
304. Hutson JR, Lubetsky A, Eichhorst J, et al. Adverse placental effect of formic acid on hCG secretion is mitigated by folic acid. *Alcohol Alcohol.* 2013 May-Jun;48(3):283-7. PMID: 23408242. Exclusion Code: X 3
305. Kallen BA, Olausson PO. Use of folic acid and delivery outcome: a prospective registry study. *Reprod Toxicol.* 2002 Jul-Aug;16(4):327-32. PMID: 12220592. Exclusion Code: X 3
306. Laurence KM, Carter CO, David PA. Major central nervous system malformations in South Wales. II. Pregnancy factors, seasonal variation, and social class effects. *Br J Prev Soc Med.* 1968 Oct;22(4):212-22. PMID: 4882275. Exclusion Code: X 3

Appendix C. Excluded Studies

307. Rosano A, Smithells D, Cacciani L, et al. Time trends in neural tube defects prevalence in relation to preventive strategies: an international study. *J Epidemiol Community Health*. 1999 Oct;53(10):630-5. PMID: 10616675. Exclusion Code: X 3
308. Van Allen MI, Boyle E, Thiessen P, et al. The impact of prenatal diagnosis on neural tube defect (NTD) pregnancy versus birth incidence in British Columbia. *J Appl Genet*. 2006;47(2):151-8. PMID: 16682757. Exclusion Code: X 3
309. Wang L, Wang F, Guan J, et al. Relation between hypomethylation of long interspersed nucleotide elements and risk of neural tube defects. *Am J Clin Nutr*. 2010 May;91(5):1359-67. PMID: 20164316. Exclusion Code: X 3
310. Lassi ZS, Bhutta ZA. Clinical utility of folate-containing oral contraceptives. *Int J Womens Health*. 2012;4:185-90. PMID: 22570577. Exclusion Code: X 3
311. Stevenson RE, Allen WP, Pai GS, et al. Decline in prevalence of neural tube defects in a high-risk region of the United States. *Pediatrics*. 2000 Oct;106(4):677-83. PMID: 11015508. Exclusion Code: X 3
312. Wild J, Sutcliffe M, Schorah CJ, et al. Prevention of neural-tube defects. *Lancet*. 1997 Jul 5;350(9070):30-1. PMID: 9217720. Exclusion Code: X 3
313. Jentink J, Bakker MK, De Jong-van Den Berg LTW. Effect of correct folic acid use on spina bifida in pregnancies exposed to valproic acid or carbamazepine in the Netherlands. *Pharmacoepidemiol Drug Saf*. 2009;18(S1):S13. Exclusion Code: X 3
314. M.R.C. trial of multivitamin prophylaxis in pregnancies at risk of neural tube defects. *Lancet*. 1982 Dec 18;2(8312):1412. PMID: 11644249. Exclusion Code: X 4
315. Medical Research Council Vitamin Study. *Lancet*. 1984 Jun 9;1(8389):1308. PMID: 11644285. Exclusion Code: X 4
316. Does folic acid decrease the risk of orofacial clefts? *Geneesmiddelenbulletin*. 1995;29(10):110-1. Exclusion Code: X 4
317. Neural tube defect surveillance and folic acid intervention--Texas-Mexico border, 1993-1998. *MMWR Morb Mortal Wkly Rep*. 2000 Jan 14;49(1):1-4. PMID: 10993565. Exclusion Code: X 4
318. From the Centers for Disease Control and Prevention. Neural tube defect surveillance and folic acid intervention--Texas-Mexico border, 1993-1998. *JAMA*. 2000 Jun 14;283(22):2928-30. PMID: 10896528. Exclusion Code: X 4
319. Ajayi G. Dehydroepiandrosterone sulphate (DHAS) in non pregnant women, during normal pregnancy and puerperium and the effect of combined iron (Chemiron(registered trademark)) and single iron therapy. *J Foetal Med*. 1993;13(1-4):31-6. Exclusion Code: X 4
320. Al-Ani ZR, Al-Hiali SJ, Al-Mehimdi SM. Neural tube defects among neonates delivered in Al-Ramadi Maternity and Children's Hospital, western Iraq. *Saudi Med J*. 2010 Feb;31(2):163-9. PMID: 20174732. Exclusion Code: X 4
321. Bailey S, Ayling J. Comparison of 5-methyltetrahydrofolic acid with folic acid for rapid folate repletion to reduce the risk for neural tube and other birth defects. *Basic and Clinical Pharmacology and Toxicology*. 2014;115:107. Exclusion Code: X 4
322. Bitsko RH, Reefhuis J, Romitti PA, et al. Periconceptional consumption of vitamins containing folic acid and risk for multiple congenital anomalies. *Am J Med Genet A*. 2007 Oct 15;143a(20):2397-405. PMID: 17853468. Exclusion Code: X 4
323. Bower C, Ryan A, Rudy E. Ascertainment of pregnancies terminated because of birth defects: effect on completeness of adding a new source of data. *Teratology*. 2001 Jan;63(1):23-5. PMID: 11169551. Exclusion Code: X 4
324. Boyles AL, Ballard JL, Gorman EB, et al. Association between inhibited binding of folic acid to folate receptor alpha in maternal serum and folate-related birth defects in Norway. *Hum Reprod*. 2011 Aug;26(8):2232-8. PMID: 21576080. Exclusion Code: X 4
325. Castrén O, Levanto A, Rauramo L, et al. Preventive iron and folic acid therapy in pregnancy. *Ann Chir Gynaecol Fenn*; 1968. p. 382-6. Exclusion Code: X 4
326. Chanarin I, Rothman D, Ward A, et al. Folate status and requirement in pregnancy. *Br Med J*; 1968. p. 390-4. Exclusion Code: X 4

Appendix C. Excluded Studies

327. Csaky-Szunyogh M, Vereczkey A, Kosa Z, et al. Risk and protective factors in the origin of conotruncal defects of heart--a population-based case-control study. *Am J Med Genet A*. 2013 Oct;161a(10):2444-52. PMID: 23950097. Exclusion Code: X 4
328. Czeizel A, Rode K. Trial to prevent first occurrence of neural tube defects by periconceptional multivitamin supplementation. *Lancet*. 1984 Jul 7;2(8393):40. PMID: 6145954. Exclusion Code: X 4
329. Dalziel K, Segal L, Katz R. Cost-effectiveness of mandatory folate fortification v. other options for the prevention of neural tube defects: results from Australia and New Zealand. *Public Health Nutr*. 2010 Apr;13(4):566-78. PMID: 19758481. Exclusion Code: X 4
330. de Smit DJ, de Jong-van den Berg LT. [Uncertain effects of folic acid on disorders other than neural-tube defects]. *Ned Tijdschr Geneesk*. 2006 Nov 18;150(46):2573; author reply PMID: 17152338. Exclusion Code: X 4
331. De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, et al. Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database Syst Rev*. 2010(10):Cd007950. PMID: 20927767. Exclusion Code: X 4
332. Diez-Ewald M, Molina RA. Iron and folic acid deficiency during pregnancy in western Venezuela. *Am J Trop Med Hyg*. 1972 Sep;21(5):587-91. PMID: 5075668. Exclusion Code: X 4
333. Forns J, Torrent M, Garcia-Esteban R, et al. Longitudinal association between early life socio-environmental factors and attention function at the age 11 years. *Environ Res*. 2012 Aug;117:54-9. PMID: 22608140. Exclusion Code: X 4
334. Fryer AA, Nafee TM, Ismail KM, et al. LINE-1 DNA methylation is inversely correlated with cord plasma homocysteine in man: a preliminary study. *Epigenetics*. 2009 Aug 16;4(6):394-8. PMID: 19755846. Exclusion Code: X 4
335. Gardiki-Kouidou P, Seller MJ. Amniotic fluid folate, vitamin B12 and transcobalamins in neural tube defects. *Clin Genet*. 1988 Jun;33(6):441-8. PMID: 3048802. Exclusion Code: X 4
336. Giebe K, Counts C. Comparison of Prenate Advance with other prescription prenatal vitamins: a folic acid dissolution study. *Adv Ther*. 2000 Jul-Aug;17(4):179-83. PMID: 11185056. Exclusion Code: X 4
337. Gildestad T, Bjorge T, Vollset SE, et al. Folic acid supplements and risk for oral clefts in the newborn: A population-based study. *Br J Nutr*. 2015;114(9):1456-63. Exclusion Code: X 4
338. Giusti B, Gori AM, Marcucci R, et al. Homocysteine as an emerging risk factor for cardiovascular disease in the elderly. *Aging Health*. 2006;2(6):983-97. Exclusion Code: X 4
339. Gjergja Juraski R, Miskov S, Cvitanovic Sojat L, et al. Prospective surveillance of Croatian pregnant women with epilepsy and their offspring - Pregnancy outcome and effects of antiepileptic drugs exposure. *Eur J Neurol*. 2010;17:180. Exclusion Code: X 4
340. Gringras M. A comparison of two combined iron-folic acid preparations in the prevention of anaemia in pregnancy. *J Int Med Res*; 1982. p. 268-70. Exclusion Code: X 4
341. Grosse SD, Collins JS. Folic acid supplementation and neural tube defect recurrence prevention. *Birth Defects Res A Clin Mol Teratol*. 2007 Nov;79(11):737-42. PMID: 17990333. Exclusion Code: X 4
342. Grossmith A, Nair M, Thompson R, et al. Observational study of the management of vitamin B12 deficiency during pregnancy. *International Journal of Gynecology and Obstetrics*. 2009;107:S417-S8. Exclusion Code: X 4
343. Guven F, Say A, Uygur Kulcu N, et al. Vitamin B12 and folate deficiency in healthy neonates and their mothers. *Arch Dis Child*. 2012;97:A220. Exclusion Code: X 4
344. Habibzadeh N, Wild J, Lucock MD, et al. One-carbon metabolism in pregnancies complicated by neural tube defects. *Lancet*. 1995 Mar 25;345(8952):791. PMID: 7891500. Exclusion Code: X 4
345. Halicioglu O, Sutcuoglu S, Koc F, et al. Vitamin B12 and folate statuses are associated with diet in pregnant women, but not with anthropometric measurements in term newborns. *J Matern Fetal Neonatal Med*. 2012 Sep;25(9):1618-21. PMID: 22185230. Exclusion Code: X 4
346. Hancock KW, Walker PA, Harper TA. Mobilisation of iron in pregnancy. *Lancet*; 1968. p. 1055-8. Exclusion Code: X 4

Appendix C. Excluded Studies

347. Hansen HA. The incidence of pernicious anaemia and the etiology of folic acid deficiency in pregnancy. *Acta Obstet Gynecol Scand.* 1967;46(7):Suppl 7:113-5. PMID: 6072971. Exclusion Code: X 4
348. Hernandez-Diaz S, Werler MM, Walker AM, et al. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med.* 2000 Nov 30;343(22):1608-14. PMID: 11096168. Exclusion Code: X 4
349. Hibbard BM, Hibbard ED. The prophylaxis of folate deficiency in pregnancy. *Acta Obstet Gynecol Scand;* 1969. p. 339-48. Exclusion Code: X 4
350. Hirahara F, Hamanoue H, Ebisawa Y, et al. Recent trends in prevalence of neural tube defects in Japan. *Birth Defects Research Part A - Clinical and Molecular Teratology.* 2010;88(6):506. Exclusion Code: X 4
351. Hobbs CA, Cleves MA, Melnyk S, et al. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. *Am J Clin Nutr.* 2005 Jan;81(1):147-53. PMID: 15640474. Exclusion Code: X 4
352. Hoyo C, Murtha AP, Schildkraut JM, et al. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics.* 2011 Jul;6(7):928-36. PMID: 21636975. Exclusion Code: X 4
353. Illsley NP, Felkner M, Fik E, et al. Autoantibody inhibition of placental microvillous folate receptor alpha (FR(alpha)) binding measured using a fluorescent folate analog. *Reprod Sci.* 2010;17(3):260A-1A. Exclusion Code: X 4
354. Iyengar L, Apte SV. Prophylaxis of anemia in pregnancy. *Am J Clin Nutr;* 1970. p. 725-30. Exclusion Code: X 4
355. Kerr MA, McNulty H, Livingstone MBE. Response to vitamin B12 intervention in lean versus obese women. *Obes Rev.* 2011;12:114. Exclusion Code: X 4
356. Khoshnood B, Loane M, De Walle H, et al. Long term trends in prevalence of neural tube defects in Europe: Population based study. *BMJ (Online).* 2015;351. Exclusion Code: X 4
357. Kocak G, Onal C, Kocak A, et al. Prevalence and outcome of congenital heart disease in patients with neural tube defect. *J Child Neurol.* 2008 May;23(5):526-30. PMID: 18056696. Exclusion Code: X 4
358. Lassi Zohra S, Salam Rehana A, Haider Batoool A, et al. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. *Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd;* 2013. Exclusion Code: X 4
359. Laurence KM, James N, Miller M. A double randomised controlled trial for preconceptional folic acid supplementation for the prevention of recurrence of neural tube defects in high risk pregnancies. *J-Med-Genet;* 1982. p. 66. Exclusion Code: X 4
360. Lee JI, Lee JA, Lim HS. Effect of time of initiation and dose of prenatal iron and folic acid supplementation on iron and folate nutriture of Korean women during pregnancy. *Am J Clin Nutr.* 2005 Oct;82(4):843-9. PMID: 16210715. Exclusion Code: X 4
361. Lu QB, Wang ZP, Gao LJ, et al. Nausea and vomiting in early pregnancy and the risk of neural tube defects: a case-control study. *Sci Rep.* 2015;5:7674. PMID: 25567703. Exclusion Code: X 4
362. Mahale AR, Shah SH. New schedule of intramuscular iron administration for pregnant women. *Asia-Oceania journal of obstetrics and gynaecology / AFOG;* 1993. p. 141-4. Exclusion Code: X 4
363. Makatsariya AD, Bitsadze VO, Baimuradova SM, et al. Low molecular weight heparin and natural progesteron in prophylaxis of recurrent pregnancy complications in women with thrombophilia. *Journal of Maternal-Fetal and Neonatal Medicine.* 2010;23:534. Exclusion Code: X 4
364. Makatsariya AD, Bitsadze VO, Baimuradova SM, et al. Low molecular weight heparin in prophylaxis of recurrent pregnancy complications in women with thrombophilia. *Pathophysiol Haemost Thromb.* 2010;37:A80. Exclusion Code: X 4
365. Milne E, Royle JA, Miller M, et al. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. *Int J Cancer.* 2010 Jun 1;126(11):2690-9. PMID: 19839053. Exclusion Code: X 4
366. Myhre R, Myking S, Pay A, et al. Dietary and supplementary folate intake before and during pregnancy and the risk for spontaneous preterm delivery. *Am J Obstet Gynecol.* 2011;204(1):S8. Exclusion Code: X 4

Appendix C. Excluded Studies

367. Nooij LS, van Eyck J, Klip H, et al. The reliability of methionine challenge test is not affected by a deficiency in pyridoxal phosphate, folic acid, or cobalamin. *Hypertens Pregnancy*. 2011;30(1):11-7. PMID: 20818966. Exclusion Code: X 4
368. Oladejo M, Bewley S. Adherence in pregnancy: A systematic review of the literature. *Fetal Matern Med Rev*. 2012;23(3-4):201-29. Exclusion Code: X 4
369. Panfilova O, Natalia M. Preconceptive treatment and principles of prophylaxis of recurrent placental abruptions in thrombophilic patients. *J Perinat Med*. 2013;41. Exclusion Code: X 4
370. Pei L, Li Z, Ye R, et al. Interaction between maternal spontaneous abortion and periconceptual folic acid use on NTDs risks. *Birth Defects Research Part A - Clinical and Molecular Teratology*. 2015;103(5):381. Exclusion Code: X 4
371. Pietrzik K, Prinz R, Reusch K, et al. Folate status and pregnancy outcome. *Ann N Y Acad Sci*. 1992 Sep 30;669:371-3. PMID: 1444051. Exclusion Code: X 4
372. Robitaille J, Carmichael SL, Shaw GM, et al. Maternal nutrient intake and risks for transverse and longitudinal limb deficiencies: data from the National Birth Defects Prevention Study, 1997-2003. *Birth Defects Res A Clin Mol Teratol*. 2009 Sep;85(9):773-9. PMID: 19350655. Exclusion Code: X 4
373. Rosenblatt DS. Folate and homocysteine metabolism and gene polymorphisms in the etiology of Down syndrome. *Am J Clin Nutr*. 1999 Oct;70(4):429-30. PMID: 10500007. Exclusion Code: X 4
374. Saravanan P, Maheswaran H, Stranges S, et al. Population levels of folic acid and vitamin B12 and their implications: Do we need to consider B12 fortification to prevent gestational diabetes and future cardiovascular risk? *Diabet Med*. 2010;27(2):80. Exclusion Code: X 4
375. Scott J, Kirke P, Molloy A, et al. The role of folate in the prevention of neural-tube defects. *Proc Nutr Soc*. 1994 Nov;53(3):631-6. PMID: 7886062. Exclusion Code: X 4
376. Scott JM, Sommerville JW. PRACTICAL EVALUATION OF THE FIGLU TEST IN PREGNANCY WITH SPECIAL REFERENCE TO THE WHITE CELL CHANGES. *J Clin Pathol*. 1965 May;18:322-6. PMID: 14304245. Exclusion Code: X 4
377. Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *Proc Natl Acad Sci U S A*. 2007 Dec 11;104(50):19995-20000. PMID: 18056804. Exclusion Code: X 4
378. Sengpiel V, Bacelis J, Myhre R, et al. Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: A prospective observational cohort study. *BMC Pregnancy Childbirth*. 2014;14(1). Exclusion Code: X 4
379. Shaw GM, Lammer EJ, Zhu H, et al. Maternal periconceptual vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida. *Am J Med Genet*. 2002 Feb 15;108(1):1-6. PMID: 11857541. Exclusion Code: X 4
380. Shaw GM, Lu W, Zhu H, et al. 118 SNPs of folate-related genes and risks of spina bifida and conotruncal heart defects. *BMC Med Genet*. 2009;10:49. PMID: 19493349. Exclusion Code: X 4
381. Shaw GM, Rozen R, Finnell RH, et al. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase, and risk for spina bifida. *Am J Epidemiol*. 1998 Jul 1;148(1):30-7. PMID: 9663401. Exclusion Code: X 4
382. Sheppard S, Nevin NC, Seller MJ, et al. Neural tube defect recurrence after 'partial' vitamin supplementation. *J Med Genet*. 1989 May;26(5):326-9. PMID: 2732994. Exclusion Code: X 4
383. Streiff RR, Little AB. Folic acid deficiency in pregnancy. *N Engl J Med*. 1967 Apr 6;276(14):776-9. PMID: 6023639. Exclusion Code: X 4
384. Swain R, Kaplan B. Vitamins as therapy in the 1990s. *J Am Board Fam Pract*. 1995 May-Jun;8(3):206-16. PMID: 7618499. Exclusion Code: X 4
385. Tamura T, Goldenberg RL, Freeberg LE, et al. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. *Am J Clin Nutr*. 1992 Aug;56(2):365-70. PMID: 1636615. Exclusion Code: X 4

Appendix C. Excluded Studies

386. Tarrant RC, Younger KM, Sheridan-Pereira M, et al. Maternal health behaviours during pregnancy in an Irish obstetric population and their associations with socio-demographic and infant characteristics. *Eur J Clin Nutr.* 2011 Apr;65(4):470-9. PMID: 21364609. Exclusion Code: X 4
387. Trigg KH, Rendall EJ, Johnson A, et al. Folate supplements during pregnancy. *J R Coll Gen Pract.* 1976 Mar;26(164):228-30. PMID: 1263177. Exclusion Code: X 4
388. Tsang B, Sandalinas F, De-Regil Luz M. Folate supplementation in women of reproductive age. *Cochrane Database of Systematic Reviews: John Wiley & Sons, Ltd; 2015.* Exclusion Code: X 4
389. Ubeda N, Reyes L, Gonzalez-Medina A, et al. Physiologic changes in homocysteine metabolism in pregnancy: a longitudinal study in Spain. *Nutrition.* 2011 Sep;27(9):925-30. PMID: 21367581. Exclusion Code: X 4
390. Van Allen MI, Kalousek DK, Chernoff GF, et al. Evidence for multi-site closure of the neural tube in humans. *Am J Med Genet.* 1993 Oct 1;47(5):723-43. PMID: 8267004. Exclusion Code: X 4
391. Watanabe H, Suganuma N, Hayashi A, et al. No relation between folate and homocysteine levels and depression in early pregnant women. *Biosci Trends.* 2010 Dec;4(6):344-50. PMID: 21248434. Exclusion Code: X 4
392. Werler MM, Cragan JD, Wasserman CR, et al. Multivitamin supplementation and multiple births. *Am J Med Genet.* 1997 Jul 11;71(1):93-6. PMID: 9215776. Exclusion Code: X 4
393. Wickramasinghe SN. The deoxyuridine suppression test: a review of its clinical and research applications. *Clin Lab Haematol.* 1981;3(1):1-18. PMID: 7014076. Exclusion Code: X 4
394. Willoughby ML, Jewell FJ. Investigation of folic acid requirements in pregnancy. *Br Med J;* 1966. p. 1568-71. Exclusion Code: X 4
395. Yang Q, Atkinson M, Erickson JD. Method of weighted proportion of reproductive-aged women taking folic acid supplements to predict a neural tube defect rate decline. *Birth Defects Res A Clin Mol Teratol.* 2003 Dec;67(12):959-67. PMID: 14745914. Exclusion Code: X 4
396. Jentink J, Vrie-Hoekstra NW, Jong-Van Den Berg LT, et al. Economic evaluation of folic acid food fortification in the Netherlands (Provisional abstract). *Eur J Public Health;* 2008. p. 270-4. Exclusion Code: X 4
397. Julvez J, Fortuny J, Mendez M, et al. Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. *Paediatr Perinat Epidemiol.* 2009 May;23(3):199-206. PMID: 19775381. Exclusion Code: X 4
398. Chitayat D, Matsui D, Amitai Y, et al. Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. *J Clin Pharmacol.* 2015 Aug 13PMID: 26272218. Exclusion Code: X 4
399. Meltzer HM, Brantsaeter AL, Nilsen RM, et al. Effect of dietary factors in pregnancy on risk of pregnancy complications: results from the Norwegian Mother and Child Cohort Study. *Am J Clin Nutr.* 2011 Dec;94(6 Suppl):1970s-4s. PMID: 21543541. Exclusion Code: X 4
400. de Walle HE, de Jong-van den Berg LT, Cornel MC. Periconceptional folic acid intake in the northern Netherlands. *Lancet.* 1999 Apr 3;353(9159):1187. PMID: 10210009. Exclusion Code: X 4
401. Bukowski R, Malone FD, Porter FT, et al. Preconceptional folate supplementation and the risk of spontaneous preterm birth: a cohort study. *PLoS Med.* 2009 May 5;6(5):e1000061. PMID: 19434228. Exclusion Code: X 4
402. Teratology Society 50th Annual Meeting - 23rd International Conference of the Organization of Teratology Information Specialists, OTIS and the 34th Annual Meeting of the Neurobehavioral Teratology Society, NBTS. *Birth Defects Research Part A - Clinical and Molecular Teratology.* 2010;88(5). Exclusion Code: X 4
403. Hamilton E, Graham P, Edwards J. Folic acid supplementation in women with epilepsy. *Epilepsia.* 2009;50:51. Exclusion Code: X 4
404. Nichols JAA, Curtis EPP, Rayman MP. Survey of total folate intake at conception and assessment of impact of fortification. *Journal of Nutritional and Environmental Medicine.* 2008;17(1):44-55. Exclusion Code: X 4

Appendix C. Excluded Studies

405. Norman M, Penman A. Neural tube defects and folic acid knowledge and use in Mississippi women. *J Miss State Med Assoc.* 2001 Sep;42(9):270-6. PMID: 11569130. Exclusion Code: X 4
406. Hamner HC, Mulinare J, Cogswell ME, et al. Predicted contribution of folic acid fortification of corn masa flour to the usual folic acid intake for the US population: National Health and Nutrition Examination Survey 2001-2004. *Am J Clin Nutr.* 2009 Jan;89(1):305-15. PMID: 19056605. Exclusion Code: X 4
407. Kim H, Cain R, Viner-Brown S, et al. Multivitamin use prior to pregnancy in Rhode Island. *Med Health R I.* 2011 Sep;94(9):276-8. PMID: 22010325. Exclusion Code: X 4
408. Walsh T, O'Broin S, Cooley S, et al. Maternal folate status and neural tube defects in Ireland: the need for a national food fortification program. *Ir Med J.* 2007 May;100(5):469-72. PMID: 17727124. Exclusion Code: X 4
409. Bouthoorn SH, Gaillard R, Steegers EA, et al. Ethnic differences in blood pressure and hypertensive complications during pregnancy: the Generation R study. *Hypertension.* 2012 Jul;60(1):198-205. PMID: 22615112. Exclusion Code: X 4
410. Sotres-Alvarez D, Siega-Riz AM, Herring AH, et al. Maternal dietary patterns are associated with risk of neural tube and congenital heart defects. *Am J Epidemiol.* 2013 Jun 1;177(11):1279-88. PMID: 23639938. Exclusion Code: X 4
411. Czeizel AE. Preterm birth preventive effect of folic acid. *Clin Chem Lab Med.* 2012;50(2):A65. Exclusion Code: X 4
412. Couto FD, Moreira LM, Dos Santos DB, et al. Folate, vitamin B12 and total homocysteine levels in neonates from Brazil. *Eur J Clin Nutr.* 2007 Mar;61(3):382-6. PMID: 16988650. Exclusion Code: X 4
413. Khodr ZG, Lupo PJ, Agopian AJ, et al. Preconceptional folic acid-containing supplement use in the national birth defects prevention study. *Birth Defects Res A Clin Mol Teratol.* 2014 Apr 7; PMID: 24706436. Exclusion Code: X 4
414. Kulier R, de Onis M, Gulmezoglu AM, et al. Nutritional interventions for the prevention of maternal morbidity. *Int J Gynaecol Obstet.* 1998 Dec;63(3):231-46. PMID: 9989892. Exclusion Code: X 4
415. Liu JM, Mei Z, Ye R, et al. Micronutrient supplementation and pregnancy outcomes: double-blind randomized controlled trial in China. *JAMA internal medicine;* 2013. p. 276-82. Exclusion Code: X 4
416. Talalaeva IN, Makatsaria AD, Kiss LN. Folate deficiency State and hyperhomocysteinemia as cause of antenatal fetal death. *J Thromb Haemost.* 2009;7(S2):445. Exclusion Code: X 4
417. Banhidy F, Acs N, Puho EH, et al. Iron deficiency anemia: pregnancy outcomes with or without iron supplementation. *Nutrition.* 2011 Jan;27(1):65-72. PMID: 20381313. Exclusion Code: X 4
418. Bailey HD, Miller M, Langridge A, et al. Maternal dietary intake of folate and vitamins B6 and B12 during pregnancy and the risk of childhood acute lymphoblastic leukemia. *Nutr Cancer.* 2012;64(7):1122-30. PMID: 22966944. Exclusion Code: X 4
419. Bortolus R, Blom F, Filippini F, et al. Prevention of congenital malformations and other adverse pregnancy outcomes with 4.0 mg of folic acid: Community-based randomized clinical trial in Italy and the Netherlands. *BMC Pregnancy Childbirth.* 2014;14(1). Exclusion Code: X 4
420. Csaky-Szunyogh M, Vereczkey A, Kosa Z, et al. Risk factors in the origin of congenital left-ventricular outflow-tract obstruction defects of the heart: a population-based case-control study. *Pediatr Cardiol.* 2014 Jan;35(1):108-20. PMID: 23843102. Exclusion Code: X 4
421. Fleming AF, Martin JD, Hahnel R, et al. Effects of iron and folic acid antenatal supplements on maternal haematology and fetal wellbeing. *Med J Aust;* 1974. p. 429-36. Exclusion Code: X 4
422. Haider BA, Yakoob MY, Bhutta ZA. Effect of multiple micronutrient supplementation during pregnancy on maternal and birth outcomes. *BMC Public Health.* 2011;11 Suppl 3:S19. PMID: 21501436. Exclusion Code: X 4
423. Hayes C, Werler MM, Willett WC, et al. Case-control study of periconceptional folic acid supplementation and oral clefts. *Am J Epidemiol.* 1996 Jun 15;143(12):1229-34. PMID: 8651221. Exclusion Code: X 4

Appendix C. Excluded Studies

424. Hininger I, Favier M, Arnaud J, et al. Effects of a combined micronutrient supplementation on maternal biological status and newborn anthropometrics measurements: a randomized double-blind, placebo-controlled trial in apparently healthy pregnant women. *Eur J Clin Nutr*; 2004. p. 52-9. Exclusion Code: X 4
425. Holmes VA, Wallace JM, Alexander HD, et al. Homocysteine is lower in the third trimester of pregnancy in women with enhanced folate status from continued folic acid supplementation. *Clin Chem*. 2005 Mar;51(3):629-34. PMID: 15615817. Exclusion Code: X 4
426. Izak G, Levy S, Rachmilewitz M, et al. The effect of iron and folic acid therapy on combined iron and folate deficiency anaemia: the results of a clinical trial. *Scand J Haematol*; 1973. p. 236-40. Exclusion Code: X 4
427. Johnson CY, Little J. Folate intake, markers of folate status and oral clefts: is the evidence converging? *Int J Epidemiol*. 2008 Oct;37(5):1041-58. PMID: 18583393. Exclusion Code: X 4
428. Merviel P, Cabry R, Temstet R, et al. Comparison between two preventive treatments in women with recurrent miscarriages carrying a C677T methylenetetrahydrofolate reductase mutation: A prospective study. *Hum Reprod*. 2011;26:i10. Exclusion Code: X 4
429. Morse NL. Benefits of docosahexaenoic acid, folic acid, vitamin D and iodine on foetal and infant brain development and function following maternal supplementation during pregnancy and lactation. *Nutrients*. 2012 Jul;4(7):799-840. PMID: 22852064. Exclusion Code: X 4
430. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, et al. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. *Am J Clin Nutr*. 1984 Sep;40(3):496-507. PMID: 6475821. Exclusion Code: X 4
431. Schmidt RJ, Tancredi DJ, Ozonoff S, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. *Am J Clin Nutr*. 2012 Jul;96(1):80-9. PMID: 22648721. Exclusion Code: X 4
432. Sengpiel V, Bacelis J, Myhre R, et al. Folic acid supplementation, dietary folate intake during pregnancy and risk for spontaneous preterm delivery: a prospective observational cohort study. *BMC Pregnancy Childbirth*. 2014 Nov 2;14(1):375. PMID: 25361626. Exclusion Code: X 4
433. Sikora J, Magnucki J, Zietek J, et al. Homocysteine, folic acid and vitamin B12 concentration in patients with recurrent miscarriages. *Neuro Endocrinol Lett*. 2007 Aug;28(4):507-12. PMID: 17693963. Exclusion Code: X 4
434. Stoltenberg C. Effects of periconceptional folate on language delay and autism spectrum disorders: The Norwegian mother and child cohort study. *Neuropsychopharmacology*. 2013;38:S38. Exclusion Code: X 4
435. Wilcox AJ, Lie RT, Solvoll K, et al. Folic acid supplements and risk of facial clefts: National population based case-control study. *Obstet Gynecol Surv*. 2007;62(7):434-5. Exclusion Code: X 4
436. Yakoob MY, Bhutta ZA. Effect of routine iron supplementation with or without folic acid on anemia during pregnancy (Provisional abstract). *BMC Public Health*; 2011. p. S21. Exclusion Code: X 4
437. Yuskiv N, Honein MA, Moore CA. Reported multivitamin consumption and the occurrence of multiple congenital anomalies. *Am J Med Genet A*. 2005 Jul 1;136(1):1-7. PMID: 15937944. Exclusion Code: X 4
438. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst Rev*. 2006(4):Cd004905. PMID: 17054223. Exclusion Code: X 5
439. Muggli EE, Halliday JL. Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006 (Structured abstract). *Med J Aust*; 2007. p. 243-8. Exclusion Code: X 5
440. Berry RJ, Li Z, Erickson JD, 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 Nov 11;341(20):1485-90. PMID: 10559448. Exclusion Code: X 6

Appendix C. Excluded Studies

441. Chen G, Song X, Ji Y, et al. Prevention of NTDs with periconceptional multivitamin supplementation containing folic acid in China. *Birth Defects Res A Clin Mol Teratol*. 2008 Aug;82(8):592-6. PMID: 18634013. Exclusion Code: X 6
442. Dean SV, Lassi ZS, Imam AM, et al. Preconception care: Nutritional risks and interventions. *Reproductive Health*. 2014;11. Exclusion Code: X 6
443. Elhusseini MA, Kassab M, Krawinkel M, et al. Folic acid intake and neural tube defects (NTDS) in a cohort of Egyptian mothers. *International Journal of Gynecology and Obstetrics*. 2012;119:S338. Exclusion Code: X 6
444. Esmaeili A, Hanaei S, Fadakar K, et al. Risk factors associated with lipomyelomeningocele: a case-control study. *Pediatr Neurosurg*. 2013;49(4):202-7. PMID: 24924296. Exclusion Code: X 6
445. Esmaeili A, Hanaei S, Fadakar K, et al. Risk Factors Associated with Lipomyelomeningocele: A Case-Control Study. *Pediatr Neurosurg*. 2014 Jun 11 PMID: 24924296. Exclusion Code: X 6
446. Fujimori E, Baldino CF, Sato AP, et al. [Prevalence and spatial distribution of neural tube defects in Sao Paulo State, Brazil, before and after folic acid flour fortification]. *Cad Saude Publica*. 2013 Jan;29(1):145-54. PMID: 23370034. Exclusion Code: X 6
447. Garcia-Casal MN, Osorio C, Landaeta M, et al. High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. *Eur J Clin Nutr*. 2005 Sep;59(9):1064-70. PMID: 16015269. Exclusion Code: X 6
448. Gotalipour MJ, Qorbani M, Mirfazeli A, et al. Risk factors of neural tube defects in northern Iran. *Iranian Red Crescent Medical Journal*. 2014;16(6). Exclusion Code: X 6
449. Gong R, Wang ZP, Gao LJ, et al. A case-control study of the effects of pregnancy planning on neural tube defects and its primary preventive measures. *Birth Defects Res A Clin Mol Teratol*. 2010 Sep;88(9):737-42. PMID: 20589881. Exclusion Code: X 6
450. Green TJ, Skeaff CM, Venn BJ, et al. Red cell folate and predicted neural tube defect rate in three Asian cities. *Asia Pac J Clin Nutr*. 2007;16(2):269-73. PMID: 17468082. Exclusion Code: X 6
451. Gu Q, Li Y, Cui ZL, et al. Homocysteine, folate, vitamin B12 and B6 in mothers of children with neural tube defects in Xinjiang, China. *Acta Paediatr*. 2012 Nov;101(11):e486-90. PMID: 22860981. Exclusion Code: X 6
452. Hibbard BM, Hibbard ED. Anaemia and folate status in late pregnancy in a mixed Asiatic population. *J Obstet Gynaecol Br Commonw*. 1972 Jul;79(7):584-91. PMID: 5043421. Exclusion Code: X 6
453. Khattak ST, Khan M, Naheed T, et al. Prevalence and management of anencephaly at Saidu Teaching Hospital, Swat. *J Ayub Med Coll Abbottabad*. 2010 Oct-Dec;22(4):61-3. PMID: 22455263. Exclusion Code: X 6
454. Kothari BV, Bhende YM. Crude liver extract, refined liver extract and folic acid in the treatment of pernicious anaemia of pregnancy. *Indian J Med Res*. 1950 Oct;38(4):385-92. PMID: 14840873. Exclusion Code: X 6
455. Krishnaveni GV, Hill JC, Veena SR, et al. Low plasma vitamin B12 in pregnancy is associated with gestational 'diabesity' and later diabetes. *Diabetologia*. 2009 Nov;52(11):2350-8. PMID: 19707742. Exclusion Code: X 6
456. Kulkarni ML, Jose S. Folic acid prevents neural tube defects in high prevalence area. *Indian Pediatr*. 1997 Jun;34(6):561-2. PMID: 9357219. Exclusion Code: X 6
457. Lehti KK. Stillbirth rates and folic acid and zinc status in low-socioeconomic pregnant women of Brazilian Amazon. *Nutrition*. 1993 Mar-Apr;9(2):156-8. PMID: 8485329. Exclusion Code: X 6
458. Mathur RK, Jain DK, Paliwal G. Review of etiology of neural tube defects: A hospital based study. *Internet J Pediatr Neonatol*. 2009;10(1). Exclusion Code: X 6
459. Mendu V, Athe R, Ku RK, et al. Maternal nutrition and adverse birth outcomes in developing countries: A systematic review and meta-analysis. *Ann Nutr Metab*. 2013;63:352. Exclusion Code: X 6
460. Metayer C, Milne E, Dockerty JD, et al. Maternal supplementation with folic acid and other vitamins and risk of leukemia in offspring: a Childhood Leukemia International Consortium study. *Epidemiology*. 2014 Nov;25(6):811-22. PMID: 25207954. Exclusion Code: X 6

Appendix C. Excluded Studies

461. Metz J, Festenstein H, Welch P. EFFECT OF FOLIC ACID AND VITAMIN B12 SUPPLEMENTATION ON TESTS OF FOLATE AND VITAMIN B12 NUTRITION IN PREGNANCY. *Am J Clin Nutr.* 1965 Jun;16:472-9. PMID: 14297280. Exclusion Code: X 6
462. Mobasheri E, Keshtkar A, Gotalipour MJ. Maternal folate and vitamin b(12) status and neural tube defects in northern iran: a case control study. *Iran J Pediatr.* 2010 Jun;20(2):167-73. PMID: 23056699. Exclusion Code: X 6
463. Nili F, Jahangiri M. Risk factors for neural tube defects: a study at university-affiliated hospitals in Tehran. *Arch Iran Med.* 2006 Jan;9(1):20-5. PMID: 16649373. Exclusion Code: X 6
464. Ogunbode O, Damole IO. Treatment of anaemia in obstetric patients with sustained-release (Ferrograd Folic 500 Plus) and conventional fersolate and folic acid as separate drugs. *Current Therapeutic Research - Clinical and Experimental.* 1984;35(6):1038-42. Exclusion Code: X 6
465. Ouarhlent YY, Boudjerra N, Bounecer H. The risk of venous thromboembolic during pregnancy. *J Thromb Haemost.* 2013;11:97. Exclusion Code: X 6
466. Pradhan M, Anand B, Malhotra M. Causes of neural tube defects: Environmental and genetic factors. *Prenat Diagn.* 2012;32:96. Exclusion Code: X 6
467. Stewart CP, Christian P, Schulze KJ, et al. Low maternal vitamin B-12 status is associated with offspring insulin resistance regardless of antenatal micronutrient supplementation in rural Nepal. *J Nutr.* 2011 Oct;141(10):1912-7. PMID: 21865563. Exclusion Code: X 6
468. Tunc T, Karadag YS, Dogulu F, et al. Predisposing factors of restless legs syndrome in pregnancy. *Mov Disord.* 2007 Apr 15;22(5):627-31. PMID: 17285614. Exclusion Code: X 6
469. Vergel RG, Sanchez LR, Heredero BL, et al. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. *Prenat Diagn.* 1990 Mar;10(3):149-52. PMID: 2343028. Exclusion Code: X 6
470. Viteri FE, Casanueva E, Tolentino MC, et al. Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women. *Reprod Toxicol.* 2012 Aug;34(1):125-32. PMID: 22507748. Exclusion Code: X 6
471. Wang M, Wang ZP, Gao LJ, et al. Maternal body mass index and the association between folic acid supplements and neural tube defects. *Acta Paediatr.* 2013 Sep;102(9):908-13. PMID: 23750819. Exclusion Code: X 6
472. Yajnik C. Intrauterine programming of adiposity and diabetes. *Obes Rev.* 2014;15:122. Exclusion Code: X 6
473. Zhang L, Ren A, Li Z, et al. Folate concentrations and folic acid supplementation among women in their first trimester of pregnancy in a rural area with a high prevalence of neural tube defects in Shanxi, China. *Birth Defects Res A Clin Mol Teratol.* 2006 Jun;76(6):461-6. PMID: 16933216. Exclusion Code: X 6
474. Blencowe H, Cousens S, Modell B, et al. Folic acid to reduce neonatal mortality from neural tube disorders (Structured abstract). *Int J Epidemiol.* 2010. p. i110-i21. Exclusion Code: X 6
475. Wehby GL, Felix TM, Goco N, et al. High dosage folic acid supplementation, oral cleft recurrence and fetal growth. *Int J Environ Res Public Health.* 2013 Feb;10(2):590-605. PMID: 23380913. Exclusion Code: X 6
476. Evidence for an association between periconceptional use of multivitamins and neural tube defects. *Nutr Rev.* 1990 Jan;48(1):15-7. PMID: 2186311. Exclusion Code: X 7
477. From the Center for Disease Control and Prevention. Prevention program for reducing risk for neural tube defects--South Carolina, 1992-1994. *JAMA.* 1995 May 17;273(19):1485. PMID: 7739059. Exclusion Code: X 7
478. Alasfoor D, Elsayed MK, Mohammed AJ. Spina bifida and birth outcome before and after fortification of flour with iron and folic acid in Oman. *East Mediterr Health J.* 2010 May;16(5):533-8. PMID: 20799554. Exclusion Code: X 7
479. Averbek P. Multivitamin prophylaxis as a cause of neural tube defect. *Lancet.* 1980 Jul 12;2(8185):101. PMID: 6105241. Exclusion Code: X 7

Appendix C. Excluded Studies

480. Barua S, Kuizon S, Junaid MA. Folic acid supplementation in pregnancy and implications in health and disease. *J Biomed Sci.* 2014;21:77. PMID: 25135350. Exclusion Code: X 7
481. Beard CM, Panser LA, Katusic SK. Is excess folic acid supplementation a risk factor for autism? *Med Hypotheses.* 2011 Jul;77(1):15-7. PMID: 21454018. Exclusion Code: X 7
482. Bendich A. Folic acid and neural tube defects. Introduction to Part II. *Ann N Y Acad Sci.* 1993 Mar 15;678:108-11. PMID: 8494253. Exclusion Code: X 7
483. Bognar M, Ponyi A, Hauser P, et al. Improper supplementation habits of folic acid intake by Hungarian pregnant women: improper recommendations. *J Am Coll Nutr.* 2008 Aug;27(4):499-504. PMID: 18978170. Exclusion Code: X 7
484. Chan AC, van Essen P, Scott H, et al. Folate awareness and the prevalence of neural tube defects in South Australia, 1966-2007. *Med J Aust.* 2008 Nov 17;189(10):566-9. PMID: 19012555. Exclusion Code: X 7
485. Clemmensen D, Thygesen M, Rasmussen MM, et al. Decreased incidence of myelomeningocele at birth: effect of folic acid recommendations or prenatal diagnostics? *Childs Nerv Syst.* 2011 Nov;27(11):1951-5. PMID: 21552997. Exclusion Code: X 7
486. Crider K, Devine O, Qi Y, et al. Predicting the impact of folic acid fortification, ready to eat cereals and supplement use on the risk of neural tube defects in the United States. *FASEB J.* 2015;29(1). Exclusion Code: X 7
487. Czeizel AE. Folic acid in the prevention of neural tube defects. *J Pediatr Gastroenterol Nutr.* 1995 Jan;20(1):4-16. PMID: 7884617. Exclusion Code: X 7
488. Dixon J. Folic acid and neural tube defects in New Zealand: a cautionary tale? *N Z Med J.* 2007;120(1254):U2533. PMID: 17515937. Exclusion Code: X 7
489. Eros E, Géher P, Gömör B, et al. Epileptogenic activity of folic acid after drug induces SLE (folic acid and epilepsy). *Eur J Obstet Gynecol Reprod Biol.* 1998. p. 75-8. Exclusion Code: X 7
490. Haggarty P, Campbell DM, Duthie S, et al. Folic acid use in pregnancy and embryo selection. *BJOG.* 2008 Jun;115(7):851-6. PMID: 18485163. Exclusion Code: X 7
491. Harrison RJ. Vitamin B12 levels in erythrocytes in anaemia due to folate deficiency. *Br J Haematol.* 1971 Jun;20(6):623-8. PMID: 5089952. Exclusion Code: X 7
492. Herbert V. Inborn errors in folate metabolism--a cause of mental retardation? *Ann Intern Med.* 1968 Apr;68(4):956-7. PMID: 5642976. Exclusion Code: X 7
493. Hernan MA, Hernandez-Diaz S, Werler MM, et al. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol.* 2002 Jan 15;155(2):176-84. PMID: 11790682. Exclusion Code: X 7
494. House JD, March SB, Ratnam MS, et al. Improvements in the status of folate and cobalamin in pregnant Newfoundland women are consistent with observed reductions in the incidence of neural tube defects. *Can J Public Health.* 2006 Mar-Apr;97(2):132-5. PMID: 16620001. Exclusion Code: X 7
495. Israels MC, Da Cunha FA. Megaloblastic anaemia of pregnancy. *Lancet.* 1952 Aug 2;2(6727):214-5. PMID: 14939891. Exclusion Code: X 7
496. Jackson IM, Doig WB, McDonald G. Pernicious anaemia as a cause of infertility. *Lancet.* 1967 Dec 2;2(7527):1159-60. PMID: 4168374. Exclusion Code: X 7
497. James SJ. Maternal metabolic phenotype and risk of Down syndrome: beyond genetics. *Am J Med Genet A.* 2004 May 15;127a(1):1-4. PMID: 15103708. Exclusion Code: X 7
498. Kar A, Kar T, Kanungo S, et al. Risk factors, organ weight deviation and associated anomalies in neural tube defects: A prospective fetal and perinatal autopsy series. *Indian J Pathol Microbiol.* 2015 Jul-Sep;58(3):285-91. PMID: 26275247. Exclusion Code: X 7
499. Klusmann A, Heinrich B, Stopler H, et al. A decreasing rate of neural tube defects following the recommendations for periconceptional folic acid supplementation. *Acta Paediatr.* 2005 Nov;94(11):1538-42. PMID: 16303691. Exclusion Code: X 7
500. Latha V, Sridhar M. Vitamin B supplementation to prevent neural tube defects? *Indian Pediatr.* 2010 Apr;47(4):361-2. PMID: 20431172. Exclusion Code: X 7

Appendix C. Excluded Studies

501. Locksmith GJ, Duff P. Preventing neural tube defects: the importance of periconceptional folic acid supplements. *Obstet Gynecol.* 1998 Jun;91(6):1027-34. PMID: 9611019. Exclusion Code: X 7
502. Matsubara S, Imai K, Murayama K, et al. Severe liver dysfunction during nausea and vomiting of pregnancy: folic acid supplement as a suggested culprit. *J Obstet Gynaecol.* 2012 Oct;32(7):701-2. PMID: 22943726. Exclusion Code: X 7
503. Navamani H. The real scenario of a preventable defect. *International Journal of Gynecology and Obstetrics.* 2009;107:S602-S3. Exclusion Code: X 7
504. Novy J, Ballhausen D, Bonafe L, et al. Recurrent postpartum cerebral sinus vein thrombosis as a presentation of cystathionine-beta-synthase deficiency. *Thromb Haemost.* 2010 Apr;103(4):871-3. PMID: 20174770. Exclusion Code: X 7
505. Poretti A, Anheier T, Zimmermann R, et al. Neural tube defects in Switzerland from 2001 to 2007: are periconceptual folic acid recommendations being followed? *Swiss Med Wkly.* 2008 Oct 18;138(41-42):608-13. PMID: 18941947. Exclusion Code: X 7
506. Richard-Tremblay AA, Sheehy O, Berard A. Annual trends in use of periconceptional folic acid and birth prevalence of major congenital malformations. *Curr Drug Saf.* 2013 Jul;8(3):153-61. PMID: 23845112. Exclusion Code: X 7
507. Rush D. Periconceptional folate and neural tube defect. *Am J Clin Nutr.* 1994 Feb;59(2 Suppl):511S-5S; discussion 5S-6S. PMID: 8304289. Exclusion Code: X 7
508. Sole-Navais P, Cavalle-Busquets P, Fernandez-Ballart JD, et al. Early pregnancy B vitamin status, one carbon metabolism, pregnancy outcome and child development. *Biochimie.* 2015 Dec 15 PMID: 26700149. Exclusion Code: X 7
509. Stephenson HE, Jr. Folic acid and neural tube defects. *South Med J.* 2005 Jan;98(1):128-9. PMID: 15678654. Exclusion Code: X 7
510. Stumpf DA. Symptoms of B(12) deficiency can occur in women of childbearing age supplemented with folate. *Neurology.* 2003 Jan 28;60(2):353; author reply PMID: 12552069. Exclusion Code: X 7
511. Evidence against an association between periconceptional use of multivitamins and neural tube defects. *Nutr Rev.* 1990 Jan;48(1):17-9. PMID: 2186312. Exclusion Code: X 7
512. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR Recomm Rep.* 1992 Sep 11;41(Rr-14):1-7. PMID: 1522835. Exclusion Code: X 7
513. Felkner M, Suarez L, Hendricks K, et al. Implementation and outcomes of recommended folic acid supplementation in Mexican-American women with prior neural tube defect-affected pregnancies. *Prev Med.* 2005 Jun;40(6):867-71. PMID: 15850889. Exclusion Code: X 7
514. Hamanoue H, Ebisawa Y, Mizusawa M, et al. For the occurrence of neural tube defects in Japan, is folate intake effective? *Congenital Anomalies.* 2010;50(4):A7. Exclusion Code: X 7
515. Mosley BS, Hobbs CA, Flowers BS, et al. Folic acid and the decline in neural tube defects in Arkansas. *J Ark Med Soc.* 2007 Apr;103(10):247-50. PMID: 17487022. Exclusion Code: X 7
516. Millar WJ. Folic acid supplementation. *Health Rep.* 2004 May;15(3):49-52. PMID: 15208890. Exclusion Code: X 7
517. Dougan C, Armstrong N, Bell S, et al. Audit of identification of antenatal patients with a raised BMI requiring high dose folate. *Arch Dis Child Fetal Neonatal Ed.* 2014;99:A127. Exclusion Code: X 7
518. Harden CL, Pennell PB, Koppel BS, et al. Management issues for women with epilepsy--focus on pregnancy (an evidence-based review): III. Vitamin K, folic acid, blood levels, and breast-feeding: Report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia.* 2009 May;50(5):1247-55. PMID: 19507305. Exclusion Code: X 7
519. Hartridge T, Illing HM, Sandy JR. The role of folic acid in oral clefting. *Br J Orthod.* 1999 Jun;26(2):115-20. PMID: 10420245. Exclusion Code: X 7
520. Interactions with periconceptional use of folic acid? *Geneesmiddelenbulletin.* 1997;31(3):36-7. Exclusion Code: X 8

Appendix C. Excluded Studies

521. Abramsky L, Botting B, Chapple J, et al. Has advice on periconceptional folate supplementation reduced neural-tube defects? *Lancet*. 1999 Sep 18;354(9183):998-9. PMID: 10501365. Exclusion Code: X 8
522. Afshar M, Golalipour MJ, Farhud D. Epidemiologic aspects of neural tube defects in South East Iran. *Neurosciences (Riyadh)*. 2006 Oct;11(4):289-92. PMID: 22266439. Exclusion Code: X 8
523. Alperin JB, Haggard ME, McGanity WJ. Folic acid, pregnancy, and abruptio placentae. *Am J Clin Nutr*. 1969 Oct;22(10):1354-61. PMID: 5344921. Exclusion Code: X 8
524. Alto WA, Czarnecki DF. Pregnancy tests and folic acid. *J Reprod Med*. 2002 Sep;47(9):776. PMID: 12380461. Exclusion Code: X 8
525. Azzena A, Sciortino R, Salmaso R. Acrania, MTHFR 677C->T mutation and role of folate intake. *Int J Gynaecol Obstet*. 1998 May;61(2):189-90. PMID: 9639227. Exclusion Code: X 8
526. Bergen N, Timmermans S, Hofman A, et al. First trimester homocysteine and folate levels are associated with increased adverse pregnancy outcomes. *Pregnancy Hypertens*. 2010;1:S22. Exclusion Code: X 8
527. Bhatla N, Kaul N, Lal N, et al. Comparison of effect of daily versus weekly iron supplementation during pregnancy on lipid peroxidation. *J Obstet Gynaecol Res*. 2009 Jun;35(3):438-45. PMID: 19527380. Exclusion Code: X 8
528. Bissenden JG. The role of nutrition in pregnancy. *Practitioner*. 1982 Feb;226(1364):313-5. PMID: 7088827. Exclusion Code: X 8
529. Chandler AL, Hobbs CA, Mosley BS, et al. Neural tube defects and maternal intake of micronutrients related to one-carbon metabolism or antioxidant activity. *Birth Defects Res A Clin Mol Teratol*. 2012 Nov;94(11):864-74. PMID: 22933447. Exclusion Code: X 8
530. Cherry FF, Bennett EA, Bazzano GS, et al. Plasma zinc in hypertension/toxemia and other reproductive variables in adolescent pregnancy. *Am J Clin Nutr*. 1981 Nov;34(11):2367-75. PMID: 6118062. Exclusion Code: X 8
531. Cornel MC, Erickson JD. Comparison of national policies on periconceptional use of folic acid to prevent spina bifida and anencephaly (SBA). *Teratology*. 1997 Feb;55(2):134-7. PMID: 9143094. Exclusion Code: X 8
532. Daniel WA, Jr., Mounger JR, Perkins JC. Obstetric and fetal complications in folate-deficient adolescent girls. *Am J Obstet Gynecol*. 1971 Sep 15;111(2):233-8. PMID: 5107222. Exclusion Code: X 8
533. De Wals P, Rusen ID, Lee NS, et al. Trend in prevalence of neural tube defects in Quebec. *Birth Defects Res A Clin Mol Teratol*. 2003 Nov;67(11):919-23. PMID: 14745929. Exclusion Code: X 8
534. DeRuiter J, Holston PL. Review of selected NMEs 2010. 2010. p. HS2-HS18. Exclusion Code: X 8
535. Elsinga J, Jong-Potjer LC, Pal-de Bruin KM, et al. The effect of preconception counselling on lifestyle and other behaviour before and during pregnancy. *Womens Health Issues*; 2008. p. S117-25. Exclusion Code: X 8
536. Fleming AF, Broquist HP. Biopterin and folic acid deficiency. *Am J Clin Nutr*. 1967 Jun;20(6):613-21. PMID: 6027584. Exclusion Code: X 8
537. Freire WB, Hertrampf E, Cortes F. Effect of folic acid fortification in Chile: preliminary results. *Eur J Pediatr Surg*. 2000 Dec;10 Suppl 1:42-3. PMID: 11214835. Exclusion Code: X 8
538. Froessler B, Cocchiario C, Saadat-Gilani K, et al. Intravenous iron sucrose versus oral iron ferrous sulfate for antenatal and postpartum iron deficiency anemia: A randomized trial. *Journal of Maternal-Fetal and Neonatal Medicine*; 2013. p. 654-9. Exclusion Code: X 8
539. Furness DL, Yasin N, Dekker GA, et al. Maternal red blood cell folate concentration at 10-12 weeks gestation and pregnancy outcome. *J Matern Fetal Neonatal Med*. 2012 Aug;25(8):1423-7. PMID: 22081889. Exclusion Code: X 8
540. Giles C. An account of 335 cases of megaloblastic anaemia of pregnancy and the puerperium. *J Clin Pathol*. 1966 Jan;19(1):1-11. PMID: 5904977. Exclusion Code: X 8
541. Giugliani ER, Jorge SM, Goncalves AL. Folate and vitamin B12 deficiency among parturients from Porto Alegre, Brazil. *Rev Invest Clin*. 1984 Apr-Jun;36(2):133-6. PMID: 6484331. Exclusion Code: X 8

Appendix C. Excluded Studies

542. Gochhait S, Gupta A, Shekhar S, et al. A cross-sectional study of nutritional markers in pregnancy. *Indian Journal of Hematology and Blood Transfusion*. 2013;29(4):398-9. Exclusion Code: X 8
543. Gomes TS, Lindner U, Tennekoon KH, et al. Homocysteine in small-for-gestational age and appropriate-for-gestational age preterm neonates from mothers receiving folic acid supplementation. *Clin Chem Lab Med*. 2010 Aug;48(8):1157-61. PMID: 20482301. Exclusion Code: X 8
544. Gordon TE, Leeth EA, Nowinski CJ, et al. Geographic and temporal analysis of folate-sensitive fetal malformations. *J Soc Gynecol Investig*. 2003 Jul;10(5):298-301. PMID: 12853092. Exclusion Code: X 8
545. Grandinetti CA. Inflammatory bowel disease. 2009. p. 48-9. Exclusion Code: X 8
546. Groenen PM, van Rooij IA, Peer PG, et al. Marginal maternal vitamin B12 status increases the risk of offspring with spina bifida. *Am J Obstet Gynecol*. 2004 Jul;191(1):11-7. PMID: 15295338. Exclusion Code: X 8
547. Haberg SE, London SJ, Nafstad P, et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. *J Allergy Clin Immunol*. 2011 Jan;127(1):262-4, 4.e1. PMID: 21094522. Exclusion Code: X 8
548. Haberg SE, London SJ, Ueland P, et al. Maternal folate levels in pregnancy and asthma at 3 years of age. *J Allergy Clin Immunol*. 2010;125(2):AB128. Exclusion Code: X 8
549. Haider Batool A, Bhutta Zulfiqar A. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database of Systematic Reviews*: John Wiley & Sons, Ltd; 2015. Exclusion Code: X 8
550. Hall MH. Folates and the fetus. *Lancet*. 1977 Mar 19;1(8012):648-9. PMID: 66447. Exclusion Code: X 8
551. Halliday JL, Riley M. Fortification of foods with folic acid. *N Engl J Med*. 2000 Sep 28;343(13):970-1; author reply 2. PMID: 11012326. Exclusion Code: X 8
552. Hambidge M, Hackshaw A, Wald N. Neural tube defects and serum zinc. *Br J Obstet Gynaecol*. 1993 Aug;100(8):746-9. PMID: 8399013. Exclusion Code: X 8
553. Hegde UM, Khunda S, GW MA, et al. Thalassemia, iron, and pregnancy. *Br Med J*. 1975 Aug 30;3(5982):509-11. PMID: 1164611. Exclusion Code: X 8
554. Hibbard BM, Hibbard ED. Neutrophil hypersegmentation and defective folate metabolism in pregnancy. *J Obstet Gynaecol Br Commonw*. 1971 Sep;78(9):776-80. PMID: 5097160. Exclusion Code: X 8
555. Hibbard ED, Spencer WJ. Low serum B12 levels and latent Addisonian anaemia in pregnancy. *J Obstet Gynaecol Br Commonw*. 1970 Jan;77(1):52-7. PMID: 5419870. Exclusion Code: X 8
556. Hogg BB, Tamura T, Johnston KE, et al. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. *Am J Obstet Gynecol*; 2000. p. 805-9. Exclusion Code: X 8
557. Huch R. Anaemia during pregnancy. *Med Hyg (Geneve)*. 1999;57(2264):1502-5. Exclusion Code: X 8
558. Imdad A, Yakoob MY, Bhutta ZA. The effect of folic acid, protein energy and multiple micronutrient supplements in pregnancy on stillbirths. *BMC Public Health*. 2011;11 Suppl 3:S4. PMID: 21501455. Exclusion Code: X 8
559. Janz D. Are antiepileptic drugs harmful when taken during pregnancy? *J Perinat Med*. 1994;22(5):367-77. PMID: 7791011. Exclusion Code: X 8
560. Johnston RB, Jr. Will increasing folic acid in fortified grain products further reduce neural tube defects without causing harm?: consideration of the evidence. *Pediatr Res*. 2008 Jan;63(1):2-8. PMID: 18043498. Exclusion Code: X 8
561. Jongbloet PH, Zielhuis SA, Pasker-de Jong PC. Outcomes in pregnancy. *Lancet*. 2002 Jun 22;359(9324):2204. PMID: 12091012. Exclusion Code: X 8
562. Joy J, McClure N, Cooke IE. A comparison of spontaneously conceived twins and twins conceived by artificial reproductive technologies. *J Obstet Gynaecol*. 2008 Aug;28(6):580-5. PMID: 19003649. Exclusion Code: X 8
563. Joy R, Dick A, Love C. Pregnant women with epilepsy: Incidence, management and outcomes. *Arch Dis Child Fetal Neonatal Ed*. 2010;95:Fa103-Fa4. Exclusion Code: X 8

Appendix C. Excluded Studies

564. Kancherla V, Druschel CM, Oakley Jr GP. Population-based study to determine mortality in spina bifida: New York State congenital malformations registry, 1983 to 2006. *Birth Defects Research Part A - Clinical and Molecular Teratology*. 2014;100(8):563-75. Exclusion Code: X 8
565. Kapur B, Soldin OP, Koren G. Potential prevention of neural tube defects by assessment of women of childbearing age through monitoring of folate. *Ther Drug Monit*. 2002 Oct;24(5):628-30. PMID: 12352934. Exclusion Code: X 8
566. Kennedy DA, Stern SJ, Moretti M, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol*. 2011 Feb;35(1):2-10. PMID: 21177150. Exclusion Code: X 8
567. Kershaw PW, Girdwood RH. SOME INVESTIGATIONS OF FOLIC-ACID DEFICIENCY. *Scott Med J*. 1964 May;9:201-12. PMID: 14146483. Exclusion Code: X 8
568. Khanna S, Dube B, Kumar S. Anaemia of pregnancy in northern India. Nature and therapeutic follow-up. *Trop Geogr Med*. 1977 Mar;29(1):24-8. PMID: 883006. Exclusion Code: X 8
569. Kim JH, Jeong KS, Ha EH, et al. Relationship between maternal mid pregnancy folate levels and the risk of allergic and respiratory diseases in early childhood: The mothers and children's environmental health (MOCEH) study. *J Allergy Clin Immunol*. 2013;131(2):AB200. Exclusion Code: X 8
570. Kim JH, Jeong KS, Ha EH, et al. Prenatal and postnatal exposure to folate and risk of allergic and respiratory diseases in children: The mothers and children's environmental health study in South Korea. *Allergy: European Journal of Allergy and Clinical Immunology*. 2013;68:54-5. Exclusion Code: X 8
571. Kirke PN, Daly LE, Molloy A, et al. Maternal folate status and risk of neural tube defects. *Lancet*. 1996 Jul 6;348(9019):67-8. PMID: 8691964. Exclusion Code: X 8
572. Kirke PN, Molloy AM, Daly LE, et al. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Q J Med*. 1993 Nov;86(11):703-8. PMID: 8265769. Exclusion Code: X 8
573. Koc A, Kocyigit A, Soran M, et al. High frequency of maternal vitamin B12 deficiency as an important cause of infantile vitamin B12 deficiency in Sanliurfa province of Turkey. *Eur J Nutr*. 2006 Aug;45(5):291-7. PMID: 16601915. Exclusion Code: X 8
574. Koch MC, Stegmann K, Ziegler A, et al. Evaluation of the MTHFR C677T allele and the MTHFR gene locus in a German spina bifida population. *Eur J Pediatr*. 1998 Jun;157(6):487-92. PMID: 9667406. Exclusion Code: X 8
575. Kosus N, Kosus A, Hizli D, et al. Can serum ferritin, vitamin B(12) and folic acid levels affect serum screening tests during pregnancy? *J Matern Fetal Neonatal Med*. 2012 Sep;25(9):1674-7. PMID: 22273004. Exclusion Code: X 8
576. Laurence KM. Prevention of neural tube defects by improvement in maternal diet and preconceptional folic acid supplementation. *Prog Clin Biol Res*. 1985;163b:383-8. PMID: 3885240. Exclusion Code: X 8
577. Lawrence C, Klipstein FA. Megaloblastic anemia of pregnancy in New York City. *Ann Intern Med*. 1967 Jan;66(1):25-34. PMID: 6066669. Exclusion Code: X 8
578. Leck I. Folates and the fetus. *Lancet*. 1977 May 21;1(8021):1099-100. PMID: 68194. Exclusion Code: X 8
579. Lindhe BA. Pregnancy anemia with special reference to folic acid deficiency. *Acta Obstet Gynecol Scand*. 1967;46(7):Suppl 7:117+. PMID: 6080709. Exclusion Code: X 8
580. Lindhout D, Wegner I. Periconceptional folic acid supplementation in pregnancies with antiepileptic drugs use. *Epilepsia*. 2013;54:131. Exclusion Code: X 8
581. Manizheh SM, Mandana S, Hassan A, et al. Comparison study on the effect of prenatal administration of high dose and low dose folic acid. *Saudi Med J*; 2009. p. 88-97. Exclusion Code: X 8
582. Matos A, Ferreira J, Portelinha A, et al. Study of C677T methylene tetrahydrofolate reductase (MTHFR) polymorphism in preeclampsia. *Pregnancy Hypertens*. 2011;1(3-4):284. Exclusion Code: X 8
583. Merialdi M, Caulfield LE, Zavaleta N, et al. Randomized controlled trial of prenatal zinc supplementation and the development of fetal heart rate. *Am J Obstet Gynecol*. 2004 Apr;190(4):1106-12. PMID: 15118650. Exclusion Code: X 8

Appendix C. Excluded Studies

584. Molen EF, Verbruggen B, Nováková I, et al. Hyperhomocysteinemia and other thrombotic risk factors in women with placental vasculopathy. *BJOG*; 2000. p. 785-91. Exclusion Code: X 8
585. Murphy M, Whiteman D, Stone D, et al. Dietary folate and the prevalence of neural tube defects in the British Isles: the past two decades. *BJOG*. 2000 Jul;107(7):885-9. PMID: 10901560. Exclusion Code: X 8
586. Nagy GR, Gyorffy B, Galamb O, et al. Use of routinely collected amniotic fluid for whole-genome expression analysis of polygenic disorders. *Clin Chem*. 2006 Nov;52(11):2013-20. PMID: 17008366. Exclusion Code: X 8
587. Nazer HJ, Cifuentes OL, Aguila RA, et al. [Effects of folic acid fortification in the rates of malformations at birth in Chile]. *Rev Med Chil*. 2007 Feb;135(2):198-204. PMID: 17406737. Exclusion Code: X 8
588. Ohya Y, Futamura M, Saito H, et al. Maternal retinol intake during pregnancy was a risk factor of offspring's atopic eczema at 18 months. *Allergy: European Journal of Allergy and Clinical Immunology*. 2010;65:310. Exclusion Code: X 8
589. Padula AM, Yang W, Carmichael SL, et al. Traffic-related air pollution, neighborhood socioeconomic factors, and neural tube defects in the San Joaquin Valley of California. *Birth Defects Research Part A - Clinical and Molecular Teratology*. 2015;103(5):382. Exclusion Code: X 8
590. Padula AM, Yang W, Carmichael SL, et al. Air Pollution, Neighbourhood Socioeconomic Factors, and Neural Tube Defects in the San Joaquin Valley of California. *Paediatr Perinat Epidemiol*. 2015;29(6):536-45. Exclusion Code: X 8
591. Sata F, Yamada H, Kishi R, et al. Maternal folate, alcohol and energy metabolism-related gene polymorphisms and the risk of recurrent pregnancy loss. *J Dev Orig Health Dis*. 2012 Oct;3(5):327-32. PMID: 25102261. Exclusion Code: X 8
592. Shah PS, Ohlsson A. Effects of prenatal multimicronutrient supplementation on pregnancy outcomes: a meta-analysis (Structured abstract). *CMAJ*; 2009. p. E99-e108. Exclusion Code: X 8
593. Simmons CJ, Mosley BS, Fulton-Bond CA, et al. Birth defects in Arkansas: is folic acid fortification making a difference? *Birth Defects Res A Clin Mol Teratol*. 2004 Sep;70(9):559-64. PMID: 15368553. Exclusion Code: X 8
594. Thame G, Guerra-Shinohara EM, Moron AF. Serum folate by two methods in pregnant women carrying fetuses with neural tube defects. *Clin Chem*. 2002 Jul;48(7):1094-5. PMID: 12089179. Exclusion Code: X 8
595. Trivedi M, Sharma S, Rifas-Shiman S, et al. Maternal intake of dietary methyl donors in pregnancy and childhood asthma at 7 years. *Am J Respir Crit Care Med*. 2014;189. Exclusion Code: X 8
596. Valera-Gran D, Garcia de la Hera M, Navarrete-Munoz EM, et al. Folic acid supplements during pregnancy and child psychomotor development after the first year of life. *JAMA Pediatr*. 2014 Nov;168(11):e142611. PMID: 25365251. Exclusion Code: X 8
597. van der Pal-de Bruin KM, van der Heijden PG, Buitendijk SE, et al. Periconceptional folic acid use and the prevalence of neural tube defects in The Netherlands. *Eur J Obstet Gynecol Reprod Biol*. 2003 May 1;108(1):33-9. PMID: 12694967. Exclusion Code: X 8
598. Volcik KA, Shaw GM, Lammer EJ, et al. Evaluation of infant methylenetetrahydrofolate reductase genotype, maternal vitamin use, and risk of high versus low level spina bifida defects. *Birth Defects Res A Clin Mol Teratol*. 2003 Mar;67(3):154-7. PMID: 12797455. Exclusion Code: X 8
599. Waller DK, Tita AT, Annegers JF. Rates of twinning before and after fortification of foods in the US with folic acid, Texas, 1996 to 1998. *Paediatr Perinat Epidemiol*. 2003 Oct;17(4):378-83. PMID: 14629320. Exclusion Code: X 8
600. Wang T, Zhang HP, Liang ZA, et al. Is folate at risk for development of asthma or other allergic diseases? *Am J Respir Crit Care Med*. 2015;191. Exclusion Code: X 8
601. Zlotogora J, Amitai Y, Leventhal A. Surveillance of neural tube defects in Israel: the effect of the recommendation for periconceptional folic acid. *Isr Med Assoc J*. 2006 Sep;8(9):601-4. PMID: 17058407. Exclusion Code: X 8

Appendix C. Excluded Studies

602. Persad VL, Van den Hof MC, Dube JM, et al. Incidence of open neural tube defects in Nova Scotia after folic acid fortification. *CMAJ*. 2002 Aug 6;167(3):241-5. PMID: 12186168. Exclusion Code: X 8
603. Haider BA, Olofin I, Wang M, et al. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis (Structured abstract). *BMJ*; 2013. p. f3443. Exclusion Code: X 8
604. Czeizel AE, Medveczky E. Periconceptional multivitamin supplementation and multimalformed offspring. *Obstet Gynecol*; 2003. p. 1255-61. Exclusion Code: X 8
605. Alwan NA, Greenwood DC, Simpson NA, et al. The relationship between dietary supplement use in late pregnancy and birth outcomes: a cohort study in British women. *BJOG*. 2010 Jun;117(7):821-9. PMID: 20353456. Exclusion Code: X 8
606. Bergen NE, Timmermans S, Hofman A, et al. First trimester homocysteine and folate levels are associated with increased adverse pregnancy outcomes. *Reprod Sci*. 2011;18(3):164A. Exclusion Code: X 8
607. Evers IM, de Valk HW, Visser GH. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ*. 2004 Apr 17;328(7445):915. PMID: 15066886. Exclusion Code: X 8
608. Thompson S, Torres M, Stevenson R, et al. Periconceptional vitamin use, dietary folate and occurrent neural tube defected pregnancies in a high risk population. *Ann Epidemiol*. 2000 Oct 1;10(7):476. PMID: 11018417. Exclusion Code: X 8
609. Thompson SJ, Torres ME, Stevenson RE, et al. Periconceptional multivitamin folic acid use, dietary folate, total folate and risk of neural tube defects in South Carolina. *Ann Epidemiol*. 2003 Jul;13(6):412-8. PMID: 12875798. Exclusion Code: X 8
610. Lindsay K, McNulty B, Brennan L, et al. Dietary intakes in obese pregnancy. *Proc Nutr Soc*. 2013;72:E163. Exclusion Code: X 8
611. Lopez-Quesada E, Vilaseca MA, Lailla JM. Plasma total homocysteine in uncomplicated pregnancy and in preeclampsia. *Eur J Obstet Gynecol Reprod Biol*. 2003 May 1;108(1):45-9. PMID: 12694969. Exclusion Code: X 8
612. Smithells RW, Sheppard S, Schorah CJ. Vitamin deficiencies and neural tube defects. *Arch Dis Child*. 1976 Dec;51(12):944-50. PMID: 1015847. Exclusion Code: X 8
613. Wasserman CR, Shaw GM, Selvin S, et al. Socioeconomic status, neighborhood social conditions, and neural tube defects. *Am J Public Health*. 1998 Nov;88(11):1674-80. PMID: 9807535. Exclusion Code: X 8
614. de Walle HE, van der Pal KM, de Jong-van den Berg LT, et al. Periconceptional folic acid in The Netherlands in 1995. Socioeconomic differences. *J Epidemiol Community Health*. 1998 Dec;52(12):826-7. PMID: 10396527. Exclusion Code: X 8
615. Ibba RM, Zoppi MA, Floris M, et al. Neural tube defects in the offspring of thalassemia carriers. *Fetal Diagn Ther*. 2003 Jan-Feb;18(1):5-7. PMID: 12566767. Exclusion Code: X 8
616. Brough L, Rees GA, Crawford MA, et al. Effect of multiple-micronutrient supplementation on maternal nutrient status, infant birth weight and gestational age at birth in a low-income, multi-ethnic population. *Br J Nutr*; 2010. p. 437-45. Exclusion Code: X 8
617. Ramakrishnan U, Grant F, Goldenberg T, et al. Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: a systematic review. *Paediatr Perinat Epidemiol*. 2012 Jul;26 Suppl 1:285-301. PMID: 22742616. Exclusion Code: X 8
618. Berg MJ, Van Dyke DC, Chenard C, et al. Folate, zinc, and vitamin B-12 intake during pregnancy and postpartum. *J Am Diet Assoc*. 2001 Feb;101(2):242-5. PMID: 11271698. Exclusion Code: X 9
619. Folic acid and neural tube defects. *Practica Pediatrica*. 1993;2(5):69-70. Exclusion Code: X 10
620. [Folic acid and neural tube defect]. *Duodecim*. 1996;112(11):983-5. PMID: 10592991. Exclusion Code: X 10
621. Folic acid during conception: Supplements or natural food? *Geneesmiddelenbulletin*. 1996;30(9):111-2. Exclusion Code: X 10
622. Ahrndt S. Folic acid during pregnancy and against depressions. *Dtsch Apoth Ztg*. 1997;137(51-52):34-47. Exclusion Code: X 10
623. Antal R, Siffel C, Czeizel E. [Prevention of anencephaly-spina bifida]. *Orv Hetil*. 1996 Jan 14;137(2):100. PMID: 8721879. Exclusion Code: X 10

Appendix C. Excluded Studies

624. Bailon ME, Landa GS. [Preventive activities in pregnancy]. *Aten Primaria*. 2003 Dec;32 Suppl 2:127-33. PMID: 15116478. Exclusion Code: X 10
625. Bodem S. Folic acid in the prevention of neural tube defects in newborns. *Pharm Ztg*. 1994;139(32):46. Exclusion Code: X 10
626. Bogнар M, Hauser P, Jakab Z, et al. [Folic acid supplementation for pregnant women in Hungary]. *Orv Hetil*. 2006 Aug 27;147(34):1633-8. PMID: 17017678. Exclusion Code: X 10
627. Burdan F. [Folic acid isn't sufficient for prevention of congenital defects. Report from the 42nd Teratologic Society Conference--6/22-27, 2002--Scottsdale Az, USA]. *Ginekol Pol*. 2002 Dec;73(12):1247-9. PMID: 14533624. Exclusion Code: X 10
628. Calvani M. [Malformations of the neural tube and vitamins: possible prevention?]. *Recenti Prog Med*. 1981 Oct;71(4):446-9. PMID: 7038803. Exclusion Code: X 10
629. Chiaffarino F, Ascone GB, Bortolus R, et al. [Effects of folic acid supplementation on pregnancy outcomes: a review of randomized clinical trials]. *Minerva Ginecol*. 2010 Aug;62(4):293-301. PMID: 20827247. Exclusion Code: X 10
630. Czeizel E. [Primary prevention of developmental abnormalities]. *Orv Hetil*. 1999 Mar 28;140(13):739-41. PMID: 10349320. Exclusion Code: X 10
631. Czeizel E, Dudas I. [Prevention of the first occurrence of anencephaly and spina bifida with periconceptional multivitamin supplementation (conclusion)]. *Orv Hetil*. 1994 Oct 16;135(42):2313-7. PMID: 7970646. Exclusion Code: X 10
632. Daltveit AK. [Prevention of neural tube defects with folic acid]. *Tidsskr Nor Laegeforen*. 2004 Sep 9;124(17):2274. PMID: 15356700. Exclusion Code: X 10
633. de la Rosa Morales V, Guzman Juarez L, Guarneros Cortes CA. [Maternal risk factors associated with anencephaly]. *Ginecol Obstet Mex*. 2000 Dec;68:476-81. PMID: 11195961. Exclusion Code: X 10
634. Eichholzer M, Weil C, Stahelin HB, et al. Folic acid to prevent spina bifida. Should wheat be fortified in combination with vitamin B6 and vitamin B12? *Schweizerische Zeitschrift fur GanzheitsMedizin*. 2002;14(2):64-74. Exclusion Code: X 10
635. Elsborg L, Norgaard-Pedersen B, Lange AP. [Folate and neural tube defect]. *Ugeskr Laeger*. 1993 Jul 19;155(29):2286-7. PMID: 8328105. Exclusion Code: X 10
636. Engin-Ustun Y, Ustun Y, Turkuoglu I, et al. Evaluation of cases with neural tube defects in an University Hospital in Eastern Anatolia. *Jinekoloji ve Obstetrik Dergisi*. 2007;21(2):84-7. Exclusion Code: X 10
637. Feferbaum R, Maspes V, Vaz FA. [Folates, vitamin b 12 and vitamin E in pregnancy and the neonatal period]. *Rev Paul Med*. 1982 Jan-Feb;99(1):19-24. PMID: 7146740. Exclusion Code: X 10
638. Fessler B. Preventive use of folic acid for women who want to become pregnant. *Dtsch Apoth Ztg*. 2005;145(36):40-2. Exclusion Code: X 10
639. Gibert MJ, Martin I, Ramos M. [Folate usage to prevent neural tube defects among population attended in a reference hospital]. *Med Clin (Barc)*. 2003 Nov 15;121(17):677-8. PMID: 14642235. Exclusion Code: X 10
640. Goeltner E. Anemia of pregnancy. *Therapiewoche*. 1981;31(5):554-9. Exclusion Code: X 10
641. Goltner E. [Folic acid deficiency and pregnancy]. *Med Klin*. 1971 Jul 2;66(27):973-6. PMID: 5089356. Exclusion Code: X 10
642. Gross N, Bruhwiler H, Luscher KP. Preconceptional folic acid prophylaxis: More promotion is required. *Gynakologie fur Hausarzte*. 2002;7(2):27-30. Exclusion Code: X 10
643. Hallmans G. [No, no mass folic acid supplementation in Sweden--not yet anyway. Incidence of cancer and cardiovascular diseases can increase and probable DNA changes are unsettling]. *Lakartidningen*. 2007 Sep 19-25;104(38):2670-2; discussion 3-4. PMID: 17969755. Exclusion Code: X 10
644. Heilmann E, Boving K, Dame WR. [Serum folic acid, vitamin b12 and iron values during pregnancy (author's transl)]. *Z Geburtshilfe Perinatol*. 1977 Dec;181(6):443-7. PMID: 602335. Exclusion Code: X 10
645. Hoffmann I. Is prevention of neural tube defects possible by means of folic acid substitution? *Fortschr Med*. 1993;111(14):53-4. Exclusion Code: X 10

Appendix C. Excluded Studies

646. Hong SC, Choi JS, Han JY, et al. Essence of preconception counseling and care. *Journal of the Korean Medical Association*. 2011;54(8):799-807. Exclusion Code: X 10
647. Jungmayr P. Folic acid administration reduces incidence of neural tube defects. *Dtsch Apoth Ztg*. 1994;134(36):49-50. Exclusion Code: X 10
648. Katan MB, Verhoef P. Uncertain effects of folic acid on disorders other than neural-tube defects [6]. *Ned Tijdschr Geneesk*. 2006;150(46):2573. Exclusion Code: X 10
649. Kharb S, Smiti N, Dipti A. Study of maternal and cord blood levels of homocysteine, vitamin B12 and folic acid in pregnancy induced hypertension. *Salud(i)Ciencia*. 2012;19(3):228-31. Exclusion Code: X 10
650. Kloosterman J, de Jong N, Rompelberg CJ, et al. [Folic acid fortification: prevention as well as promotion of cancer]. *Ned Tijdschr Geneesk*. 2006 Jul 1;150(26):1443-8. PMID: 16875264. Exclusion Code: X 10
651. Koletzko B, Von Kries R. Prevention of neural tube defects by folic acid supplementation in early pregnancy. *Monatsschr Kinderheilk*. 1995;143(10):1003-5. Exclusion Code: X 10
652. Konopka L. [The role of folic acid in megaloblastic anemias]. *Pol Tyg Lek*. 1970 Jun 22;25(25):945-8. PMID: 4913927. Exclusion Code: X 10
653. Kwee-Zuiderwijk WJM. Standard on pregnancy and puerperium of the Dutch College of General Practitioners: Information, stimulation and surveillance. *Pharm Weekbl*. 2003;138(44):1538-42. Exclusion Code: X 10
654. Lee WH, Park CH, Ko HY, et al. Two cases of pulmonary thromboembolism in young patients with hyperhomocysteinemia. *Tuberculosis and Respiratory Diseases*. 2008;64(6):460-5. Exclusion Code: X 10
655. Leemans L. [Does 5-methyltetrahydrofolate offer any advantage over folic acid?]. *J Pharm Belg*. 2012 Dec(4):16-22. PMID: 23350208. Exclusion Code: X 10
656. Levy S, Rachmilewitz EA, Izak G, et al. A therapeutic trial in anemia of pregnancy. *Isr J Med Sci*. 1968 Mar-Apr;4(2):218-22. PMID: 5650052. Exclusion Code: X 10
657. Lung-Kurt S, Boos R, Schmidt W. Toxoplasmosis during pregnancy. *Arch Gynecol Obstet*. 1993;254(1-4):637-8. Exclusion Code: X 10
658. Mathias L, Nobile L, Mathias R. Anticonvulsivants in pregnancy - fetal effects. *Revista Brasileira de Clinica e Terapeutica*. 1981;10(10):755-60. Exclusion Code: X 10
659. Metneki J, Dudas I, Czeizel AE. Higher rate of multiple births after periconceptual multivitamin supplementation. *Orv Hetil*; 1996. p. 2401-5. Exclusion Code: X 10
660. Metneki J, Dudas I, Czeizel E. [Periconceptual multivitamin administration may result in higher frequency of twin pregnancies]. *Orv Hetil*. 1996 Oct 27;137(43):2401-5. PMID: 8992436. Exclusion Code: X 10
661. Meyer R. Folic acids in the prevention of spina bifida. *Pharm Ztg*. 1991;136(33):30-2. Exclusion Code: X 10
662. Molins A. [Antiepileptic drugs in the elderly , pregnant women, children and in systemic disorders]. *Rev Neurol*. 2000 May 1-15;30(9):865-72. PMID: 10870202. Exclusion Code: X 10
663. Nexo E. [Folate and neural tube defect]. *Ugeskr Laeger*. 1993 May 17;155(20):1567-8. PMID: 8316992. Exclusion Code: X 10
664. Olivier D. [Prevention of neural tube defects: prescription of folic acid before conception]. *J Gynecol Obstet Biol Reprod (Paris)*. 2004 Apr;33(2):168. PMID: 15052183. Exclusion Code: X 10
665. Orn P. [Reduced number of neural tube defects after folic acid supplementation in China]. *Lakartidningen*. 1999 Dec 1;96(48):5336. PMID: 10612975. Exclusion Code: X 10
666. Potier de Courcy G. [Folic acid (vitamin B 9). Consequences of a deficiency, of excessive vitamin B 9, and value of systematic supplementation]. *J Gynecol Obstet Biol Reprod (Paris)*. 1997;26(3 Suppl):75-83. PMID: 9471469. Exclusion Code: X 10
667. Potier De Courcy G, Frelut ML, Gourchala F, et al. Folate status and fetal development. *Gazette Medicale*. 1993;100(29):23-7. Exclusion Code: X 10
668. Queisser-Luft A, Spranger J. Congenital malformations. *Deutsches Arzteblatt*. 2006;103(38):2464-71. Exclusion Code: X 10
669. Rasmussen LB, Andersen NL, Ovesen LF. [Folacin and neural tube defect]. *Ugeskr Laeger*. 1993 Jul 19;155(29):2287-8. PMID: 8328106. Exclusion Code: X 10

Appendix C. Excluded Studies

670. Reinke C. Prevention of neural tube defects: Folic acid during pregnancy. *Schweizerische Apotheker Zeitung*. 1993;131(10):317. Exclusion Code: X 10
671. Rincon-Pabon D, Ramirez-Velez R. Social determinants, prenatal care and postpartum depression: Results from the 2010 National Nutrition Survey: A cross sectional study. *Progresos de Obstetricia y Ginecologia*. 2013;56(10):508-14. Exclusion Code: X 10
672. Ritvanen A. [Neural tube defects and folic acid]. *Duodecim*. 1996;112(11):975-82. PMID: 10592990. Exclusion Code: X 10
673. Roegholt LP. [Folic acid for all fertile women?]. *Ned Tijdschr Geneesk*. 1993 Aug 14;137(33):1683; author reply -4. PMID: 7741809. Exclusion Code: X 10
674. Rosch C, Steinbicker V, Kolbe M, et al. [Prevention of folic acid-induced malformations--recommendations and reality]. *Gesundheitswesen*. 1999 Feb;61(2):82-5. PMID: 10226395. Exclusion Code: X 10
675. Schneider KTM, Qattawi O. Folic acid substitution before and during pregnancy. *Tagliche Praxis*. 2002;43(4):908-10. Exclusion Code: X 10
676. Steegers-Theunissen RP, Boers GH, Eskes TK. [Prevention of neural tube defects using folic acid]. *Ned Tijdschr Geneesk*. 1993 Jun 26;137(26):1294-8. PMID: 7688446. Exclusion Code: X 10
677. Suske-Zirpel VP. Prevention during pregnancy and lactation: More intensive advice on folate. *Pharm Ztg*. 2009;154(11). Exclusion Code: X 10
678. Teichmann A, Schlicht E. [Folic acid in pregnancy]. *Med Monatsschr Pharm*. 1995 Nov;18(11):333-4. PMID: 8544791. Exclusion Code: X 10
679. Thamm M, Mensink GB, Hermann-Kunz E. [Folic acid status]. *Gesundheitswesen*. 1998 Dec;60 Suppl 2:S87-8. PMID: 10063729. Exclusion Code: X 10
680. Tonz O. Folic acid prophylaxis in the prevention of neural tube defects. *Schweizer Apothekerzeitung*. 1996;134(6):124-7. Exclusion Code: X 10
681. Van Veen WA. Folic acid for expectant mothers. *Huisarts en Wetenschap*. 1994;37(3):90-3. Exclusion Code: X 10
682. Vargas MH, Campos-Bedolla P, Segura P. [Inverse association between asthma and neural tube defects: a binational ecological study]. *Salud Publica Mex*. 2012 Jul-Aug;54(4):418-24. PMID: 22832834. Exclusion Code: X 10
683. Vollmer H. Prevention of neural tube defects by folic acid. *Z Allgemeinmed*. 1995;71(23):1818. Exclusion Code: X 10
684. Wijnja L. [Folic acid for all pregnant women?]. *Ned Tijdschr Geneesk*. 1993 Oct 16;137(42):2164-5. PMID: 7661891. Exclusion Code: X 10
685. Zabala R, Waisman I, Corelli M, et al. [Folic acid for neural tube defects prevention: consumption and information in fertil-age women in Centro Cuyo Region]. *Arch Argent Pediatr*. 2008 Aug;106(4):295-301. PMID: 18766275. Exclusion Code: X 10
686. Anneren G. [Folic acid therapy recommended for women with increased risk of giving birth to children with neural tube defects]. *Lakartidningen*. 1991 Nov 27;88(48):4110. PMID: 1956246. Exclusion Code: X 10
687. The significance of folic acid during pregnancy. *Med Welt*. 1993;44(1):88-90. Exclusion Code: X 11
688. Bense A, Harmos C, Endrodi T, et al. Prevention of anaemia in pregnancy with combined iron supplementation containing ferro-sulfate, vitamin B12 and folic acid. *Magyar Noorvosok Lapja*. 2005;68(1):33-7. Exclusion Code: X 11
689. Bortolus R. Exclusion Code: X 11
690. Hajnzic TF. Macrocytic anaemias in children. *Paediatrica Croatica, Supplement*. 2002;46(2):31-5. Exclusion Code: X 11
691. Sarella A, Glynou A, Tasiopoulou I. The role of folic acid in pregnancy. *Epiteorese Klinikes Farmakologias kai Farmakokinetikes*. 2014;32(1):41-4. Exclusion Code: X 11

Appendix D Table 1. Quality Assessments for All Included Systematic Reviews and Meta-Analyses (Part 1)

First Author, Year	Comprehensive literature search performed?	Was the status of publication used as an inclusion criterion?	Was an a priori design provided?	Was a list of studies provided?	Was there explicit inclusion/exclusion criteria for the selection of studies?	Were the characteristics of the included studies provided?	Was the likelihood of publication bias assessed?	Was there duplicate study selection and data extraction?
Brown et al, 2014 ¹⁴³	No	Unclear	No	No	No	Yes	No	Unclear
Crider et al, 2013 ¹⁰¹	Yes	No	Unclear	No	Yes	Yes	Yes	Yes
Goh et al, 2006 ⁸⁴	Yes	No	No	No	No	No	Yes	Yes
Wang et al, 2015 ¹⁰⁹	Yes	No	Yes	Yes	Unclear	Yes	Yes	Yes
Wolff et al, 2009 ³⁶ Wolff et al, 2009 ⁸⁶	Yes	Unclear	Yes	Yes	Yes	Yes	No	Yes
Yang et al, 2014 ¹⁰⁸	Yes	No	Unclear	No	Yes	Yes	Yes	Unclear

Appendix D Table 2. Quality Assessments for All Included Systematic Reviews and Meta-Analyses (Part 2)

First Author, Year	Was the scientific quality of the included studies assessed and documented?	Was the conflict of interest included?	Was the scientific quality of the included studies used appropriately in formulating conclusions?	Were the methods used to combine the findings of studies appropriate?	Were the authors' conclusions supported by the evidence they presented?	Quality Rating	Comments
Brown et al, 2014 ¹⁴³	No	No	NA	Unclear	No	Poor	Although the review is titled a systematic review the crucial aspects of a systematic review including details on searches, review, risk of bias appraisal, and synthesis are NR.
Crider et al, 2013 ¹⁰¹	Yes	Unclear	Yes	Yes	Yes	Good	
Goh et al, 2006 ⁸⁴	No	No	No	Yes	Yes	Poor	The quality of studies is not assessed. The characteristics of studies included in the meta-analysis were not presented; Includes an appropriate synthesis and statistical testing, but does not include a discussion of publication bias.
Wang et al, 2015 ¹⁰⁹	Yes	Yes	Yes	Yes	Yes	Fair	A list of excluded studies and the inclusion criteria was not provided.
Wolff et al, 2009 ³⁶ Wolff et al, 2009 ⁸⁶	Yes	no	Yes	NA	Yes	Good	
Yang et al, 2014 ¹⁰⁸	Yes	Unclear	Unclear	Yes	Yes	Fair	Unclear how authors used risk of bias assessments in the analysis. The study noted that they included high quality studies.

NA = not applicable

Good: Recent, relevant review with comprehensive sources and search strategies; explicit and relevant selection criteria; standard appraisal of included studies; and valid conclusions.

Fair: Recent, relevant review that is not clearly biased but lacks comprehensive sources and search strategies.

Poor: Outdated, irrelevant, or biased review without systematic search for studies, explicit selection criteria, or standard appraisal of studies.

Appendix D Table 3. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 1)

First Author, Year	Were eligibility criteria described clearly?	Are the inclusion/exclusion criteria measured using valid and reliable measures, implemented across all study participants?	Was the symptom status of subjects determined using valid and reliable methods, implemented across all study participant?	Was the intervention or exposure clearly defined, across all study participants?	Was method of randomization adequate?	Was allocation concealment adequate?
Abe et al, 2014 ¹⁴⁴	No	Unclear	NA	Unclear	NA	NA
Abe et al, 2013 ¹⁴⁵	No	Unclear	NA	No	NA	NA
Abe et al, 2015 ¹⁴⁶	No	Unclear	NA	Unclear	NA	NA
Agopian et al, 2013 ⁹	Yes	Yes	Yes	No	NA	NA
Ahrens et al, 2011 ¹¹	Yes	Yes	Unclear	Yes	NA	NA
Berry et al, 2004 ¹⁴⁷	Yes	Yes	Yes	No	NA	NA
Botto et al, 2002 ¹⁴⁸	Yes	Yes	Yes	Yes	NA	NA
Bower et al, 1989 ¹⁴⁹	Yes	Yes	Yes	No	NA	NA
Bower, 1992 ¹⁵⁰						
Brescianini et al, 2012 ¹⁵¹	No	Unclear	Yes	Unclear	NA	NA
Carmichael et al, 2010 ¹⁵²	Yes	Yes	Yes	No	NA	NA
Chandler et al, 2012 ¹⁵³	Yes	Yes	Yes	No	NA	NA
Charles et al, 2004 ¹⁵⁴	Yes	Yes	Yes	Yes	No	Yes
Charles et al, 2005 ¹⁵⁵						
Taylor et al, 2015 ¹⁵⁶						
Correa et al, 2012 ²⁸	Yes	Yes	Yes	No	NA	NA
Czeizel et al, 2004 ¹⁵⁷	Yes	Yes	Yes	Yes	NA	NA
Czeizel et al, 2004 ⁸¹	Yes	Yes	NA	Yes	NA	NA
Czeizel et al, 1992 ⁸⁸ , Czeizel et al, 1993 ⁸⁹ , Czeizel et al, 1993 ⁹² , Czeizel et al, 1994 ⁹⁰ , Czeizel et al, 1994 ⁹¹ , Czeizel et al, 1996 ⁹⁴ , Czeizel et al, 1998 ⁹³	Yes	Yes	NA	Yes	Yes	Unclear
Czeizel et al, 1996 ¹⁵⁸	Yes	Yes	Yes	Yes	NA	NA
De Marco et al, 2011 ¹⁵⁹	Yes	Yes	Yes	Yes	NA	NA
DeSoto et al, 2012 ¹⁶⁰	Yes	Yes	Yes	No	NA	NA
Ericson et al, 2001 ¹⁶¹	No	No	NA	No	NA	NA
Gildestad et al, 2013 ¹⁶²	Unclear	Unclear	Unclear	Unclear	NA	NA
Haberg et al, 1994 ¹⁶³	Unclear	Unclear	NA	Unclear	NA	NA
Hernandez et al, 2001 ⁹⁵	Yes	Yes	Yes	Unclear	NA	NA
Kallen et al, 2004 ¹⁶⁴	Yes	Yes	Yes	No	NA	NA
Kallen et al, 2007 ¹⁶⁵	Yes	Yes	NA	No	NA	NA
Kondo et al, 2015 ¹⁶⁶	No	Yes	Unclear	No	NA	NA
Medvezky et al, 2003 ¹⁶⁷	Yes	Yes	Yes	No	NA	NA
Mills et al, 1989 ⁹⁶	No	Yes	Yes	Yes	NA	NA

Appendix D Table 3. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 1)

First Author, Year	Were eligibility criteria described clearly?	Are the inclusion/exclusion criteria measured using valid and reliable measures, implemented across all study participants?	Was the symptom status of subjects determined using valid and reliable methods, implemented across all study participant?	Was the intervention or exposure clearly defined, across all study participants?	Was method of randomization adequate?	Was allocation concealment adequate?
Moore et al, 2003 ⁹⁸ Milunsky et al, 1989 ⁹⁷	Yes	Yes	NA	Yes	NA	NA
Mosley 2009 et al, ⁸⁷	Yes	Yes	Yes	Yes	NA	NA
Mulinare et al, 1988 ¹⁶⁸	Yes	Yes	Yes	Yes	NA	NA
Ohya et al, 2011 ¹⁶⁹	No	Unclear	NA	Unclear	NA	NA
Shaw et al, 2002 ¹⁷⁰	Yes	Yes	Yes	No	NA	NA
Shaw et al, 1995 ⁸²	Yes	Yes	Yes	Yes	NA	NA
Suarez et al, 2000 ²²	Yes	Yes	Unclear	Yes	NA	NA
Veeranki et al, 2014 ¹⁷¹	Yes	Yes	NA	No	NA	NA
Veeranki et al, 2014 ¹⁷²	No	Unclear	NA	No	NA	NA
Veeranki et al, 2015 ¹⁷³	Yes	Yes	NA	No	NA	NA
Vollset et al, 2005 ⁸³	Yes	Yes	Yes	No	NA	NA
Werler et al, 1993 ⁹⁹	Yes	Unclear	Yes	Yes	NA	NA

NA = not applicable

Appendix D Table 4. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 2)

First Author, Year	Was the strategy for recruiting participants into the study the same across study groups?	Do start of followup and start of intervention coincide?	Are baseline characteristics similar between groups?	Did the study control for baseline differences between groups?	Were the participants and the administrators of the intervention blinded to the intervention or exposure status of participants?	Were the outcome assessors blinded to the outcome status of participants?
Abe et al, 2014 ¹⁴⁴	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Abe et al, 2013 ¹⁴⁵	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Abe et al, 2015 ¹⁴⁶	Unclear	Unclear	Unclear	Unclear	No	Unclear
Agopian et al, 2013 ⁹	No	NA	No	Yes	NA	NA
Ahrens et al, 2011 ¹¹	Unclear	NA	No	Yes	NA	NA
Berry et al, 2004 ¹⁴⁷	NA (registry)	No	Unclear	Unclear	NA	Unclear
Botto et al, 2002 ¹⁴⁸	No	NA	Yes	Yes	NA	Yes
Bower et al, 1989 ¹⁴⁹ Bower et al, 1992 ¹⁵⁰	No	NA	Unclear	Unclear	NA	NA
Brescianini et al, 2012 ¹⁵¹	Unclear	No	Unclear	Unclear	NA	NA
Carmichael et al, 2010 ¹⁵²	Yes	NA	No	Yes	NA	NA
Chandler et al, 2012 ¹⁵³	No	NA	No	Yes	NA	NA
Charles et al, 2004 ¹⁵⁴ Charles et al, 2005 ¹⁵⁵ Taylor et al, 2015 ¹⁵⁶	NA	NA	No	Yes	No	Unclear
Correa et al, 2012 ²⁸	No	NA	No	Yes	NA	NA
Czeizel et al, 2004 ¹⁵⁷	Yes	No	No	No	NA	NA
Czeizel et al, 2004 ⁸¹	No	no	No	Yes	No	Unclear
Czeizel et al, 1992 ⁸⁸ , Czeizel et al, 1993 ⁸⁹ , Czeizel et al, 1993 ⁹² Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³	NA	Yes	Yes	NA	Unclear	Unclear
Czeizel et al, 1996 ¹⁵⁸	Yes	No	Unclear	Unclear	NA	NA
De Marco et al, 2011 ¹⁵⁹	Yes	NA	No	Yes	NA	NA
DeSoto et al, 2012 ¹⁶⁰	Yes	NA	Yes	Yes	NA	NA
Ericson et al, 2001 ¹⁶¹	Unclear	No	Unclear	Unclear	Unclear	Unclear
Gildestad et al, 2013 ¹⁶²	Unclear	No	Unclear	Unclear	Unclear	Unclear
Haberg et al, 1994 ¹⁶³	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Hernandez et al, 2001 ⁹⁵	Yes	NA	No	Yes	NA	Unclear
Kallen et al, 2004 ¹⁶⁴	NA (registry)	No	Unclear	Unclear	NA	Unclear
Kallen et al, 2007 ¹⁶⁵	Yes	No	No	Yes	Unclear	Unclear
Kondo et al, 2015 ¹⁶⁶	No	NA	No	Yes	NA	NA
Medvezky et al, 2003 ¹⁶⁷	Yes	NA	Unclear	Unclear	NA	NA

Appendix D Table 4. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 2)

First Author, Year	Was the strategy for recruiting participants into the study the same across study groups?	Do start of followup and start of intervention coincide?	Are baseline characteristics similar between groups?	Did the study control for baseline differences between groups?	Were the participants and the administrators of the intervention or exposure status of participants blinded to the outcome status of participants?	Were the outcome assessors blinded to the outcome status of participants?
Mills et al, 1989 ⁹⁶	No	NA	No	Yes	NA	Unclear
Moore et al, 2003 ⁹⁸ Milunsky et al, 1989 ⁹⁷	Yes	No	No	Yes	No	No
Mosley et al, 2009 ⁸⁷	Yes	NA	Yes	Yes	NA	Unclear
Mulinare et al, 1988 ¹⁶⁸	No	NA	Unclear	Unclear	NA	Unclear
Ohya et al, 2011 ¹⁶⁹	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Shaw et al, 2002 ¹⁷⁰	No	NA	Unclear	Unclear	NA	NA
Shaw et al, 1995 ⁸²	No	NA	No	Yes	NA	NA
Suarez et al, 2000 ²²	No	NA	Unclear	Yes	NA	NA
Veeranki et al, 2014 ¹⁷¹	Yes	No	No	Yes	Unclear	Unclear
Veeranki et al, 2014 ¹⁷²	Yes	No	Unclear	Unclear	No	Unclear
Veeranki et al, 2015 ¹⁷³	Yes	No	No	Yes	No	Yes
Vollset et al, 2005 ⁸³	Yes	Unclear	Unclear	Yes	Unclear	Unclear
Werler et al, 1993 ⁹⁹	Unclear	Yes	Unclear	No	NA	NA

NA= not applicable

Appendix D Table 5. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 3)

First Author, Year	Were outcome assessors blinded to the exposure?	Was intervention fidelity adequate?	Was there a risk of recall bias?	Did the study focus on the time period that we are interested in?	Did researchers rule out any impact from a concurrent intervention or an unintended exposure that might bias results?	Did variation from the study protocol compromise the conclusions of the study?
Abe et al, 2014 ¹⁴⁴	NA	Unclear	Unclear	Unclear	Unclear	Unclear
Abe et al, 2013 ¹⁴⁵	NA	Unclear	Yes	Unclear	No	NA
Abe et al, 2015 ¹⁴⁶	NA	Unclear	Yes	Yes	Unclear	NA
Agopian et al, 2013 ⁹	Unclear	Unclear	Unclear	Yes	Yes	NA
Ahrens et al, 2011 ¹¹	Yes	Unclear	Unclear	Yes	Yes	NA
Berry et al, 2004 ¹⁴⁷	NA	Unclear	Unclear	No	No	NA
Botto et al, 2002 ¹⁴⁸	Unclear	Yes	Unclear	Yes	Yes	NA
Bower et al, 1989 ¹⁴⁹ Bower et al, 1992 ¹⁵⁰	Unclear	Unclear	Unclear	Unclear	Yes	NA
Brescianini et al, 2012 ¹⁵¹	Unclear	Unclear	Unclear	Unclear	No	NA
Carmichael et al, 2010 ¹⁵²	Unclear	Unclear	Unclear	No	Yes	NA
Chandler et al, 2012 ¹⁵³	Unclear	Unclear	Yes	Yes	Yes	NA
Charles et al, 2004 ¹⁵⁴ Charles et al, 2005 ¹⁵⁵ Taylor et al, 2015 ¹⁵⁶	NA	Yes	No	Yes	No	No
Correa et al, 2012 ²⁸	Unclear	Unclear	Yes	No	No	NA
Czeizel et al, 2004 ¹⁵⁷	Unclear	Unclear	Unclear	Unclear	No	NA
Czeizel et al, 2004 ⁸¹	Unclear	Unclear	No	Yes	No	No
Czeizel et al, 1992 ⁸⁸ , Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1993 ⁹² Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³	NA	Yes	No	Yes	No	No
Czeizel et al, 1996 ¹⁵⁸	Unclear	Unclear	Unclear	Yes	No	NA
De Marco et al, 2011 ¹⁵⁹	Unclear	Yes	Unclear	No	No	NA
DeSoto et al, 2012 ¹⁶⁰	Unclear	Unclear	Yes	No	No	NA
Ericson et al, 2001 ¹⁶¹	NA	Yes	Unclear	Unclear	No	NA
Gildestad et al, 2013 ¹⁶²	NA	Unclear	Unclear	Unclear	No	NA
Haberg, 1994 ¹⁶³	NA	Unclear	Unclear	Yes	Unclear	NA
Hernandez et al, 2001 ⁹⁵	Unclear	Yes	Unclear	No	No	NA
Kallen et al, 2004 ¹⁶⁴	NA	Unclear	Unclear	No	No	NA
Kallen et al, 2007 ¹⁶⁵	NA	Yes	Unclear	Unclear	No	NA
Kondo et al, 2015 ¹⁶⁶	No	Unclear	Yes	Unclear	No	NA
Medvezky et al, 2003 ¹⁶⁷	Unclear	Unclear	Unclear	No	Unclear	NA
Mills et al, 1989 ⁹⁶	Yes	Yes	Unclear	Yes	Yes	NA
Moore et al, 2003 ⁹⁸	NA	Unclear	Unclear	No	Yes	NA

Appendix D Table 5. Quality Assessments for All Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 3)

First Author, Year	Were outcome assessors blinded to the exposure?	Was intervention fidelity adequate?	Was there a risk of recall bias?	Did the study focus on the time period that we are interested in?	Did researchers rule out any impact from a concurrent intervention or an unintended exposure that might bias results?	Did variation from the study protocol compromise the conclusions of the study?
Milunsky et al, 1989 ⁹⁷						
Mosley et al, 2009 ⁸⁷	Unclear	Yes	Unclear	Yes	Yes	NA
Mulinare et al, 1988 ¹⁶⁸	Yes	Unclear	Yes	No	No	NA
Ohya et al, 2011 ¹⁶⁹	NA	Unclear	Unclear	Unclear	Unclear	Unclear
Shaw et al, 2002 ¹⁷⁰	Unclear	Unclear	Yes	No	Yes	NA
Shaw et al, 1995 ⁸²	Yes	Unclear	Unclear	Yes	Yes	NA
Suarez et al, 2000 ²²	Unclear	Yes	Unclear	Yes	Unclear	NA
Veeranki et al, 2014 ¹⁷¹	NA	Unclear	No	Unclear	No	NA
Veeranki et al, 2014 ¹⁷²	NA	Unclear	No	Yes	No	NA
Veeranki et al, 2015 ¹⁷³	NA	Unclear	No	Unclear	No	NA
Vollset et al, 2005 ⁸³	NA	Unclear	No	Unclear	No	NA
Werler et al, 1993 ⁹⁹	Unclear	Yes	Yes	Yes	Yes	NA

NA = not applicable

Appendix D Table 6. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 4)

First Author, Year	What was the overall attrition/overall response rate?	What was the overall differential attrition?	Did the study have high attrition or low response rate raising concern for bias?	Is the analysis conducted on an intention-to-treat basis?	Did the analysis adjust for potential confounders?	Did the study have cross-overs or contamination raising concern for bias?
Abe et al, 2014 ¹⁴⁴	Unclear	Unclear	Unclear	NA	Unclear	Unclear
Abe et al, 2013 ¹⁴⁵	Unclear	Unclear	Unclear	NA	Unclear	Unclear
Abe et al, 2015 ¹⁴⁶	Unclear	Unclear	Unclear	NA	Yes	Unclear
Agopian et al, 2013 ⁹	Response rate (overall sample in original study; see Yoon et al, 2001 companion article) G1+G2: ~74 (NR out of 7,470) G2: ~63 (NR out of 3,821)	NA	Unclear	NA	Unclear	NA
Ahrens et al, 2011 ¹¹	G1: 66% G2: 53%	NA	No	NA	Yes	NA
Berry et al, 2004 ¹⁴⁷	Unclear	Unclear	Unclear	NA	Yes	Unclear
Botto et al, 2002 ¹⁴⁸	Overall sample G1: 69% G2: 71% NTD analysis G1: NR G2: NR	NA	Unclear	NA	Unclear	NA
Bower et al, 1989 ¹⁴⁹ Bower et al, 1992 ¹⁵⁰	G1: 93% G2: 88% G3: 84%	Response rate G1: 93 (77/83) G2: 88 (77/87) G3: 84 (154/183)	No	NA	No	NA
Brescianini et al, 2012 ¹⁵¹	G1: 93% G2: 88% G3: 84%	NA	No	NA	No	NA
Carmichael et al, 2010 ¹⁵²	G1: 73% (146/200) G2: 79% (191/241) G3: 80% (626/786)	NA	No	NA	Yes	NA
Chandler et al, 2012 ¹⁵³	62% for anencephaly; 76% for spina bifida and 71% for controls	NA	No	NA	Unclear	NA
Charles et al, 2004 ¹⁵⁴ Charles et al, 2005 ¹⁵⁵ Taylor et al, 2015 ¹⁵⁶	Unclear	Unclear	Unclear	No	NA	No
Correa et al, 2012 ²⁸	Overall, 70% among mothers of case infants and 67% among mothers of control infants. Response rate NR for NTD mothers.	NA	Unclear	NA	Yes	NA

Appendix D Table 6. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 4)

First Author, Year	What was the overall attrition/overall response rate?	What was the overall differential attrition?	Did the study have high attrition or low response rate raising concern for bias?	Is the analysis conducted on an intention-to-treat basis?	Did the analysis adjust for potential confounders?	Did the study have cross-overs or contamination raising concern for bias?
Czeizel et al, 2004 ¹⁵⁷	Unclear	NA	Unclear	NA	Yes	NA
Czeizel et al, 2004 ⁸¹	Overall attrition unclear	G1: 3,069/3,981 (77.1%) G2: Unclear	Yes	No	Yes	Unclear
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1993 ⁹² Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³	1%	0.10%	No	NA	NA	No
Czeizel et al, 1996 ¹⁵⁸	63% for negative controls, rate for positive controls NR	NA	Unclear	Na	No	NA
De Marco et al, 2011 ¹⁵⁹	Response rate G1: 92% (133/145) G2: 82% (273/332)	NA	No	NA	Unclear	NA
DeSoto et al, 2012 ¹⁶⁰	Response rate G1: 48.1% (321/668) G2: 31.7% (774/2444)	NA	Yes	NA	Unclear	NA
Ericson et al, 2001 ¹⁶¹	Unclear	Unclear	Unclear	NA	Yes	No
Gildestad et al, 2013 ¹⁶²	Unclear	NA	Unclear	NA	Unclear	Unclear
Haberg et al, 1994 ¹⁶³	Unclear	Unclear	NA	Unclear	Unclear	Unclear
Hernandez et al, 2001 ⁹⁵	Response rate G1: 84% (1,242/NR) G2: 83% (6,600/NR) G3: 80% (1,626/NR) G4: NR (2,138/NR)	NA	No	NA	No	NA
Kallen et al, 2004 ¹⁶⁴	Unclear	Unclear	Unclear	NA	Yes	Unclear
Kallen et al, 2007 ¹⁶⁵	Unknown	Unclear	Unclear	NA	Yes	No
Kondo et al, 2015 ¹⁶⁶	Response rate G1: 79% G2: 56%	NA	No	NA	Yes	NA
Medvezky et al, 2003 ¹⁶⁷	96.9% cases; 96% other non-NTD cases; 83.1% controls	NA	Yes (was unclear)	NA	Unclear	NA
Mills et al, 1989 ⁹⁶	Response rate G1: 64.8%-82% (571/NR) G2: NR (546/NR) G3: NR (573/NR)	NA	No	NA	Unclear	NA

Appendix D Table 6. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 4)

First Author, Year	What was the overall attrition/overall response rate?	What was the overall differential attrition?	Did the study have high attrition or low response rate raising concern for bias?	Is the analysis conducted on an intention-to-treat basis?	Did the analysis adjust for potential confounders?	Did the study have cross-overs or contamination raising concern for bias?
Moore et al, 2003 ⁹⁸ Milunsky et al, 1989 ⁹⁷	3% (715/23,491)	Unclear	No	NA	Unclear	No
Mosley et al, 2009 ⁸⁷	62% anencephaly; 76% spina bifida; 71% controls	NA	No	NA	Yes	NA
Mulinare, 1988 ¹⁶⁸	G1: 347/519 (66.9%) G2: 2,829/4,043 (69.9%)	NA	No	NA	Yes	NA
Ohya et al, 2011 ¹⁶⁹ Shaw et al, 2002 ¹⁷⁰	Response rate G1 (NTD only): 84% G2: 76% from both control cohorts	Unclear	Unclear	NA	Unclear	Unclear
Shaw et al, 1995 ⁸²	88% both groups	NA	No	NA	Yes	NA
Suarez et al, 2000 ²²	72% cases; 53% controls	NA	No	NA	Yes	NA
Veeranki et al, 2014 ¹⁷¹	Unclear	Unclear	Unclear	NA	Yes	Unclear
Veeranki et al, 2014 ¹⁷²	Unclear	Unclear	Unclear	NA	Yes	Unclear
Veeranki et al, 2015 ¹⁷³	Unclear	Unclear	Unclear	NA	Yes	Unclear
Vollset et al, 2005 ⁸³	Unclear	Unclear	Unclear	NA	Yes	Unclear
Werler et al, 1993 ⁹⁹	G1: 567-436/567=76.9% G2: 3672-2,615/3,672=71.2%	NA	No	NA	Yes	NA

G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4; ITT = intent to treat; NA = not applicable; NR = not reported; NTD = neural tube defect

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Abe et al, 2014 ¹⁴⁴	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Abe et al, 2013 ¹⁴⁵	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Abe et al, 2015 ¹⁴⁶	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Agopian et al, 2013 ⁹	Yes	NA	NA	NA	Unclear	Fair	Most of the major confounders were adjusted for in the analyses. However, there was still a possibility of residual confounding because some NTD-specific variables not controlled for, specifically, previous NTD pregnancy or having or having partner(s) with NTDs. Cases and controls selected from different populations. Specifically, cases could be stillborn infants or therapeutic abortions, while all controls were liveborn infants. Definition of exposure not defined clearly. Positive response to folic acid supplement use question could have indicated any frequency of usage.
Ahrens et al, 2011 ¹¹	Yes	NA	NA	NA	Unclear	Fair	Ascertainment of cases for non-live births is not routine. Unclear how missing data was handled (although “all women were included” in the analysis).
Berry et al, 2004 ¹⁴⁷	Yes	Yes	Yes	Yes	Unclear	Poor	The study itself does not describe the data source well, but it cites other studies that do. Based on these other studies, we infer that the exposure period starts before pregnancy and extends to first attendance (in 90% of cases before the end of the first trimester and usually around week 10) with lack of clarity on degree of exposure, difficult to clearly distinguish exposure from non-exposure; authors did not specify definition of exposure clearly (defined as any vs. no supplement), so the degree of adherence is unclear; although the study does not appear to account for fetal deaths, the resulting selection bias would serve to mute rather than exaggerate the effect of FA on twinning; study uses probabilistic simulations to assess bias caused by misclassification of the use of IVF.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Botto, 2002 ¹⁴⁸	es	NA	NA	NA	Unclear	Poor	Cases and controls selected from different populations. Cases could be liveborn or stillborn infants, while all controls were liveborn infants. No information about 1) timing of data collection given to assess recall bias; 2) whether potential NTD-specific confounders were measured or adjusted for in analyses; 3) exposure to dietary folate; 4) response rates for patients analyzed for analysis of NTD outcomes; or 5) whether MVs contained similar and clinically effective doses of FA. Missing data not accounted for in an ITT analysis; covers a 6 month period of exposure (3 months before to 3 months after pregnancy); no controls for concurrent interventions such as exposure to dietary folate, however, this sample was drawn before dietary supplementation; significant risk of recall bias because women asked to remember for a period ranging from 1968-1980. Only still births, no information on terminations. Cases included live an still-borns controls only
Bower et al, 1989 ¹⁴⁹ Bower et al, 1992 ¹⁵⁰	Yes	NA	NA	NA	Unclear	Poor	Cases and controls selected from different populations. Cases could be stillborn infants or elective terminations following antenatal NTD diagnosis, while Control group 1 included liveborn infants and terminated pregnancies with non-NTD malformations and Control group 2 included live born infants only. Possibility of residual confounding because no NTD-specific variables controlled for in analyses. Definition of exposure not defined clearly. Positive response to folic acid supplement use question could have indicated any frequency of usage. Risk of recall bias because mothers interviewed up to 99 weeks after last menstrual period. Also, minor risk of interviewer bias during 5 interviews because interviewers unintentionally learned case-control status.
Brescianini et al, 2012 ¹⁵¹	Unclear	NA	NA	NA	Unclear	Unclear	Meeting abstract with very little information to base judgment on most domains. Although study does not appear to account for fetal deaths, the resulting selection bias would serve to mute rather than exaggerate the effect of folic acid on twinning.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Carmichael et al, 2010 ¹⁵²	Yes	NA	NA	NA	Unclear	Poor	Unclear how missing data was handled; time period extends to 2 months before and 2 months after and with lack of clarity on degree of exposure, difficult to clearly distinguish exposure from non-exposure; authors did not specify definition of exposure clearly (defined as any vs. no supplement), so the degree of adherence is Unclear. The control group only looked at live births and not fetal deaths.
Chandler et al, 2012 ¹⁵³	Yes	NA	NA	NA	Unclear	Fair	See Yoon et al. (2001) for recruitment information. Cases and controls selected from different populations. Cases could be stillborn infants or therapeutic abortions, while all controls were liveborn infants. Also, definition of exposure not defined clearly. Positive response to folic acid supplement use question could have indicated any frequency of usage. Possibility of residual confounding because some NTD-specific variables not controlled for, specifically, previous NTD pregnancy or having or having partner(s) with NTDs. In addition, analyses not adjusted for comparisons across different centers.
Charles et al, 2004 ¹⁵⁴ Charles et al, 2005 ¹⁵⁵ Taylor et al, 2015 ¹⁵⁶	Yes	Yes	Yes	Yes	NA	Poor	Randomization was inadequate: "tablets were kept in numbered drawers and distributed in sequence." Unclear whether administrators or outcome assessors were blinded because "The patients' notes were marked with a sticker the same colour as the tablets they were receiving." Some proportion of deaths not linked to patient files (occurring before 1980) but followup N not reported, so rate of overall attrition and differential attrition is Unclear. Because N at followup is not reported, it does not appear that the analysis used an intention-to-treat analysis for the missing data.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Correa et al, 2012 ²⁸	Yes	NA	NA	NA	No	Poor	Unclear how missing data was handled but appears to have been excluded in some tables. Definition of exposure does not account for adherence to meds ("any use during the month before conception or during the first 3 months of pregnancy) so exposed vs. non-exposed not clearly demarcated; additionally, the time period of recall is 1 month before conception to 3 months after, so folic acid supplementation after becoming aware of pregnancy would be misclassified as pre-pregnancy exposure; mothers interviewed up to 24 months after birth so risk of recall bias; authors did not specify definition of exposure clearly, so the degree of adherence is Unclear. Controls were only live borns.
Czeizel et al, 2004 ¹⁵⁷	Yes	NA	NA	NA	Unclear	Poor	Does not mention how missing data was handled. Unclear how authors determined who were clearly users of folic acid. Unable to determine response rate due to limited information. Women continued folic acid usage until at least 3rd trimester. There was only one product of folic acid at the time of the study (3mg) and required a prescription. Unclear on baseline differences and if they were controlled for.
Czeizel et al, 2004 ⁸¹	Yes	Yes	Yes	Yes	Unclear	Fair	The trial recruited patients to each arm differently. Supplemented women were recruited before pregnancy and asked to take vitamins and were followed up for several months. This recruitment before exposure and continuous measurement would have meant that all pregnancies and terminations would have been counted. Unsupplemented women were identified at 8–12 weeks of pregnancy, by which time, early pregnancy losses would have occurred (possibly due to lack of folic acid). Because one arm differentially identified women, this could have potentially led to a high and differential risk of selection bias, but the study restricted the analysis for supplemented cases with a pregnancy at 14 weeks. Thus the risk of differential selection bias was reduced but the risk of attrition bias was increased. A second potential source of bias arises from the residual confounding effects of having a higher proportion in the supplemented

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
							group of previous fetal deaths and in fact mortality because of congenital abnormalities.
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 Czeizel et al, 1993 ⁹² Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹ Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³	Yes	Yes	Yes	Yes	NA	Fair	Does not include fetal death in the analysis (but provides data for that calculation; Unclear allocation concealment and blinding processes (participants blinded, Unclear whether administrators or outcome assessors were blinded); does not conduct ITT but dropout extremely low so risk of bias low from dropout. Study did not consider diet and did not mention how missing data was handled.
Czeizel et al, 1996 ¹⁵⁸	Yes	NA	NA	NA	No	Poor	Does not include fetal deaths; study does not control for dietary intake but this study predates food fortification; relevant analysis (supplementation vs. no supplementation in NTD in critical period vs. healthy births) does not control for confounding; does not measure adherence. Unclear on how missing data was handled.
De Marco et al, 2011 ¹⁵⁹	Yes	NA	NA	NA	Unclear	Poor	Well-defined outcomes; exposure is slightly outside our period of interest (i.e., 3 months prior to pregnancy, rather 2 months prior to pregnancy). Possibility of residual confounding because some NTD-specific variables not controlled for, specifically, use of antiepileptic drugs or having or having partner(s) with NTDs. Investigators measured percentages of mothers with previous NTD-affected pregnancies, but relationship to outcomes of interest unlikely (see pg. 1080). In addition, dietary folate intake either not assessed or not taken into account in analyses. Risk of recall bias because mothers completed interviews 18-24 months after childbirth. Unclear how missing data affected findings.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
DeSoto et al, 2012 ¹⁶⁰	Yes	Yes	NA	NA	Unclear	Poor	Definition of folic acid exposure not defined clearly. Positive response to folic acid supplement use question could have indicated any frequency of usage. High risk of recall bias because mothers asked about FA use 6-13 years after childbirth. In addition, dietary folate not taken into account. Unclear if important confounders related to ASD included in statistical analyses.
Ericson et al, 2001 ¹⁶¹	Yes	Yes	Unclear	Yes	Unclear	Poor	Participation rates not reported; unclear whether the two groups are actually comparable-one group included multivitamin use only but whether folic acid was in vitamins and the amount is unclear; also unclear whether those taking folic acid tablets were or were not also taking multivitamins with folic acid.
Gildestad et al, 2013 ¹⁶²	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Haberg et al, 1994 ¹⁶³	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Hernandez et al, 2001 ⁹⁵	Yes	NA	NA	NA	Unclear	Fair	Possibility of residual confounding because numerous NTD-specific variables not controlled for, specifically, diabetes, family history of NTDs, prior NTD-affected pregnancy, or having or having partner(s) with NTDs. Dietary folate intake not accounted for in analysis of interest. Authors make point in Discussion that unaccounted effects of folate intake would reduce the magnitude of their findings, which were statistically significant. Outcome assessors blind to study hypothesis, but that does not mean they were blind to women's' case-control status.
Kallen et al, 2004 ¹⁶⁴	Yes	Yes	Yes	Yes	Unclear	Poor	Exposure period starts before pregnancy and extends to first attendance (in 90% of cases before the end of the first trimester and usually around week 10) with lack of clarity on degree of exposure, difficult to clearly distinguish exposure from non-exposure; authors did not specify definition of exposure clearly (defined as any vs. no supplement), so the degree of adherence is unclear; although study does not appear to account for fetal deaths, the resulting selection bias would serve to mute rather than exaggerate the effect of FA on twinning; study

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
							does not control for dietary intake but this study predates food fortification; study does not control for dietary intake but this study predates food fortification; study controls for use of ovarian stimulation drugs, but this control variable is insufficient because fertility treatment includes several other options
Kallen et al, 2007 ¹⁶⁵	Yes	Yes	Yes	Yes	Unclear	Poor	Response rate unclear; percentage of women approached who agreed to participate is unclear. Also, total number of eligible women is unclear. Women were recruited at the first antenatal visit, but the total number of women presenting for care during the time period is unclear. Also, the extent of folic acid not ascertained from participants.
Kondo et al, 2015 ¹⁶⁶	Yes	NA	NA	NA	No	Poor	Selection bias from being limited to live births. Definition of folic acid exposure not defined clearly. Positive response to folic acid supplement use question could have indicated any frequency of usage. High risk of recall bias because half of the control and case mothers asked about folic acid use 6–12 years after childbirth. In addition, dietary folate not taken into account. Controls and cases not matched on year or place of birth, statistically significant differences in knowledge of FA benefits.
Medvezky et al, 2003 ¹⁶⁷	Yes	NA	NA	NA	Unclear	Poor	Unclear how missing data handled. Exposure not well defined or quantified.
Mills et al, 1989 ⁹⁶	Yes	NA	NA	NA	Unclear	Fair	Eligibility criteria for cases Unclear because they do not clarify whether infants or fetuses were eligible if stillborns or had been aborted. Possibility of residual confounding because numerous NTD-specific variables not controlled for, specifically, diabetes, family history of NTDs, prior NTD-affected pregnancy, having or having partner(s) with NTDs, or treatment with folic acid antagonists. Unclear how high response rates for control groups were. Unclear how missing data was handled.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Moore et al, 2003 ⁹⁸ Milunsky et al, 1989 ⁹⁷	Yes	Unclear	Yes		Unclear	Fair	Physicians provided 76.5% of the outcome data, but if physicians did not respond, mothers completed the outcome questionnaires; information provided by mothers may not have been entirely accurate in terms of prenatal test results, presence of birth defects or chromosomal abnormalities, complications of pregnancy or delivery, complications of the newborn, or perinatal maternal illnesses. Treatment fidelity not entirely clear, specifically weekly frequency of folic acid supplementation. Possibility of residual confounding because use of folic acid antagonists not taken into account in analyses. Not enough information provided to calculate differential attrition, but overall attrition rate was very low for full sample and therefore unlikely to bias findings
Mosley et al, 2009 ⁸⁷	Yes	NA	NA	NA	Yes	Fair	Exposure not well defined or quantified. Response rates didn't approached 80% but relevant, without major apparent selection or diagnostic work-up bias.
Mulinare et al, 1988 ¹⁶⁸	Yes	NA	NA	NA	No	Poor	Missing data not accounted for in an ITT analysis; covers a 6 month period of exposure (3 months before to 3 months after pregnancy); No controls for concurrent interventions such as exposure to dietary folate, however, this sample was drawn before dietary supplementation; significant risk of recall bias because women asked to remember for a period ranging from 1968-1980. Only still births, no information on terminations. Cases included live an still-borns controls only.
Ohya et al, 2011 ¹⁶⁹	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Not enough information in the publication to assess quality.
Shaw et al, 2002 ¹⁷⁰	Yes	NA	NA	NA	Unclear	Poor	Unclear how missing data was handle, did not adjust for potential NTD confounders; wide range between those who actually used vitamin (did they use vitamin or mineral supplements during 4 month period but does not say how they divided group out), study does consider cereal usage but not other forms of dietary folate. Potential recall bias 3.7 to 3.9 years later. Does not mention miscarriages and stillbirths.

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Shaw et al, 1995 ⁸²	Yes	NA	NA	NA	Unclear	Fair	3 months before and 3 months after; did not control for all of the confounders for folate antagonist medications; does not mention miscarriages and stillbirths. Differences based on ethnicity, age, and education but study controlled for. No mention of how missing data was handled.
Suarez et al, 2000 ²²	Yes	NA	NA	NA	Unclear	Fair	1) Response rate less than 80%; 2) some selection bias as controls do not include recruitment at all of the same centers; 3) study collects data on dietary folate intake, but no data shown of association of folic acid with NTDs, adjusted for dietary intake. Strength is adjustment for other confounders. Several issues: (1) very low prevalence of folic acid supplements, limited power (2) food frequency questionnaire doesn't distinguish 3 months prior to conception (3) differential recall period produced by not matching case and control infants/ fetuses for gestational age (control women recalling exposures further in past than case women); (4) different response rate between case (72%) and control (53%)
Veeranki et al, 2014 ¹⁷¹	Yes	Yes	Yes	Yes	Unclear	Poor	Exposure to multivitamin supplement defined as filling rather than consuming prenatal vitamins; assumes no misclassification from consumption of over-the-counter supplements; also needed only 1 day of fill in period of exposure to count as exposed; differences between exposed (G1) and non-exposed (G3) in prenatal care, maternal asthma, number of siblings: does not separate out the potential effect of folic acid specifically from other micronutrients that may have an independent effect on respiratory outcomes; does not control for dietary folate exposure; does not control for environmental exposure; excludes stillbirths and miscarriages so risk of selection bias; because of definition of exposure, Unclear whether time period of fill for first trimester covers the first month of pregnancy); loss through poor response rate or missing data NR so cannot judge attrition bias

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
Veeranki et al, 2014 ¹⁷²	Unclear	No	Unclear	Yes	Unclear	Poor	Exposure to multivitamin supplement defined as filling rather than consuming prenatal vitamins; assumes no misclassification from consumption of over-the-counter supplements; does not separate out the potential effect of folic acid specifically from other micronutrients that may have an independent effect on allergic rhinitis; does not control for dietary folate exposure; does not control for environmental exposure; excludes stillbirths and miscarriages so risk of selection bias; because of definition of exposure, Unclear whether time period of fill for first trimester covers the first month of pregnancy); may not include all cases of allergic rhinitis
Veeranki et al, 2015 ¹⁷³	Yes	No	Yes	Yes	Unclear	Poor	Exposure to multivitamin supplement defined as filling rather than consuming prenatal vitamins; assumes no misclassification from consumption of over-the-counter supplements; also needed only 1 day of fill in period of exposure to count as exposed; differences between exposed (G1) and nonexposed (G3) in prenatal care, maternal asthma, number of siblings: does not separate out the potential effect of folic acid specifically from other micronutrients that may have an independent effect on respiratory outcomes; does not control for dietary folate exposure; does not control for environmental exposure; excludes stillbirths and miscarriages and preterm, so risk of selection bias; because of definition of exposure, Unclear whether time period of fill for first trimester covers the first month of pregnancy; loss through poor response rate or missing data NR so cannot judge attrition bias; may not include all cases of asthma
Vollset et al, 2005 ⁸³	Yes	Yes	Yes	Yes	Unclear	Fair	Risk of recall bias in original data assumed to be high - based on estimates of underreporting of folate use, they estimated that 45% of women who took folate before conception were registered as nonusers and adjusted potential misclassification as a result; however, periconceptional use not defined, as a result, cannot tell if exposed vs. non-exposed is clearly defined; looks at pregnancies, but Unclear how stillbirths and terminations were handled; no adjustment for dietary folate; because of

Appendix D Table 7. Quality Assessments for all Included Randomized, Controlled Trials, Case Control Studies, and Cohort Studies (Part 5)

First Author, Year	Were outcomes prespecified/ defined and adequately described?	Were outcome measures valid and reliable?	Were all important outcomes considered?	Was the duration of followup adequate to assess the outcome?	Was an appropriate method used to handle missing data?	Quality Rating	Comments
							lack of definition of exposure, Unclear whether time period of fill for first trimester covers the first month of pregnancy); loss through poor response rate or missing data NR so cannot judge attrition bias
Werler et al, 1993 ⁹⁹	Yes	NA	NA	NA	No	Fair	Cases and controls identified by “systematic contact” at tertiary and birth hospitals, details NR, as a result, unable to determine whether cases and controls came from similar populations and had a similar chance of selection; looked at the effect of dietary intake of folate, but only for those with no use of supplements, so does not fully control for concurrent interventions

G1 = group 1; G3 = group 3; NA = not applicable; NR = not reported; NTD = neural tube defect

Good: Meets all criteria: Comparable groups are assembled initially and maintained throughout the study (follow-up at least 80%; reliable and valid measurement instruments are used and applied equally to the groups; interventions are spelled out clearly; all important outcomes are considered; and appropriate attention to confounders in analysis. In addition, for RCTs, intention to treat analysis is used.

Fair: Studies will be graded “fair” if any or all of the following problems occur, without the fatal flaws noted in the “poor” category below: Generally comparable groups are assembled initially but some question remains whether some (although not major) differences occurred with follow-up; measurement instruments are acceptable (although not the best) and generally applied equally; some but not all important outcomes are considered; and some but not all potential confounders are accounted for. Intention to treat analysis is done for RCTs.

Poor: Studies will be graded “poor” if any of the following fatal flaws exists: Groups assembled initially are not close to being comparable or maintained throughout the study; unreliable or invalid measurement instruments are used or not applied at all equally among groups (including not masking outcome assessment); and key confounders are given little or no attention. For RCTs, intention to treat analysis is lacking.

Appendix E Table 1. Overview of 2009 Included Studies and Inclusion/Exclusion Status in Current Report

First Author, Year	Status in Current Report	Reasons for Exclusion
Czeizel et al, 2004 ⁸¹	Included	
Goh et al, 2006 ⁸⁴	Excluded	Excluded due to poor quality.
Shaw et al, 1995 ⁸²	Included	
Thompson et al, 2003 ⁸⁵	Excluded	Excluded for wrong intervention. Unable to separate out the effects of supplementation from diet.
Vollset et al, 2005 ⁸³	Included	

Appendix E Table 2. Overview of Studies Excluded From the 2009 Report Due to Quality and Inclusion/Exclusion Status in Current Report

First Author, Year	Reasons for 2009 Exclusion	Status in Current Report	Reasons for Exclusion
Czeizel et al, 1996 ¹⁵⁸	Retrospective exposure assessment poses potential recall bias. Differential measurement of exposure causes potential measurement bias. Lower response rate in controls No adjustment for smoking	Excluded	Excluded for high risk of bias
Locksmith et al, 1998 ¹⁷⁴	Study type not included in review (not a systematic review)	Excluded	Excluded for wrong design
Kallen et al, 2002 ¹⁷⁵	Used involuntary childlessness as proxy for infertility Exposure assessed by questionnaire at gestational week 10–12: drugs taken “since she became pregnant” No information about dose, timing	Excluded	Excluded for wrong comparator
Lumley et al, 2001 ¹⁷⁶	Studies included were not recent (many published prior to 1995 and included in USPSTF previous evidence report).	Excluded	Excluded for wrong population
Medveczky et al, 2004 ¹⁶⁷	No information on overall effect of folic acid on NTDs	Excluded	Excluded for high risk of bias
Moore et al, 2003 ⁹⁸	This was a study of dose-response re-examining data from study reviewed in 1996 USPSTF report; no new information about overall benefits of folic acid supplementation	Included	Even though the previous report listed it as an exclude “after abstraction and quality rating”, the notes in the abstraction form indicate “This was a study of dose-response re-examining data from study reviewed in 1996 USPSTF report; no new information about overall benefits of folic acid supplementation
Shaw et al, 2002 ¹⁷⁰	No information on overall effect of folic acid on NTDs	Excluded	Excluded for high risk of bias
Shaw et al, 1998 ¹⁷⁷	No information on overall effect of folic acid on NTDs	Excluded	Excluded for wrong outcome
Shaw et al, 2001 ¹⁷⁸	No information on overall effect of folic acid on NTDs	Excluded	Excluded during title/abstract review
Shaw 1996 ¹⁷⁹	No information on overall effect of folic acid on NTDs	Excluded	Excluded during title/abstract review
Suarez et al, 2000 ²²	Study performed in high-risk population	Included	Even though the previous report listed it as an exclude “after abstraction and quality rating”, the notes in the abstraction form indicate that was excluded for “high-risk populations” - that is, the population of Mexican-Americans have a higher risk of NTDs
Ericson et al, 2001 ¹⁶¹	Potential confounding by patients undergoing IVF or ovulation stimulation; subgroup analysis on women without “period of involuntary childlessness”, but authors reported known underreporting of infertility history (40% of women who underwent IVF or ovulation stimulation did not report involuntary childlessness.) Measurement validity issues: exposure	Excluded	Excluded for high risk of bias

Appendix E Table 2. Overview of Studies Excluded From the 2009 Report Due to Quality and Inclusion/Exclusion Status in Current Report

First Author, Year	Reasons for 2009 Exclusion	Status in Current Report	Reasons for Exclusion
	measured at 10 weeks; reported folic acid use was 0.6% in this study based on Birth Registry, as compared to 8% in concurrent study. No information on doses or timing of initiation of folic acid Potential differential recall based on knowledge of twin gestation by 8–10 weeks		
Czeizel et al, 2004 ¹⁵⁷	No adjustment for possible confounders: IVF, ovulation induction, smoking No information on doses or timing of initiation of folic acid Potential differential recall based on knowledge of twin gestation early in pregnancy or twin delivery	Excluded	Excluded for high risk of bias
Kallen et al, 2004 ¹⁶⁴	Incomplete information on doses (women likely took either 400 micrograms or 5 mg) or whether prenatal vitamins with folic acid were included in analysis No information on timing of initiation or duration of exposure Initial comparability of groups unknown Potential differential recall based on knowledge of twin gestation by 8–10 weeks Residual confounding possible if incomplete reporting of fertility treatments. Unclear how many women were included in the final analysis.	Excluded	Excluded for high risk of bias

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
Agopian et al, 2012 ⁹ National Birth Defects Prevention Study Case-control United States Centers for Disease Control and Prevention and Texas Department of State Health Services Medium	Included: National Birth Defects Prevention Study (NBDPS), data collected from population-based surveillance systems located in 10 states: Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah. Cases included live births, fetal deaths, and elective pregnancy terminations. Live born controls without major birth defects were ascertained through birth certificate data or hospital birth logs. Controls were selected at random among infants delivered in the study regions. Excluded: Cases with additional major birth defects that were unlikely to be secondary to the NTD. Excluded potential cases with single-gene disorders or chromosome abnormalities	G1: Spina bifida or anencephaly live births, fetal deaths, and elective pregnancy terminations (n=1,239) G2: Live born controls without major birth defects (n=8,494)	Folic acid supplementation before pregnancy through first month of pregnancy Population-based surveillance systems in 10 states. Data collected from medical records, birth certificate data, or hospital birth logs.	NR	42%
Ahrens et al, 2011 ¹¹ Slone Birth Defects Study Case-control United States Centers for Disease Control and Prevention Medium	Included: Slone Birth Defects Study, infants with birth defects were identified from discharge records of participating hospitals serving the areas surrounding Boston; Philadelphia; San Diego; and Toronto; in addition, cases have been identified through birth defect registries in Massachusetts and parts of New York. Nonmalformed controls have been randomly selected each month from study hospitals' discharge lists or from statewide birth records. Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation were eligible as cases for our study. Only live-born nonmalformed infants were eligible as controls.	G1: Malformed live-born infants, therapeutic abortions after 12 weeks' gestation, and fetal deaths after 20 weeks' gestation (n=205) G2: Live-born nonmalformed infants (n=6,357)	Folic acid supplements 2 months before the last menstrual period and 1 month after last menstrual period. Cases identified from discharge records of participating hospitals serving the areas surrounding Boston, Philadelphia, San Diego, and Toronto and through birth defect registries in Massachusetts and New York. Nonmalformed controls selected each month from study hospitals' discharge lists or from statewide birth records.	Median age at conception G1: 28 G2: 30	29%
Czeizel et al, 1992 ⁸⁸ Czeizel et al, 1993 ⁸⁹ Czeizel et al, 1993 ⁹² Czeizel et al, 1994 ⁹⁰ Czeizel et al, 1994 ⁹¹	Included: Women planning a pregnancy, did not have delayed conception or infertility, not currently pregnant. During the first four years of trial women had to be less than 35 years old and no previous	G1: Vitamin supplement (0.8 folic acid and 12 vitamins, 4 minerals, 3 trace elements) ⁹³ (n=2793) G2: Trace-element	28 days before conception and at least until the date of the second missed menstrual period ⁹¹	27	NR

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
Czeizel et al, 1996 ⁹⁴ Czeizel et al, 1998 ⁹³ Hungarian RCT RCT Hungary NR Medium	wanted pregnancy. ⁹⁰ Excluded: Patients with genetically determined syndromes, including those involving NTDs (e.g., Meckel's syndrome or Patau's syndrome). ⁸⁸	supplement (copper, manganese, zinc, low dose of vitamin C) ⁹⁴ (n=2660)	Hungarian Preconceptional Service (HPS) began 3 months before a pregnancy is planned and continues for the first 3 months after conception. HPS provided information and counseling, exams, and interventions during all trimesters by qualified nurses.		
Czeizel et al, 2004 ⁸¹ Hungarian Cohort-Controlled Trial Cohort-controlled trial Hungary NR Medium	Included: Supplemented cohort was recruited from the Hungarian Periconceptional Service between May 1, 1993 and April 30, 1996. Routine care subjects for an unsupplemented cohort were recruited during their first visit at an antenatal care clinic between the 8th week and 12th week of gestation. Excluded: Supplemented group: did not conceive within 1 year. Unsupplemented group: multivitamin and/or folic acid use during the periconceptional period and before first visit.	G1: Women supplemented with multivitamin (n=3,056) G2: Nonsupplemented women (n=3,056)	Before conception and at least until first missed menstrual period. Supplemented cohort was recruited from the Hungarian Periconceptional Service (HPS). HPS provides information and counseling, exams, and interventions during all trimesters by qualified nurses. Unsupplemented cohort was recruited during their first visit at an antenatal care clinic.	27	NR

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
<p>Hernandez-Diaz et al, 2001⁹⁵</p> <p>Slone Epidemiology Unit Birth Defects Study</p> <p>Case-control</p> <p>United States</p> <p>Pharmacoepidemiology Teaching and Research Fund of the Harvard School of Public Health; the National Center for Environmental Health, Centers for Disease Control and Prevention; the Massachusetts Department of Public Health; the National Institute of Child Health and Human Development and the National Heart, Lung, and Blood Institute</p> <p>Medium</p>	<p>Inclusion: Slone Epidemiology Unit Birth Defects Study. Cases, infants and fetuses with anencephaly, spina bifida, encephalocele, or other NTDs. Controls, infants with malformations other than NTDs.</p> <p>Excluded: Infants with chromosomal or Mendelian-inherited anomalies or with amniotic bands, caudal regression, or twin disruption. Subjects with oral clefts, urinary tract defects, limb reduction, heart defects, and conditions related to NTD (hydrocephalus, microcephalus, and other anomalies of the brain, spinal cord, or nervous system).</p>	<p>G1: Cases with NTDs (spina bifida, anencephaly, encephalocele, or other NTD) (n=1,242)</p> <p>G2: Infants with malformations not related to vitamin supplementation (n=6,660)</p>	<p>Any time during the 2 months after the last menstrual period.</p> <p>Participants of the Slone Epidemiology Unit Birth Defects Study. Study interviewed mothers of malformed children born in the greater metropolitan areas of Boston, Philadelphia, Toronto, and between 1983 and 1985, part of Iowa. Subjects identified through review of admissions and discharges at major referral hospitals and clinics and through regular contact with newborn nurseries in community hospitals.</p> <p>A random sample of nonmalformed infants was identified at the birth hospitals as potential controls (only after 1993).</p>	<p>Overall percentages</p> <p>< 24: 28.11%</p> <p>25-29: 36.71%</p> <p>30-34: 25.87%</p> <p>>35: 9.3%</p>	<p>2%</p>

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
Mills et al, 1989 ⁹⁶ National Institute of Child Health and Human Development Neural Tube Defects Study Case-control United States Funding NR, conducted by NIH. Medium	Inclusion: National Institute of Child Health and Human Development Neural Tube Defects Study based in CA and IL. Cases were mothers of an infant or fetus with an NTD diagnosis prenatally or postnatally between June 15, 1985 and April 30, 1987 in IL or between August 1, 1985 and April 30, 1987 in CA. NTDs included anencephaly, meningocele, myelomeningocele, encephalocele, rachischisis, iniencephaly, and lipomeningocele. Two control groups, one mothers of a normal infant or fetus and one mothers of an abnormal or still-born infant or fetus. Exclusion: Cases excluded insolated hydrocephalus, hydranencephaly, dermal sinus, and spina bifida occulta. Abnormal control group excluded mothers of infants with malformations related to vitamin use. Mothers with a history of NTDs in a first-degree relative were excluded from both control groups.	G1: Cases, mothers of an infant or fetus with an NTD (n=571) G2: Controls, mothers of normal infants (n=573) G3: Controls, mothers of an abnormal or stillborn infant or fetus (n=546)	Vitamin use 30 days before the first day of the last menstrual period and ending about 45 days thereafter. Study based in CA and IL. Cases included mothers of an infant or fetus with an NTD diagnosis prenatally or postnatally between June 15, 1985 and April 30, 1987 in IL or between August 1, 1985 and April 30, 1987 in CA.	< 21: 11.60% 21-25: 25.92% 26-30: 34.14% 31-35: 20.18% 36-40: 7.04% 41-45: 1.01% Unknown: 0.12%	39%
Milunsky et al, 1989 ⁹⁷ Moore et al, 2003 ⁹⁸ Cohort United States Study 1: National Institute of Neurological Disorders and Stroke Study 2: March of Dimes Birth Defects Foundation National Institute of Neurological Disorders and Stroke Medium	Inclusion: Women were identified and recruited when they had a MSAFP screen or amniocentesis. They were receiving prenatal care and routine MSAFP screening in the practices of over 100 participating obstetricians. MSAFP and amniotic fluid samples were analyzed at the Center for Human Genetics of Boston University School of Medicine. Remaining amniocenteses were performed and analyzed at other genetic centers throughout the country and the results were made available to the researchers. Exclusion: Interviews of mothers pregnant a 2nd or 3rd time during study were excluded.	G1: Use of multivitamins containing folic acid G2: No use of multivitamins containing folic acid (or use less than 1 a week) Total: (n=22,715; multivitamin use information available)	3 months prior to pregnancy through 1st 3 months of pregnancy Women were identified and recruited when they had a MSAFP screen or amniocentesis at 16 weeks of pregnancy between October 1984 and June 1987. Women were receiving prenatal care and routine MSAFP screening in the practices of over 100 participating obstetricians.	Study 1 <20: 2% 20-29: 44% 30-39: 52% ≥40: 2% Study 2 <30: 2% 30-39: 44% ≥40: 2%	Study 1: 4% Study 2: NR

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
Moseley et al, 2009 ⁸⁷ National Birth Defects Prevention Study Case-control United States Centers for Disease Control and Prevention Medium	Inclusion: National Birth Defects Prevention Study, began in 1997 and includes participants from 10 population-based birth defects surveillance systems (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, or Utah). Cases, pregnancy affected by anencephaly or spina bifida that did not result from a single gene or chromosomal abnormality. Controls, random sample of women from each center site who delivered a liveborn infant without a structural birth defect. Included pregnancies were conceived on or after July 1, 1998. Exclusion: Women with type 1 or 2 diabetes or use of periconceptional use of any folate antagonist medication. Pregnancies resulting in multiple births. Women with incomplete food frequency questionnaires or supplement use information.	G1: Women with a pregnancy affected by spina bifida or anencephaly that did not result from a single gene or chromosomal abnormality G2: Women who delivered a live-born infant without a structural birth defect	Folic acid supplementation 3 months before pregnancy through 1st month of pregnancy Population-based surveillance systems in 10 states. Data collected from medical records, birth certificate data, or hospital birth logs.	Median age at conception G1: 26 G2: 27	42%
Shaw et al, 1995 ⁸² California Birth Defects Monitoring Program Case-control United States NR Medium	Inclusion: California Birth Defects Monitoring Program (CBDMP): birth years between June 1, 1989 and May 31, 1991. Cases: women who had liveborn and stillborn infants with NTDs; place of delivery in California county other than Los Angeles, Ventura, or Riverside; mother was a resident of California; and those who had NTD-affected pregnancies that were terminated. Controls: singletons born alive in the specified month and year in that hospital; mother was a resident of California; and no reportable birth defect. Exclusion: Women who spoke only languages other than English or Spanish. Previous NTD affected pregnancies.	G1: Singleton liveborn infants and electively terminated fetuses with an NTD (anencephaly, spina bifida cystica, craniorrhachischisis, and iniencephaly) (n = 538) G2: Singleton live births without a reportable birth defect (n = 539)	3 months before pregnancy and/or first 3 months after conception California Birth Defects Monitoring Program (CBDMP), birth years between June 1, 1989 and May 31, 1991. Cases, women who had live- and stillborn infants with NTDs and those who had NTD-affected pregnancies that were terminated after prenatal diagnosis (February 1, 1989-January 31, 1991). Controls, an equal number of singleton live births randomly selected in proportion to hospital's contributions to total	Overall <20: 10.86% 20-24: 27.21% 25-29: 29.80% 30-34: 22.47% ≥35: 9.66%	54%

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
			population of infants born alive in California.		
Suarez et al, 2000 ²² Texas Department of Health's Neural Tube Defect Project United States Centers for Disease Control and Prevention Medium	Inclusion: Texas Department of Health's Neural Tube Defect Project, projects occurs in 14 Texas counties along the US-Mexico border. Cases, infants or fetuses with anencephaly (including craniorachischisis and iniencephaly), spina bifida, or encephalocele identified at birth or prenatally between January 1995 and February 1999. Cases included diagnoses made among liveborns, stillborns, and fetuses at all gestational ages, and included abortions, whether induced or spontaneous. Control, women from study area who had normal births during the same time period. Exclusion: Controls were ineligible if they were not a resident of the area or if they had an infant with an apparent or prenatally diagnosed congenital abnormality	G1: Infants or fetuses who had anencephaly (including craniorachischisis and iniencephaly), spina bifida, or encephalocele identified at birth or prenatally (n=148) G2: Normal live births (n=158)	3 months before conception to 3 months after conception Texas Department of Health's Neural Tube Defect Project, occurrence of NTDs in 14 Texas counties along the US-Mexico border identified at birth or prenatally between January 1995 and February 1999. Surveillance included hospitals, birthing centers, genetics clinics, ultrasound centers, licensed abortion centers, and approximately midwives in the region.	< 20: 25.50% 20-24: 32.68% 25-29: 24.84% >30-39: 16.99%	100%
Vollset et al, 2005 ⁸³ Medical Birth Registry of Norway Case-control Norway NR Medium	Inclusion: Medical Birth Registry of Norway, women who gave birth from December 1998 through the end of 2001. Information on IVF pregnancies obtained by contacting fertility clinics in Denmark and Sweden. Exclusion: NR	G1: Preconceptional use of folate (n=11,077) G2: No preconceptional use of folate (n=164,965)	Preconception Medical Birth Registry of Norway, women who gave birth from December 1998 through the end of 2001. Information on IVF pregnancies obtained by contacting fertility clinics in Denmark and Sweden.	NR	NR

Appendix F Table 1. Characteristics of Included Randomized, Controlled Trials, Case-Control Studies, and Cohort Studies

First Author Study Name Design Country Funding Risk of Bias	Inclusion Exclusion Criteria	Groups	Timing Setting	Mean Age	% Nonwhite
Werler et al, 1993 ⁹⁹ Case-control United States and Canada Maternal and Child Health Resources Development grant; Marion Merrell Dow; Food and Drug Administration Cooperative Agreement; Hoffmann-LaRoche Medium	Inclusion: Study subjects (liveborn and stillborn infants and therapeutic abortuses) recruited from tertiary and birth hospitals in greater metropolitan Boston, MA, Philadelphia, PA, and Toronto, Ontario. Primary physicians of potential subjects were asked for permission to contact mothers. Cases: subjects with anencephaly, spina bifida, or encephalocele. Controls: subjects with other major malformations. Exclusion: Subjects with chromosomal anomalies or mendelian-inherited disorders; recurrent NTD cases; oral clefts.	G1: Liveborn, stillborn infants, and therapeutic abortions with anencephaly, spina bifida, or encephalocele (n= 436) G2: Liveborn, stillborn infants, and therapeutic abortions with other major malformations (n=2615)	Periconceptional period (interval from 28 days before the last menstrual period (LMP) through the 28 days after the LMP (the first lunar month). Study subjects recruited from tertiary and birth hospitals in greater metropolitan Boston, MA, Philadelphia, PA, and Toronto, Ontario. Primary physicians of potential subjects were asked for permission to contact mothers.	NR	NR

G1 = group 1; G2 = group 2; G3 = group 3; N = sample; NR = not reported; NTD = neural tube defect

Appendix F Table 2. Characteristics of Included Systematic Reviews and Meta-Analyses

First Author Study Design Search Dates Country Funding	Eligibility Criteria	Studies Included in Review	Included Study Designs	Intervention Dose Time Period	Overall Sample Size	Countries Included
Crider et al, 2013 ¹⁰¹ SR/meta-analysis Inception of database to March 2012 United States CDC	1) Randomized, controlled trial, cohort, case-control, or cross-sectional study; 2) report the exposure of natural food folate intake, folic acid intake from fortified foods, total folate intake from foods (e.g., dietary folate equivalents), folic acid intake from supplements, or maternal or cord blood serum, plasma, or red blood cell folate concentrations; 3) have an exposure timing during the periconceptional period or during pregnancy; 4) provide results on at least one allergic or respiratory outcome; and 5) include an evaluation of the direct association between folic acid exposure and one of the outcomes of interest	5 in meta-analysis Håberg et al, 2009 ¹⁰² Kiefte-de Jong et al, 2012 ¹⁰³ Magdelijns et al, 2011 ¹⁰⁴ Martinussen et al, 2012 ¹⁰⁵ Whitrow et al, 2009 ¹⁰⁶	10 cohort studies, 3 nested case-control studies, 1 case-control, 2 cohort, 2 case-control	Folic acid supplementation 400-500 µg/d Prepregnancy and first trimester	45,642	The Netherlands, Norway, Australia, United States
Yang et al, 2015 ¹⁰⁸ Meta-analysis Earliest available date to May 2013 China NR	Exposure was maternal folic acid supplementation during pregnancy; outcome was infant asthma; analytical study (case-control studies or cohort studies); available multivariate-adjusted relative risks (RRs), hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CIs); unrelated case and control groups or exposed and unexposed groups in a cohort study and all subjects from the same temporally and geographically defined underlying population.	Bekkers et al, 2012 ¹⁰⁰ Granel et al, 2008 ¹⁰⁷ Magedlijns et al, 2011 ¹⁰⁴ Martinussen et al, 2012 ¹⁰⁵ Whitrow et al, 2009 ¹⁰⁶	5 cohort studies	Folic acid supplementation Range NR Prepregnancy	14,438	Australia, the Netherlands, United Kingdom

G1 = group 1; G2 = group 2; N = sample; NR = not reported; NTD = neural tube defect.

Appendix G. Ongoing Trials

Principal investigators	Location	Population	Approximate size	Investigations	Outcomes	Status as of 2015
Renata Bortolus, MD	Italy	Women 18-44 who intend to become pregnant	5,000	4 vs. 0.4 mg/day	Number of congenital malformations Rate of selected congenital malformations Miscarriages and recurrent abortions Pre-eclampsia Abruptio placenta Intrauterine growth restriction Pre-term delivery Multiple births	Recruiting; Estimated Study completion: September 2016
Fenneke Blom, PhD	The Netherlands	Women 18-45 who want to become pregnant within 12 months	5,000	4 vs. 0.4 mg/day	Folic acid related congenital anomalies Preterm birth Birth weight Preeclampsia Compliance with intervention	Recruiting; Estimated Study completion: December 2016