National Toxicology Program
U.S. Department of Health and Human Services

DRAFT NTP MONGRAPH FOR THE EXPERT PANEL:

IDENTIFYING RESEARCH NEEDS FOR ASSESSING SAFE USE OF HIGH INTAKES OF FOLIC ACID

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Office of Health Assessment and Translation
Division of the National Toxicology Program
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ABBREVIATIONS

Abbreviation	Name
CDC	Centers for Disease Control and Prevention
CSFII	Continuing Survey of Food Intakes by Individuals
DFE	Dietary folate equivalent
DNTP	Division of the National Toxicology Program
HAWC	Health Assessment Workspace Collaborative
HOMA	Homeostatic model assessment
IOM	Institute of Medicine
MeSH	Medical Subject Heading
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NORVIT	Norwegian Vitamin Trial
NTD	Neural tube defect
NTP	National Toxicology Program
ODS	Office of Dietary Supplements
OHAT	Office of Health Assessment and Translation
OLPR	Office of Liaison, Policy and Review
OSIM	Office of Science Information Management
PECO	Population, Exposure, Comparator and Outcome
PICO	Population, Intervention, Comparator and Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red blood cell
RDA	Recommended Dietary Allowance
UL	Tolerable upper intake level
WENBIT	Western Norway B Vitamin Intervention Trial
WHO	World Health Organization

ABSTRACT

Folate is a water-soluble B-complex vitamin required for cell growth and division, and adequate folate intake is necessary to prevent a wide variety of health conditions. Some published studies have raised concerns about the safe use of folic acid, a synthetic form of folate, above the Recommended Dietary Allowance (RDA) of 400 μg. In order to identify potential research needs for evaluating the safe use of folic acid at an intake level higher than the current RDA (referred to as "high intake" in this document), the National Toxicology Program (NTP) partnered with the NIH Office of Dietary Supplements (ODS) to convene an expert panel to evaluate the current state of the science.

As background material for the expert panel, published literature relevant for evaluating the potential for health effects associated with high doses of folic acid was collected and summarized. Due to the large number of published studies on folate and folic acid, screening of the literature using systematic review methodology was undertaken to transparently identify, select, and group the studies by potential health effects areas.

A steering committee of individuals knowledgeable about the folic acid health literature was formed to suggest areas where data indicate potential adverse health effects associated with high intakes or blood levels of folic acid. Four general health effect categories were identified (cancer, cognition, hypersensitivity, and endocrine/metabolic). This document includes an explanation of the methods used to identify and collect the relevant literature. The human study data for these health effects were summarized and are available online (http://ntp.niehs.nih.gov/go/730864) and in the Supplementary Material as a resource for the expert panel. Supporting literature from relevant animal and *in vitro* studies are listed in Supplementary Material. This document also presents the reasoning of the steering committee as to why other health effects identified in the literature were not considered high priority areas of focus for this review.

The expert panel will use this document, supplementary material, and the online resource to to determine the state of the science for four general health effects areas associated with high intakes of folic acid. Specifically, the expert panel will (1) identify the areas of consistency and areas of uncertainty in the available science, (2) identify research needs based on review of the available science, and (3) propose research approaches for addressing the research needs and gaps in the available science. This NTP Monograph and the expert panel report will be published together. Information from this project will help inform development of a research agenda for evaluating the safe use of high intakes of folic acid.

1.0 INTRODUCTION

The National Toxicology Program (NTP)[1] in conjunction with the NIH Office of Dietary Supplements (ODS)[2] is holding an expert panel meeting to identify research needs based on consideration of the state of the science from published literature related to the safe use of high intakes of folic acid. The benefit of supplemental folic acid for pregnant women to prevent neural tube defects in their children is well established; at the same time, there is interest in identifying and understanding any potential adverse health impacts from high intakes of folic acid. This project aims to inform the development of a research agenda for evaluating the safe use of high intakes of folic acid.

Due to the vastness of the research on folate and folic acid,[3] screening of the literature using systematic review methodology was undertaken to identify potential adverse health effects for which further research might be warranted. This document (1) outlines the approach used to identify the literature, select relevant studies, and group data by health effect categories; (2) describes how high priority health effect categories were identified; and (3) summarizes the human literature in the high priority and other health effect categories, including discussion of why the health effects were or were not considered high priority areas of focus for this evaluation.

1.1 Overall Objective

The objective of this project is to identify research needs and outline research approaches for evaluating the safe use of high intakes of folic acid. This objective was developed by the NTP Office of Health Assessment and Translation, National Institute of Environmental Health Sciences and ODS with input from the steering committee and staff in the Centers for Disease Control and Prevention Division of Birth Defects and Developmental Disabilities. This effort will be informed by this state-of-the-science literature review and facilitated by a panel of qualified experts convened by NTP and ODS. Following the expert panel meeting, the NTP Monograph will be finalized with the expert panel's report as an appendix and published on the NTP Website (http://ntp.niehs.nih.gov/go/730864).To achieve this objective, the expert panel will:

- Identify the areas of consistency and areas of uncertainty in the available science
- Identify research needs based on review of the available science
- Propose research approaches for addressing the research needs and gaps in the available science

[1] The NTP is a federal, interagency program whose goal is to safeguard the public by identifying substances in the environment that may affect human health. NTP is headquartered at the National Institute of Environmental Health Sciences, which is part of the National Institutes of Health. For more information about NTP and its programs, visit http://ntp.niehs.nih.gov/

[2] The mission of the ODS is to strengthen knowledge and understanding of dietary supplements by evaluating scientific information, stimulating and supporting research, disseminating research results, and educating the public to foster an enhanced quality of life and health for the U.S. population. For more information about ODS and its programs, visit http://ods.od.nih.gov/.

[3] Over 29,000 references identified in Pubmed search using the MeSH term "folic acid" in January 2015

1.2 Organization of Background Material

This document was prepared by the NTP Office of Health Assessment and Translation for use as background material for the expert panel and public. The focus is on how the human health effects literature was collected and includes a brief summary of the identified literature. The health benefit of folic acid in preventing neural tube defects is well established, and this document does not include a review of that literature or consider dose-related effects of this protective effect. This document also does not include information on sources of folic acid or current intake or measured blood levels. In summarizing only the human literature, this background document also does not include relevant information on biological plausibility provided by relevant animal and *in vitro* experimental studies. Lists of such relevant studies are provided in the Supplementary Material. The Supplementary Material provides bibliographic lists of relevant supporting studies. This document has been reviewed internally for clarity and accuracy prior to release to the public.

1.2.1 Methods

This document has been prepared using the principles of systematic review methodology to comprehensively identify relevant studies. As such, the Methods section provides a description of this process in a manner similar to a systematic review protocol. Scientific judgments made by NTP, ODS, and the steering committee during the development of this project are documented with scientific justification for the decisions.

1.2.2 Health Effects Summaries

Chapter **4.0 High Priority Health Effect Categories** includes summaries of the information collected for the four health effect categories of focus for the expert panel. Each section includes a brief introduction to the topic, what literature was identified, why the topic was considered high priority, and potential issues the expert panel may discuss when considering the consistency and uncertainty in the literature. There is also a figure of the number of citations by year since 1980 and an example graph of results across studies with similar endpoints.

For health effect categories not considered high priority, chapter **5.0 Other Health Effect Categories** includes a brief description of why these health effects were not considered as pressing topics for the expert panel's consideration.

1.2.3 Supplementary Material

For each of the high priority health effect categories, **Study Summaries** provide detailed information about the design and results as reported in the publications. Presenting each study in a consistent manner facilitates comparison of results across the literature base.

Reference Lists are provided for the studies captured in the literature search and screen:

- Human studies of High Priority Health Effects
- Animal studies relevant to the High Priority Health Effect Categories
- *In vitro* studies relevant to the High Priority Health Effect Categories
- Human studies in the Other Health Effect Categories
- Pooled and meta-analyses for High Priority and Other Health Effect Categories

1.2.4 Web-based Health Effects Data

Health Assessment Workspace Collaborative (HAWC, https://hawcproject.org/) is an online content management system for conducting human-health risk assessments. HAWC allows users to have a standardized and transparent presentation of the data from each study and create customized data presentations in both graphical and textual formats. As a freely available online resource, it also allows the public to view the studies in the same manner as the expert panel.

As part of the background materials, NTP extracted data on health outcomes within each of the four high priority health effect categories. Details of study design, folate measurement (intake or blood level), outcome assessment and results are presented in HAWC as four projects (one for each category). Endpoint Data Pivots graph results from across studies (see **Figure 1**) and are available within each project in HAWC. The expert panel members and the public can use this online tool to browse graphs of results across studies and interactively explore additional details about each study.

The four folic acid projects are publically accessible https://hawcproject.org/assessment/public/. Additional information about the HAWC resource can be found here (https://hawcproject.org/user/new/).

An example of an Endpoint Data Pivot graph available in HAWC is provided (**Figure 1**). Additional graphs will be created and made available to meet the needs of the expert panel in reviewing subsets of the data. The graphs are interactive allowing users to click on results and text to access additional information in a pop-up window (e.g., to see how the outcome was diagnosed, what adjustments were made in the statistical analysis, etc.) or navigate to the full study summary information. All of the data included in these graphs are also available in the Study Summaries in the Supplementary Material.

Study	Population Name	Exposure Measure	Exposure Comparison	Dose (min.)
Nwaru, 2011	Finnish Type 1 Diabetes Prediction and Prevention Nutrition Study	Maternal intake of folate during pregnancy- dietary	Dietary Folic Acid Intake	
Dunstan, 2012	Pregnant women in Western Australia	Maternal folic acid intake from supplements in pregnancy	Tertile 1 (<200 µg/day)	0.0
Miyake, 2011	Osaka Maternal and Child Health Study	Maternal intake of folate during pregnancy	Q1 (median = 206.8)	150
Dunstan, 2012	Pregnant women in Western Australia	Maternal folic acid intake from supplements in pregnancy	Tertile 2 (200-499 µg/day)	200
Miyake, 2011	Osaka Maternal and Child Health Study	Maternal intake of folate during pregnancy	Q2 (median = 255.1)	225
Miyake, 2011	Osaka Maternal and Child Health Study	Maternal intake of folate during pregnancy	Q3 (median = 291.2)	275
Miyake, 2011	Osaka Maternal and Child Health Study	Maternal intake of folate during pregnancy	Q4 (median = 370.6)	350
Dunstan, 2012	Pregnant women in Western Australia	Maternal folic acid intake from supplements in pregnancy	Tertile 3 (500+ µg/day)	500

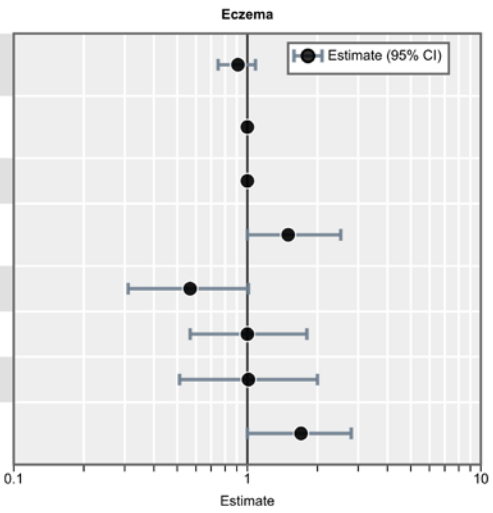

Figure 1: Example figure. Eczema studies of maternal folate intake, ordered by increasing dose (No dose reports for (Nwaru *et al.* 2011); total folate intake results reported as not statistically significant.)

2.0 BACKGROUND

Folate is a general term for this water-soluble B-complex vitamin, which humans require for the synthesis of nucleic acids and to provide methyl groups for biochemical reactions within cells (National Research Council 1998). These functions are needed for everyday growth and cell division, including during critical periods of rapid growth and cell division such as embryonic development. Thus, folate is necessary for all individuals, and is especially important for women who may become pregnant.

In 1998, the Food and Nutrition Board of the Institute of Medicine (IOM) set Dietary Reference Intakes that included the Recommended Dietary Allowances (RDAs) and tolerable upper intake levels (ULs) — the highest level of daily intake likely to pose no risk of adverse health effects to almost all of the population(National Research Council 1998). Folic acid (pteroylmonoglutamic acid) is rarely present in food, but as the most stable form of folate it is used in fortified food products and most vitamin supplements (National Research Council 1998). The folic acid UL (1000 µg for adults[4]) was established with the paucity of data available to the committee at the time, and based on limited but suggestive evidence that excessive folic acid intake may precipitate or exacerbate neuropathy in vitamin B_{12}-deficient individuals. Since the 1998 IOM report set the UL for folic acid, many research publications have reported health effects over a range of folic acid intakes. Some studies have raised concerns that high intake of folic acid may be associated with potential adverse health effects.

In a discussion of folate and folic acid intake, it is important to note that folate is present in the diet through its natural occurrence in food, as a food additive, and as an ingredient in dietary supplements. Naturally occurring folate is unlikely to be associated with potential adverse effects because it has lower bioavailability than folic acid and its consumption is also limited by the bulk and caloric content of foods. Therefore, the primary substance of interest for considering the safety of high intake is folic acid, the form of folate commonly added to foods and dietary supplements. Another form of folate available as a dietary supplement is “methylfolate” which is chemically distinct from folic acid. While methylfolate was included in the comprehensive literature search strategy, it is not the focus of this review and no studies of methylfolate were identified within the high priority categories.

3.0 METHODS

Research publication rates are rapidly increasing, and folic acid research is no exception (see **Figure 2**). For example, of the more than 28,000 publications considered for this document 20,000 (>70%) were published after 1998 when the IOM last evaluated the RDA and UL for folic acid. Given this vast literature, systematic review methods offer an approach to comprehensively consider a large literature, as an alternative to author-directed narrative reviews.

[4] The RDA for folate is 400 µg dietary folate equivalents (DFEs) for adults and 600 µg DFEs for pregnant women. 1 µg DFE = 1 µg of food folate = 0.5-0.6 µg of folic acid (depending on if it is ingested with food) (National Research Council 1998).

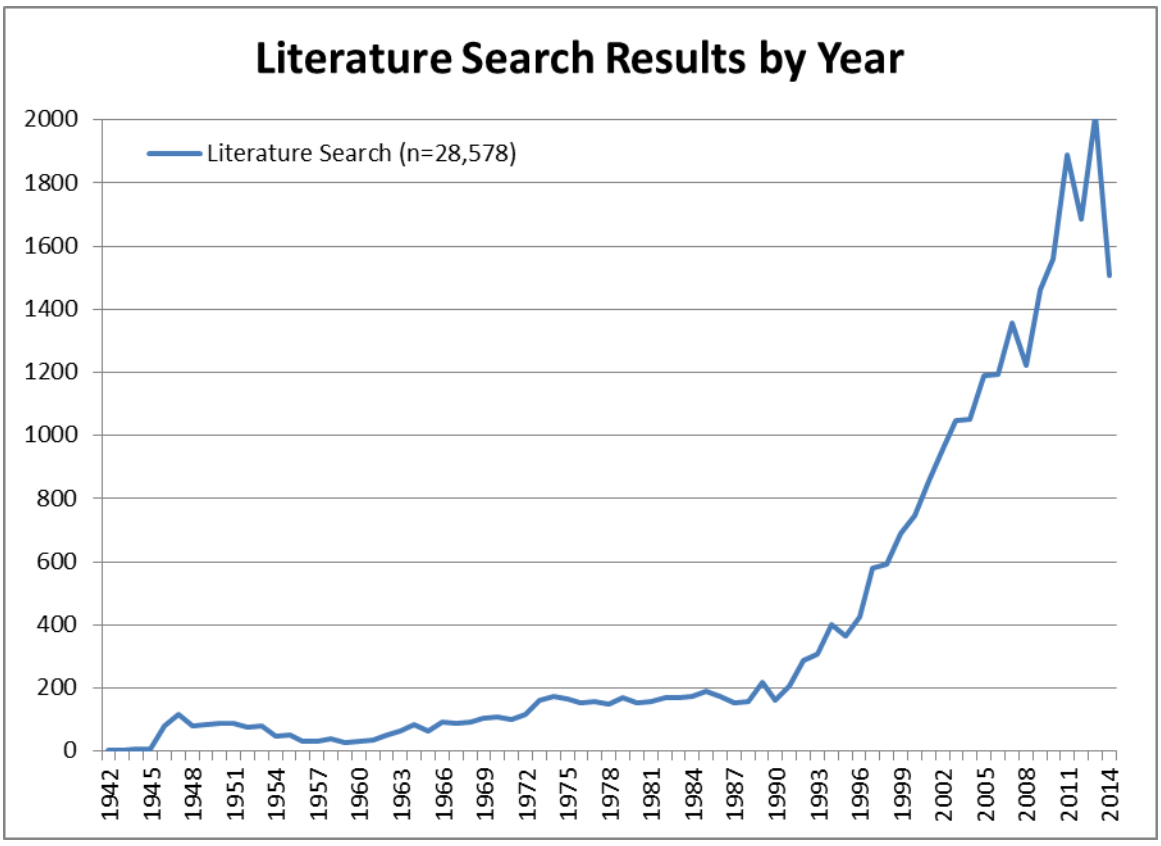

Figure 2. Publication rates over time. Number of studies identified by the literature search per year (cumulative total = 28,578).

This document presents the data collected on potential health effects reported in studies with high intakes of folic acid for consideration by the expert panel in evaluating the state-of-the-science and identifying research needs within four health effect categories. To comprehensively and objectively identify relevant studies, this document was prepared using systematic review methodology. Such an approach to the literature search and data extraction provides increased transparency and objectivity to the process of identifying, selecting, and summarizing results of relevant studies. Systematic review methods do not eliminate the need for scientific judgment – judgments made by NTP, ODS, and the steering committee are documented including the scientific justification for these decisions.

A detailed description of the methodology used is provided below, including procedures for each of the 4 steps in the process of assembling the literature for this document:

3.1 Literature Screen: searching for and selecting relevant studies following PICO/PECO criteria as in a systematic review

3.2 Detailed Tagging of Human Studies: collecting additional information on exposure(s) and outcome(s) to identify high priority topics

3.3 Outcome Prioritization: identifying high priority health effect categories for consideration by the expert panel

3.4 Data Extraction: summarizing information from the selected human studies into HAWC and Supplementary Material study summaries

3.1 Literature Screen

3.1.1 Criteria for Identifying Relevant Studies

Systematic review procedures use a precise statement to define the information that is relevant for addressing the research question, in this case, the state of the science on the safe use of high intakes of folic acid. That statement is outlined in PICO criteria[5] (Population, Intervention or exposure, Control or comparator and Outcomes of interest) that were used to guide the review process. These criteria are broad by design, as the primary objective of the screening effort is to identify areas of highest priority for assessing safe use of high intakes of folic acid. See **Appendix 1: Literature Search Method** and **Appendix 2: Criteria for Screening of Studies** for additional details on screening of the search results.

Population

Humans, experimental animals, and *in vitro* model systems exposed to folate or folic acid were considered relevant.

Studies were excluded if subjects had comorbidities likely to contribute to a folate deficiency, including impaired renal function, alcoholism, or gastrointestinal disorders impairing folate absorption such as celiac disease.

Intervention or Exposure

Exposure to folate, folic acid, folacin, folinic acid, tetrahydrofolate, methyltetrahydrofolate, and 5-methylfolate were considered relevant. The Institute of Medicine defined the unit of measurement for folate as dietary folate equivalents (DFEs) (National Research Council 1998). However, many studies do not report intakes as DFEs. Information on the proportion of total intake from naturally occurring folate sources vs. from synthetic forms of folate is necessary to convert reported intakes into DFE units. Exposure to "total folate" was included because many studies report intakes in this form rather than specifying only the intake of folic acid. Additionally, baseline folate status is likely to affect response to supplemental or fortified sources of folic acid. Although folic acid is currently the primary form of added folate, other forms may have been evaluated and may gain use in the future.

Studies were excluded that focus on evaluating the effect of folic acid supplementation after an intervention such as surgery or medication. This included the exclusion of studies of chemotherapeutic agents where the focus is on the impact of folic acid on treatment efficacy, and there is a known interaction between folic acid and some chemotherapeutics.

Although the focus of this review is assessing safe use of high intakes of folic acid, there were no a *priori* exclusions in the initial screening process based on a dose level of folic acid or a specific blood folate concentration, because these doses are not uniformly reported in the title or abstract. Therefore a consistent screening by dose could not be made without evaluating the full article text. Studies considered for full data extraction were selected by focusing on exposure to folic acid above 400 µg per day (or total folate above 600 µg/d) or circulating folate concentrations above 10 nmol/L (4ng/mL) for serum folate or 340 nmol/L (151ng/mL) for red blood cell (RBC) folate, when specified. These cut-offs are based on cut-offs for assessing folate

[5] The Cochrane Collaboration: Asking an Answerable Question (http://ph.cochrane.org/sites/ph.cochrane.org/files/uploads/Unit_Five.pdf)

status as identified by authoritative bodies. That is, the Institute of Medicine's highest recommended intake is for women of childbearing age: "400 µg of folic acid daily from fortified foods, supplements, or both in addition to consuming food folate from a varied diet" (National Research Council 1998). The World Health Organization (WHO) defined folate deficiency as below 10 nmol/L (4 ng/mL) for serum or 340 nmol/L (151 ng/mL) for RBC folate based on elevations in total plasma homocysteine in the US National Health and Nutrition Examination Survey III (1988-1994) and is slightly more conservative than the standards used in clinical practice (305 nmol/L for RBC folate) (de Benoist 2008). These levels were used as a practical cut-offs for the evaluation of the safety of folic acid as they are recent guidelines for assessing folate status. By including studies in this review that were at or above recommended intakes or clinical indicators of adequate status, information on the availability of evidence defining safe ranges of intake as well as intakes associated with potential adverse effects can be captured.

Control or Comparator

No *a priori* restrictions were made on the type of control or comparator groups considered or on specific study designs. If the same amount of folic acid was given to all participants in a randomized controlled trial (both treated and placebo groups), it was not included.

Outcomes of Interest

Studies that focused on evaluating the association between folic acid or folate and a health outcome relevant to human health were considered relevant at the screening stage with the following exceptions:

1. Birth defects where the benefit of folic acid in prevention is established; there are many studies showing benefit and very few studies that show adverse effects.
2. Bone outcomes where no studies of adverse effects were identified after a preliminary screen of the PubMed literature between 1992 and 2011.
3. Kidney disease which is associated with folate deficiency from increased requirements.
4. Gastrointestinal disorders leading to deficiency from impaired folate absorption.
5. Homocysteine blood concentration without an additional health effect .
6. Infectious disease studies conducted in areas with endemic infectious diseases where folate deficiency is more of a concern. While it is acknowledged that treatment of folate deficiency with folic acid may increase susceptibility to infectious diseases in these environments, these health effects are not a high priority for evaluating folic acid safety in the United States. Studies that focus on the management of infectious disease rather than a concern that folic acid may increase the risk of contracting an infectious disease are also excluded.
7. Liver outcomes where no studies of adverse effects were identified after a preliminary screen of the PubMed literature between 1992 and 2011.

3.1.2 Search Methods for Identification of Studies

The initial literature search was performed in November of 2011 and was not limited by language or publication date. As noted above, the literature search was designed to exclude studies of co-treatment with folic acid and chemotherapeutic agents. For the purposes of refining the scope of this review the PubMed literature was initially screened back to 1992, well before the last review of folic acid by the Institute of Medicine and these preliminary screening results were used to amend the inclusion criteria for health outcomes considered. In addition to PubMed, Embase, Scopus and Web of Science were

searched with no limits on publication date or language. Given the content overlap among the databases, all duplicate records were removed. Two search updates were conducted in May 2013 and December 2014 so that additional references could be screened and data extracted prior to the expert panel meeting. In addition to the initial databases, the Cochrane Library was also searched for both updates. Cochrane content was added to ensure that no clinically-based systematic reviews or trials were missed.

The details of the electronic database searches are presented in **Appendix 1: Literature Search Method**. The PubMed search includes both MeSH and text words. The Embase search used both the Emtree controlled vocabulary terms as well as text words. The Web of Science and Scopus searches used text words only.

Additional published data were included as identified by NTP staff, reference lists of review articles, and subject matter experts.

Literature from alternative sources ("grey literature" not published in books or journal articles) was not identified for inclusion. Meeting abstracts and unpublished data from personal author communication would have been considered as a supplement to a peer-reviewed publication, but a study that was entirely unpublished or otherwise not peer-reviewed was not considered.

3.1.3 Selection of Studies

First, two reviewers independently screened titles and abstracts for relevance. **Appendix 2: Criteria for Screening of Studies** details the screening approach. Those studies considered relevant or uncertain in the title/abstract screen moved forward. Studies that did not fulfill the criteria were excluded and their bibliographic details listed with the reason for exclusion. Next, the full text of references deemed relevant or uncertain in the title/abstract screen was reassessed by one reviewer with the same inclusion/exclusion criteria as in the title/abstract screen. If this reviewer indicated that a reference should be excluded, a second reviewer independently confirmed the exclusion. Any discrepancies were resolved by consensus of the two reviewers. The number of studies retained at each step in this process is diagramed in **Figure 3** following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement criteria (Moher *et al.* 2009). The number and type of studies within each included health effect category is detailed in **Table 1**, with the number of references per year by category graphed in **Figure 4**.

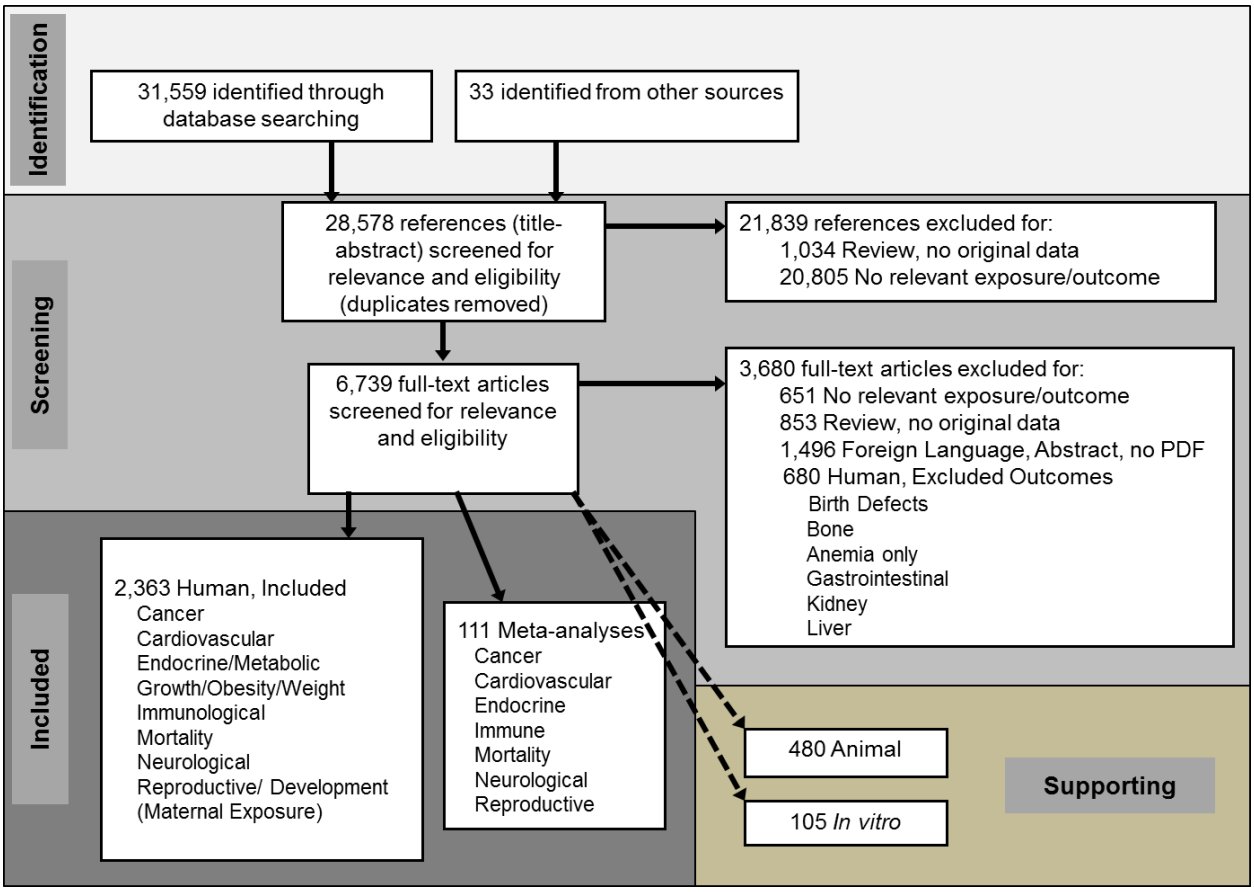

Figure 3. Selection of Studies. Diagrams the flow of studies through the screening process, including reasons for the exclusion of studies (adapted from Moher *et al.* 2009).

Table 1. Number of studies identified within each health effect category. Studies could be classified under more than one category so the sum across categories will be higher than the overall total.

Health Effect Category	Human – Primary (n=2,363)	Human – Meta-analyses (n=111)	Animal (n=480)	*In Vitro* (n=105)
Cancer	604	50	95	62
Neurological	540	14	78	20
Cardiovascular	486	39	79	15
Reproductive/Developmental	290	12	99	16
Immunological	146	1	29	12
Endocrine/Metabolic	207	1	76	4
Growth/Obesity/Weight	132	7	64	3
Mortality	104	16	9	2
Maternal Exposure*	255	12	127	

*Maternal folate exposure includes outcomes in offspring across multiple categories, and this tabulation does not include studies of birth defects or other excluded outcomes.

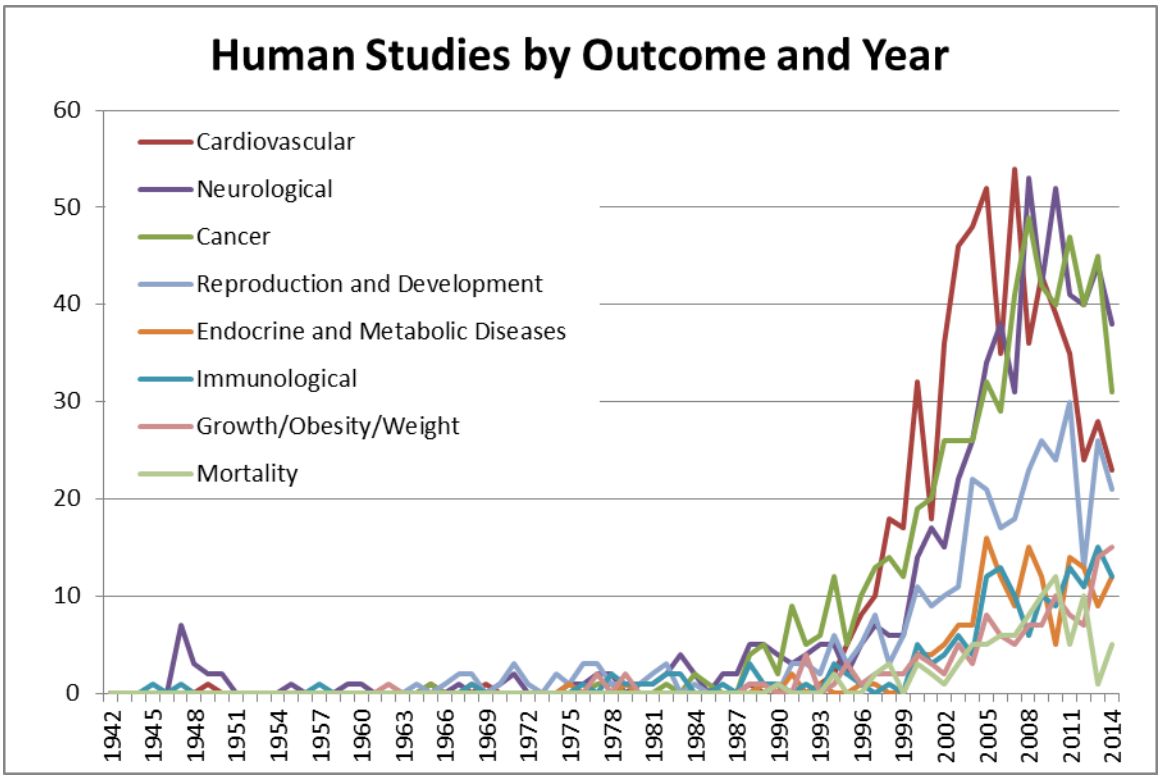

Figure 4. Number of studies by year for the included studies and by each major health effect category.

3.2 Detailed Tagging of Human Studies

After completion of the initial screening, the body of human studies was still quite large (>2,000 studies). A second level of more detailed "tagging" was undertaken to obtain more information about studies within the included health outcome areas. For example, instead of considering all "reproductive effects" as captured in the full text screening, results were considered by specific outcome, such as "preterm birth". This approach captured outcome-specific details and whether the publication reported any statistically significant results indicating an adverse effect from folic acid. Beneficial effects were not considered separately from studies reporting no effect because safety is the focus of this evaluation, not efficacy. The degree of "lumping" or "splitting" of outcomes was based on the available studies, and some outcomes overlapped considerably (e.g., cognition, memory, dementia).

It is important to note that reporting of adverse effects may be inconsistent, particularly in intervention studies, so the lack of an adverse report cannot be interpreted as evidence that there was no adverse effect in a study. For example, an intervention study that only reported results for cardiovascular effects and made no mention of respiratory effects was only considered within the body of cardiovascular studies and not as evidence of no respiratory effects, even if it included a statement that no adverse events were reported.

While this approach is relatively crude, and admittedly not a detailed assessment of the internal validity of the study, it enabled prioritization on topics with potential safety concerns. It also captured the reported folate measurements and level of exposure (intake vs. blood measurement) to assess if studies reporting an effect had subjects in the high range. In addition, inclusion of vitamin B_{12} level was also considered for studies of cognitive effects. As covered in **5.0 Other Health Effect Categories**, the vast majority of literature was designed to assess beneficial effects of folic acid and there are few reports of adverse effects for many health effects.

3.3 Outcome Prioritization

Results from the detailed tagging identified four categories of high priority health outcomes based on the presence of adverse reports in studies of intake over 400ug/day or blood levels above the deficient range. These decisions were made in conjunction with the steering committee based on the information available up to the May 2013 literature search, while the numbers of studies listed below also include those identified in the December 2014 search update. Additional information about why the health effect categories were considered high priority is included in **4.0 High Priority Health Effect Categories**. Human studies in each category are included in the Study Summaries in the Supplementary Material.

4.1 Cancer Pooled and Meta-analyses: pooled and meta-analyses (n=43) across 12 cancer subtypes. There were ~600 primary human studies identified, not extracted, and made available to the expert panel as supplemental material.

4.2 Cognition and Vitamin B12: cognitive outcomes where vitamin B_{12} level or intake was included in the analysis includes primary studies (n=27) and meta-analyses (n=2).

4.3 Hypersensitivity-related Outcomes: a collection of related outcomes (n=40) often with multiple outcomes per study and many considering maternal exposure [including respiratory infection (n=16), asthma (n=14), allergy and atopic disease (n=12), wheeze (n=8), hypersensitivity test (n=5), eczema (n=5), and food allergy (n=2); and one meta-analysis of asthma and wheeze with maternal exposure].

4.4 Thyroid and Diabetes-related Disorders: includes primary studies (n=72) with thyroid outcomes (n=10) as well as diabetes (n=38), insulin resistance (HOMA, n=21) and metabolic syndrome (n=12); and one meta-analysis of Hb1Ac levels.

The health effect categories not considered high priority topics for the assessment of safe use are summarized in **5.0 Other Health Effect Categories** and include: cardiovascular outcomes, twinning and multiple births, autism, other neurological outcomes, other immunological outcomes, other endocrine and metabolic disease outcomes, other reproductive outcomes, and mortality.

3.4 Data Extraction

Data extraction was completed with the web-based tool Health Assessment Workspace Collaborative (HAWC, hawcproject.org), which is publically accessible and allows for interactive exploration of the assembled data in addition to the Study Summary format provided in the Supplementary Material. Links to electronic database records (e.g., PubMed) are available in the public version, and the expert panel has access to the full text of all papers. Relevant studies were extracted into HAWC in a standardized manner by one reviewer and independently checked for accuracy and consistency across studies by a second reviewer. Details of specific elements of studies captured in HAWC are listed in **Appendix 3: Data Extraction Elements**. The HAWC summary is not meant to entirely recapitulate the full information provided in the publication, but to facilitate evaluation of consistency and uncertainty across studies by summarizing key aspects of design and results in a consistent manner.

Data on folate intakes was captured as reported because there is no standard reporting format for folate and folic acid intakes. Ideally, intakes would be converted to dietary folate equivalents (DFEs) a common unit of measurement to facilitate comparisons among studies, however, few studies reported results in DFEs and sufficient information in individual studies was typically not available for conversion.

4.0 HIGH PRIORITY HEALTH EFFECT CATEGORIES

High priority health effect categories were chosen in consultation with the steering committee based on the results from the detailed tagging available up to the May 2013 literature search. Four categories of high priority were identified based on reported adverse effects in studies of intake over 400ug/day or blood levels above the deficient range. Sub-panels of the expert panel were formed and each focuses on one high priority health effect category. The Supplementary Material and HAWC resource contain the Study Summaries for the human studies in each category and lists of supporting references from the animal and *in vitro* literature are also included in Supplementary Material.

4.1 Cancer Pooled and Meta-analyses

The link between cancer and folate metabolism has a long history, including the development of antifolates, aminopterin and methotrexate, as early chemotherapeutic agents used in the 1950s. While initially studied for a role in cancer prevention, the potential for cancer risks from high intake of folic acid was raised when the Norwegian Vitamin Trial (NORVIT) and the Western Norway B Vitamin Intervention Trial (WENBIT) were stopped early due to preliminary analysis showing no improvement in cardiovascular outcomes and possible increases in cancer risks (Ebbing *et al.* 2008, Ebbing *et al.* 2009). There is biological plausibility to the paradoxical role of folate in both cancer prevention and promotion (Mason 2009).

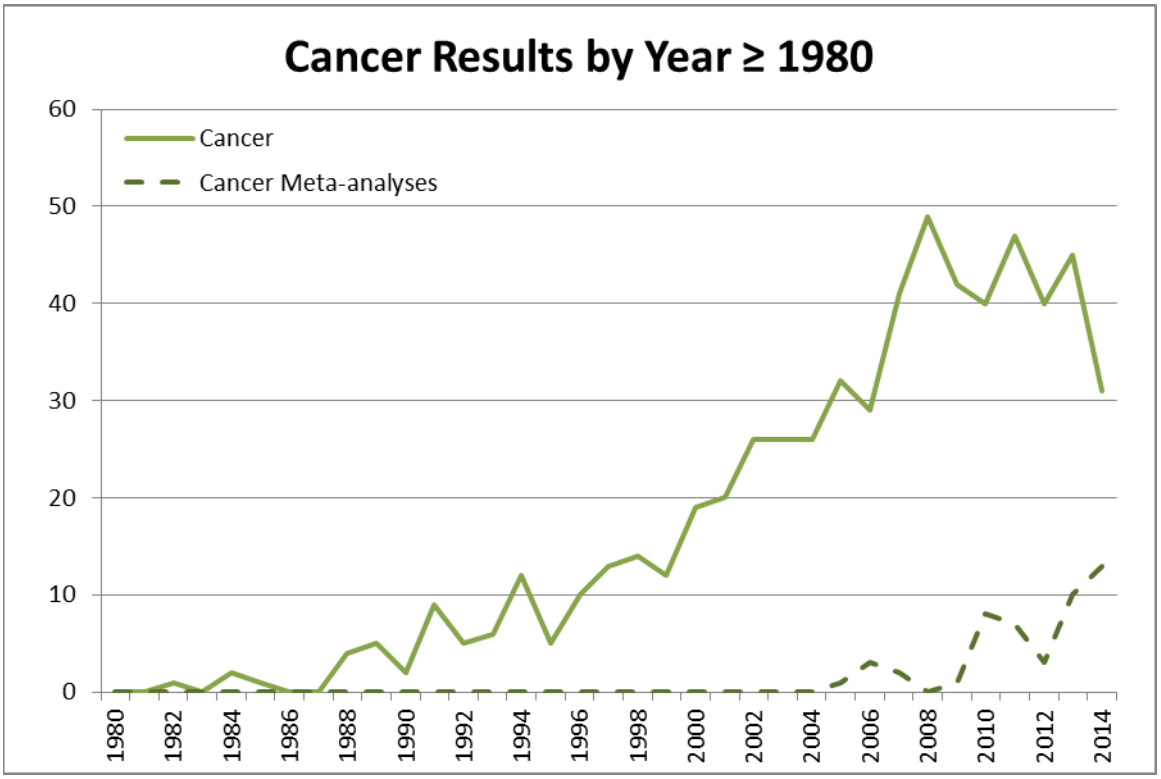

Figure 5. Cancer studies identified by year (since 1980) including the number of meta-analyses.

The literature search captured 604 primary studies addressing cancer and folate (Note: not all were at high intake levels). These studies included most major cancer subtypes, with colorectal, breast, cervical, lung, and prostate being the most frequently studied. There were also studies of childhood cancers

considering *in utero* folate exposures, which predominantly reported no increased risks from maternal folate intake during pregnancy. The cancer subtypes with the highest proportion of studies reporting increases in risk associated with folic acid are skin, leukemia, prostate, breast, and bladder/urogenital cancer.

Given the wealth of primary research in this area, numerous meta-analyses have been conducted for cancer outcomes and are also captured in the literature screen (see **Figure 5**). The meta-analyses utilized a range of different inclusion/exclusion criteria (PICO/PECOs) and analytical approaches. Although the use of the systematic review methodology is growing, the uptake of objective tools to assess internal validity or risk of bias is still in process (Higgins *et al.* 2011). Most of the identified meta-analyses did not conduct risk-of-bias analyses to evaluate potential sources of bias from the studies' design and conduct.

For this review, cancer was considered a high priority health effect category based on the presence of numerous studies of high folic acid intake in a wide variety of populations with inconsistent results across multiple cancer types. Pooled and meta-analyses provide an approach to quantitatively synthesize results across multiple studies; however, the results across the pooled and meta-analyses identified for this review are inconsistent. Exploring the areas of consistency and uncertainty across this large literature base was considered a high-priority.

Given the availability of numerous recent pooled analyses and systematic reviews with meta-analyses, NTP chose to take advantage of these synthesized results and focus the data extraction for cancer on the 43 available pooled and meta-analyses. Most of these studies focused on intervention studies or observational studies on intake, although a few considered blood folate levels as well. Three publications considered pediatric cancers from maternal folic acid exposure, and none found evidence of increased risks. The HAWC project "Folic Acid - Cancer Pooled and Meta-analyses (2015)" summarizes the protocols used for these pooled and meta-analyses and their results. Endpoint Data Pivots graphically display results across studies with similar endpoints. All individual human, animal, and *in vitro* studies identified relevant to cancer endpoints are listed in the Supplementary Material and were made available to the panel, but not included in the Study Summaries or the HAWC project.

The expert panel is tasked with using the pooled and meta-analysis Study Summaries and primary studies listed to consider the areas of consistency or uncertainty in this diverse literature and what additional research could address unresolved scientific questions. There are many potential reasons for inconsistency of results in this large literature base, most of which are not unique to cancer studies. When discussing areas of uncertainty the expert panel may consider the types of folate exposures or measurements (interventions, dietary intake, blood levels) and the study designs employed (randomized trials, cohorts, case-control, cross-sectional and ecological studies of cancer rates pre/post fortification are all included in the cancer literature). The populations in these studies varied in their level of folate intake; if and when the food supply was fortified with folic acid; and in prevalence of potential confounders, such as smoking. Length of follow-up of subjects in these studies is also important to consider given the likely lifetime exposure to folic acid at some level and the potentially long period of time from the development of a neoplasm to a diagnosis of cancer.

4.2 Cognition and Vitamin B_{12}

In 1998, the Food and Nutrition Board of the Institute of Medicine set Dietary Reference Intakes and based the tolerable upper intake level (UL) of 1mg folic acid on an increased risk of neurological effects from folic acid administration in individuals with an underlying vitamin B_{12} deficiency(National Research Council 1998). The studies that contributed to this decision were generally older case reports and case series studies (no study with more than 50 subjects; all but one published prior to 1962). Several studies have been published in recent years with more diverse study designs and exposure assessment methods that jointly consider the relationship between folic acid, vitamin B_{12}, and cognition – particularly in elderly populations with high rates of vitamin B_{12} deficiency.

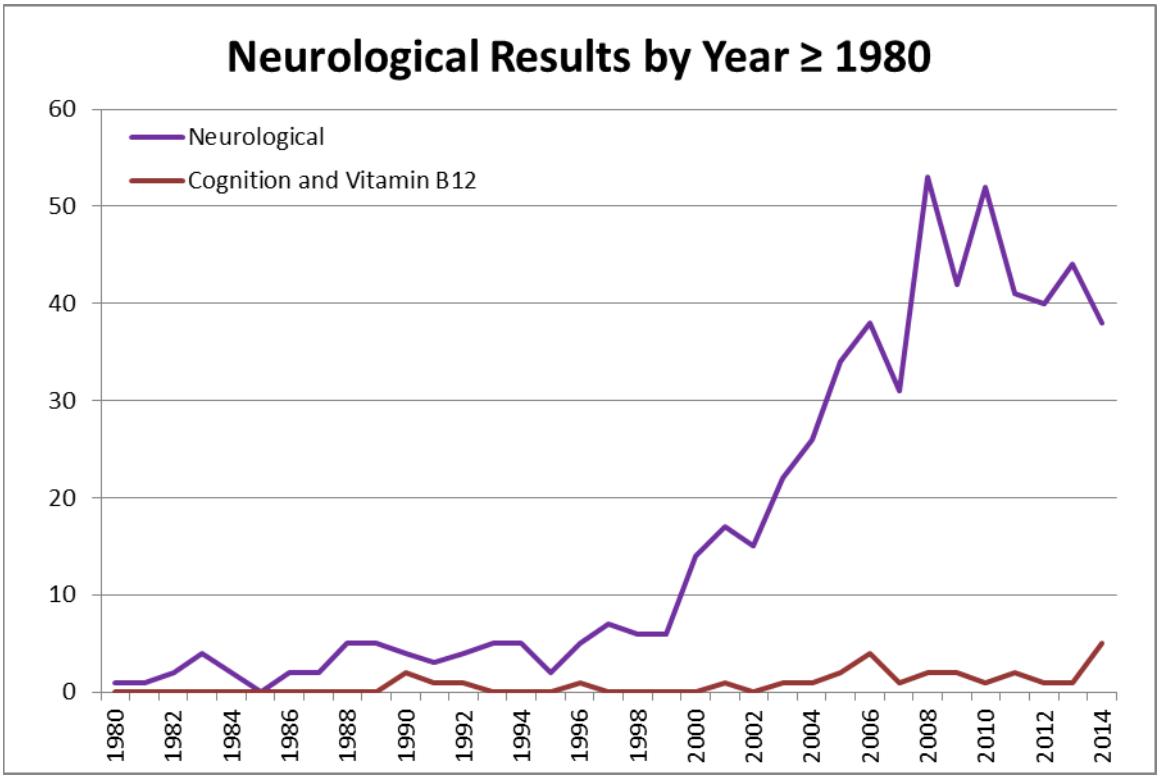

Figure 6. Number of neurological and cognition and vitamin B_{12}-related studies by year since 1980 .

In the literature search and screening for this review, over 500 studies investigating various neurological effects of folic acid (including the studies that were the basis of the UL) were identified (see **Figure 6**), the vast majority of which explored beneficial effects of folic acid on neurological endpoints (see **5.0 Other Health Effect Categories** for additional discussion of other neurological endpoints). More than 100 studies including cognitive tests as endpoints were identified, yet very few also considered vitamin B_{12} level. In the subset of studies that considered both B vitamins, several studies were identified reporting adverse effects of high folic acid in subjects with low vitamin B_{12} – as considered by the IOM Board in 1998.

Given the long-standing concern for the potential impact of high folic acid intake in individuals with an underlying vitamin B_{12} deficiency, cognition including vitamin B_{12} assessment was considered a high priority health effect category for this review. The data extraction focused on the studies of cognitive

effects that jointly considered vitamin B_{12} levels or deficiencies. There are 27 human studies and two meta-analyses that considered folate (both intake and blood levels), vitamin B_{12}, and cognitive endpoints summarized in the Supplementary Material.

The HAWC project "Folic Acid - Cognition and Vitamin B12 (2015)" graphically display results across these studies in Endpoint Data Pivots and includes descriptions of the study design, exposure assessments, and outcome assessments as well. Experimental animal and *in vitro* model system studies identified relevant to cognitive endpoints are listed in the Supplementary Material and made available to the expert panel, although not included in the Study Summaries or the HAWC project.

The expert panel is tasked with considering how the approaches to addressing these inter-B vitamin relationships in the available studies inform consistency or uncertainty in this literature and propose what additional research, if any, would address unresolved scientific questions about the impact of high folic acid intake on cognition.

4.3 Hypersensitivity-related Outcomes

The role of folate in the development of childhood allergy, asthma, and other hypersensitivity-related immunological outcomes has emerged relatively recently in the literature. Other than a few case reports of anaphylactic reactions to folic acid, there were no publications prior to the 1998 IOM report. Given increasing intakes of folic acid and rates of allergic disease, this area of research has grown considerably over the last 15 years.

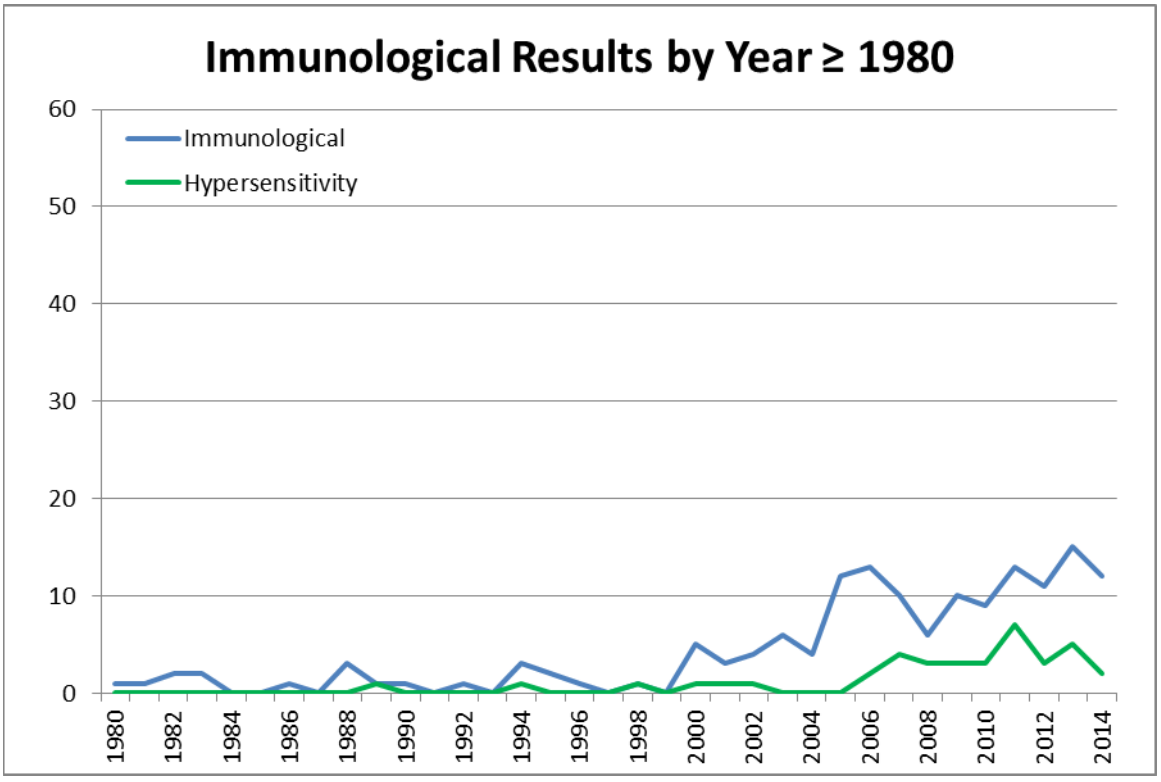

Figure 7. Number of immunological and hypersensitivity-related studies by year since 1980.

Within the relatively small literature considering immunological effects of folic acid (n=146), the studies reporting more potential adverse effects of folic acid were for hypersensitivity-related outcomes (e.g., allergy, atopic disease, and asthma) as opposed to auto-immunity or other immunological outcomes (see **Figure 7**). Many of these studies considered maternal folic acid intake or levels during pregnancy, a sensitive developmental window for later-in-life immune effects in children. Folate's role in DNA methylation and inflammation are two biological pathways by which it is thought to influence the development of asthma or allergy in children (Brown *et al.* 2014).

Given the recommendations to take folic acid during pregnancy to prevent birth defects and theoretical epigenetic effects, the potential impact on hypersensitivity-related endpoints is a high priority topic when considering the safe use of high intakes of folic acid. This review includes hypersensitivity as a high priority health effect category. Forty studies of folate level, folate intake, and folic acid interventions that assessed immunological endpoints associated with hypersensitivity were identified and included in the Study Summaries. Outcomes included are: respiratory infection (n=16), asthma (n=14), allergy and atopic disease (n=12), wheeze (n=8), hypersensitivity test (n=5), eczema (n=5), and food allergy (n=2). These studies include a range of study designs, several are from large prospective birth cohorts where

prenatal exposure was considered in relationship to hypersensitivity outcomes in childhood, and many reported multiple outcomes per publication. A meta-analysis has been published which found no increased risk of asthma in the children of mothers who took folic acid supplements during pregnancy (Crider *et al.* 2013).

These 40 studies are included in the project "Folic Acid – Hypersensitivity-related Outcomes (2015)" accessible on the HAWC website. Endpoint Data Pivots graphically display results for each endpoint across these studies and additional information on the study design, exposure assessment, and outcome assessment is available in pop-up windows with the graphs. Relevant *in vitro*, experimental animal and human studies of other immunological endpoints are listed in the Supplementary Material for consideration by the expert panel, but not included in the Study Summaries.

The expert panel is tasked with reviewing this literature and proposing additional research approaches that could potentially clarify the relationship between folic acid and hypersensitivity-related endpoints, including consideration of developmental windows of susceptibility. Potential unmeasured confounding factors associated with both hypersensitivity and if and when a woman might take supplements during pregnancy, would include maternal factors during pregnancy and post-natal factors in the children's environment. Several animal studies have also been published exploring the epigenetic mechanisms by which folic acid might influence hypersensitivity (Palmer *et al.* 2014), and the expert panel may consider this literature as well.

4.4 Thyroid and Diabetes-related Disorders

As discussed in the hypersensitivity section, metabolism later in life might also be "preprogramed" by *in utero* nutrition. While much of this literature has focused on famine or nutrient deficiencies (Ravelli *et al.* 1998, Li *et al.* 2010), there is some evidence in human and animal studies of increased risk of diabetes and adiposity with increased maternal exposure to folic acid, particularly in conjunction with vitamin B_{12} deficiency (Finer *et al.* 2014). Postnatal exposure to folic acid may also impact metabolism.

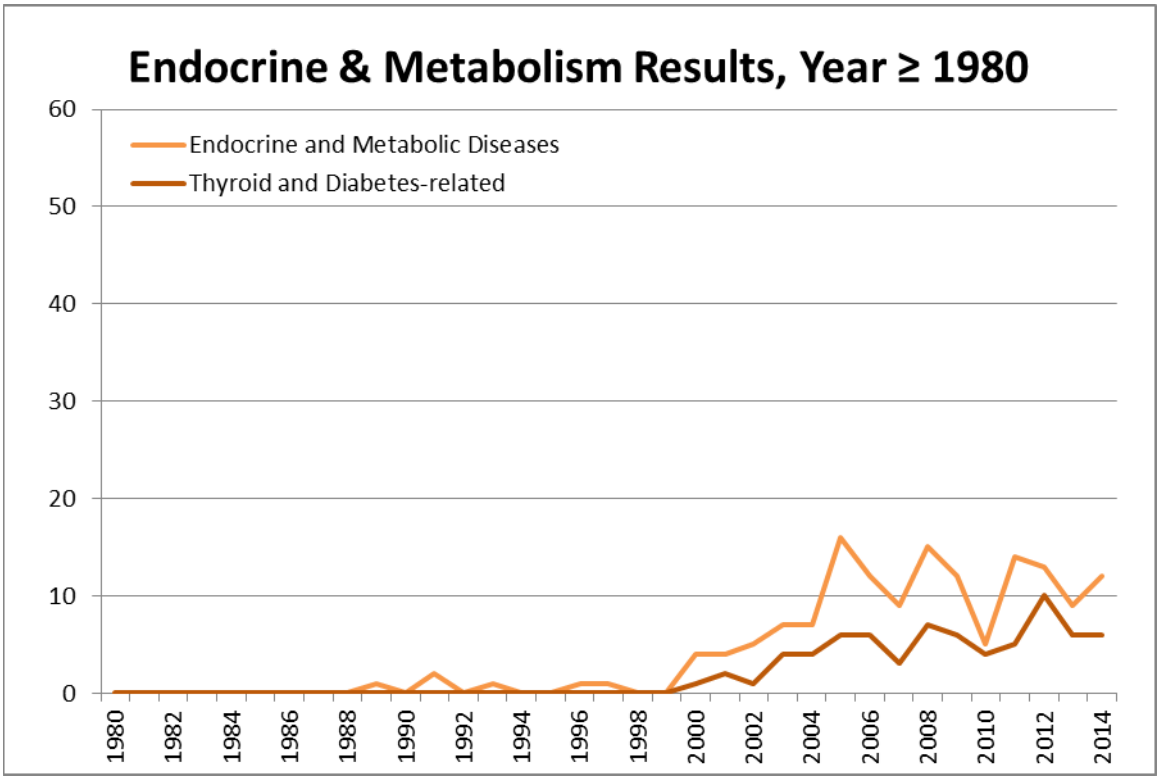

Figure 8. Number of endocrine and metabolism studies by year since 1980.

Metabolic or endocrine diseases, such as diabetes and thyroid function, have not been extensively studied for potential effects of high folic acid intake as other areas of focus for the expert panel (see **Figure 8**). Within the ~200 studies identified with metabolic or endocrine endpoints, reports of higher folic acid were identified in some studies of diabetes-related outcomes and thyroid disorders. Only one meta-analysis was identified; the study found no effect of folic acid in randomized control trials on glycemic control in type 2 diabetics (Sudchada *et al.* 2012).

Diabetes and other metabolic disorders are a major public health concern with established environmental risk factors (Thayer *et al.* 2012).This review includes throid disorders, diabetes, and diabetes-related outcomes as a high priority health effect category. The HAWC project "Folic Acid – Thyroid and Diabetes-related Disorders (2015)" includes 72 primary studies: thyroid (n=10), diabetes (n=38), insulin resistance (HOMA, n=21) and metabolic syndrome (n=12). Endocrine and metabolic disease studies not included were not directly related to diabetes or thyroid function (such as focusing only on body mass index or body composition), or the folate levels were in the low/deficient range. However for many of these studies the primary focus of the publication was not potential adverse endocrine effects of folate, and these studies reported only folate levels in diabetics and non-diabetics –

the minimum requirement for inclusion in this review. The meta-analysis of glycemic control in type 2 diabetics is also summarized in HAWC (Sudchada *et al.* 2012). Of the 10 studies of thyroid function identified, 4 contained potential reports of adverse effects, but no reviews or commentaries highlighting this as a potential risk from high intake of folic acid were identified.

When the results are appropriate for graphing (not just p-values), Endpoint Data Pivots display inter-study results with additional information accessible via the graphs including aspects of study design and exposure or outcome assessment. Experimental animal and *in vitro* studies relevant to endocrine and metabolic health effects are not included in the Study Summaries but are listed in the Supplementary Material.

The potential impact of folic acid on metabolism may not represent a mature field of research, particularly when compared to the hundreds of studies of cancer. There is also the potential for confounding or reverse causation given recommendations for diabetics to take folic acid supplements, particularly during pregnancy. These challenging issues will be explored by the expert panel when considering of areas of consistency and proposing additional research that may clarify uncertainties for this health effect category.

5.0 OTHER HEALTH EFFECT CATEGORIES

Due to the vastness of the research on folate and folic acid, large areas of research were captured in this literature search and screening process that were not deemed to be high priority categories for assessing safe use of high intakes of folic acid. Some of the health outcomes excluded during the literature screening process include: gastrointestinal, renal/kidney, infectious disease, and birth defects. All other outcomes underwent detailed tagging to collect additional information on the exposures, outcomes, and findings reported in each study. Under each health outcome, results were tagged as "adverse" or "no effect/beneficial effect" only for reported outcomes, such that a statement that no adverse events were reported could not be considered as evidence of no effect if no specific outcomes were included as being monitored.

The expert panel will not evaluate the literature for other health effects not identified as high priority category by the NTP and the steering committee based on the information available up to the May 2013 literature search. This chapter summarizes the state-of-the-science for each category as captured in this literature review and briefly discusses why these other health effects were not selected as high priority topics for evaluation. Lists of studies identified in each of the categories are included in the Supplementary Material.

5.1 Cardiovascular Outcomes

Cardiovascular endpoints represented one of the largest bodies of research captured in the literature screen (over 400 human studies). Many of the controlled trials of B vitamin interventions were designed with the expectation that folic acid treatment would be beneficial to cardiovascular health by lowering homocysteine and reducing adverse cardiovascular effects. The detailed tagging identified very few studies reporting increases in adverse cardiovascular effects associated with folic acid treatments, use, or blood level (likely within the expected false positive rate, though no formal test was performed). None of the 39 identified meta-analyses reported any adverse effects associated with folic acid intake. Due to the dearth of reported adverse associations, cardiovascular endpoints were not considered a high priority topic for assessing safe use of high intakes of folic acid.

5.2 Twinning and Multiple Births

Of the 18 studies identified that assessed the role of folic acid in the incidence of twins or multiple births, 11 reported significantly increased rates. However, some initial studies reporting an association did not fully account for fertility treatment as a confounder (Czeizel *et al.* 1994b, Ericson *et al.* 2001). One meta-analysis of randomized controlled trials reported a significant association between folic acid and multivitamins and multiple pregnancy in two randomized controlled trials [RR 1.36, 95%CI 1.00 to 1.85, two trials (Czeizel *et al.* 1994a, ICMR and Unit. 2000), 5141women] but did not consider this fertility treatment in the analysis (Rumbold *et al.* 2011). Since fertility treatments can increase the risk of multiple births and women undergoing such treatments are also likely to be taking recommended doses of supplemental folic acid, these findings could be spurious if not properly controlled for (Berry *et al.* 2005). Subsequent studies that accounted for this confounder showed that the association was greatly reduced or eliminated (Li *et al.* 2003, Signore *et al.* 2005, Vollset *et al.* 2005). While it may be biologically plausible that periconceptional vitamin use plays a role in the incidence of multiple births, the available evidence has been well explored – the most recent human study identified was published in 2006 – so this was not determined to be a high priority topic for this review.

5.3 Autism

Neurological outcomes of concern with high levels of folic acid were not limited only to cognitive effects in conjunction with B_{12} deficiency, but also to neurodevelopmental outcomes in children. On the population level, folic acid intake during pregnancy and autism rates have both increased over the last 15-20 years. Of the 11 publications of autism and folate that were identified, three reported an adverse association, although all three studies had potential weaknesses in study design. One reported a positive correlation between availability of prenatal vitamins containing 1mg or greater folic acid and autism incidence (Beard *et al.* 2011), but could not consider confounding factors because it did not have information on individual cases and vitamin intake. The other two studies reported higher intake of folic acid or higher folate levels in autism cases (Lowe *et al.* 1981, Hyman *et al.* 2012), which could be due to reverse causation - Lowe et al. (1981) noted that more than 50% of autistic children were taking a multivitamin in their study. Conversely, publications from a large prospective birth cohort and two case-control studies showed significant, protective effects of maternal folic acid intake (Ali *et al.* 2011, Schmidt *et al.* 2011, Schmidt *et al.* 2012, Al-Farsi *et al.* 2013, Suren *et al.* 2013). A meta-analysis found no association with blood folate levels in case-control studies of autism (Frustaci *et al.* 2012). Due to weaknesses in the design of studies reporting adverse effects, the currently available literature did not support consideration of autism as a high priority outcome for this review.

5.4 Other Neurological Outcomes

Neurological outcomes were the second largest area of research identified with over 500 studies. Very few of the specific neurological outcomes reported adverse effects of folic acid, for instance, none of the 70 studies of Alzheimer's disease and only 3 of almost 100 studies of depression reported any adverse associations. None of the 10 identified meta-analyses reported adverse effects of folic acid. Cognitive effects in the context of vitamin B_{12} deficiency was considered as a high priority category and the small literature on autism was discussed previously, however no other neurological outcomes were considered further by the steering committee.

5.5 Other Immunological Outcomes

The majority of other immunological outcomes which were not considered hypersensitive-related, such as autoimmune diseases, did not suggest any adverse effects of folic acid and were not considered a high priority category. Few of these studies focused on children, unlike the hypersensitivity studies which included early life outcomes such as allergy and asthma, and these studies did not consider prenatal windows of exposure. One meta-analysis of multiple sclerosis was identified and showed no adverse effect of folic acid (Zhu *et al.* 2011). While respiratory infections were included with the hypersensitivity studies due to the overlap with studies of wheeze and asthma, no other infections were considered.

In considering other immunological findings not considered related to hypersensitivity, such as natural killer cell cytotoxicity, the steering committee proposed highlighting those studies and providing the PDFs as related evidence for consideration, and not including them in the studies for data extraction, which focused on hypersensitivity-related immune endpoints.

5.6 Other Endocrine and Metabolic Disease Outcomes

Few endocrine and metabolic disease studies not directly related to diabetes or thyroid function were identified. Body weight, body composition, and BMI constituted the largest group of studies (~50), with only 2 studies reporting any significant relationship between higher folate intake or level and increased body weight. No studies of folate and polycystic ovary syndrome or pancreatitis reported any adverse associations. These outcomes were not considered a high priority area, and the review focused only on thyroid and diabetes-related endpoints.

5.7 Other Reproductive Outcomes

Given the extensive investigation of folic acid in birth defects prevention, other reproductive effects have been widely studied as well and the vast majority reports no adverse effects (~ 300 studies). Most of these studies focus on the baby's growth (e.g., 80 studies of birth weight), although this category also includes studies on effects in the pregnant mother such as preeclampsia, as well as male and female fertility. None of the 9 meta-analyses reported an adverse effect of folic acid, so reproductive effects were not considered a high priority category. Note that studies of birth defects were not included in the detailed tagging process, as this project did not aim to summarize the literature on this well-established public health benefit.

5.8 Mortality

Mortality-related outcomes were identified in ~100 primary studies. Often mortality was reported by life stage (e.g., childhood) or cause (e.g., cardiovascular) and in conjunction with multiple outcomes. The specific types of mortality where >20% of the studies reported an adverse effect associated with folic acid had few published studies (e.g., 1 out of 4 or 5 studies reported any adverse result for those outcomes). 18 meta-analyses have been conducted for several mortality outcomes with a sufficient number of available studies (e.g., all-cause, cardiovascular, cancer, perinatal) and none report any statistically significant adverse meta-estimates. Based on these findings, mortality was not considered as a high priority category for further focus.

6.0 SUMMARY

This background document presents the methods used to compile the literature for this state-of-the-science review of the published literature related to the safe use of high intakes of folic acid for four high priority health effect categories. The expert panel will rely on these materials to identify research needs based on consideration of the human literature provided in the Study Summaries and HAWC database, as well as the supporting materials from animal and *in vitro* studies included in the Supplementary Material. Following the expert panel meeting, this document will be published as an NTP monograph and include the expert panel's report.

NTP has led this effort in partnership with the NIH Office of Dietary Supplements ODS. NTP and ODS would like to acknowledge the valuable input of the steering committee and thank the expert panel members in advance for their service.

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APPENDIX 1: LITERATURE SEARCH METHOD

Concept	PubMed *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Embase *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Scopus and Web of Science *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Cochrane *Initial search: May 7, 2013* *Update 1: Dec. 1, 2014*
Publication Years	Initial search: up through 2011; Update 1: 2011-2013: Update 2: 2013-2014	Initial search: up through 2011; Update 1: 2011-2013: Update 2: 2013-2014	Initial search: up through 2011; Update 1: 2011-2013: Update 2: 2013-2014	Initial search: up through May 2013; Update: 2013-2014
Folic Acid	**#1**: Folic acid[mh] OR "Folic acid"[tiab] OR "Pteroylglutamic Acid"[tiab] OR "pteroylmonoglutamic acid" [tiab] OR tetrahydrofolate*[tiab] OR "5-Methyltetrahydrofolic acid"[tiab] OR "5-methyltetrahydrofolate"[tiab] OR leucovorin[tiab] OR "folinic acid"[tiab] OR folate*[tiab]	**#1**: 'folic acid'/exp AND ('diet supplementation'/exp OR 'vitamin'/exp)	**#1**: "Folic acid" OR "Pteroylglutamic Acid" OR "pteroylmonoglutamic acid" OR tetrahydrofolate* OR "5-Methyltetrahydrofolic acid" OR "5-methyltetrahydrofolate" OR leucovorin OR "folinic acid" OR folate*	**#1**: "Folic acid" OR "Pteroylglutamic Acid" OR "pteroylmonoglutamic acid" OR tetrahydrofolate* OR "5-Methyltetrahydrofolic acid" OR "5-methyltetrahydrofolate" OR leucovorin OR "folinic acid" OR folate*
Dietary Supplement	**#2**: Dietary supplements[mh] OR "food, fortified"[mh] OR vitamins[mh] OR Supplement*[tiab] OR fortif*[tiab] OR diet[tiab] OR dietary[tiab] OR food[tiab] OR feed*[tiab] OR fed[tiab] OR enrich*[tiab] OR intake[tiab] OR ingest*[tiab] OR vitamin*[tiab] OR consum*[tiab]	**#2**: (("Folic acid" OR "Pteroylglutamic Acid" OR "pteroylmonoglutamic acid" OR tetrahydrofolate* OR "5-Methyltetrahydrofolic acid" OR "5-methyltetrahydrofolate" OR leucovorin OR "folinic acid" OR folate*) NEAR/6 (Supplement* OR fortif* OR diet* OR food OR feed* OR fed OR enrich* OR intake OR ingest* OR vitamin* OR consum* OR administrat* OR administer*)):ti,ab	**#2**: Supplement* OR fortif* OR diet* OR food OR feed* OR fed OR enrich* OR intake OR ingest* OR vitamin* OR consum* OR administrat* OR administer*	**#2:** Supplement* OR fortif* OR diet* OR food OR feed* OR fed OR enrich* OR intake OR ingest* OR vitamin* OR consum* OR administrat* OR administer*
Folic Acid AND Dietary Supplement	**#3**: #1 AND #2	**#3**: #1 AND #2	**#3**: #1 AND #2	**#3**: #1 AND #2

Concept	PubMed *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Embase *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Scopus and Web of Science *Initial Search: Nov. 25, 2011* *Update 1: May 7, 2013* *Update 2: Dec. 1, 2014*	Cochrane *Initial search: May 7, 2013* *Update 1: Dec. 1, 2014*
Chemotherapy	**#4:** Antineoplastic Combined Chemotherapy Protocols[mh] OR carboplatin[mh] OR methotrexate[mh] OR fluorouracil[mh] OR oxaliplatin[Supplementary Concept] OR irinotecan[Supplementary Concept] OR chemotherapy[mh] OR radiation[mh] OR "antineoplastic agents"[mh] OR camptothecin[mh] OR Chemotherapy[tiab] OR monotherapy[tiab] OR carboplatin[tiab] OR fluorouracil[tiab] OR oxaliplatin[tiab] OR irinotecan[tiab] OR radiation[tiab] OR antineoplas*[tiab] OR camptothecin[tiab] OR methotrexate[tiab] OR “drug target”[tiab] OR “drug targets”[tiab] OR drug delivery systems[mh] OR “drug delivery”[tiab]	**#4:** 'antineoplastic agent'/exp OR 'carboplatin'/exp OR 'methotrexate'/exp OR 'fluorouracil'/exp OR 'oxaliplatin'/exp OR 'irinotecan'/exp OR 'chemotherapy'/exp OR 'radiation'/exp OR 'camptothecin'/exp OR 'drug delivery system'/exp OR 'nanomaterial'/exp OR ((chemotherapy OR Monotherapy OR carboplatin OR fluorouracil OR oxaliplatin OR doxorubicin OR irinotecan OR radiation OR antineoplas* OR camptothecin OR methotrexate OR “drug target” OR “drug targets” OR “drug delivery” OR nano*):ti,ab)	**#4:** chemotherapy OR Monotherapy OR carboplatin OR fluorouracil OR oxaliplatin OR doxorubicin OR irinotecan OR radiation OR antineoplas* OR camptothecin OR methotrexate OR “drug target” OR “drug targets” OR “drug delivery” OR nano*	**#4:** chemotherapy OR Monotherapy OR carboplatin OR fluorouracil OR oxaliplatin OR doxorubicin OR irinotecan OR radiation OR antineoplas* OR camptothecin OR methotrexate OR "drug target" OR "drug targets" OR "drug delivery" OR nano*
Remove chemotherapy results.	**#5: # 3 NOT #4**	**#5: # 3 NOT #4**	**#5: # 3 NOT #4**	**#5: # 3 NOT #4**

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APPENDIX 2: CRITERIA FOR SCREENING OF STUDIES

Does this article pertain to health effects of folic acid above reference values for assessing the adequacy of folate status or adequate folate intakes in animal model studies?

Studies Included:

- Human studies that assess health effects of folic acid (including folate, folacin, or folinic acid) above 400 µg per day or above 10 nmol/L (4 ng/mL) for serum or 340 nmol/L (151 ng/mL) for red blood cell (RBC) concentrations[6], when intake is not specified, including mixtures containing folate. If a study only reports intakes as total folate, then intakes above 600 µg/day will be considered relevant.
- Animal studies that include adverse effects, tolerability, safety or mechanisms of action of folic acid (including folate, folacin, or folinic acid) above the standard dose (not of deficiency)[7], including studies of mixtures containing folic acid
- *In vitro* studies that include a higher than standard dose of folic acid (possibly in mixtures)
- Methylation/Epigenetic studies if they pertain to a health effect of folic acid

Exposures Excluded:

- Studies of methotrexate (or other chemotherapeutics).
- Studies focused on folic acid's role in the efficacy of clinical interventions including surgery, dialysis, or medication. This would include studies of the effect of folic acid after heart transplant or studies of valproic acid in conjunction with folic acid in birth defects.
- Studies (including case reports) where folic acid is part of the therapeutic treatment giving equal amounts to all participants.
- Alcohol and tobacco smoke exposure are linked to folate deficiency, so studies of co-exposure of folic acid and alcohol are not relevant to the topic of higher daily intakes of folic acid. Studies of co-exposure with alcohol and tobacco smoke will only be included if an independent folic acid effect is examined as well.
- If plasma or serum folate is only considered as an adjustment factor for another exposure-outcome analysis, the study is not relevant to the question.
- Studies of dietary patterns, such as vegetarian or Western diet, where folate is measured but not directly analyzed for association with a health effect.

Outcomes Excluded:

- Homocysteine concentration alone will not be considered as an independent heath outcome, but it will be considered in the context of disease.

[6] Based on WHO definition of folate deficiency (de Benoist 2008), this level is slightly more conservative than the standards used in clinical practice (305 nmol/L for RBC).
[7] Standard dose is considered the control diet amount

- Gastrointestinal outcomes where impaired folate absorption is the primary concern in gastrointestinal disorders, and they are unlikely to be examined for risk from higher daily intakes of folic acid.
- Renal/Kidney where impaired kidney function (particularly end stage disease) contributes to folate deficiency and there are many studies of greater supplement use in these populations that do not pertain to primary health effects of higher daily intakes of folic acid.
- Infectious diseases where folate has been examined in association with malaria or other infectious diseases more common in populations where folate deficiency is the primary concern. *Helicobactor pylori* and Human papillomavirus will be considered in the context of cancer promotion.
- Any condition present at birth (Down's, cystic fibrosis, etc.) will only be considered if maternal folate intake or exposure is assessed, as management of these conditions with folic acid is not related to the primary question.

APPENDIX 3: DATA EXTRACTION ELEMENTS

Individual Epidemiology Studies

Citation

- Full citation and abstract (if available)
- Hyperlink to PubMed, pdf (reviewers only)
- Report of Conflicts of Interest
- Funding Source
- Summary of results to be extracted

Population

- Study design (prospective, cross-sectional, etc.)
- Location (country, region)
- Population demographics (gender, ethnicity, age)
- Sample size: Overall N (primary one referenced, possibly in the abstract for entire group) and Starting N (a larger sample before narrowing to the main study N, if applicable)
- Inclusion, Exclusion, and Confounding Criteria (for the overall study, not specific analyses)

Exposure

- Route of exposure (for folic acid, primarily oral or *in utero*)
- Exposure measurement (type, units, description of method)
- Description of control, if applicable
- Levels of Exposure as presented in the study (including gender, ethnicity, N, and age if provided)

Outcome

- Short name for outcome and location of results in the text (e.g., “Table 3”)
- Diagnostic used and description
- Outcome N (number included in analysis, not number affected)
- Summary, can be used for details not captured in the exposure level results (P-trend, other information)
- Prevalence incidence, if provided or applicable
- Adjustment factors (in the final model, and any considered)
- Dose Response (shape of trend and details, if applicable)
- Statistical power (if reported)
- Statistical metric and method, as reported in the paper

Results

- For each exposure group, N, estimate, SE, confidence interval, and p-value (as reported)

Pooled or Meta-analyses

Citation

- Full citation and abstract (if available)
- Hyperlink to PubMed (all) and full-text pdf (reviewers only)
- Report of Conflicts of Interest
- Funding Source
- Summary of results to be extracted

Protocol Details

- Type of analysis (meta-analysis or pooled-analysis)
- Literature search (strategy, dates, details)
- Total number of studies found, and total number included overall
- Inclusion/exclusion criteria

Results

- Health outcome (name and description)
- Exposure (name and description)
- Number of studies for this result
- Statistical method (metric and description)
- Number of subjects overall
- Result (estimate, confidence interval)
- Test of Heterogeneity result
- Adjustment factors, if included