



# National Toxicology Program

U.S. Department of Health and Human Services

## **NTP MONOGRAPH:**

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

August 1, 2015

Office of Health Assessment and Translation

Division of the National Toxicology Program

National Institute of Environmental Health Sciences

National Institutes of Health

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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## CONTRIBUTORS

### **Office of Health Assessment and Translation (OHAT), NIEHS/DNTP**

*Conducted scientific evaluation, screened literature, and prepared the NTP Monograph*

Abee L. Boyles, PhD (Project Lead)  
Andrew A. Rooney, PhD (Deputy Director, OHAT)  
Vickie R. Walker  
Kristina A. Thayer, PhD (Director, OHAT)

### **Office of Dietary Supplements (ODS), NIH/OD**

*Provided input on project development*

Paul M. Coates, PhD (Director)  
Elizabeth A. Yetley, PhD (Scientific Consultant)

### **Office of Scientific Information Management (OSIM), NIEHS/ODD**

*Developed and conducted the initial literature search and updates*

Stephanie D. Holmgren, MSLIS, MBA

### **Program Operations Branch (POB), NIEHS/DNTP**

*Developed and maintained the HAWC resource*

Andy Shapiro, MPH

### **Office of Liaison, Policy and Review (OLPR), NIEHS/DNTP**

*Managed expert panel meeting*

Yun Xie, PhD (Designated Federal Official)  
Denise Lasko  
Anna Lee Mosley  
Mary Wolfe, PhD (Director, OLPR and Deputy Division Director for Policy)

### **Integrated Laboratory Systems, Inc.**

*Performed detailed tagging of human studies*

Claudine A. Gregorio, MA  
Neepa Y. Choski, PhD

### **MDB, Inc.**

*Developed and conducted the initial literature search*

Lesley Skalla, MSLS, PhD

### **Social & Scientific Systems**

*Performed detailed tagging and extracted data from studies into the HAWC database*

Grace Megumi Sotherden, MS  
Anna Ciesielski Jones, MPH  
Fikri Yucel, MA

## **Steering Committee**

*Provided input on the prioritization of topics and expert panel composition*

Nicole F. Dowling, PhD	Associate Director for Science, Division of Birth Defects and Developmental Disabilities, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA
Amanda MacFarlane, PhD	Research Scientist, Section Head, Micronutrient Research, Nutrition Research Division, Health Canada, Ottawa, Ontario
Edward McCabe, MD, PhD	Senior Vice President and Chief Medical Officer, March of Dimes Foundation, White Plains, NY
Linda D. Meyers, PhD	Senior Science Advisor, American Society for Nutrition, Bethesda, MD
Robert M. Russell, MD	Professor Emeritus of Medicine and Nutrition, Tufts University, Medford, MA
Yu (Janet) Zang, PhD, DABT	Review Toxicologist, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, U. S. Food and Drug Administration, College Park, MD

## **Rapporteurs**

*Assisted subpanels with summarizing recommendations and preparing presentations*

Kara Koehn, MEM	Program Analyst, Toxics Release Inventory; US Environmental Protection Agency, Washington, DC
Adam J. Kuszak, PhD	Science and Technology Policy Fellow at AAAS, Office of Dietary Supplements, National Institutes of Health, Bethesda, MD
Katherine E. Pelch, PhD	Postdoctoral Fellow, Office of Health Assessment and Translation, National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Durham, NC
Paul R. Thomas, EdD, RDN	Scientific Consultant, Office of Dietary Supplements, National Institutes of Health, Bethesda, MD

## **Expert Panel**

*Made research recommendations based on review of the literature*

Joseph M. Braun, PhD	Assistant Professor, Department of Epidemiology, School of Public Health, Brown University, Providence, RI
Tim Byers, MD	Associate Dean for Public Health Practice, Director, Center for Public Health Practice, Colorado School of Public Health, University of Colorado Denver, Aurora, CO
Robert Clarke, MD	Professor of Epidemiology and Public Health, Clinical Trial Service Unit and Epidemiological Studies Unit, Course Director of MSc in Global Health Science, Nuffield Department of Population Health, University of Oxford, Oxford, Oxfordshire, UK
Todd M. Gibson, PhD	Assistant Member, Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, TN

Jesse F. Gregory, III, PhD	Professor, Food Science and Human Nutrition Department, University of Florida, Gainesville, FL
Paul F. Jacques, DSc	Director, Professor, and Senior Scientist, Nutritional Epidemiology Program, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA
Young-In Kim, MD	Professor of Medicine and Nutritional Sciences, University of Toronto; Senior Scientist, Keenan Research Center for Biomedical Science of St. Michael's Hospital; Staff Gastroenterologist, St. Michael's Hospital Toronto, ON, Canada
Joel B. Mason, MD	Professor of Medicine, School of Medicine, Professor of Nutrition, School of Nutritional Science and Policy, Tufts University, Boston, MA
Elizabeth Matsui, MD	Associate Professor of Pediatrics, Epidemiology, and Environmental Health Sciences, Johns Hopkins University, Baltimore, MD
Joshua W. Miller, PhD	Professor and Chair, Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ
James L. Mills, MD, MS	Senior Biomedical Research Service Scientist, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
Anne M. Molloy, PhD	Associate Professor, School of Medicine, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland
Martha C. Morris, ScD	Director, Section of Nutrition and Nutritional Epidemiology, Professor, Department of Internal Medicine, Rush University, Chicago, IL
Jeanne I. Rader, PhD	Retired December 2013, Office of Regulatory Science, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD
Barry Shane, PhD	Professor of Nutrition, Department of Nutritional Sciences and Toxicology, University of California, Berkeley, Berkeley, CA
Patrick J. Stover, PhD	Professor and Director, Division of Nutritional Sciences, Cornell University, Ithaca, NY
Miroslav Stýblo, PhD	Professor, Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC
Henk van Loveren, PhD	Professor of Immunotoxicology, Centre for Health Protection, National Institute of Public Health and the Environment, Bilthoven, the Netherlands; Department of Toxicogenomics, Maastricht University, Maastricht, the Netherlands

### **Expert Panel Chair**

*Moderated the meeting and developed the Expert Panel Report*

Cutberto Garza, MD, PhD	Visiting Professor, Bloomberg School of Public Health, Johns Hopkins University; Visiting Professor, Milken Institute School of Public Health, George Washington University; University Professor, Boston College Chestnut Hill, MA
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## ABBREVIATIONS

<b>Abbreviation</b>	<b>Name</b>
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 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 in conjunction with vitamin B<sub>12</sub> deficiency, hypersensitivity-related outcomes, and thyroid and diabetes related disorders). 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 (<https://hawcproject.org/assessment/public/>) and in the Supplementary Material (<http://ntp.niehs.nih.gov/go/749003>) 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.

On May 11-12, 2015, the National Toxicology Program (NTP) and Office of Dietary Supplements (ODS) convened an expert panel to identify research needs related to the safe use of high intakes of folic acid based on consideration of the state of the science. The expert panel was charged to (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. The Panel's recommendations did not require consensus, included minority opinions, and do not necessarily represent the opinion of NTP or ODS. This NTP Monograph includes the expert panel report as Chapter 6.0.

\*Text was changed from "a synthetic form of folate, above the Recommended Dietary Allowance (RDA) of 400 µg" to "a synthetic form of folate, above 400 µg" on September 16, 2015.

## 1.0 INTRODUCTION

The National Toxicology Program (NTP)<sup>1</sup> in conjunction with the NIH Office of Dietary Supplements (ODS)<sup>2</sup> held 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 aimed 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,<sup>3</sup> 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 was 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 was 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 was finalized with the expert panel's report as Chapter 6.0 and published on the NTP website (<http://ntp.niehs.nih.gov/go/38144>). To achieve this objective, the expert panel was charged to:

- 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

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<sup>1</sup> 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/>.

<sup>2</sup> 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/>.

<sup>3</sup> 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 (<http://ntp.niehs.nih.gov/go/749003>). 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 Expert Panel Report

Chapter [6.0](#) is the report written and approved by the Expert Panel and does not necessarily represent the opinion of NTP or ODS. The panel voted unanimously (18 yes, 0 no, 0 abstain) to accept the research recommendations and issues relevant to all four subpanel's recommendations after some revision in response by comments from the full panel.

### 1.2.4 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

Supplementary Materials are available online (<http://ntp.niehs.nih.gov/go/749003>).

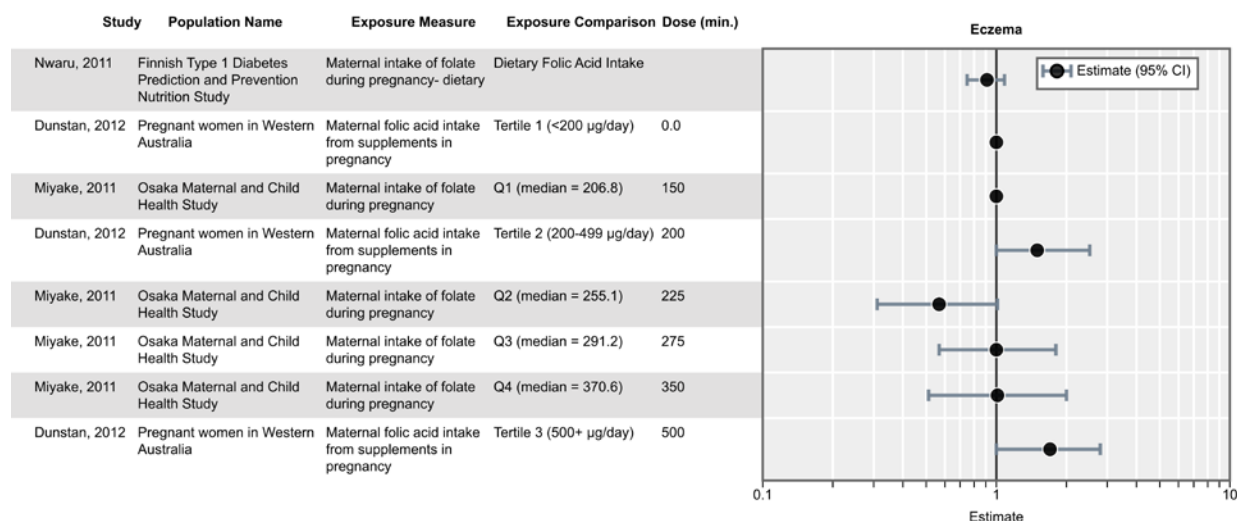
### 1.2.5 Web-based Health Effects Data

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). Visualizations includes graphs of results from across studies (see [Figure 1](#)) and are available within each project in HAWC. The expert panel members used 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 a graph available in HAWC under Visualizations is provided ([Figure 1](#)). 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 (<http://ntp.niehs.nih.gov/go/749003>).



**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<sup>4</sup>) 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<sub>12</sub>-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.

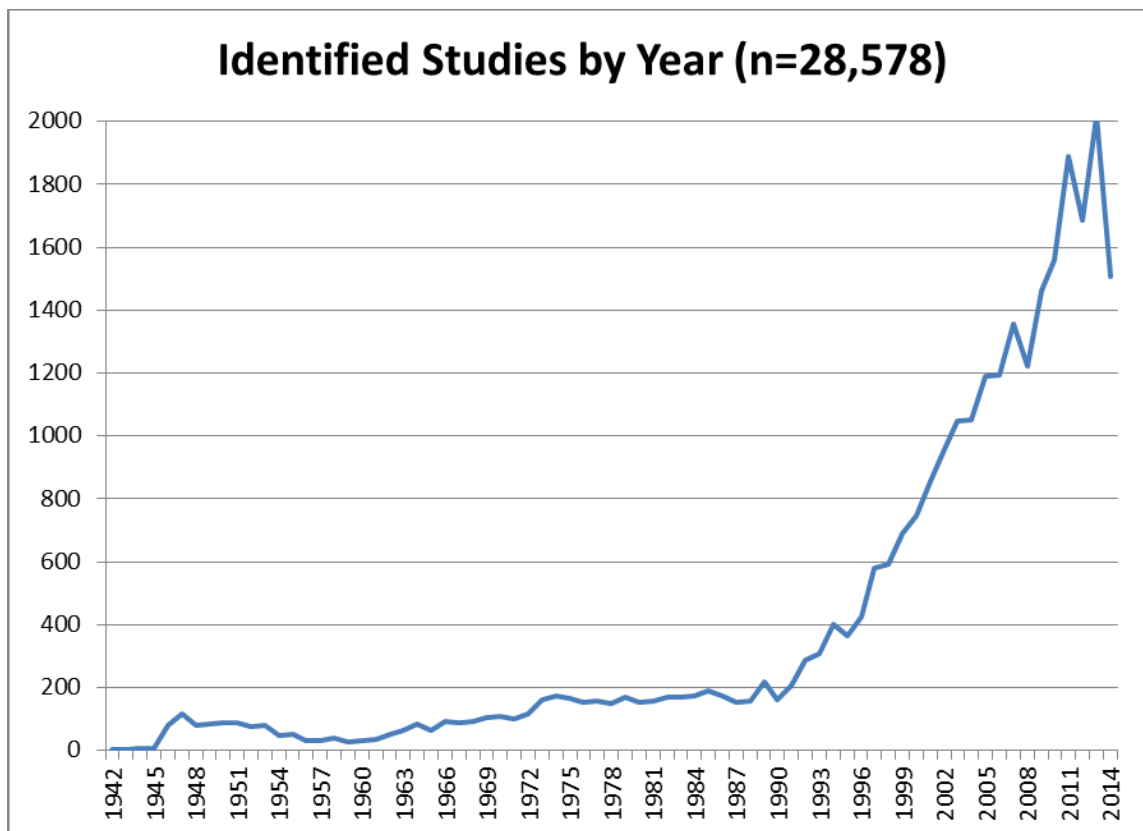
In a discussion of folate and folic acid intake, it is important to note that folate is present in the diet through its natural occurrence in food, as a food additive, and as an ingredient in dietary supplements. Naturally occurring folate is unlikely to be associated with potential adverse effects because 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.

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<sup>4</sup> 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 and hand collected per year (cumulative total = 28,580).

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<sup>5</sup> (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

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<sup>5</sup> The Cochrane Collaboration: Asking an Answerable Question  
([http://ph.cochrane.org/sites/ph.cochrane.org/files/uploads/Unit\\_Five.pdf](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.

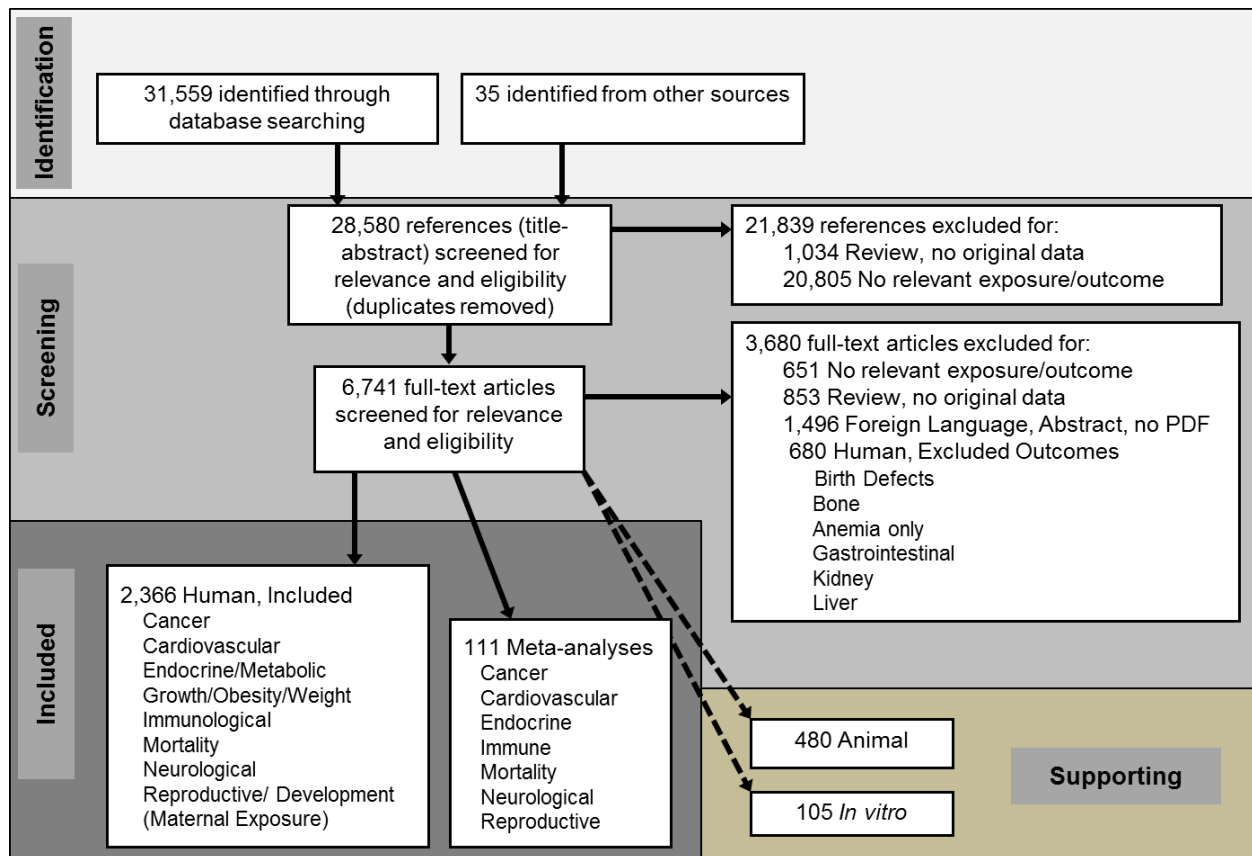
The details of the electronic database searches are presented in [Appendix 1: Literature Search Method](#). The PubMed search includes both MeSH and text words. The Embase search used both the Emtree controlled vocabulary terms as well as text words. The Web of Science and Scopus searches used text words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

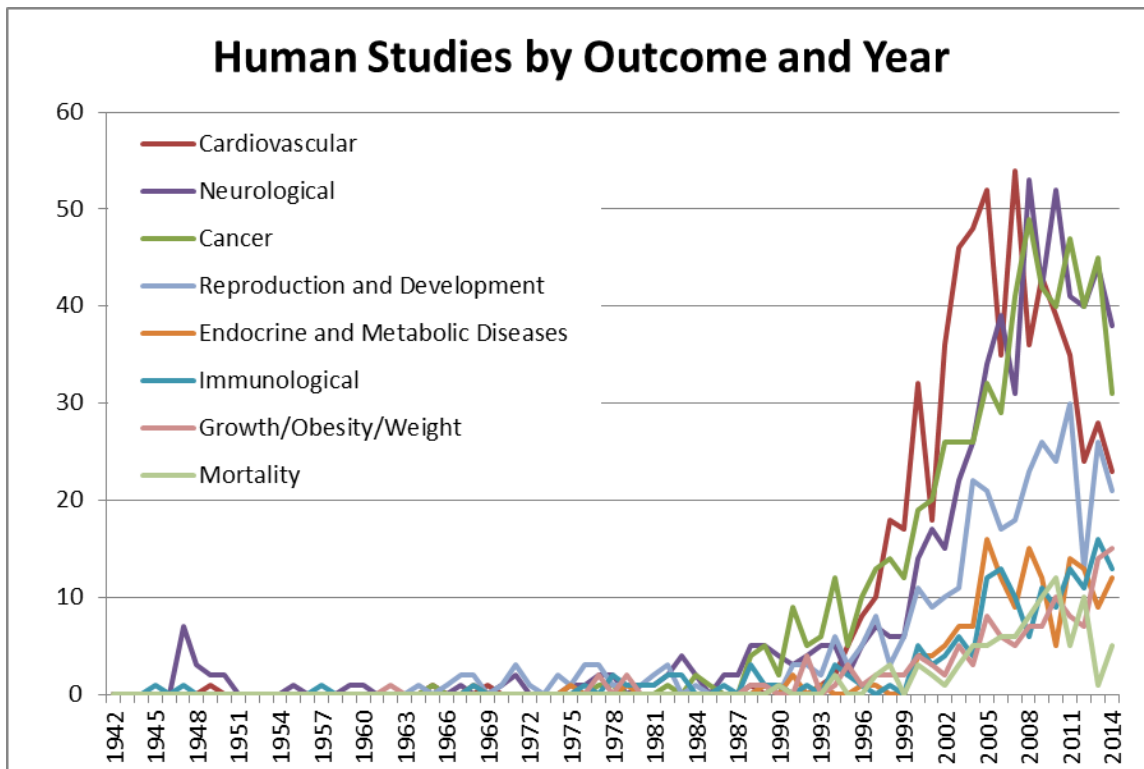


**Figure 3.** Selection of Studies. Diagrams the flow of studies through the screening process, including reasons for the exclusion of studies (adapted from Moher *et al.* 2009).

**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	149	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<sub>12</sub> 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 (<http://ntp.niehs.nih.gov/go/749003>).

**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 B<sub>12</sub>:** cognitive outcomes where vitamin B<sub>12</sub> level or intake was included in the analysis includes primary studies (n=28) and meta-analyses (n=2).

**4.3 Hypersensitivity-related Outcomes:** a collection of related outcomes (n=43) often with multiple outcomes per study and many considering maternal exposure [including respiratory infection (n=16), asthma (n=15), allergy and atopic disease (n=14), wheeze (n=9), hypersensitivity test (n=6),

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](http://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 (<http://ntp.niehs.nih.gov/go/749003>). 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.

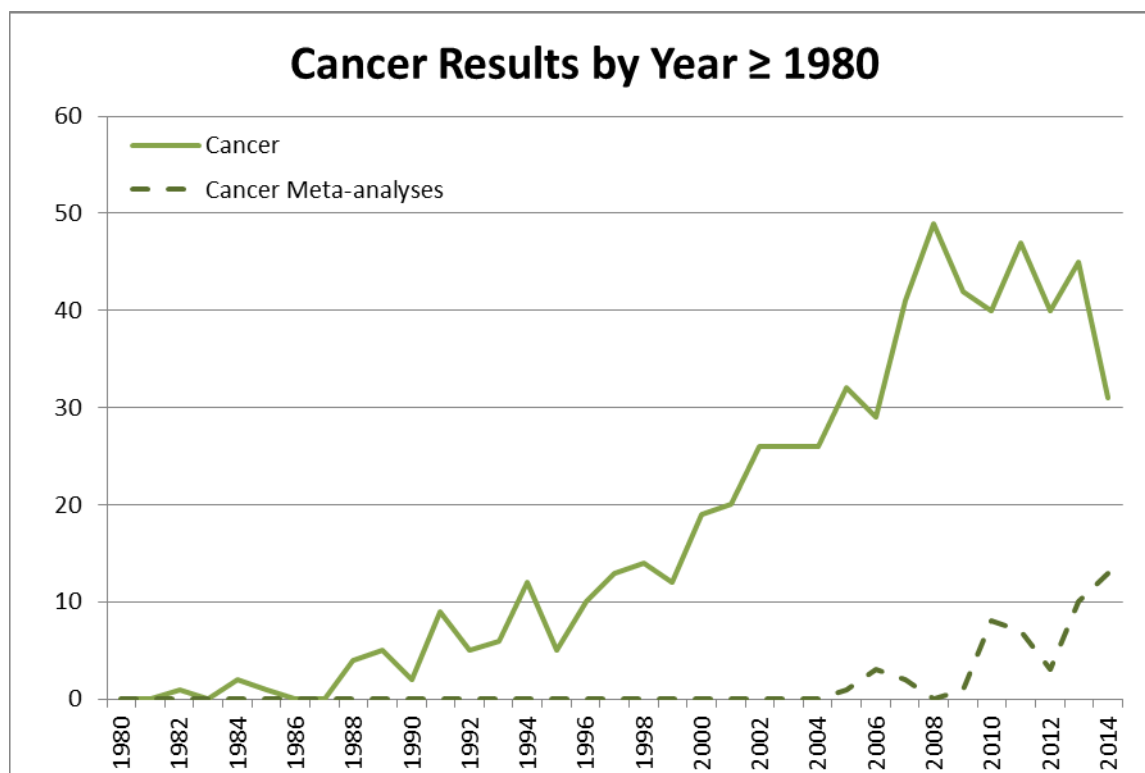
Data on folate intakes was captured as reported because there is no standard reporting format for folate and folic acid intakes. Ideally, intakes would be converted to dietary folate equivalents (DFEs) a common unit of measurement to facilitate comparisons among studies, however, few studies reported 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 (<http://ntp.niehs.nih.gov/go/749003>).

### 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.

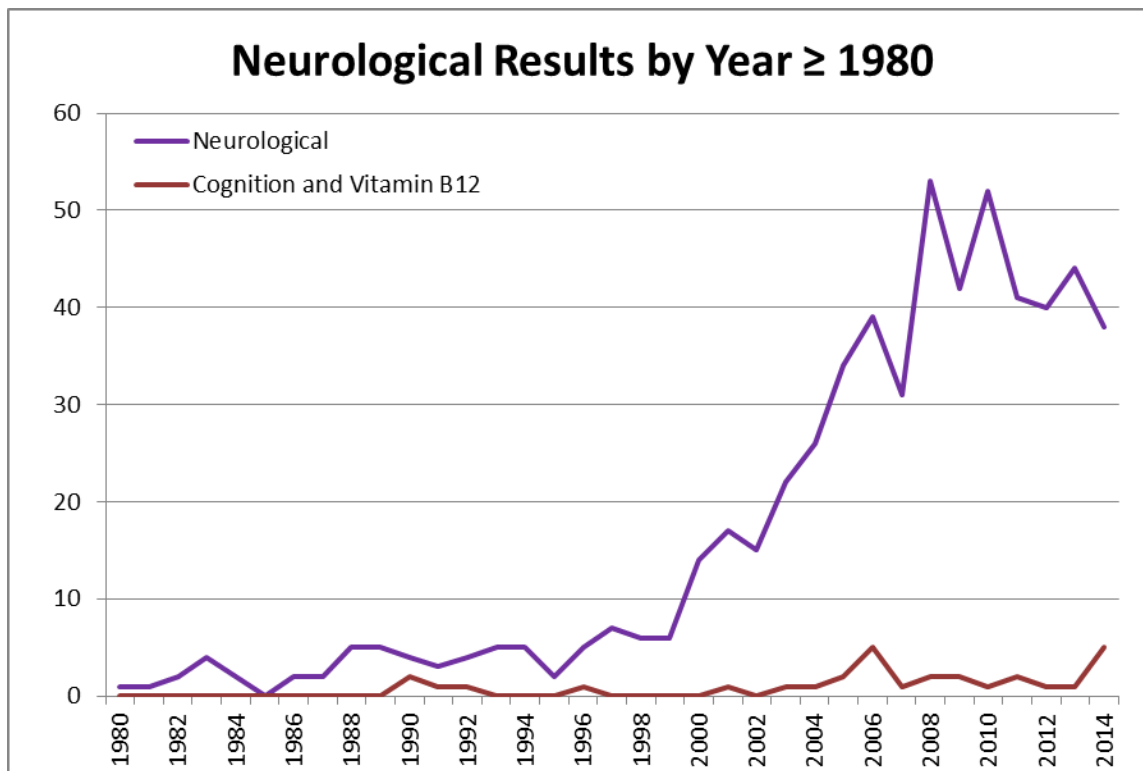
For this review, cancer was considered a high priority health effect category based on the presence of numerous studies of high folic acid intake in a wide variety of populations with inconsistent results across multiple cancer types. Pooled and meta-analyses provide an approach to quantitatively synthesize 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. Visualizations 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<sub>12</sub>

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<sub>12</sub> 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<sub>12</sub>, and cognition – particularly in elderly populations with high rates of vitamin B<sub>12</sub> deficiency.



**Figure 6.** Number of neurological and cognition and vitamin B<sub>12</sub>-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<sub>12</sub> 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<sub>12</sub> – 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<sub>12</sub> deficiency, cognition including vitamin B<sub>12</sub> assessment was considered a high priority health effect category for this review. The data extraction focused on the studies of cognitive



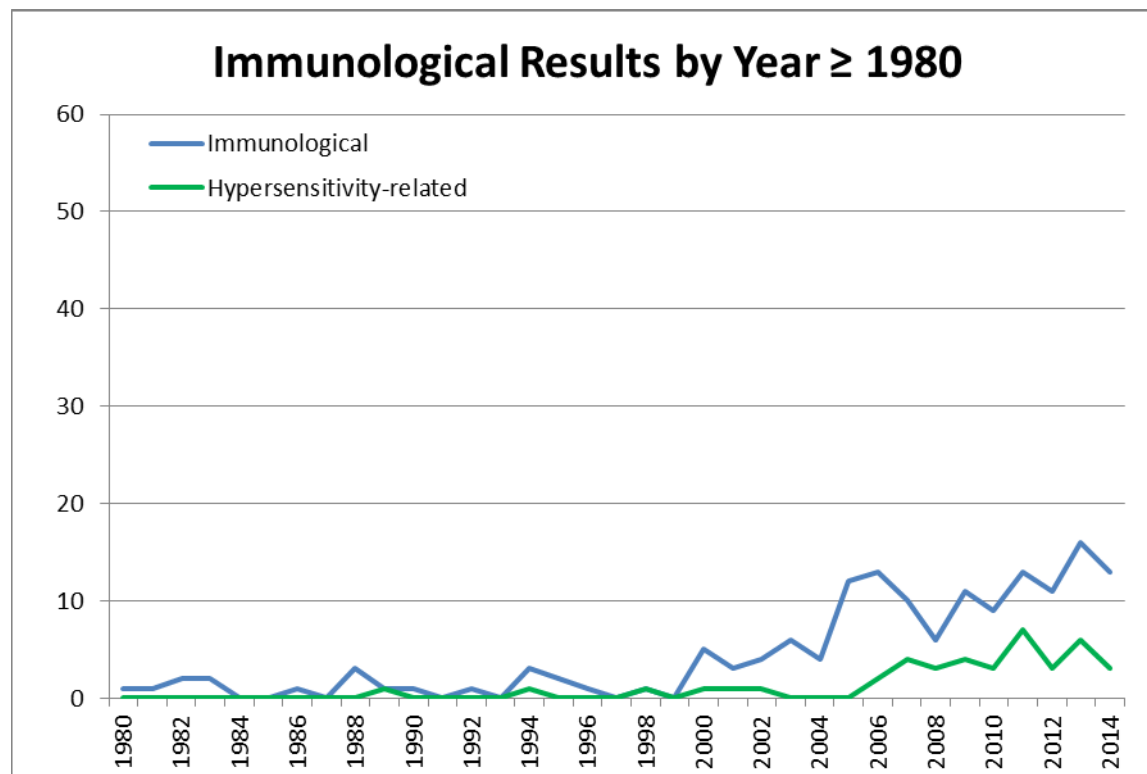
effects that jointly considered vitamin B<sub>12</sub> levels or deficiencies. There are 28 human studies and two meta-analyses that considered folate (both intake and blood levels), vitamin B<sub>12</sub>, and cognitive endpoints summarized in the Supplementary Material (<http://ntp.niehs.nih.gov/go/749003>).

The HAWC project “Folic Acid - Cognition and Vitamin B12 (2015)” graphically display results across these studies in Visualizations 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-two 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=15), allergy and atopic disease (n=12), wheeze (n=9), hypersensitivity test (n=6), eczema (n=5), and food allergy (n=2). These studies include a range of study designs, several are from large prospective

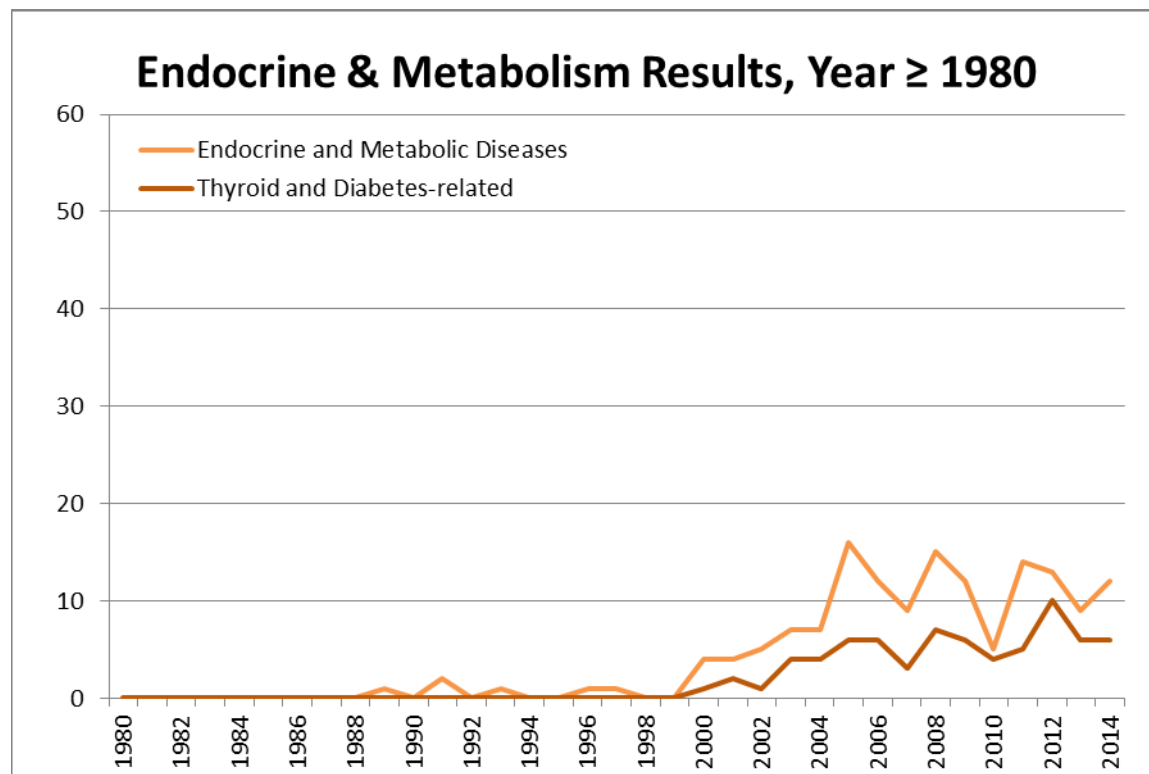
birth cohorts where prenatal exposure was considered in relationship to hypersensitivity outcomes in childhood, and many reported multiple outcomes per publication. A meta-analysis has been published which found no increased risk of asthma in the children of mothers who took folic acid supplements during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

These 43 studies are included in the project “Folic Acid – Hypersensitivity-related Outcomes (2015)” accessible on the HAWC website. Visualizations 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 “preprogrammed” 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<sub>12</sub> 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 thyroid 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), Visualizations 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 (<http://ntp.niehs.nih.gov/go/749003>).

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 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 (<http://ntp.niehs.nih.gov/go/749003>).

### 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<sub>12</sub> 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<sub>12</sub> 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 hypersensitivity-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 EXPERT PANEL REPORT

### 6.1 Introduction and Expert Panel Charge

On May 11-12, 2015, the National Toxicology Program (NTP) and the NIH Office of Dietary Supplements (ODS) convened an expert panel to identify research needs related to the safe use of high intakes of folic acid based on consideration of the state of the science. Dr. Garza, chair of the expert panel, welcomed everyone to the meeting and asked all attendees to introduce themselves.<sup>6</sup> Designated Federal Officer Dr. Yun Xie read the conflict of interest policy statement.

The expert panel was charged to carry out a state-of-science evaluation for four general health effect categories to identify areas for further research. To address this charge, the expert panel was asked to:

- 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.

The panel was divided into four subpanels, one for each health effect category: cancer, cognition in conjunction with vitamin B<sub>12</sub> deficiency, thyroid and diabetes-related disorders, and hypersensitivity-related outcomes. Each subpanel was asked to evaluate the literature assembled for its health effect category.

### 6.2 Introductory Presentations

Introductory presentations provided background on folic acid's recognized beneficial roles in birth defects prevention, sources of folic acid, and assessments of serum and red blood cell folate levels. The full presentations are available on the meeting's webpage: <http://ntp.niehs.nih.gov/go/751400>.

#### ***Dr. Gary Shaw, Stanford University: Role of Folic Acid in Birth Defects Prevention – Epidemiologic Perspectives***

Dr. Shaw said that during the past two decades, there has been an increasing body of data supporting the use of multivitamins containing folic acid and folic acid only supplements for the prevention of neural tube defects. He noted that while it is unclear how folic acid prevents birth defects, research has shown that folic acid supplementation before and in early pregnancy is effective in achieving this targeted aim. To understand how folic acid prevents neural tube defects, researchers have examined folic acid's roles in DNA methylation and nucleotide synthesis. Several research groups also are examining if meeting increased folate needs imposed by known genetic polymorphisms, which code for critical enzymatic pathways in folate metabolism, may be involved in the prevention of neural tube defects.

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<sup>6</sup> The expert panel roster and the list of attendees are available in [Appendix 4: Expert Panel Members and Meeting Attendees](#).

\*A header was added to Chapter 6 on September 22, 2015.

The panel asked Dr. Shaw if folic acid is associated with the prevention of other birth defects. Dr. Shaw responded that there are studies suggesting that folic acid also reduces the risk of orofacial clefts. In addressing a question about the roles of environmental pollutants in the occurrence of neural tube defects, Dr. Shaw responded that the role of environmental pollution has not been well studied. The panel and Dr. Shaw acknowledged that there are animal models that demonstrate specific mechanisms that explain folates' roles in reducing neural tube defects; however, additional work is needed on animal models to better understand folate metabolism in humans, most notably in early fetal development.

***Dr. Regan Bailey, ODS: Sources of Folic Acid and Supplement Use***

Dr. Bailey said folic acid is the synthetic compound added to foods to enrich and fortify the food supply and is the form most commonly found in dietary supplements. There is little folate deficiency in children and adults in the U.S. The tolerable upper intake level (UL) for folic acid set by the Institute of Medicine in 1998 is 1000 micrograms per day; there is no UL for natural forms of folate. The recommended daily allowance is 400 micrograms per day. The National Health and Nutrition Examination Survey (NHANES) tracks the use of nutrient supplements that contain folic acid in adults and children in different groups of people (e.g., ethnicity, age) and measures serum and red blood cell (RBC) folate. Based on NHANES data from 2003-2006, about 10 percent of children (2-8 years) exceed the UL of folic acid from food alone. Although there are discrepancies between dietary intake and biomarker measures of folate, these measures are very different constructs. Correlations between folate intake and biomarkers of folate status are higher at upper levels of total folate intakes than at lower ends of the range of U.S. intakes.

The panel asked for clarification of the definition of high levels of serum folic acid. Dr. Bailey noted that 45 nmol/L or greater is considered "high." When asked about data on pregnant women, Dr. Bailey cited published data reporting that 78 percent of pregnant women in the U.S. use nutrient supplements containing folic acid. The panel and Dr. Bailey raised the need for comparative data to determine if there is underreporting of folic acid levels in fortified foods.

***Dr. Christine Pfeiffer, Centers for Disease Control and Prevention: Blood Levels of Folate Over Time, Current U.S. Levels, and Differences Between Assessment Methods***

Dr. Pfeiffer spoke about advantages and disadvantages of three main laboratory methods for measuring serum and RBC folate. Assays that measure folate levels in serum or RBCs have not been standardized. Thus, folate measurements often are not comparable across assays or laboratories. She presented results of assays used in NHANES over the years and how conversions have been made when assays were changed. NHANES data show that fortification has increased serum and RBC folate levels significantly in the U.S. population. Post-fortification serum and RBC folate levels have been fairly constant over a period of ~15 years.

In response to a question about how self-reported folate intakes compare to serum and RBC measurements, Dr. Pfeiffer said that either measurement is objectively made with no influence from individual intake recall. The panel asked how much of the variation in the serum folate is explained by dietary intake. Dr. Pfeiffer explained that based on a recent analysis that accounted for demographic, socioeconomic, and lifestyle factors, roughly 30-40 percent of observed variation in serum levels is explained by dietary intake. In addressing the scientific or technological reasons for why there are no standardized folate assays, Dr. Pfeiffer noted that folate has many forms and automated assays are usually developed for only one specific folate form.

### ***Dr. Abee Boyles, NIEHS/Division of NTP: Background on Literature Evaluation***

Dr. Boyles provided background information about screening the literature and preparing the draft monograph describing what and how published literature was assembled. Prior to the Expert Panel's meeting, NTP staff screened the literature to identify potential adverse health effects related to blood measures and/or intake of folate for which further research might be warranted. The literature screening identified more than 28,000 publications. NTP considered prioritized outcomes in reports of adverse effects from studies of folate intake over 400 µg/day or biomarkers of folate in blood above the deficient range.<sup>7</sup> The draft monograph was prepared on the basis of four health outcome areas: (1) cancer, (2) cognition in conjunction with vitamin B12 deficiency, (3) hypersensitivity-related outcomes, and (4) thyroid and diabetes-related disorders. The literature review was conducted using systematic review methodology to comprehensively and objectively identify relevant studies. Because systematic review methods do not eliminate the need for scientific judgment, the literature review required inclusion and exclusion decisions by NTP, ODS, and the steering committee. The scientific justifications for such decisions were documented in the draft monograph.

### **6.3 Subpanels' Reports to the Full Panel and Discussion**

On the afternoon of May 11, 2015, the panel split into four subpanels to discuss the scientific literature identified in the NTP monograph relative to the charge, develop research recommendations, and prepare presentations summarizing their discussions. On May 12, 2015, each subpanel presented its summary and research recommendations to the full panel for deliberation. Each subpanel's presentation is found on the meeting's webpage: <http://ntp.niehs.nih.gov/go/751400>.

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<sup>7</sup> For more details about how studies were identified, see [3.1.1 Criteria for Identifying Relevant Studies, Intervention or Exposure](#).

### 6.3.1 Cancer Subpanel

#### Summary of Responses to the Charge and Research Recommendations

##### Pre-Clinical Summary

- Although inadequate dietary folic acid intake increases colon cancer risk in rodent model systems, there also is consistency in showing an acceleration of colon cancer development in the few studies that have tested the effects of folic acid intake above basal requirements for total folate in those systems (National Research Council 1998).

##### Pre-Clinical Research Recommendations

- Study the effects of folic acid above basal requirements on the growth of cancers other than in the colon, especially those for which there is a suggestion of a clinical effect in humans (e.g., prostate).
- Compare the effects of folic acid to those of reduced folates and overall folate status on cancer growth, define the dose response of each, evaluate the interplay of other one-carbon nutrients, study mechanisms of effects, and evaluate the modification by other covariates (e.g., alcohol, age, genetics, sex).
- Identify and employ those animal models that are most relevant to human folate metabolism and carcinogenic processes.
- Better define critical life stages and other timing effects of exposure.
- Explore maternal and paternal effects of folates and folic acid intakes on their offspring's cancer risk.

##### Clinical Summary

- Although inadequate dietary folate intake increases colorectal cancer risk in humans, there is no benefit for cancer reduction from supplements among people whose baseline folate status is adequate.
- There is a consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental folic acid to justify further research.

##### Clinical Research Recommendations

- There is a need to clarify whether the existing suggestive evidence from clinical trials for increased risk is driven by effects in specific subgroups (e.g., by age, pre-existing neoplasia, genetics, other factors), and to assess long-term outcomes among subjects from prior folic acid trials.
- New studies are needed to identify subgroups that are at risk of tumor acceleration linked to high folic acid intakes.
- Define whether the form of ingested folate impacts risk and whether other one-carbon nutrients are modifiers of the potential pro-tumorigenic effects of excess folic acid.
- Define the above issues in other implicated cancer sites in addition to colorectal cancer (e.g., prostate).

### Areas of Consistency and Research Recommendations

In the pre-clinical areas, there is consistent evidence that inadequate folic acid intakes increase colon cancer risk in rodent model systems. There is also consistency in evidence acquired in rodent model

systems for accelerated colon cancer development from a few studies that have tested the effects of folic acid intakes above basal requirements for total folate.

Several priority, pre-clinical, research needs were identified. In cancers other than cancer of the colon, additional studies are needed for examining the effects of folic acid intakes above basal folate requirements on the growth of diverse tumors (National Research Council 1998), especially those for which there are human data suggesting adverse effects of high folate intakes on cancer progression (e.g., prostate and mammary). Experimental animal studies are needed comparing the effects of folic acid, reduced folates, and overall folate status on cancer progression.

The field should better define dose-response relationships for specific outcomes, such as different types of cancer in animal models. There is a need to identify and employ appropriate animal models that are most relevant to human folate metabolism and processes related to cancer initiation, promotion, and progression. Animal models used in published studies may not be sufficiently informative of risks relevant to cancer in humans. It is important to better define critical life stages and other timing effects of exposure in relevant animal systems, particularly carcinogen administration. There is also a need to better understand possible interplay and interactions among folic acid and other one-carbon nutrients most likely to act in concert. Some of the evidence for adverse effects in humans occurred in trials that administered one-carbon nutrients in addition to folic acid. It is also important to study effect modification by covariates, such as alcohol, age, genetics, and sex. Finally, the field also should explore cancer risk in offspring of mothers and fathers with histories of diverse folate intakes.

For clinical research, there are three main areas of consistency: (1) inadequate dietary folate intakes increase colorectal cancer risk in humans, (2) there is no cancer reduction benefit from supplements in people with adequate baseline folate status, and (3) there is a consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental folic acid to justify further research.

For clinical research needs, it is important to clarify whether existing, suggestive evidence from clinical trials for increased risk is driven by effects in particular subgroups. Although some work has been done to clarify such relationships, more *de novo* research is needed, especially to sort out differences by age, pre-existing neoplasia, and genetics. In addition, there is a need for extended follow-up of participants in randomized trials in post-treatment phases to evaluate the effects of folic acid on site-specific cancers and in relevant subgroups.

New observational studies are needed to identify subgroups that are at risk of tumor acceleration enabled by high folic acid intakes. Similar to a pre-clinical research recommendation, the field should determine if other one-carbon nutrients might be modifiers of potential adverse folic acid effects. It is important to define the form of ingested folate and its impact on risk.

Another clinical research need is to determine the effects of withdrawal from folic acid supplementation on colon polyp growth. One approach would be to identify individuals with adenomas who are supplement users with relatively high folate intakes, to determine whether reducing folic acid intakes over a period of three years reduces polyp growth.

Finally, there is a need to apply the clinical research recommendations noted above to other implicated cancer sites, such as prostate cancer. There is suggestive evidence that folic acid has an adverse effect on the development of prostate cancer (initiation, promotion, or progression).

### 6.3.2 Cognition in Conjunction with Vitamin B<sub>12</sub> Deficiency Subpanel

#### Summary of Responses to the Charge and Research Recommendations

##### Summary

- The hypothesis that high folic acid/folate in the presence of low vitamin B<sub>12</sub> status exacerbates neurological problems based on early case reports has supportive evidence from observational studies, but available data are limited.
- Data from epidemiological studies are difficult to interpret because of heterogeneity in vitamin status cut points, inconsistencies in the cognitive outcomes that were followed, and the omission of key neurological outcomes.
- Existing intervention studies were not designed to address this question.
- Mechanisms by which folic acid exacerbates vitamin B<sub>12</sub> deficiency remain unclear.

##### Recommendations

- Meta-analysis of existing large observational studies should be conducted to quantify effect sizes in various groups.
- Mendelian randomization studies can help inform causal relevance of putative associations.
- Pending results of meta-analyses and Mendelian randomization studies, animal and in vitro studies using appropriate models should be conducted to identify potential biological mechanisms linking vitamin B<sub>12</sub> status, folate excess, and cognitive function. Human investigations also are needed for examining the impact of timing of exposure (e.g., *in utero*, weaning, older).

#### Areas of Consistency and Research Recommendations

In evaluating effects on cognition of high intakes of folic acid, areas of evidentiary consistency were noted for two health outcomes: (1) vitamin B<sub>12</sub> status, assessed biochemically e.g., by measurements of homocysteine and methylmalonic acid, and (2) neurological outcomes, such as attained cognitive function or changes in cognitive function. One area of consistency in research findings was the association between the combination of high folic acid intakes and the presence of low vitamin B<sub>12</sub> status with adverse neurological outcomes. These findings, however, were confined to observational studies. The observed consistency also is difficult to evaluate because the number of available studies is limited and available studies followed different cognitive outcomes and used disparate vitamin status cutoffs. Another area of evidentiary consistency from randomized controlled trials is that cognitively intact individuals consuming high levels of folate are not at increased risk of cognitive impairment. However, available randomized controlled trials were not designed to answer whether the combination of high folic acid intakes and low vitamin B<sub>12</sub> status is beneficial or deleterious to neurological outcomes.

There are some areas of consistency where additional research would clarify available findings. First, meta-analyses of observational study data are needed to investigate the interactions among low vitamin B<sub>12</sub> status and high folic acid intake using biochemical markers, dietary intake data, and other indicators of vitamin status. Mendelian randomization studies based on markers of vitamin B<sub>12</sub> and folate status in relation to cognitive outcomes could further clarify areas of evidentiary consistency.

There are recognized ethical issues associated with intervention studies that enroll individuals with untreated vitamin B<sub>12</sub> deficiency; however, it may be possible to conduct randomized trials to study

potential beneficial and adverse responses to vitamin B<sub>12</sub> supplementation with or without added folic acid/folate in populations with low vitamin B<sub>12</sub> status. The ethical issue involves studying people who are vitamin B<sub>12</sub> deficient and leaving them deficient.

Importantly, it is necessary to exploit past studies to investigate effect modifications due to interactions between low vitamin B<sub>12</sub> status and high folic acid intakes. Such factors include hematologic measures (e.g., anemia), ethnicity, alcohol consumption, aspirin use, smoking, fatty acid levels, genotypes, mood and depression, age, antacid use, and source of folic acid (e.g., fortification, enrichment, or supplement use).

Several animal models could be used to examine effects of folic acid supplementation in the presence of vitamin B<sub>12</sub> deficiency, such as surgical models of total gastrectomy, the administration of inhibitors of vitamin B<sub>12</sub> metabolism, targeted genetic knockouts, and nitrous oxide exposure. These models could examine dose-response relationships, effect of diverse folate forms, and impacts of timing of folate exposure in the presence of vitamin B<sub>12</sub> deficiency of varying duration. For studies of timing of exposure, animals could be *in utero*, nurslings, weaned, or older. The outcomes measured could include behavioral (e.g., learning and memory) and biochemical, such as gene expression and DNA methylation. It is important that animal models selected for study recapitulate human neurological phenotypes of vitamin B<sub>12</sub> deficiency. In addition, differences in folic acid metabolism between candidate animal models and humans should be considered with great care.

In considering systematic reviews, meta-analyses of observational study data should be done to investigate interactions between low vitamin B<sub>12</sub> status and high folic acid intakes on the basis of biochemical and dietary intake data and vitamin status levels. Meta-analyses should be done for studies of older populations with low vitamin B<sub>12</sub> status and high folate intakes to investigate effects on cognitive function, such as cognitive declines. Comparisons would be to individuals with normal vitamin B<sub>12</sub> status and non-high folate intake levels.

### **Areas of Uncertainty and Research Recommendations**

Health outcomes for which there are uncertainties include attained cognitive function and cognitive changes, as well as dementia types. There are also uncertainties regarding relationships between high folate intakes and hematologic measures, such as anemia, mean corpuscular volume, and red cell distribution width; mood, depression, and peripheral neuropathy; and visual and somato-sensorial evoked potentials. Cognitive development in children also has not been adequately investigated.

With regard to un- or under-studied outcomes, there needs to be an investigation of the effects of different cut points for high serum folate in the presence of vitamin B<sub>12</sub> deficiency. The various definitions of vitamin B<sub>12</sub> deficiency or insufficiency, including multiple or combined measures of vitamin B<sub>12</sub> and its metabolites should be examined. Research also is needed on the effect size estimates of cognitive impairments for different levels or cut points. In addition, different folate forms should be investigated, such as folic acid versus reduced folates. Research should be done on the duration of folic acid exposures, the importance of concomitant hematologic problems (e.g., anemia), and effect modification by severity or stage of vitamin B<sub>12</sub> deficiency or the other potential effect modifiers mentioned previously.

There is also a need for observational studies that focus on high folic acid intakes in the presence of low vitamin B<sub>12</sub> status to examine unstudied outcomes of public health relevance so that future randomized trials can be better designed. These include prospective studies to examine changes in outcomes, such

as longitudinal changes in cognitive batteries or hematologic variables. Future studies should consider the list of factors that were identified earlier as potential confounders or effect modifiers (e.g., ethnicity and alcohol consumption). Additional randomized trials would be required to examine such areas of uncertainty using a design similar to that described earlier of vitamin B<sub>12</sub> supplementation with or without folic acid/folate in populations with low vitamin B<sub>12</sub> status. The need for additional animal investigations of previously unstudied health outcomes is uncertain. In addition, sufficient data do not exist to conduct systematic reviews of areas characterized by evidentiary uncertainty.

### 6.3.3 Hypersensitivity-Related Outcomes<sup>8</sup> Subpanel

#### Summary of Responses to the Charge and Research Recommendations

##### Sensitization and Asthma

- Address the limited health effects data on “high” folic acid exposure levels for both prenatal and postnatal time periods.
- Need to better understand how folic acid/folate functions in biological pathways in sensitization and asthma.
  - Well-designed studies of human maternal and cord blood, human cell culture, mast cell and basophil responses, and animal studies (RCT, prospective or observational) to identify pathways and biomarkers.
  - Complementary animal and human cell culture studies.
- More well-controlled human studies, especially in pregnant women and in children, are needed to elucidate the effect of folic acid exposure on sensitization and asthma.
  - Better assessment of confounders (e.g., diet, other nutrients, environmental exposures).
  - Better assessment of effect modifiers (age, dose, genetics, nutrients, disease).

##### Eczema/Atopic Dermatitis and Respiratory Infection

- Unless new data emerge, these are not priority areas for future research.

### Challenges Related to the Literature Base

The literature base for hypersensitivity-related outcomes is much smaller than for cancer and cognition in conjunction with vitamin B<sub>12</sub> deficiency. There are major challenges to identifying areas of consistency and uncertainty and determining research recommendations. Studies in this literature base were entirely observational, and there were many confounding factors that were not assessed in the studies. Also, a handful of animal studies had mixed results. For the folic acid exposures that were evaluated in the studies, there was a lack of high exposure. Thus, it is difficult to determine when there was no effect of high folic acid exposure on the outcome, and it is difficult to rule out an increased risk of allergic disorders with high folic acid exposure, especially because studies have shown both positive and negative dose-response relationships between folic acid exposure/blood folate in the low-moderate range and allergic conditions. In addition, not all studies used the same measurements of folic acid exposure. Questionnaires were used in some studies to determine folic acid exposure; other studies

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<sup>8</sup> Hypersensitivity-related outcomes encompass a collection of effects observed in the available studies of allergy and atopy, including respiratory infection, asthma, wheeze, hypersensitivity tests, eczema, and food allergy.



measured biomarkers of folate in blood. Different methods also were used to quantify folate in blood. The effect sizes were small in the available studies, posing the question whether the small effect size is due to a lack of a real effect, or if there is a true effect and the effect is actually small. In some studies, a correction for multiple comparisons was lacking. For the most part, investigators took advantage of existing cohorts or bio-specimen banks and examined allergic disease endpoints, such as asthma and allergy, as secondary outcomes. Finally, some studies dealt with folic acid deficiency rather than excess folic acid exposure.

### **Areas of Consistency/Uncertainty and Research Recommendations**

There are some common themes for hypersensitivity-related outcomes, which can be grouped. One group of outcomes is atopy or allergy. Atopy is the genetic predisposition to make immunoglobulin E (IgE) or an allergic antibody response to something in the environment that is typically ignored by the immune system. Studies to evaluate atopy included outcomes such as total IgE and food and aeroallergen-specific IgE. Other groups of outcomes included asthma (and related outcomes, such as wheeze), eczema/atopic dermatitis, and respiratory infection. Although there were different groups of outcomes, they are all interrelated. For example, wheeze and allergic sensitization are both risk factors and predictors of childhood asthma.

For measures of atopy, such as IgE, there was mixed, scientific evidence for a relationship between folic acid intakes or folate status and total and specific serum IgEs levels. For example, while one nationally representative cross-sectional study of adults found an inverse association between higher serum folate levels and total IgE and allergen-specific IgE, other studies found positive associations between higher serum folate and incident allergic sensitization. This is an important public health issue to resolve because atopic diseases are the most common chronic childhood diseases (i.e., asthma, allergic rhinitis, eczema, and food allergy); therefore, more research is recommended. Neither high folic acid intake nor high levels of biomarkers of folate in blood have been shown to induce asthma in either adults or children. Wheezing itself is not a singular biological condition and is typically measured when asthma is evaluated as an outcome, and most, but not all, studies found no association between folic acid intake or levels of biomarkers of folate in blood and this outcome. Overall, the preponderance of this literature does not support a relationship between folic acid exposure and wheezing at the studied doses.

For eczema and atopic dermatitis, all studies (including pre- and post-natal exposure), except a multivitamin study, showed no association between eczema/atopic dermatitis and high levels of folic acid intakes or high levels of biomarkers of folate in blood. Although pathway and mechanistic insights are lacking, this is a low priority research area. For respiratory infection, the preponderance of evidence does not suggest a relationship between folic acid intakes and respiratory infection susceptibility.

There are few published studies, so there are few data. These few studies are also quite heterogeneous. Thus, for all outcomes, no new systematic reviews are warranted.

For sensitization and asthma, there is a need to address the limited health effects data on high folic acid/ folate exposure levels for both prenatal and postnatal time periods. A better understanding of how folic acid/folate functions in biological pathways leading to sensitization and asthma is also needed. This could be accomplished by well-designed, human randomized controlled trials and prospective or observational studies to investigate maternal and cord blood, human cell culture, mast cell, and basophil responses, as well as animal studies to identify pathways and biomarkers. Such animal and human cell culture studies should be complementary.

Better-controlled human studies are especially needed in pregnant women and in young children to elucidate the effect of prenatal and early life folic acid exposure on sensitization and asthma. Many of the observational studies had inadequate assessment of confounders, because folate intakes or levels of biomarkers of folate in blood were viewed as a secondary predictor and allergic outcomes were seen as secondary outcomes. Better assessments of confounders such as diet, other nutrients, and environmental exposures are needed. There were some studies that suggested non-linear dose-response relationships between folic acid and hypersensitivity-related outcomes. Thus, there is a need to better assess dose-response relationships, as well as other effect modifiers, such as age, genetics, other nutrients, and disease status. For example, would high folate intakes act differently in someone with established asthma compared to someone who is at risk of developing asthma?

The other two outcomes, eczema and respiratory infection, are not priority areas for *de novo* research unless new data emerge. However, this statement does not mean research related to these outcomes should be avoided. Some of these outcomes could be evaluated without significant expense and commitment of resources if there were opportunities to take advantage of current bio-banks or cohort studies already in process.

### 6.3.4 Thyroid and Diabetes-Related Disorders Subpanel

#### Summary of Responses to the Charge and Research Recommendations

##### Summary of Available Research

- Diabetes: Based on limited data, there is no consistent evidence for any effects of high folic acid intakes or high folate status on diabetes risk or glucose/insulin metabolism.
- Prenatal exposures: There are inconsistent results between trial and observational studies, but evidence linking high folic acid intakes/folate status to increasing fat mass and insulin resistance from a single observational study should be investigated further.
- Thyroid: Available evidence does not address possible adverse effects of high folic acid intakes on thyroid disease.

##### Recommendations (Diabetes)

- Studies that could be done now
  - Use existing prospective cohorts with valid and reliable measurements of exposure and outcome.
  - Use existing GWAS studies to do Mendelian randomization studies focused on candidate genes.
  - Follow-up with adults and children from maternal folic acid supplementation trials.
- Future studies
  - Use valid and reliable prospective measurements of folic acid intakes or folate status.
  - Need to consider baseline intakes and deficiency prevalence.
  - Consider the impact of confounders that affect folate metabolism and diabetes risk (e.g., medications).
  - Examine subgroup susceptibilities according to genetic polymorphisms, environmental exposures (e.g., environmental diabetogens), and nutritional factors.
  - Consider different periods of vulnerability, especially preconception and prenatal.
  - Look at folic acid and total folate intakes in assessing risk of toxicities related to thyroid disorders and diabetes.

#### Areas of Consistency and Inconsistency and Research Recommendations

In the diabetes literature, there is no evidence that folates (including folic acid supplementation, folate intake, and biochemical measures of folate status) affect type 1 diabetes in adults, type 2 diabetes, and measures of impaired glucose or insulin homeostasis or metabolism in adults. Notably, there is limited reporting on type 1 diabetes. The literature predominantly involves case control and cross-sectional studies, measuring folate biomarkers or folic acid intakes in adults or children with type 2 diabetes and controls. There is the potential for reverse causality of people altering their diets in response to their disease diagnosis or for pharmacological interventions having an effect on folate metabolism. One high-quality randomized trial in the U.S. evaluated folic acid supplementation as well as other B vitamins. This randomized trial found no evidence of increased or decreased risk of type 2 diabetes with folic acid

supplementation. Thus, there seems to be no evidence of an adverse effect of folic acid supplementation in a controlled setting on diabetes risk.

For prenatal exposures, there is inconsistent evidence from the results of a randomized trial done in Nepal compared to the results of an observational study from India. The randomized trial reported that folic acid supplementation was associated with a decreased risk of metabolic syndrome. However, the observational study reported statistically significant associations of erythrocyte folate levels during pregnancy with altered insulin resistance in children, as well as increased body fat. Thus, this result should be further investigated. In terms of thyroid disorders, the available evidence does not address possible adverse effects of folic acid supplementation on thyroid disease.

For research recommendations, it would be expedient to use existing genome-wide association studies (GWAS) to conduct Mendelian randomization experiments with the candidate gene approach to select genes that are known to be involved with folic acid or folate metabolism. These Mendelian experiments could elucidate whether or not certain alleles of folate metabolic pathway genes are associated with type 2 diabetes risks.

There is a lack of large and well-established cohort studies that have examined relationships between dietary folic acid intake and type 2 diabetes. The Women's Health Initiative has data on folic acid intake and diabetes; however, the data are not reported in the literature. Thus, another avenue of research would be to examine prospective studies with relevant outcome and exposure measurements (e.g. Nurses' Health Study or Women's Health Initiative) to study the relationship between folic acid intakes and diabetes risk as measured by dietary questionnaires or biobanked samples. For existing prenatal studies, additional follow-up of children from randomized trials, such as the one conducted in China, could be done. Children should be assessed for cardiometabolic outcomes, fat mass, and other end points that might be related to diabetes. Follow-up should also be done in adults who participated in similar studies conducted in western populations.

There are several limitations in the current literature that should be addressed if future studies are conducted. First, it is important to distinguish folic acid from various other forms of folate provided by supplementation, fortification, enrichment, and found naturally in foods. Second, measurements of folate should be prospective and consider unmetabolized folic acid and total folate. Third, many of the case studies examined were controlled or cross-sectional in design, so it was difficult to disentangle temporal relationship between exposure and outcomes. Fourth, there is a need to consider baseline intakes and deficiency prevalence in study populations. Finally, it is important to consider the impact of confounders that may affect folate metabolism and diabetes risk, such as medications. For example, in observational studies, there is indication that metformin, a drug used to treat type 2 diabetes, can lower vitamin B<sub>12</sub> status and thus may be a potential confounder in studies of associations between folic acid intake and type 2 diabetes.

Subgroup susceptibility according to genetic polymorphisms, environmental exposures (e.g., environmental diabetogens), and other modifiers such as nutritional factors should be examined. For example, some studies that examined risk for diabetes with folic acid supplementation were conducted in areas where the probability of multiple micronutrient deficiencies is not insignificant or characterized by diets (e.g., vegetarian diets) not externally generalizable; thus, nutritional factors could have a confounding effect. It is also important to consider different periods of vulnerability, such as preconception and prenatal periods, given that there is evidence that folate intakes in the preconception period may affect cardiometabolic risk.

Currently, human studies do not provide additional rationales for supplementary animal studies examining folic acid alone as a risk factor for diabetes. However, animal studies that investigate modifying factors (e.g., diabetogenic chemicals) may provide additional insight into susceptible human subgroups and lead to human studies with high quality designs. Animal studies should consider different stages of vulnerability and genetic or epigenetic mechanisms, because animal studies could do this in a more robust manner than what is feasible in human studies due to limited access to target tissues of interest in humans.

At this time, systematic reviews are not recommended, because the design of most of the studies would not be conducive to such an evaluation and the lack of published prospective studies or trials.

### **Areas of Uncertainty and Research Recommendations**

The available scientific literature does not address the effect of folic acid on thyroid disease. Most of the studies examined hyperthyroidism or hypothyroidism, as well as measuring controls and the biomarkers for folic acid intake at the time of diagnosis. Thus, there is a question of whether the exposure or outcome occurred first. There are well-documented effects of thyroid disease on folate metabolism, as well as hyperthyroidism and hypothyroidism on appetite and food intake. Thus, there are no compelling reasons to study this particular endpoint over others. In addition, systematic review is not recommended at this time to evaluate thyroid disorders.

## **6.4 Discussion of Common Themes**

Relevant to all four subpanels' recommendations is the need for (1) better methods for estimating exposure/intake (total folates and specific forms of the vitamin), such as improved methods to ascertain timing and duration of exposure, (2) standardization of folate assays, and (3) enhanced reliability of food and supplement composition tables. However, there was general agreement that addressing these needs should not supersede recommendations made by the four subpanels or assume priority for funding over those recommendations.

In addition, the four subpanels touched upon research needs related to identifying those individuals who are most likely to be at risk or to benefit from high folate intakes. However, there were no unifying themes in approaches to identifying such subgroups, because subgroups would vary depending on the health outcome being studied.

## **6.5 Public Comments**

### ***May 11: Written and Oral Public Comments***

#### **Written Public Comments**

Two public written comments were received and shared with the panel and posted to the meeting webpage (<http://ntp.niehs.nih.gov/go/730864>):

- Brett Bartel of Buriva, LLC sent written comments on April 17, 2015.
- Gretchen DuBeau, Esq.; Robert Verkerk, BSc, MSc, DIC, PhD, FACN; and Ze'eva Banks sent written comments on behalf of Alliance for Natural Health USA on May 4, 2015.

## **Public Oral Comments by Phone**

No one registered to provide public oral comments by phone.

## **In-Person Public Oral Comments**

Dr. Ralph Green of the University of California, Davis Department of Pathology and Laboratory Medicine noted the importance of addressing vitamin B<sub>12</sub> status in addition to folic acid intakes in all four of the health effect categories examined by the panel, i.e., not just in evaluations of cognition in conjunction with vitamin B<sub>12</sub> status. Dr. Green recommended that the panel consider the metabolic link between vitamin B<sub>12</sub> status and folate intakes when making research recommendations.

In response to a question from the panel about experimental data that show any metabolic or physiologic readouts demonstrating the exacerbation of vitamin B<sub>12</sub> deficiency due to high levels of folate intakes, Dr. Green responded that he did not know of any studies in vitamin B<sub>12</sub> deficient animal models in which the amount of administered folate resulted in detrimental effects, no effects, or beneficial effects. Dr. Green was asked by the panel to elaborate on the history of studies and clinical reports of the masking of vitamin B<sub>12</sub> deficiency. Dr. Green provided a brief background on masking and concluded that giving folic acid to someone who is vitamin B<sub>12</sub> deficient can eliminate or ameliorate anemia; however, folic acid would not treat the adverse neurologic effects associated with vitamin B<sub>12</sub> deficiency and may potentially have a negative consequence on the neurologic status of B<sub>12</sub> deficient patients.

## ***May 12: Oral Public Comments***

### **Dr. Yu (Janet) Zang, U.S. Food and Drug Administration**

Dr. Zang had a comment for the Cognition in Conjunction with Vitamin B<sub>12</sub> Deficiency subpanel. She noted from the first day's introductory presentations that U.S. children have high folic acid intake and noted the subpanel mentioned that there are no studies on the effect of folic acid on cognitive development in children. She asked the subpanel why this lack of studies is not reflected in the subpanel's final recommendation slide.

The subpanel noted that finding a baseline level of folate in absence of fortification for such studies would be difficult because the U.S. has mandated fortification of selected foods with folic acid since 1996, so all these generations of children have grown up with fortification. People in their twenties have been exposed to folic acid since they were *in utero*. The subpanel's current recommendations address this indirectly. The recommendations address the issue of timing, such as different time periods of exposure with respect to future human studies.

### **Dr. Ralph Green, University of California, Davis**

Dr. Green commented that the current medical practice is to administer folate to individuals who are vitamin B<sub>12</sub> deficient as long as it is ensured that the individuals also are being treated adequately to remedy their B<sub>12</sub> deficiency. There is nothing that proscribes the use of folate in conjunction with vitamin B<sub>12</sub>. Thus, he proposed that it is important to conduct randomized trials to study the response to vitamin B<sub>12</sub> supplementation with or without folic acid/folate in populations with low vitamin B<sub>12</sub> status to clarify the level of folic acid that is detrimental to those with low and deficient B<sub>12</sub> status.

The Cognition in Conjunction with Vitamin B<sub>12</sub> Deficiency subpanel noted that they did not include a recommendation to conduct the randomized trials mentioned by Dr. Green because of the ethical issues. However, that point is still included in the subpanel's report, because it is important to look at vitamin B<sub>12</sub> responsiveness to supplementation by lowering folic acid levels.

### **Dr. Johanna Dwyer, ODS**

Dr. Dwyer asked for clarification on using the terms folic acid, dietary supplement folic acid, folic acid in food, fortified food, and food folate. She asked how these terms are defined in the subpanels' reports.

The panel noted that they were not restricting their report to high intakes of folic acid, meaning folic acid in fortified foods and dietary supplements. They also included high folate status or high intakes of other forms of folate, such as those found in fruits and vegetables. Although the expert panel specifically addressed folic acid, they also considered total folate levels to understand the role of folic acid.

## **6.6 Full Panel Votes**

### ***Full Panel Vote on Each Subpanel's Summary of Responses to Charge and Research Recommendations***

The panel unanimously voted (18 yes, 0 no, 0 abstain) to accept each subpanel's summary of responses to the charge and research recommendations, which are listed below.

### **Cancer**

#### **Pre-Clinical Summary/Research Recommendations**

- Although inadequate dietary folic acid intake increases colon cancer risk in rodent model systems, there is consistency in showing an acceleration of colon cancer development in the few studies that have tested the effects of folic acid intake above basal requirements in these systems.
- Study effects of folic acid above basal requirements on the growth of cancers other than the colon, especially those where there is a suggestion of a clinical effect in humans (e.g., prostate).
- Compare the effects of folic acid to those of reduced folates and overall folate status on cancer growth, define the dose response of each, evaluate the interplay of other 1-carbon nutrients, study mechanisms of effects, and evaluate the modification by other covariates (e.g., alcohol, age, genetics, sex).
- Identify and employ those animal models that are most relevant to human folate metabolism and carcinogenic processes.
- Better define critical life stages and other timing effects of exposure.
- Explore maternal and paternal effects of folates and folic acid on offspring cancer risk.

#### **Clinical Summary/Research Recommendations**

- Although inadequate dietary folate intake increases colorectal cancer risk in humans, there is no benefit for cancer reduction from supplements among people whose baseline folate status is adequate.
- There is a consistent enough suggestion in human studies of an adverse effect on cancer growth from supplemental folic acid to justify further research.
- There is a need to clarify whether the existing suggestive evidence from clinical trials for increased risk is driven by effects in specific subgroups (e.g.: by age, pre-existing neoplasia,

genetics, other factors), and to assess long-term outcomes among subjects from prior folic acid trials.

- New studies are needed to identify subgroups that are at risk of tumor acceleration.
- Define whether the form of ingested folate impacts on risk and whether other 1-carbon nutrients are modifiers of the potential pro-tumorigenic effects of excess folic acid.
- Define the above issues in other implicated cancer sites in addition to colorectal cancer (e.g., prostate).

## **Cognition in Conjunction with Vitamin B12 Deficiency**

### Summary

- The hypothesis that high folic acid/folate in the presence of low B12 exacerbates neurological problems based on early case reports has some supportive evidence from observational studies, but the data are limited.
- The data from epidemiological studies are difficult to interpret because of heterogeneity in vitamin status cut points and in the cognitive outcomes and the omission of other neurological outcomes.
- Existing intervention studies were not designed to address this question.
- The mechanisms by which folic acid may exacerbate B12 deficiency are unclear.

### Recommendations

- Meta-analysis of the existing larger observational studies to quantify effect sizes in various groups.
- Mendelian randomization studies can help inform causal relevance of these associations.
- Pending results of meta-analyses and Mendelian randomization studies, conduct animal and in vitro studies using appropriate models to identify potential biological mechanisms, as well as further human investigations including studies that examine the timing of exposure (e.g., in utero, weaning, older).

## **Hypersensitivity-Related Outcomes**

### Sensitization and Asthma

- Address limited health effects data on “high” folic acid exposure levels for both prenatal and postnatal time periods.
- Need to better understand how folic acid/folate functions in biological pathways in sensitization and asthma.
  - Well-designed human maternal and cord blood, human cell culture, mast cell and basophil responses and animal studies (RCT, prospective or observational) to identify pathways and biomarkers.
  - Complementary animal and human cell culture studies.
- More well-controlled human studies, especially in pregnant women and in children, to elucidate the effect of folic acid exposure on sensitization and asthma.
  - Better assessment of confounders (diet, other nutrients, environmental exposures)
  - Better assessment of effect modifiers (age, dose, genetics, nutrients, disease)

### Eczema/Atopic Dermatitis and Respiratory Infection

- Unless new data emerge, these are not priority areas for future research.



## Thyroid and Diabetes-Related Disorders

### Summary of available research

- Diabetes: Based on limited data, there is not consistent evidence for an effect of high folic acid intakes or high folate status on diabetes risk or glucose/insulin metabolism
- Prenatal exposures: Inconsistent results between the trial and observational studies, but increasing fat mass and insulin resistance in the observational study should be further investigated
- Thyroid: Available evidence does not address the effect of folic acid on thyroid disease

### Recommendations (diabetes)

- Studies that could be done now
  - Use existing prospective cohorts with valid and reliable measurements of exposure and outcome
  - Use existing GWAS studies to do Mendelian randomization studies with candidate genes
  - Follow-up with adults and children from maternal folic acid supplementation trials
- Future studies
  - Use valid and reliable prospective measurements of folic acid intake or folate status.
  - Need to consider baseline intake and deficiency prevalence
  - Consider the impact confounders that affect folate metabolism and diabetes risk (e.g., medications)
  - Examine subgroup susceptibility according to genetic polymorphisms, environmental exposures (e.g., environmental diabetogens), and nutritional factors
  - Consider different periods of vulnerability, especially preconception and prenatal
  - Look at folic acid as well as total folate in assessing risk of toxicities

### ***Full Panel Vote on Issues Relevant to All Four Subpanels' Recommendations***

The panel voted unanimously (18 yes, 0 no, 0 abstain) to accept as relevant to all four subpanel's recommendations the need for: (1) better methods for estimating exposure/intake (total folates and specific forms of the vitamin), such as improved methods to ascertain timing and duration of exposure, (2) standardization of folate assays, and (3) enhanced reliability of food and supplement composition tables.

## **6.7 Approval of the Expert Panel Report by the Chair**

This expert panel report was reviewed by all panelists, and then read and approved by the Chair of the May 11-12, 2015, National Toxicology Program and NIH Office of Dietary Supplements' expert panel to identify research needs related to the safe use of high intakes of folic acid based on consideration of the state of the science.

Cutberto Garza, M.D., Ph.D.

Chair, Expert Panel: Identifying Research Needs for Assessing Safe Use of High Intakes of Folic Acid

Date: July 28, 2015

## 7.0 REFERENCES

- Al-Farsi YM, Waly MI, Deth RC, Al-Sharbati MM, Al-Shafae M, Al-Farsi O, Al-Khaduri MM, Gupta I, Ali A, Al-Khalili M, Al-Adawi S, Hodgson NW, Ouhtit A. 2013. Low folate and vitamin B12 nourishment is common in Omani children with newly diagnosed autism. *Nutrition* 29(3): 537-541.
- Ali A, Waly MI, Al-Farsi YM, Essa MM, Al-Sharbati MM, Deth RC. 2011. Hyperhomocysteinemia among Omani autistic children: a case-control study. *Acta Biochim Pol* 58(4): 547-551.
- Beard CM, Panser LA, Katusic SK. 2011. Is excess folic acid supplementation a risk factor for autism? *Med Hypotheses* 77(1): 15-17.
- Berry RJ, Kihlberg R, Devine O. 2005. Impact of misclassification of in vitro fertilisation in studies of folic acid and twinning: modelling using population based Swedish vital records. *BMJ* 330(7495): 815.
- Brown SB, Reeves KW, Bertone-Johnson ER. 2014. Maternal folate exposure in pregnancy and childhood asthma and allergy: a systematic review. *Nutr Rev* 72(1): 55-64.
- Crider KS, Cordero AM, Qi YP, Mulinare J, Dowling NF, Berry RJ. 2013. Prenatal folic acid and risk of asthma in children: a systematic review and meta-analysis. *Am J Clin Nutr* 98(5): 1272-1281.
- Czeizel AE, Dudas I, Metneki J. 1994a. Pregnancy outcomes in a randomised controlled trial of periconceptional multivitamin supplementation. Final report. *Archives of Gynecology and Obstetrics* 255(3): 131-139.
- Czeizel AE, Metneki J, Dudas I. 1994b. The higher rate of multiple births after periconceptional multivitamin supplementation: an analysis of causes. *Acta Genet Med Gemellol (Roma)* 43(3-4): 175-184.
- de Benoist B. 2008. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull* 29(2 Suppl): S238-244.
- Ebbing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen EK, Nygard O. 2008. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA* 300(7): 795-804.
- Ebbing M, Bona KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njolstad I, Refsum H, Nilsen DW, Tverdal A, Meyer K, Vollset SE. 2009. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA* 302(19): 2119-2126.
- Ericson A, Kallen B, Aberg A. 2001. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res* 4(2): 63-66.
- Finer S, Saravanan P, Hitman G, Yajnik C. 2014. The role of the one-carbon cycle in the developmental origins of Type 2 diabetes and obesity. *Diabet Med* 31(3): 263-272.
- Frustaci A, Neri M, Cesario A, Adams JB, Domenici E, Dalla Bernardina B, Bonassi S. 2012. Oxidative stress-related biomarkers in autism: systematic review and meta-analyses. *Free Radic Biol Med* 52(10): 2128-2141.
- Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA. 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343: d5928.
- Hyman SL, Stewart PA, Schmidt B, Cain U, Lemcke N, Foley JT, Peck R, Clemons T, Reynolds A, Johnson C, Handen B, James SJ, Courtney PM, Molloy C, Ng PK. 2012. Nutrient intake from food in children with autism. *Pediatrics* 130 Suppl 2: S145-153.
- ICMR, Unit. CCaCTC-o. 2000. Multicentric study of efficacy of periconceptional folic acid containing vitamin supplementation in prevention of open neural tube defects from India. *Indian J Med Res* 112: 206-211.
- Li Y, He Y, Qi L, Jaddoe VW, Feskens EJ, Yang X, Ma G, Hu FB. 2010. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. *Diabetes* 59(10): 2400-2406.

- Li Z, Gindler J, Wang H, Berry RJ, Li S, Correa A, Zheng JC, Erickson JD, Wang Y. 2003. Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet* 361(9355): 380-384.
- Lowe TL, Cohen DJ, Miller S, Young JG. 1981. Folic acid and B12 in autism and neuropsychiatric disturbances of childhood. *Journal of the American Academy of Child Psychiatry* 20(1): 104-111.
- Mason JB. 2009. Folate, cancer risk, and the Greek god, Proteus: a tale of two chameleons. *Nutr Rev* 67(4): 206-212.
- Moher D, Liberati A, Tetzlaff J, Altman DG. 2009. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Journal of Clinical Epidemiology* 62(10): 1006-1012.
- National Research Council. 1998. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*, Washington, DC: The National Academies Press.
- Nwaru BI, Erkkola M, Ahonen S, Kaila M, Kronberg-Kippila C, Ilonen J, Simell O, Knip M, Veijola R, Virtanen SM. 2011. Intake of antioxidants during pregnancy and the risk of allergies and asthma in the offspring. *European Journal of Clinical Nutrition* 65(8): 937-943.
- Palmer DJ, Huang RC, Craig JM, Prescott SL. 2014. Nutritional influences on epigenetic programming: asthma, allergy, and obesity. *Immunology and allergy clinics of North America* 34(4): 825-837.
- Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. 1998. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 351(9097): 173-177.
- Rumbold A, Middleton P, Pan N, Crowther CA. 2011. Vitamin supplementation for preventing miscarriage. *Cochrane Database of Systematic Reviews*(1).
- Schmidt RJ, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tancredi DJ, Tassone F, Hertz-Picciotto I. 2011. Prenatal Vitamins, One-carbon Metabolism Gene Variants, and Risk for Autism. *Epidemiology* 22(4): 476-485.
- Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, Schmidt LC, Tassone F, Hertz-Picciotto I. 2012. 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* 96(1): 80-89.
- Signore C, Mills JL, Cox C, Trumble AC. 2005. Effects of folic acid fortification on twin gestation rates. *Obstet Gynecol* 105(4): 757-762.
- Sudchada P, Saokaew S, Sridetch S, Incampa S, Jaiyen S, Khaithong W. 2012. Effect of folic acid supplementation on plasma total homocysteine levels and glycemic control in patients with type 2 diabetes: a systematic review and meta-analysis. *Diabetes Res Clin Pract* 98(1): 151-158.
- Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjolberg S, Davey Smith G, Oyen AS, Susser E, Stoltenberg C. 2013. Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *JAMA* 309(6): 570-577.
- Thayer KA, Heindel JJ, Bucher JR, Gallo MA. 2012. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environ Health Perspect* 120(6): 779-789.
- Vollset SE, Gjessing HK, Tandberg A, Ronning T, Irgens LM, Baste V, Nilsen RM, Daltveit AK. 2005. Folate supplementation and twin pregnancies. *Epidemiology* 16(2): 201-205.
- Zhu Y, He ZY, Liu HN. 2011. Meta-analysis of the relationship between homocysteine, vitamin B, folate, and multiple sclerosis. *J Clin Neurosci* 18(7): 933-938.

## APPENDIX 1: LITERATURE SEARCH METHOD

<b>Concept</b>	<b>PubMed</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Embase</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Scopus and Web of Science</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Cochrane</b> <i>Initial search: May 7, 2013</i> <i>Update 1: Dec. 1, 2014</i>
<b>Publication Years</b>	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
<b>Folic Acid</b>	<b>#1:</b> 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]	<b>#1:</b> 'folic acid'/exp AND ('diet supplementation'/exp OR 'vitamin'/exp)	<b>#1:</b> "Folic acid" OR "Pteroylglutamic Acid" OR "pteroylmonoglutamic acid" OR tetrahydrofolate* OR "5-Methyltetrahydrofolic acid" OR "5-methyltetrahydrofolate" OR leucovorin OR "folinic acid" OR folate*	<b>#1:</b> "Folic acid" OR "Pteroylglutamic Acid" OR "pteroylmonoglutamic acid" OR tetrahydrofolate* OR "5-Methyltetrahydrofolic acid" OR "5-methyltetrahydrofolate" OR leucovorin OR "folinic acid" OR folate*
<b>Dietary Supplement</b>	<b>#2:</b> 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]	<b>#2:</b> ("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	<b>#2:</b> 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*	<b>#2:</b> 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*
<b>Folic Acid AND Dietary Supplement</b>	<b>#3:</b> #1 AND #2	<b>#3:</b> #1 AND #2	<b>#3:</b> #1 AND #2	<b>#3:</b> #1 AND #2

<b>Concept</b>	<b>PubMed</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Embase</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Scopus and Web of Science</b> <i>Initial Search: Nov. 25, 2011</i> <i>Update 1: May 7, 2013</i> <i>Update 2: Dec. 1, 2014</i>	<b>Cochrane</b> <i>Initial search: May 7, 2013</i> <i>Update 1: Dec. 1, 2014</i>
<b>Chemotherapy</b>	<b>#4:</b> 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]	<b>#4:</b> '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)	<b>#4:</b> 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*	<b>#4:</b> 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*
<b>Remove chemotherapy results.</b>	<b>#5: # 3 NOT #4</b>	<b>#5: # 3 NOT #4</b>	<b>#5: # 3 NOT #4</b>	<b>#5: # 3 NOT #4</b>

## 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<sup>9</sup>, 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)<sup>10</sup>, 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 health outcome, but it will be considered in the context of disease.
- 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.

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<sup>9</sup> 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).

<sup>10</sup> Standard dose is considered the control diet amount

- 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. Helicobacter 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

## APPENDIX 4: EXPERT PANEL MEMBERS AND MEETING ATTENDEES

### Expert Panel Members:

#### Panel Chair

Cutberto Garza

#### Cancer Subpanel

Tim Byers (Subpanel chair)

Todd M. Gibson

Jesse F. Gregory

Young-In Kim

Joel B. Mason

#### Cognition in Conjunction with Vitamin B12 Deficiency Subpanel

Martha C. Morris (Subpanel chair)

Robert Clarke

Paul F. Jacques

Joshua W. Miller

Jeanne I. Rader

#### Hypersensitivity-Related Outcomes Subpanel

Patrick J. Stover (Subpanel chair)

Elizabeth Matsui

James L. Mills

Anne M. Molloy

Henk van Loveren

#### Thyroid and Diabetes-Related Disorders Subpanel

Joseph M. Braun (Subpanel chair)

Barry Shane

Miroslav Stýblo

#### NTP Board of Scientific Counselors Representative:

Sonya Sobrian, Howard University

### **National Institute of Environmental Health Sciences (NIEHS) Staff:**

Linda Birnbaum  
Abee Boyles  
John Bucher  
Mark Miller  
Katie Pelch (Rapporteur)  
Andrew Rooney  
Kris Thayer  
Mary Wolfe  
Yun Xie (Designated Federal Officer)

### **Office of Dietary Supplements (ODS) Staff**

Regan Bailey  
Paul Coates  
Cindy Davis  
Johanna Dwyer  
Adam Kuszak (Rapporteur)  
Leila Saldanha  
Barbara Sorkin  
Christine Taylor  
Paul Thomas (Rapporteur)

### **Other U.S. Federal Agency Staff:**

Krista Crider, Centers for Disease Control and Prevention (CDC)  
Nicole Dowling, CDC  
Kara Koehn, Environmental Protection Agency (Rapporteur)  
Cynthia Moore, CDC  
Christine Pfeiffer, CDC  
Nancy Potischman, National Institutes of Health (NIH)/National Cancer Institute (NCI)  
Yu (Janet) Zang, Food and Drug Administration (FDA)

**International Federal Agency Staff:**

Amanda MacFarlane, Health Canada

**Public Attendees:**

Brett Bartel, Burvia, LLC

Ralph Green, University of California, Davis

Linda Meyers, American Society for Nutrition

Ed McCabe, March of Dimes

Andrea Wong, Council for Responsible Nutrition