Office of the Prime Minister's
Chief Science Advisor

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The Health Benefits and Risks of Folic Acid Fortification of Food

A report by the Office of the Prime Minister's Chief Science Advisor and the Royal Society Te Apārangi

June 2018

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26 June 2018

Mr Andrew Forsyth
Manager, Public Health Capability
Public Health Group
Ministry of Health

Dear Mr Forsyth,

The following report is provided in response to a request by the Ministry of Health (the Ministry) in April 2017 to the Prime Minister's Chief Science Advisor (PMCSA), Sir Peter Gluckman, and the Royal Society Te Apārangi (the Society), to review the health benefits and risks of folic acid fortification of food.

Aim

Many countries mandate the fortification of staple foods with folic acid to reduce the rates of neural tube defects. However, due to consumer and commercial concerns, New Zealand currently relies on industry-led voluntary fortification of bread. Therefore, this report aims to provide government decision makers and the general public with a comprehensive and up-to-date understanding of the available scientific evidence on the health benefits and risks to human health of folic acid fortification of food (including voluntary and mandatory fortification). The report was co-chaired by Sir Peter and Emeritus Professor Robert Beaglehole, who was appointed by the Society.

Process

The scientific review was conducted in accord with a general process agreed between the PMCSA and the Society for such reports. The Society appointed a Panel of six experts across the relevant disciplines, and a respected member of civil society as a lay observer. The PMCSA appointed an experienced Research Fellow to undertake the primary research and literature reviews, and interface with the Panel. A Panel meeting was held during which intensive discussions took place on the state-of-the-science, areas of consensus and debate in the literature, issues of potential controversy, and broader issues/concerns. Following an initial scoping that included an extensive reading of the literature (informal, grey, and peer reviewed) on the subject, and further input by the Panel, a table of contents was agreed between the co-chairs and the Ministry.

Based on these headings, a draft synthesis report was prepared that underwent numerous revisions in an iterative process between the Research Fellow, co-chairs, and Panel members. During this

time, a Panel teleconference was held to provide an opportunity for further discussion on outstanding issues. The Ministry then provided extensive written and verbal feedback on the draft. The final draft report was sent out for national and international peer review by seven scientists with relevant expertise. The peer reviewers were identified by the co-chairs and Panel, and agreed to by the Ministry. Following receipt and consideration of all peer review comments, the report underwent additional revisions which were discussed by Panel members via a further teleconference. The final report was then approved by the Panel.

Findings and conclusions

There is compelling evidence that mandatory folic acid fortification is associated with lower rates of neural tube defects, and that taking folic acid supplements at the recommended doses in pregnancy has no adverse effects on pregnancy outcome or the child's health.

No evidence was found to link folic acid supplements to increased risks of neurological/cognitive decline, diabetes, or cardiovascular disease; nor was there evidence that unmetabolised folic acid that remains within the body's circulation is harmful. The Panel reviewed data related to potential effects on cancer risk. Most data suggest no effect, but some limited evidence from genetic studies of people with different folate metabolism suggests that higher folate levels may be associated with reduced risks overall cancer rates and lower risks of breast cancer in particular, but may also be associated with higher risks of prostate and colorectal cancer. The Panel discussed this issue in great depth over an extended period of time, and took this into account in preparing its unanimous advice.

Based on an overall assessment of the evidence, and also considering the need to ensure that disadvantaged people including Māori receive benefit, the Expert Panel concludes that the benefits of mandatory fortification of packaged bread with folic acid outweigh any potential adverse effects.

In addition, the Panel strongly encourages the continued use of folic acid supplements by pregnant women as recommended by their healthcare professionals, and encourages all women of childbearing age to ensure that their folate intakes are adequate.

Yours sincerely,

Sir Peter Gluckman
Prime Minister's Chief Science Advisor
Co-chair

Emeritus Professor Robert Beaglehole
Co-chair

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Acknowledgements

This report is a joint undertaking by the Office of the Prime Minister's Chief Science Advisor (OPMCSA) and the Royal Society Te Apārangi (the Society) to review the health benefits and risks of folic acid fortification of food. It was commissioned and funded by the Ministry of Health. An Expert Panel, including a Panel Lay Observer, was appointed by the Society to oversee the review. The project was co-chaired by Professor Sir Peter Gluckman and Emeritus Professor Robert Beaglehole, who was appointed by the Society. National and international peer reviewers were selected by the co-chairs. Research, analysis, and writing was undertaken by Dr Felicia Low, PhD, of the OPMCSA, working in close collaboration with the Expert Panel and co-chairs.

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List of abbreviations

AHMAC	Australian Health Ministers' Advisory Council
AIHW	Australian Institute of Health and Welfare
AP	adenomatous polyps
CVD	cardiovascular disease
DFE	dietary folate equivalent
EAR	estimated average requirement
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FSAI	Food Safety Authority of Ireland
IOM	Institute of Medicine
MPI	Ministry for Primary Industries
MR	Mendelian randomisation
5-MTHF	5-methyltetrahydrofolic acid
MTHFR	methylenetetrahydrofolate reductase
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council of Australia
NK	natural killer
NRV	nutrient reference value
NTD	neural tube defect
NTP	National Toxicology Program
NZEO	New Zealand European/other
RBC	red blood cell
RCT	randomised controlled trial
RR	risk ratio
SACN	Scientific Advisory Committee on Nutrition
THF	Tetrahydrofolate
UL	tolerable upper intake level
UMFA	unmetabolised folic acid
USPSTF	United States Preventive Services Task Force
VKM	Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet)
WCBA	women of childbearing age
WHO	World Health Organization

Plain language summary

Folate, a natural B vitamin, is essential for good health. It can also be taken as a synthetic form known as folic acid. Many countries, including the New Zealand Ministry of Health, recommend that women take the recommended dose of folic acid supplements before and during early pregnancy. This considerably reduces the risk of their baby developing neural tube defects, a type of birth defect. However, for various reasons many women are not able to follow this practice. To increase folate intake among women, many countries have made it mandatory to fortify some staple foods with folic acid. This has been shown to reduce the rates of neural tube defects, in some cases to a substantial degree. However, due to commercial and consumer concerns over the safety of folic acid fortification, New Zealand currently relies on industry-led voluntary fortification of bread.

This report reviews the scientific literature on the health benefits and risks of folic acid, particularly in relation to food fortification and consumption of higher-dose supplements. It concludes that mandatory fortification is unequivocally associated with lower rates of neural tube defects, and that taking folic acid supplements at the recommended doses in pregnancy has no adverse effects on pregnancy outcome or the child's health. There is no evidence that folic acid supplements increase the risk of neurological/cognitive decline, diabetes, or cardiovascular disease, or that unmetabolised folic acid that remains within the body's circulation is harmful. There is no strong evidence of adverse effects on risks of some common cancers, or total cancers. There is limited evidence from genetic studies involving people born with lower blood folate levels, that relatively higher blood folate may be associated with lower risks of breast and total cancers, but higher risks of prostate and colorectal cancer. This is an ongoing area of research which should be monitored.

Based on an overall assessment of the evidence, and also considering the need to ensure that disadvantaged people including Māori receive benefit, the Expert Panel concludes that the benefits of mandatory fortification of packaged bread with folic acid outweigh any potential adverse effects.

In addition, the Panel strongly encourages the continued use of folic acid supplements by pregnant women as recommended by their healthcare professionals, and encourages all women of childbearing age to ensure that their folate intakes are adequate.

Executive summary

Introduction

Folate is an important B vitamin naturally present in some foods including leafy green vegetables, legumes, and liver. Its synthetic form, known as folic acid, is used to fortify food and is a component of many dietary supplements. This report reviews the evidence for the health benefits and risks of folic acid fortification of food. It was commissioned by the New Zealand Ministry of Health to provide decision makers and the public with an updated understanding of the scientific literature on the health outcomes that are relevant to fortification of food with folic acid.

Many countries make fortification of some food with folic acid mandatory. Joint Australia and New Zealand legislation for the mandatory fortification of wheat flour or bread with folic acid was set to come into effect in 2009. The purpose was to reduce rates of a specific form of disabling congenital malformation, known as neural tube defects (NTDs), in both countries. While mandatory fortification was introduced in Australia, this was not implemented by the New Zealand government, which instead encouraged increased voluntary fortification by the baking industry.

In 2016, the efficacy of the mandatory fortification scheme in Australia for reducing NTD rates was confirmed in a report by the Australian government. It also determined that there were no health risks posed by excessive folic acid intake resulting from the introduction of mandatory fortification. In particular, it found no evidence in the scientific literature for any direct association of folic acid consumption with cancer incidence or all-cause mortality. The New Zealand Ministry of Health has chosen to evaluate the latest scientific evidence on the health benefits and risks of folic acid, and commissioned this report jointly from the Office of the Prime Minister's Chief Science Advisor and the Royal Society Te Apārangi. This report constitutes part of wider efforts to reassess the effectiveness of the New Zealand voluntary bread fortification programme in reducing NTD rates.

Neural tube defects in New Zealand

NTDs are severe birth defects that can lead to miscarriage, stillbirth, or to lifelong and usually serious disabilities, with consequent costs to the affected individual, their family/whānau, and society. The most recent complete NTD data for New Zealand show that in 2013, 18 babies were born with NTDs, with a further 6 babies being stillborn with an NTD. Because NTDs can be detected by ultrasound scan during pregnancy, many affected pregnancies are electively terminated, and these cases have been recorded since 2008. Taking into account termination numbers, the total number of NTD-affected pregnancies in New Zealand in 2013 was 51. This equates to a prevalence in 2013 of about 3.0 live births/10,000 births, 4.1 live and stillbirths/10,000 births, and 8.6 pregnancies/10,000 births. The estimated average rate of NTD pregnancies in 2008–2015 is 10.3/10,000 births. These figures are likely to be underestimates as they exclude spontaneous miscarriages, of which a proportion could be due to neural tube defects. Ethnicity data are only available for NTD live births; combined data from 2000 to 2015 show that Māori (but not Pacific) women have a higher live birth prevalence of NTDs (4.58/10,000 live births) compared to New Zealand European and other women (2.81/10,000 live births).

A 2014/15 population-based survey showed that only 16% of New Zealand women of childbearing age had blood folate levels above the World Health Organization recommended level for being at minimal risk of having an NTD-affected pregnancy.

Mandatory fortification of food with folic acid

There is overwhelming evidence that taking folic acid supplements before and during early pregnancy can prevent many cases of NTDs. The New Zealand Ministry of Health's policy is for women planning a pregnancy to start taking 800 µg folic acid at least four weeks before conception, and continuing through the first twelve weeks of pregnancy. However, many women do not follow this practice. There are a variety of reasons for this, with one of the major factors being that approximately 40% of pregnancies in New Zealand are unplanned. NTDs arise in the first few weeks of pregnancy, usually before many women are aware that they are pregnant or have sought medical advice. Therefore, starting folic acid supplementation once pregnancy has been confirmed may be too late to reduce the risk of an NTD being developed.

In countries where it has been effectively implemented and evaluated, mandatory folic acid fortification of staple food to provide women of childbearing age with additional folate has been shown to reduce rates of NTD-affected pregnancies. Analyses from 2012 suggest that moving from a hypothetical voluntary program in which 50% of all packaged[1] bread in New Zealand is fortified, to a mandatory program in which 100% of packaged bread is fortified, would prevent approximately 5–15 extra NTD pregnancies annually. These figures may be an underestimate for the present situation in New Zealand, as less than half of packaged bread is currently being fortified. However, the potential implementation of mandatory fortification in New Zealand has generated some debate mainly relating to commercial concerns and the perceived lack of safety of folic acid. These concerns have arisen mostly from high-dose supplementation studies that have claimed associations between folic acid supplementation and increased risks of cancer, cognitive impairment, some adverse effects on children whose mothers took folic acid supplements during pregnancy, and possible adverse effects of excess folic acid that is not metabolised to folate by the body.

Evaluating the evidence of health effects of folic acid

Methods

This report discusses the health benefits and risks found in clinical trials, observational studies, and genetic association studies, and those associated with voluntary or mandatory food fortification. Recent reports were reviewed and supplemented by additional comprehensive literature searches performed through to July 2017, with a primary focus on recent human studies. Due to the fast-moving literature in this area, key papers published between July 2017 and March 2018 arising from a supplementary search were also included. The Panel's conclusions are based on an overall assessment of the current evidence.

[1] Refers to packaged, sliced loaf breads. High-volume loose breads in supermarkets may also need to be taken into account during implementation.

Health effects of folic acid

NTDs

A large number of studies from countries that have mandatory flour fortification categorically show reductions in NTD rates following introduction of the programme, although the extent of reduction varies depending on initial NTD rates. As a whole, NTD prevalence has not decreased in countries or regions that have not implemented mandatory fortification. Among NTD cases that do occur in countries with mandatory fortification, there may be a shift towards less severe types of NTDs. In New Zealand, the NTD rates of live births and live-and-stillbirths from 2000–2013 has declined. Insufficient data, together with the small population size, present difficulties in demonstrating any impact of increased voluntary fortification on NTD rates in New Zealand since 2009.

Pregnancy and offspring childhood effects

Taking folic acid supplements before and during pregnancy is not associated with increased twinning or multiple births. Children of women who took folic acid supplements during pregnancy are not at increased risk of asthma, wheezing, eczema, other hypersensitivity-related outcomes, or childhood cancer.

Cancer

Interventional (clinical trial) studies and observational studies find no consistent evidence for adverse effects of folic acid on the risks of developing prostate, breast, colorectal, or total (all) cancers, but follow-up durations are too short to detect cases that develop after a long lag time. Genetic association studies suggest that higher blood folate levels may be associated with decreased risks of breast and total cancers, and increased risks of prostate and colorectal cancer. However, the interpretation of these results, and their applicability to supplementation and fortification, are areas for further research. All the study types have different strengths and weaknesses, and different scientific disciplines weigh different forms of evidence differently; in the end judgement is required on the totality of the evidence.

Other health outcomes

There is no overall evidence that folic acid supplementation increases the risk of neurological/cognitive decline, diabetes, or cardiovascular disease. No evidence is available to suggest any adverse effects of unmetabolised folic acid.

Conclusions

Mandatory folic acid fortification of food unequivocally reduces the prevalence of NTDs; the evidence is strong and convincing, and recent authoritative reviews have concluded that its implementation has not had any adverse impact at the population level. It is noteworthy that the beneficial effects of a reduction in the incidence of NTDs are not limited to pregnant women—entire families, whānau and society as a whole benefit. There is no evidence of harmful health effects of folic acid supplementation in adults, at least at low doses in the range suggested for fortification.

Genetic studies comparing people with genetic mutations that affect blood folate levels have provided limited evidence suggesting that higher folate levels may be associated with a possible

reduction in risk of breast and total (all) cancers, but also with a possible increase in prostate and colorectal cancer risk in certain population subgroups. As this is an emerging type of analysis that does not directly test fortification effects, and different scientific disciplines weigh different forms of evidence differently, these findings must be considered in the context of all the evidence on benefits and risks of fortification.

The Panel notes an added potential complexity in interpreting the genetic studies. Even if the findings are accepted as valid for extrapolation to fortification, then the effects on public health are complex, with any potential increases in risks of colorectal and prostate cancer needing to be considered alongside the potential *benefits* of decreases in risks of breast and overall cancer rates. In this case, the undoubted immediate benefits of mandatory fortification, the possible longer-term risks, and possible longer-term benefits will each impact on different demographic groups of the population. Reduced NTD rates benefit women and their families/whānau through prevented terminations and babies being born with disabling congenital abnormalities, whereas the possible alterations in cancer risk are borne by an older population, with women potentially receiving benefit through decreased breast cancer risk, but men potentially placed at greater risk in terms of prostate cancer. Whilst it is possible to weigh up the various potential outcomes against each other using modelling approaches, these still require the use of assumptions and values-based judgements, and are inherently complex.

The Panel notes that the unavailability of recent, complete, and robust New Zealand data poses a challenge in evaluating the efficacy of New Zealand's current voluntary regime. It further recognises that mandatory fortification of packaged bread alone may not adequately reach the most vulnerable populations, given changing demographics and dietary habits of New Zealand women of childbearing age. Just 16% of these women have blood folate levels sufficient to place them at a minimal risk of carrying an NTD-affected pregnancy.

On balance and considering the totality of the current evidence, the Panel concludes that, with respect to NTD reduction, the benefits to parents, child, family/whānau and society as a whole of introducing mandatory fortification of packaged[2] bread outweighs any potential adverse effects. At the same time, the Panel acknowledges the importance of ongoing monitoring and evaluation of new evidence, particularly from genetic studies with improved methodologies, together with other international science and policy developments in this field. At-risk individuals (such as those with personal or familial histories of colorectal cancer) may require additional advice from their medical professional in monitoring their overall folic acid intake. The Panel strongly supports greater public health and educational efforts to ensure all women of childbearing age have adequate folate intakes, as well as the continued use of folic acid and other micronutrient tablets for pregnant women as recommended by their healthcare professionals.

The Panel also notes that in fulfilment of Treaty of Waitangi obligations, Māori should be centrally involved in the decision-making process on folic acid fortification, and that every effort is made to ensure that they receive equal benefit from the decision.

[2] Comprising packaged sliced loaves at a minimum, but ideally including high-volume in-store supermarket breads.

1 Background on folic acid fortification

Folate is a naturally occurring B vitamin found in many foods such as leafy green vegetables and yeast spreads, while folic acid is a synthetic form of folate that is used in the manufacture of dietary supplements and the fortification of food[3] (Appendix 5.3). Folic acid is more stable and readily absorbed, and can be converted into folate in the body.

Folate is essential for embryonic development, and an adequate folate status before and during early pregnancy reduces the risk of the fetus developing a neural tube defect (NTD). NTDs, including spina bifida and anencephaly, are major birth defects involving the spinal cord that can lead to miscarriage, stillbirth, or to lifelong and usually severe disabilities. NTDs incur substantial costs that impact on the individuals, families, and wider society. Therefore, international health policy recommendations encourage folic acid supplements during the periconceptional period (from before conception to early pregnancy) as a safe and effective means of reducing the prevalence of NTDs [1, 2].

A sufficient level of blood folate before conception is crucial because NTDs arise well before a woman is aware that she is pregnant. However, even with public health promotional strategies, adherence to these guidelines is often low. There are many reasons for this, including high proportions of unplanned pregnancies, lack of awareness, and the requirement for active behavioural change.

Therefore, many countries have adopted the mandatory fortification of staple foods with folic acid as a complementary public health approach to maximise the proportion of women of childbearing age (WCBA[4]) having optimal folate status. Since the first national-scale fortification of wheat flour in Oman in 1996, a further 80 countries including the US, Canada, and Australia have implemented this measure, which has invariably resulted in reductions in NTD rates, often to a substantial extent [5, 6].

1.1 The scope of the problem

Folic acid is a common component of dietary supplements targeted at the general consumer. It has been taken by pregnant women in supplemental form for decades, and by the general population in fortified food for 20 years. Yet, folic acid fortification of food has not received unequivocal acceptance: New Zealand and nearly all countries in Europe currently favour voluntary rather than mandatory regimes. There are several reasons for this (detailed in Appendix 5.6); a primary concern in any public health intervention is of safety, which is the focus of this report. The safety of mandatory folic acid fortification is assessed by the absence of evidence of harm based on expected levels of folic acid intake. It should be noted that absence of evidence is not evidence of a lack of effect. Table 1 lists some of the health benefits and potential[5] risks of mandatory fortification of food with folic acid. It should be noted that many of the potential risks have been

[3] The term folate is often used interchangeably with folic acid. In this Report we use the former to refer collectively to the bioactive forms, and the latter to refer to the synthetic form.

[4] The age range varies slightly by country and/or study. The New Zealand Ministry of Health and Statistics New Zealand define it as age 15–49 [3, 4].

[5] 'Risk' in its strict sense is probabilistic, and so by definition all risk is potential in nature. In this report, 'potential' is used as a descriptor of risk to convey theoretical concerns, or indicative or unclear evidence.

raised by studies involving high-dose folic acid supplements and not mandatory fortification *per se*.

Table 1: Several health benefits and possible risks of implementing mandatory fortification of folic acid

Benefits	**Possible risks that have been raised**
• Reduction in prevalence of NTD-affected pregnancies • Reduction in prevalence of folate deficiency	• Potential for increased risk of certain cancer types • Potential for cognitive/neurological impairment in the presence of vitamin B_{12} deficiency • Potential effects on diabetes and thyroid disorders • Potential effects on children whose mothers took supplements during pregnancy • Unknown effects of unmetabolised folic acid (UMFA)

The decision to introduce any public health measure generally involves a high level of uncertainty, necessitating the use of risk assessments to aid policy making [7, 8]. The key question raised by possible mandatory folic acid fortification of bread in New Zealand is whether it is safe and does no harm. Because mandatory fortification exposes the majority of the population beyond the target group to the fortificant, albeit at lower levels than supplements, a rigorous evaluation of the potential health risks posed is demanded.

Evaluating the health benefits and risks of mandatory fortification requires an understanding of how the magnitude of the potential health benefits of folic acid (primarily a reduction in NTD rates) weighs up against the magnitude of potential health risks in the general population (Table 1). Numerous reports from governments and other reputable organisations have found no convincing overall evidence for adverse health effects associated with folate/folic acid. There has also been no evidence to suggest that the recommended maximum intake of folic acid should be lowered, or that supplementation and fortification guidelines in any jurisdiction should be made more conservative. However, there is an inherent degree of uncertainty in nearly any aspect of scientific research. The latter is pertinent because of the complex biological role of folate, and its potential for both beneficial and adverse health effects depending on dose and timing of exposure. These factors have continued to stimulate debate surrounding its safety, and influence interpretation of the evidence (e.g. [9, 10]).

Since 1996, New Zealand food manufacturers have been permitted to add folic acid to certain foods, including breakfast cereals and yeast-based spreads for nutritional purposes. In 2009, legislative plans to include bread among the fortified products on a mandatory basis were deferred—and, in 2012, revoked—with the Government instead encouraging manufacturers to voluntarily increase fortification coverage (Appendix 5.2.1). This arrangement operates to the present day.

This report provides a formal review of the health benefits and risks of folic acid fortification by surveying the latest peer-reviewed scientific literature with a particular focus on the New Zealand context. Because the aim is part of a wider objective to facilitate decision making on the implementation of mandatory fortification of bread for public health purposes, the report also considers related key issues that may inform an overall risk-benefit assessment of such a policy. These are further discussed in Section 3.

1.1.1 Neural tube defects in New Zealand

The prevalence of NTDs can be assessed by live births, total births (live and stillbirths), and all pregnancies (resulting in live births, stillbirths, or terminations following antenatal diagnosis). The latter provides the most accurate estimate of all cases of NTDs, but is also likely still an underestimate as miscarried pregnancies are neither recorded nor subject to autopsy in New Zealand. It is estimated that about 1 in 5 known pregnancies ends in spontaneous miscarriage [11], and a proportion of these may be due to an NTD.

The availability of NTD data for New Zealand is variable. The most recent complete dataset, shown in Table 2, is for 2013 [12]. The estimated average rates of NTDs per year from 2008 to 2015 are also shown. Figure 1 and Figure 2 (Appendix 5.2.2) show recent trends in NTD rates.

Table 2: New Zealand NTD statistics

Outcome	**Number of cases in 2013**	**Prevalence (per 10,000 births[6])**	
		2013	**2008–2015**
Live birth	18	3.0	3.8
Stillbirth	6		
Live and stillbirth	24	4.1	5.5
Terminated pregnancy†	27		
Total	51	8.6	10.3

† Recorded terminations due to central nervous system defects, the majority of which are NTDs.

Because mandatory fortification is implemented at a specific time point, its health effects can be assessed by monitoring folic acid intake, folate status, and other health outcomes before and after the regulations are in place. However, in New Zealand, there is no clearly delineated time point to evaluate the impact of voluntary fortification. Folic acid has been a permitted fortificant since 1996; in 2009–2011 it was added to an increased number of bread and breakfast cereal products [13], and thereafter to a wider bread product range at higher levels.

To estimate the effects of voluntary fortification, data obtained prior to the Government's encouragement in 2009 of increased voluntary fortification by the baking industry in lieu of implementing a mandatory policy [14] are generally regarded to represent the baseline. Data on health outcomes can give an indication of trends over time but causal relationships[7] cannot usually be assigned.

From 2005 to 2009, the estimated[8] rate of NTD pregnancies had halved from 17.2 to 8.6/10,000 births, for an average rate of 12.9/10,000 births [15]. From 2008 to 2015, during which termination

[6] Unless otherwise stated, this Report expresses all NTD rates as per 10,000 total births, that is, comprising live and stillbirths.

[7] This type of analysis can usually only suggest that health outcomes are *associated* with, but not necessarily *caused* by an intervention. Other types of studies may provide more reliable indications of causal effects (Appendix 5.7.2).

[8] As termination data were not available for this time period, Australian termination rates for NTD pregnancies (about 60%) were used to calculate estimates.

data are available, no clear trend in NTD pregnancy rates can be discerned (Figure 1, Appendix 5.2.2). Excluding terminations, there have been statistically significant declines in rates of NTD-affected live births from 2000 to 2015, and of affected live-and-stillbirths from 2000 to 2013 [16]. There does not appear to be any clear change in NTD rates before and after the start of increased voluntary fortification in 2009, but any effect may be obscured by the relatively small numbers involved, and data fluctuations limit firm conclusions.

NTD rates by maternal ethnicity are only available for live births (Figure 2, Appendix 5.2.2) [12]. Statistical analysis of combined data from 2000 to 2015 showed that rates of NTD-affected live births were higher in Māori women than in New Zealand European/other (NZEO) women (4.58 vs 2.81/10,000 live births, respectively). Pacific women also appeared to have a higher rate (4.09/10,000 live births) than NZEO women, but this was not statistically significant (i.e. it may be a chance finding due to data variability). There appears to be a trend towards decreased rates in NTD-affected live births among Māori (but not apparent in Pacific) women over time, albeit in the context of larger fluctuations within this small dataset (Figure 2).

The most recent modelling analysis to estimate the potential impact of mandatory fortification on NTD prevalence in New Zealand, reported by MPI in 2012, compared NTD reductions by proportion of fortified bread products and level of fortification [17].[9] This estimated that, compared to a complete absence of fortification, the current industry voluntary target of 50% fortification coverage at 200 µg/100 g bread would prevent 9–13 NTD cases per year. Having 100% coverage (akin to mandatory fortification) would prevent 18–24 cases. This equates to the prevention of about 10 *additional* cases per year (range of 5–15) when comparing voluntary with mandatory fortification. Preventing 10 cases was estimated to avoid an economic burden[10] ranging from $37 million (if all cases result in live births) to $50 million (if all lead to stillbirths or pregnancy terminations) [17]. The protective effect of fortified bread became even greater as fortification coverage reached higher proportions and benefited a larger proportion of the population. Updated modelling analyses to estimate the numbers of NTD pregnancies prevented and preventable under voluntary and mandatory regimes are warranted in view of changing population demographics and food consumption patterns, and the availability of updated NTD and blood folate data.

1.1.2 Folate status in New Zealand women of childbearing age

Provisional data on the 2014/2015 folate status in New Zealand WCBA, collected as part of a Ministry of Health population-based survey [4], show that only 16.2% of women were folate sufficient—that is, they had red blood cell (RBC) folate levels that are internationally accepted to confer minimal risk of NTDs[11]. At these target levels, most folate-responsive NTD pregnancies are likely to be prevented, bringing the estimated NTD pregnancy rate down to 8/10,000 births [20]

[9] An analysis published in 2018 has estimated the numbers of preventable live-born cases of spina bifida and anencephaly in 71 countries lacking mandatory fortification [18]. It calculated that mandatory fortification could prevent 72 NTD births per year in New Zealand, but this is highly likely to be an overestimate as it was based on an undated estimate, from a 2006 report, of 90 NTD births per year [19]. The number of NTD live and stillbirths in New Zealand has not exceeded 49 since 2000 [12].

[10] Data reflect the lifetime cost of the disease, discounted to the year of birth. The greater burden presented by stillbirths and terminations is due to a higher assigned value of life and suffering.

[11] The RBC folate levels among 1,567 women aged 15–49, in nmol/L, were: mean 544 (95% CI 528–560), and median 496 (95% CI 478–521). 16.2% of women (95% CI 13.5–19.2) had levels exceeding the target cut-off level for minimal NTD risk (748 nmol/L).

(Appendix 5.3.6.4). The prevalence of folate insufficiency was greater in women aged 15–24 compared to those aged 25–44, and in women of Asian ethnicity compared to non-Asian women. Women residing in the most deprived neighbourhoods had lower mean RBC folate levels than those in the least deprived areas.

1.1.3 Bread fortification and consumption

In 2016, 38% of packaged bread was fortified, a higher proportion than in previous years (Figure 3, Appendix 5.2.4) [21]. This meets the baking industry's target of 25–50% coverage, although the setting of this target was not based on an optimal effect on NTD reduction.

Audits of folic acid levels in bread samples from 2013–2016 have continued to show high variability in fortification levels. In 2016, 47% of bread samples contained folic acid at levels greater than the target of 200 µg/100 g [21] (Figure 4, Appendix 5.2.4).

In 2010, half of New Zealand WCBA surveyed were aware of the importance of increasing folate/folic acid intake during and before pregnancy, and of concern one-third were unaware of any need for increased intake at all [22]. Thirty percent of all respondents were aware that bread was fortified with folic acid; just 3% actively chose products that had folate/folic acid, while 1% actively avoided products containing folic acid.

A 2011 survey found that, although a large majority of WCBA were bread consumers, three-quarters of them ate bread that may not have been fortified [13, 23]. Fortified bread products are not necessarily prominently labelled or marketed as such; they can be identified from the ingredient list but not always from the Nutrition Information Panel [21]. A 2017 consumer survey found that 78% of WCBA ate packaged sliced bread, but it is not certain what proportion of this was fortified [16]. Between 1997 and 2011, there has been a clear decrease in bread consumption among New Zealand WCBA, from 3–4 slices/d to under 2 [24]. 2017 data show that this decrease appears to be sustained, with WCBA consuming an average of 1.9 slices/d [16]. The consumption among all adults was 2.5 slices/d.

In the 2011 survey, consumption of fortified bread was associated with 25% higher serum folate levels, but unchanged RBC folate levels, compared to non-consumers of this food. The improved folate status following increased voluntary fortification was therefore likely to be partially—but not completely—accounted for by consumption of fortified bread, and may reflect the increase in numbers of fortified bread and breakfast cereal products on the market in 2010/11.

Summary

- There were 51 NTD-affected pregnancies in New Zealand in 2013—a prevalence of 8.6/10,000 total births. The average rate of NTD pregnancies during 2008–2015 is estimated at 10.3/10,000 births.
- In 2014/15, 16% of New Zealand WCBA had sufficient blood folate levels for minimal risk of having an NTD-affected pregnancy.
- In 2016, 38% of packaged bread was fortified, and folic acid levels were highly variable. Bread consumption among New Zealand WCBA may be declining.
- The latest available estimates, from 2012, suggest that implementing mandatory fortification of bread may prevent an additional 10 cases of NTDs per year compared to voluntary fortification at the current industry target of 50% coverage.

2 Health outcomes of folic acid exposure

This section synthesises the findings and conclusions from recent, comprehensive literature searches undertaken by scientific committees and public health organisations [25-31] (Appendix 5.7.1). These health outcomes are derived from interventional, observational, and genetic studies (Appendix 5.7.2). The genetic studies investigate the effects of a mutation in a gene known as *MTHFR*.[12] The presence of this mutation leads to lower levels of blood folate compared to those without the mutation, so comparing health outcomes between individuals with and without the mutation can indicate a potential causal effect of folate.

Several health outcomes are further discussed in the context of mandatory or voluntary fortification. Data from high-income countries are reported because population characteristics such as prevalences of folate deficiency or insufficiency, other health indicators, nutritional profile, and economic development are likely to be more reflective of that in New Zealand, and provide more comparable outcomes.

The maximum intake of folic acid that is unlikely to cause an adverse effect (known as the Tolerable Upper Intake Level, or UL) is 1 mg (1,000 µg) per day (Appendix 5.3.5). This is the internationally recognised benchmark for assessing its potential health risk.

2.1 Neural tube defects

NTD data from New Zealand are discussed in Section 1.1.1.

Systematic reviews of studies performed in multiple countries have unequivocally demonstrated that mandatory fortification is associated with reductions in spina bifida, anencephaly, and overall NTD prevalence [5] (Appendix 5.7.4.1). Greater declines were seen in countries with a higher baseline prevalence.

In the US, NTD prevalence decreased during the transition period to mandatory fortification, and remained relatively stable for at least a further 13 years [32] (Appendix 5.7.4.1). In terms of change in rates, the largest effect was seen in the Hispanic population, which had the highest baseline prevalence, and the smallest effect in the non-Hispanic black population, which had the lowest baseline prevalence.

In Australia, prevalence of NTD pregnancies fell by 14.4% following implementation of mandatory fortification [33, 34] (Appendix 5.7.4.1). There was a decline of 54.8% among teenagers, and of 74.2% among Indigenous women compared to 9.1% in non-Indigenous women. It is estimated that about 14 NTD cases per year were directly prevented by the introduction of mandatory fortification [31].

Mandatory fortification in the US was associated with slightly increased survival of live-born spina bifida-affected infants [35] (Appendix 5.3.6.3). In Canada, the intervention was associated with a decrease from 32% to 13% in the proportion of newborns with severe spina bifida [36], and an 11% reduction in the prevalence of overall congenital heart defects among all pregnancies, with decreases of up to 27% for some congenital heart defect subtypes [37]. A recent meta-analysis of

[12] International research indicates that the prevalence of this mutation depends on ethnicity and geographical region, but the proportion of the New Zealand population that has this mutation is not known (Appendix 5.3.2).

studies found a decreased risk of cleft lip with or without cleft palate in association with fortification [38] (Appendix 5.7.4.1).

Studies have shown that across the UK and Europe, where fortification is not mandatory and is generally undertaken at low levels on a voluntary basis, NTD prevalence has not fallen since the 1990s [39, 40] (Appendix 5.7.4.2), demonstrating that alternative measures to prevent NTD occurrence, including guidelines for periconceptional supplementation and the provision for voluntary fortification, have been ineffective. Extensive practice of voluntary fortification in Ireland was associated with NTD prevalence decrease, but fortification levels have dropped and NTD rates have recently increased (Appendix 5.7.4.2). Modelling analyses have conservatively estimated that about 87% of folic acid-responsive NTD cases that occurred globally in 2015 could have been prevented by mandatory fortification [41].

Summary

- Mandatory fortification in many countries is strongly associated with reduced prevalence of NTDs.
- In the US, NTD prevalence decreased with the transition to mandatory fortification and has remained at a lower rate since mandatory fortification was introduced. The decrease in prevalence has been most pronounced in ethnic groups that had been at higher NTD risk pre-fortification.
- In Australia, NTD prevalence decreased following mandatory fortification, with the greatest decline seen in teenagers and in Indigenous women.
- In addition to lowering NTD rates, mandatory fortification may shift the severity distribution of NTD cases such that cases tend to be less severe.
- As a whole, NTD prevalence has not decreased in countries or regions that have not implemented mandatory fortification.

2.1.1 Dietary intake/blood folate status

The blood folate status of New Zealand WCBA is discussed in Section 1.1.2.

In Australia, following mandatory fortification in 2009, the proportion of individuals with intakes exceeding the UL remained unchanged in adults but increased in children. The exceedance in children was not considered to be of concern [33, 42] (Appendix 5.7.4.3). WCBA with inadequate folate intake decreased markedly [42], but the proportion with folate sufficiency was unable to be determined (Appendix 5.7.4.3). A separate study of an Aboriginal population pre- and post-fortification found increases in RBC folate levels and a 68% fall in NTD prevalence [43]. The change in NTD risk is similar to that found in the nationwide monitoring survey discussed in Section 2.1.

In the US, mandatory fortification introduced in 1998 greatly improved blood folate levels and reduced folate deficiency to negligible levels. A low proportion of the population exceeded the UL, but supplement takers under eight years of age were more susceptible [44] (Appendix 5.7.4.3). The proportion of WCBA who were folate sufficient increased from 41% to 77%.

Across the UK, between 2008 and 2013, 75% of WCBA had RBC folate levels lower than the cut-off for folate sufficiency (Appendix 5.7.4.3) [45]. In Ireland, where voluntary fortification had increased since 1997, dietary surveys between 1997–1999 and 2008–2010 showed that Irish WCBA increased

their folic acid intake without exceeding the UL, but two-thirds of the women were still folate insufficient (Appendix 5.7.4.3).

Summary

- Mandatory fortification in Australia enabled most WCBA to achieve adequate folate intake, but no robust data are available on improvements in blood folate status. No UL exceedances of concern were found in the general population.
- Mandatory fortification in the US reduced the proportion of folate insufficient WCBA. UL exceedances were low except in some children.
- Most WCBA in the UK, where fortification is not mandatory, are folate insufficient.

2.2 Cancer

The evidence suggests that folate may have both protective and detrimental health effects in relation to cancer, and its ultimate effect depends on both the level of intake (i.e. *dose*), and the absence or presence of precancerous lesions[13] and established tumours (i.e. *timing* of exposure) [46]. Thus, adequate folate is essential to maintain normal function of healthy cells, but high folate levels may accelerate the growth of pre-existing tumours (Appendix 5.7.5.1).

Cancer—in particular colorectal, prostate, and breast—has been the primary focus of studies that aim to monitor the safety of folic acid supplementation and fortification. This report focuses on these three types of cancer.

The literature on health effects of folate predominantly encompasses four types of studies:

1. Clinical studies (including RCTs) involving folic acid supplementation;
2. Observational studies of dietary folate intake or blood folate levels;
3. Genetic studies involving mutations in folate metabolism enzymes such as MTHFR; and
4. Ecological studies comparing pre/post-fortification health outcomes, and other data relating to the fortification of food.

Each of the study types has strengths and limitations, all of which need to be taken into account in interpreting the overall evidence base for health effects (Appendix 5.7.2). Broadly speaking, RCTs provide the best evidence for causal effects, but tend to use higher doses than would be encountered in fortified food, and involve shorter-term exposures and follow-up durations that may not reveal long-term benefits or harms. Genetic studies can assist in determining causality as they may reflect more realistic exposure levels and durations, but they may be biased for many reasons, such as if the *MTHFR* gene also affects other biochemical and physiological processes that in turn affect cancer risk (Section 5.7.2.3) [47, 48]. Observational and ecological studies are only able to suggest an association (and not an effect) which may arise from confounding factors.

[13] A precancerous lesion is an area of abnormal tissue that is likely (but not always) to turn into cancer. This includes polyps in the colon.

2.2.1 Summary of recent reports

For the 2017 UK ***Scientific Advisory Committee on Nutrition (SACN)*** update report on the potential adverse effects of folic acid on cancer, 54 meta-analyses and systematic reviews relating to prostate, breast, colorectal, and total cancer risk were evaluated [30]. For each cancer site, evidence from interventional randomised controlled trials (RCTs), observational studies on dietary folate intake or serum/plasma folate levels, and genetic studies on mutations in the *MTHFR* gene were evaluated[14]. Folic acid doses used in the trials were generally much higher than those used in food fortification (doses ranged from 0.5–40 mg/d, with a median of 2.0 mg/d; fortification is estimated to increase intakes by around 0.1 mg/d (Table 10, Appendix 5.4.1).

The SACN found that the effect of folic acid on risk of each cancer type appeared to differ by the type of studies being reviewed. For example, RCTs assessing prostate cancer generally found no adverse effect, but genetic association studies generally found a weak association with increased risk. Across the four different study types for prostate cancer, two summaries indicated no effect while two suggested increased risk. Complicating the prostate picture is that incidence data tend to reflect the more common indolent (slow growing) prostate cancers detected through screening, whereas the important outcome is aggressive and fatal cancers. The overall evidence base for all the other cancer types examined was similarly inconsistent.

The available RCTs suggested no consistent effect on risk for all cancer types, while genetic studies suggested that the risk depends on cancer type: having two copies of the mutation (which results in lower blood folate levels) was associated with higher breast and total cancer risk, but with lower prostate and colorectal cancer. That is, higher blood folate was inferred to be linked to greater risk of prostate and colorectal cancer, but lower risk of breast cancer and total cancer. It remains uncertain if the increased risk of prostate and colorectal cancer is a true reflection of the effect of folate alone, rather than other factors such as the *MTHFR* gene having other functions unrelated to folate metabolism that affect cancer risk (Section 5.7.2.3). Nonetheless, it should be noted that for each of the cancer types reviewed by SACN, the genetic studies suggested relatively consistent, but weak, associations. These genetic associations are not necessarily causal and will be further investigated using better genetic instruments.[15] Until then, their public health significance remains uncertain, especially when compared with the undoubted benefits of mandatory folate fortification.

The US ***National Toxicology Program (NTP)*** evaluated 43 pooled and meta-analyses that covered 12 cancer types across different human populations [27]. A visual representation of individual risk estimates for each human study for prostate, breast, and colorectal cancer is shown in Appendix 5.9. Based on the reviewed clinical data, the NTP concluded that:

- Colorectal cancer risk is higher in humans with inadequate dietary folate intake (this is relatively consistent with SACN's findings, but may still be due to confounding and not an adverse effect of low folate *per se*);
- Supplements confer no added protection against cancer if folate status was adequate;
- Human studies provided a "consistent enough suggestion" of an adverse effect of folic acid supplementation on cancer growth that warrants continued research.

[14] Among the recent reports, only SACN reviewed genetic studies.

[15] New genetic studies enabling more robust analyses of the one-carbon pathway and various cancers will be undertaken at the University of Bristol (Prof R. Martin, pers. comm., 11 June 2018).

These conclusions were not further assessed in depth.

The NTP made note of "suggestive evidence" that folic acid may adversely affect the onset and progression of prostate cancer. Three publications examining the consequences of maternal folic acid intake with respect to paediatric cancers found no evidence of increased risk.

A systematic search by the ***Norwegian Scientific Committee for Food Safety (VKM)*** yielded 5 studies and 8 meta-analyses relating to cancer [26]. The VKM was unable to find any new evidence associating folic acid with risk of cancer of any type, including colorectal cancer, colorectal adenoma, overall cancer, or brain tumour and childhood leukaemia in children of women taking pregnancy supplements. In drawing up the summary evidence, it concluded that despite data limitations there was no substantial support to change the existing UL for folic acid supplementation.

The ***European Food Safety Authority (EFSA)*** review similarly found no consistent association of folate or folic acid with cancer risk, and it noted that potentially adverse effects tended to manifest at intake levels in excess of the UL of 1 mg/d [25]. The ***Food and Drug Administration (FDA)*** review found that based on overall available evidence, cancer risk was unclear and could not be substantiated [29]. The ***Australian Health Ministers' Advisory Council (AHMAC)*** found that meta-analyses of RCTs for colorectal, prostate, other cancer sites, and total cancer consistently demonstrated no increase in cancer risk associated with supplementation at a population level [31].

An updated meta-analysis of gene association studies has corroborated SACN's finding that higher blood folate confers a lower risk of breast cancer [49]. A recent systematic review of health outcomes associated with higher blood folate levels found increased risk of prostate cancer (in agreement with SACN), and decreased risk of colorectal and breast cancer (SACN found no effects) [50]. Other recent studies on the role of folate/folic acid in cancer risk that had not been available for consideration in previously published reports are described in Appendix 5.7.5.3. In general, there is at least partial agreement with the SACN evidence summaries.

An overall assessment of the cancer literature, based on the reports summarised above and other studies, is given in Section 3.2.

Summary

- Much of the evidence across different study types is inconsistent (Table 3). However, findings from genetic studies suggest that higher blood folate is weakly associated with increased risks of colorectal and prostate cancer, and with decreased risks of breast and total cancer.
- The associations seen in the genetic studies are not necessarily causal, and their public health significance remains uncertain especially when compared with the undoubted benefits of mandatory folate fortification.

Table 3: Overall summary of recently published major reports assessing the potential effects of folic acid on cancer risk

	Prostate	**Breast**	**Colorectal**	**Total (all cancers)**
SACN	• Inconsistent results across study types • Genetic studies suggest increased risk from higher blood folate • Overall evidence is inconclusive	• Inconsistent results across study types • Genetic studies suggest decreased risk from higher blood folate • Overall evidence does not suggest an adverse effect, and may imply benefit	• Inconsistent results across study types • Genetic studies suggest increased risk from higher blood folate • Overall evidence is inconclusive	• Inconsistent results across study types • Genetic studies suggest decreased risk from higher blood folate • Overall evidence does not suggest an adverse effect, and may imply a net benefit at the population level
NTP†	No specific conclusion		Adequate dietary folate is protective	Suggestion of adverse effect on cancer growth
VKM	No new robust evidence of risk	No new evidence of risk		
FDA	Overall evidence is unclear; adverse effects could not be substantiated			
EFSA	Possible adverse effects relate to higher intakes ≥ UL			
AHMAC	No increase in risk			

† See Appendix 5.9 for the NTP's visual summaries of the effect reported by individual studies.

2.2.2 Cancer trends

Latest data from the US show falls in incidence of total cancers (from 1998–2013), colorectal cancer (1998–2013), and prostate cancer (2000–2013) [51] (Figure 10, Appendix 5.7.5.6). Female breast cancer rates have remained stable from 2004–2013. The pre-fortification era ended in 1996; optional fortification occurred from 1996–1998, and mandatory fortification began in 1998.

In Canada, where mandatory fortification began in 1998, cancer trends from 1988–2017 show that breast cancer rates have remained steady through this period, while prostate cancer has decreased since 2007 (Figure 11a, Appendix 5.7.5.6) [52]. Statistical analysis has revealed slight decreases in

colorectal cancer since 2000 (females) and 2008 (males), while incidence of all cancers has either increased (in females) or decreased (in males) by less than 1% annually since 1992 (Figure 11b).

As mandatory fortification in Australia was introduced in 2009, later than in the US and Canada, less post-fortification data are available. The most recent data suggest that rates of colorectal cancer have remained steady, breast cancer rates have continued to increase according to pre-fortification trends, and prostate cancer has decreased [53] (Figure 12, Appendix 5.7.5.6). However, the generally long period of onset for these common cancers make it difficult to establish associations. Rates of overall cancer appear to be relatively steady.

The most recent incidence data from New Zealand (2005–2015) suggest no substantial changes for each of the three cancer types or for total cancer [54, 55] (Figure 13, Appendix 5.7.5.6).

These trends are suggestive of a lack of notable adverse impact at the population level, but it is not possible to directly relate the data to folic acid fortification.

Summary

- Incidence of several common cancers and total cancer in US, Canada, and Australia has mostly remained stable or decreased since the introduction of mandatory fortification. No substantial changes in cancer incidence has been seen in New Zealand since increased efforts at voluntary fortification. These population level data, while reassuring, are not conclusive because of the possible impact of many factors other than folic acid fortification,

2.2.3 Overall summary

Clinical trials, which generally provide higher quality evidence on causality, suggest that there is no evidence of adverse health effects of folic acid supplementation at low dose in adults. Observational studies mostly (but not consistently) suggest a lack of harm, while ecological studies do not suggest any increase in cancer rates following mandatory fortification in other countries. However, evidence from both observational and ecological studies, especially the latter, is considered weaker as it only indicates associations and not cause-and-effect. Genetic studies, which are an emerging tool to study possible causal effects, suggest that life-long relatively higher blood folate levels may be weakly associated with increased risk of prostate and colorectal cancer, but decreased risk of breast and total cancer. These findings are not unequivocally accepted because the current data may be subject to several biases, such as the *MTHFR* gene having multiple biological impacts.

Thus, the overall evidence across all the studies and study types is inconsistent. However, taking into account the relative quality of each of the findings and especially the need to interpret the genetic studies cautiously, the Panel concluded that any potential increase in cancer risk would likely be outweighed by the strong and convincing evidence on the benefits of mandatory fortification.

2.3 Neurological/cognitive impairment

Within the 28 studies and 2 meta-analyses assessed by the ***NTP***, observational studies provided limited evidence that high folic acid coupled with low vitamin B_{12} status was associated with poor

neurological function, while RCTs found no increased risk in individuals with normal cognitive abilities.

The ***SACN***'s evaluation of recent systematic reviews relating folate status/intake with cognitive ability found either inconsistent effects on general or specific measures of cognition (in intervention studies), or a lack of association (in cohort studies). There was an indication from the latter studies that relatively higher folate levels were associated with lower risks of cognitive decline and dementia. Three systematic reviews on RCTs in infants and children found no evidence for an effect of folic acid irrespective of vitamin B_{12} status.

The ***FDA*** found "suggestive evidence" for masking of B_{12} deficiency and exacerbation of B_{12}-deficiency-related neurological/cognitive decline, and identified those aged >50 years as the at-risk group for both health outcomes. However, it found no evidence for a need to amend the UL. The ***AHMAC*** found no evidence for a role of folic acid in increasing cognitive impairment on a population-wide basis. A recent systematic review found that on the whole, higher blood folate levels were associated with a decreased risk of cognitive impairment [50].

Summary

- Folic acid is unlikely to have an adverse effect on neurological or cognitive impairment at intakes below the UL (Table 4).

Table 4: Summary of recently published reports assessing the potential effects of folic acid on risk of neurological/cognitive impairment.

SACN	NTP	VKM	FDA	EFSA	AHMAC
Either no association, or lower risk of cognitive decline	Very limited supportive evidence for exacerbation of neurological decline	Not assessed	Intakes ≤ UL unlikely to pose risk	UL is appropriate	No evidence of increased risk

2.4 Diabetes

The NTP evaluated one meta-analysis of glycaemic control in type 2 diabetics, and 62 human studies on diabetes and related disorders. There was no consistent evidence that folic acid supplements, high dietary folate intake, or high folate status had adverse effects on risk of type 1 diabetes in adults or type 2 diabetes, or on impaired glucose/insulin metabolism in adults.

Summary

- There is no evidence of adverse effects on diabetes.

2.5 Folate in pregnancy – effects on offspring

Hypersensitivity outcomes comprise a range of effects including asthma, wheezing, eczema, food allergy, and respiratory infection. In assessing these outcomes, the ***NTP*** reviewed one meta-analysis on prenatal folic acid intake and asthma risk in children, and 39 human studies that

involved pre- and postnatal exposure. The association between folic acid intake/folate status and measures of atopic disease was inconsistent, and no association was observed with asthma in adults or children, wheezing, eczema and atopic dermatitis, or susceptibility to respiratory infection. The ***AHMAC*** noted an absence of consistent evidence of risk. A recent review for the ***US Preventive Services Task Force*** captured two additional meta-analyses and found no association between periconceptional folic acid supplementation and risk of hypersensitivity [56, 57]. A large study has recently suggested a slight increase in risk of offspring asthma at the highest levels of maternal folate/folic acid intake [58] (Appendix 5.7.8).

A recent nation-wide cohort study of 687,406 Norwegian children found no association between maternal folic acid supplementation and risk of six major types of childhood cancers [59].

Two systematic reviews have found no consistent evidence for an association between maternal exposure to folic acid and autism spectrum disorders [60, 61].

The ***FDA*** review did not find any overall evidence for adverse effects of prenatal exposure on health outcomes in childhood.

Summary

- There is no evidence for adverse effects of maternal supplementation on atopy, asthma, wheezing, eczema and atopic dermatitis, susceptibility to respiratory infection, childhood cancer, and autism spectrum disorders.

2.6 Other effects

Thirty-nine meta-analyses reviewed by the ***NTP*** reported no adverse effect of folic acid on cardiovascular disease (CVD) risk. A more recent meta-analysis of RCTs showed that folic acid supplementation led to modest reductions in risks of stroke and CVD (10% and 4% respectively), but not coronary heart disease [62] (Appendix 5.7.9). It was recently reported that levels of homocysteine, a component closely linked to folate metabolism, in elderly Australians substantially decreased post-fortification [63]. Elevated homocysteine levels have been associated with both low folate status and higher cardiovascular disease risk.

The NTP observed a lack of association between maternal folic acid supplementation and twinning or multiple births in studies that controlled for use of fertility treatments, a key risk factor for multiple births. The ***AHMAC*** determined that there was no evidence for increase in twinning.

The NTP's literature review for neurological outcomes unrelated to vitamin B_{12} deficiency, immunological outcomes unrelated to hypersensitivity, other endocrine and metabolic disorders, other reproductive outcomes, and mortality yielded few or no reports of any adverse associations.

Very recent systematic reviews and meta-analyses of studies in countries with and without mandatory fortification suggest that this policy has had no effect on risk of Down Syndrome pregnancies [64], and may be associated with a small decrease in risk of multiple births [65].

Summary

- There is no evidence for adverse effects on risk of cardiovascular disease, twinning/multiple births, other neurological outcomes, other immunological outcomes, other endocrine and metabolic disorders, or Down Syndrome pregnancies.
- Folic acid may be associated with a small decrease in risk of stroke and CVD.

2.7 Unmetabolised folic acid

As humans have a limited capacity to metabolise large amounts of folic acid, the excess folic acid—known as unmetabolised folic acid (UMFA)—can remain in the circulation (Appendix 5.7.10).

The ***SACN*** identified three studies relating health outcomes to unmetabolised folic acid (UMFA) levels. Two studies were unable to identify any associations between UMFA and risk of colorectal cancer or overall cancer [66, 67], while a third study found that detectable UMFA was associated with lower cognitive score only among participants with low vitamin B_{12} [68]. No clear relationship between folic acid intake and UMFA levels could be defined (Appendix 5.7.10). Uncertainties regarding homeostatic mechanisms of folate metabolism [69], of the metabolic provenance and fate of UMFA, and of its toxicological effects have led the SACN to conclude that there is insufficient evidence to determine the long-term effect, if any, of UMFA on health outcomes.

The ***FDA*** similarly concluded that there was an absence of consistent evidence implicating UMFA in adverse health effects. The ***VKM*** has adopted a more cautious position, noting that the health impact of UMFA remains "of concern", but also that the six studies assessed in their review did not provide support for amending the UL of folic acid. The ***NTP*** did not evaluate UMFA.

Summary

- There is no evidence for adverse effects of UMFA (Table 5).

Table 5: Summary of recently published reports assessing the potential effects of unmetabolised folic acid.

SACN	NTP	VKM	FDA	EFSA	AHMAC
Insufficient evidence	Not assessed	No evidence of adverse effects but still of concern	Inconsistent evidence	Health effects uncertain	No specific conclusion†

† Reported on one RCT that was also evaluated by SACN

2.8 At-risk subgroups

Recent data show that in Canada, the proportion of people exceeding the UL ranged from 2% to 14% depending on sex and age group; all of these individuals were supplement users [70] (Appendix 5.7.11). Of the 2.7% of US adults who exceeded the UL daily, 98.5% were consumers of folic acid supplements, and had intake levels that skewed towards higher doses (e.g. 47% of

supplement users took ≥800 µg/d) [71]. However, no individual exceeded the UL through consumption of mandatorily fortified food alone. No specific health outcomes for supplement users who have exceeded their UL have been reported. It should be noted that supplement use among the elderly, in whom prostate and colorectal cancer are more prevalent, tends to be higher (Appendix 5.7.11). In New Zealand, a 2008/9 population-wide survey found that about 10% of respondents self-reported daily folic acid supplement intake, and that red blood cell folate levels in these individuals were 28% higher than in those who did not take folic acid supplements [72]. However, UL exceedances cannot be determined as total folate/folic acid intakes were not estimated for this survey.

Children have not generally been identified as being directly at risk of specific health outcomes, other than via their potential to exceed the UL for folic acid [73]. A 2006 SACN report found no suggestive data for negative health outcomes in children exposed to high levels of folic acid [74] (Appendix 5.7.11).

Summary

- Supplement users are at greater risk of exceeding the UL, but no specific adverse health outcomes have been noted.

3 Weighing up the risks and benefits of mandatory folic acid fortification

3.1 Health benefits

There is strong and convincing evidence that mandatory folic acid fortification reduces rates of NTDs, and this has been unanimously observed across different countries. However, while folic acid substantially reduces NTD risk, it does not completely eliminate all risk, with genetic and other environmental factors likely contributing to cases that occur despite periconceptional supplementation. These cases are also referred to as non-folate responsive NTDs. The association between mandatory fortification and NTD risk is not linear—the extent of NTD reduction following mandatory fortification tends to be less substantial in populations with lower baseline NTD rates and/or higher levels of serum folate [6, 20, 75]. In other words, mandatory fortification provides diminishing returns at lower NTD rates.

It has been noted from interventional trials and in many countries mandating fortification that the rates of NTDs have not declined below a level of about 5 births or 7–8 pregnancies per 10,000 births [76, 77]. These figures have been proposed to comprise a so-called 'floor' level of non-folate responsive NTDs.

The average NTD rate in New Zealand in 2008–2015 (an estimated 5.5 live and stillbirths or 10.3 pregnancies/10,000 births) has been declining and may be near the apparent floor level. If mandatory fortification were to reduce NTD rates to the floor level of 7–8 pregnancies/10,000 births, this would equate to approximately 14–20 fewer NTD pregnancies per year. From 2000 to 2015, Māori women had a higher overall rate of NTD live births than NZEO women, but no data

are available on the totality of NTDs, that is, including terminated pregnancies. Indigenous women in Australia, and Hispanic women in the US, have had higher reported NTD rates pre-mandatory fortification and experienced the greatest decline post-fortification. The available New Zealand data suggest that Māori women may, similarly, receive benefit from mandatory fortification, although the extent of the potential impact is not clear.

The proportion of folate-sufficient WCBA in New Zealand—16% in 2014/15—is very low. Evidently, there is room for substantial improvements to be made in raising the folate status in this population and thus further reducing the risk of NTDs. It will also be important to determine the extent to which both folate sufficiency and NTD rates are impacted by the greater increases in bread fortification coverage in 2015–2016 (Figure 3).

In terms of folate deficiency (where an individual has very low folate levels that may have metabolic and clinical consequences; Appendix 5.3.3), rates in New Zealand are relatively low (2.8%)[16] [78], suggesting that any further beneficial effects on this health measure from introducing mandatory fortification may be small.

With respect to cancer, genetic studies provide weak and inconsistent evidence of an association between higher blood folate levels and decreased risk of breast cancer and total cancers. However, this evidence has yet to receive universal acceptance (Sections 3.2 and 5.7.2.3).

3.2 Health risks

Among the range of considered health outcomes, the only possible concern related to the potential for some impact of folic acid fortification on the risks of some types of cancer. However, while the totality of the evidence has some residual ambiguity in this regard, both other expert bodies and ourselves find that the current balance of evidence supports mandatory fortification of staple foods.

RCTs examining cancer risk do not find evidence of increased risk, but are limited by relatively short (~one decade long) durations of both supplementation and follow-up; this may be sufficient only to detect rapid effects such as formation of polyps (a precursor to most colorectal cancer cases) [79] (Appendix 5.7.5.4), or effects that accelerate the later stages of carcinogenesis. In this regard, the reviewed RCTs are generally reassuring (other than [80], but see Appendix 5.7.5.4). However, extended follow-up durations may reveal altered effects over time, as illustrated by previous RCTs that only found a benefit of aspirin on colorectal cancer incidence after at least 10 years' follow-up [81]. Hence, the generally null findings of the RCTs cannot absolutely inform how folic acid supplementation may affect cancer rates over the long-term. Nonetheless, the inability of the reviewed RCTs to detect any statistically significant effect suggests that, if there were a true effect occurring during the trials that had not been revealed due to other study limitations, the effect size is not likely to be substantial (but see following discussion on extrapolation to absolute numbers of affected individuals).

Observational studies suggest on balance a protective association of folate/folic acid on many outcomes, and ecological studies on cancer trends after mandatory fortification do not suggest

[16] As determined from RBC folate levels. The prevalence of folate deficiency did not vary by ethnicity or socioeconomic status.

any substantial impact. However, both study types have major limitations as they cannot determine causal effects.

Genetic studies more consistently infer that higher blood folate levels lead to greater risks of colorectal and prostate cancer, and a lower risk of breast cancer and overall cancer rates. However it is not known how the degree of change in blood folate expected in a population exposed to mandatory fortification compares to *MTHFR* mutation-related differences in blood folate[17]. The activity of folate at the intracellular level—where any biological effect on cancer processes would potentially occur—could be more substantive with genetic differences as opposed to fortification. The *MTHFR* gene is relatively well studied and is not known to have effects unrelated to folate metabolism that in turn influence cancer risk. However, the mechanisms of folate metabolism in relation to cancer remain incompletely understood [83], and the possibility that the genetic studies are biased by an as-yet unidentified effect of the *MTHFR* gene cannot be ruled out. Therefore, these studies raise concerns, but remain far from conclusive.

In summary, the available evidence on cancer suggests that:

- In the shorter term, there is no effect of folic acid supplementation or fortification on cancer incidence and mortality.
- In the longer term, the genetic studies provide weak and inconsistent evidence that there may be increased colorectal cancer rates especially among people at particular risk because of the presence of polyps. However, the same evidence suggests a possible reduction in rates of breast cancer and overall cancers in association with higher folate levels.

Within the context of mandatory fortification, the health risks can be distilled to an uncertain increase in risk of prostate and colorectal cancer. This putative risk for a subgroup of the population should be considered against a background of decreased risks in breast cancer, and in total cancer within the wider population. The same caveats on the uncertainty of the evidence apply to both possibilities.

3.3 Can health benefits and risks be compared?

When considered strictly from the absolute number of cases involved, the number of NTD-affected pregnancies actually prevented by folic acid fortification (whether voluntary or mandatory) is small given the relative rarity of the disease, with the number potentially being no higher than about 20 per year and more likely around 10 per year. On the other hand, a small increase in risk of prostate or colorectal cancer could adversely affect a larger number of people.

One argument from an ethical perspective is that the smaller number of beneficiaries does not justify placing a greater number at potentially increased risk of cancer or other adverse health outcomes [84].

Yet, considerations of risk purely in terms of number of cases involved clearly ignores the enormous burden and distress imposed by NTDs on affected women and their families/whānau, as well as NTD-affected individuals themselves, over their entire life course. At the same time, it is worth

[17] Median RBC folate levels in individuals with two copies of the *MTHFR* mutation or with no *MTHFR* mutation are 636 nmol/L and 759 nmol/L, respectively (a difference of 19%) [82].

noting that New Zealand has a well-established national breast screening programme for breast cancer, and a national bowel cancer screening programme is being rolled out.

The Panel also notes that, from a toxicological perspective, it is not possible to evaluate risk acceptability of mandatory fortification because this would require assessing the risk and benefits in the same population or cohort. However, this concern is diminished by the weak evidence of an adverse effect on some older people.

Table 6 summarises the state of the scientific evidence on folic acid supplementation and the health benefits and risks of folic acid.

Table 6: Summary of key findings relating to folic acid supplementation or fortification, and the health benefits and risks of folate/folic acid

Knowns	Uncertainties
• Folic acid supplementation during the periconceptional period reduces the risk of NTDs. • Folic acid supplementation during the periconceptional period does not have widespread uptake and adherence. • Mandatory folic acid fortification in other countries has led to marked decreases in NTD prevalence. The strength of the evidence is conclusive. • There is no evidence that folate/folic acid is associated with adverse health effects other than (possibly) some types of cancer.	• With respect to cancer, clinical trials do not consistently point to any net benefit or harm. However, there is a lack of evidence for very long-term effects. • Genetic studies suggest that having higher blood folate levels may be associated with increased colorectal and prostate cancer rates, and decrease breast and total cancer rates. This evidence is not universally endorsed and the associations are not necessarily causal.

3.4 Addressing health inequities

While folic acid fortification is a strategy to improve the folate status of WCBA in general, it may confer especial benefits to women who are at social and economic disadvantage, as seen in the US and Australia (Section 5.5.2). Live birth data have identified a difference in NTD rates between Māori and NZEO women, but no information is available for stillbirths and pregnancies. There is also a suggestion that NTD live birth rates in Māori women are declining at a greater rate than that for NZEO women.

Treaty of Waitangi

While strictly beyond the Panel's terms of reference, we wish to acknowledge the broader context in which the deliberations must be placed. Mandatory fortification of staple food can be viewed as an infringement on individual autonomy, especially if alternative products are unavailable or prohibitively expensive. Further, within the New Zealand context it is important that all public policies are designed and implemented within a Treaty of Waitangi framework [85]. In this context, mandatory fortification may be interpreted as a reduction of power and control for Māori if it is undertaken without appropriate Māori involvement in the decision-making process. At the same time, it must be recognised that Māori WCBA are likely to have lower levels of both access to folic acid supplements and awareness of its importance. Hence, foregoing mandatory fortification with this knowledge also raises rights-based issues on the need to ensure equitable healthcare services and outcomes. Irrespective of the policy decision on folic acid fortification, a Treaty perspective requires that Māori be involved in the decision-making process, and that every effort is made to ensure that they receive equal benefit from the decision.

3.5 Unresolved issues

Apart from the uncertainties presented by the limited evidence from the genetic studies, there are several other key issues relating to exposure of the population to folic acid that need to be considered.

This report has considered fortification levels at the current voluntary target of 200 µg folic acid /100 g bread. Mandatory fortification at a level of 135 µg/100g bread, as initially proposed by the 2007 food Standard that was revoked in 2012, should remain under consideration. This lower level of fortification is in line with that practised in Australia and the US (120 µg and 140 µg/100 g food product, respectively), and will result in lower folic acid exposure within the general population compared to mandatory fortification at 200 µg/100 g bread. Modelling work from 2012 has estimated that mandatory fortification at a level of 135 µg/100 g could prevent 17 (range of 14–20) NTD pregnancies when compared to 2005–2009 NTD rates [17].

The level of intake of folic acid-containing supplements within the general New Zealand population needs to be determined, as this can inform on the size of the subgroup that may be at higher risk of exceeding the UL for folic acid under mandatory fortification. In addition, the widespread folic acid fortification of breakfast cereals in New Zealand suggests the need to monitor consumption levels and cumulative folic acid intake from all fortified food.

There are consistent indications in other countries of ethnic inequities in factors such as NTD-related health outcomes, folic acid supplement use, fortified food consumption, and NTD-related genetic risk. In New Zealand, there is evidence for social inequities in supplement use and awareness of its importance. Assuming rates for stillbirths and terminations are similar among all New Zealand women, there appears to be inequities in NTD rates between Māori and non-Māori women. Furthermore, NTD rates are not known for other socially disadvantaged women and other ethnic minorities that consume less (packaged) bread such as Asians and Indians, who comprise an increasing proportion of the New Zealand population.

More comprehensive dietary intake data for consumption of bread and other folic acid-fortified food by New Zealand WCBA is essential in determining the potential efficacy of mandatory fortification. Unlike most other countries in which wheat or other grain flour is the vehicle for fortification, in New Zealand folic acid is added to bread for trade and manufacturing reasons. This means that the range of fortified food products is much narrower. Changing demographics and food consumption patterns in New Zealand provide an impetus to reconsider the fortification vehicle.

Lastly, the practice of periconceptional folic acid supplement intake among New Zealand WCBA is low (Appendix 5.5.1). Mandatory folic acid fortification is not intended as a replacement for periconceptional supplementation (Appendix 5.3.4). Therefore, greater public health and educational efforts to raise levels of both awareness and practice of periconceptional supplement intake are fundamental to minimising NTD risk.

4 Conclusions

4.1 Latest New Zealand data

Folate insufficiency in pregnant women is a cause of NTDs and increasing blood folate levels is a proven preventive measure at a population level. In New Zealand, voluntary folic acid fortification of bread has been in place since 2009 and approximately 40% of packaged bread is now fortified with the goal being 50% coverage. Australia has had mandatory fortification since 2009 and this programme was endorsed by a 2016 review.

The most recent complete NTD data for New Zealand found that in 2013, 18 babies were born with an NTD, a further 6 were stillborn, and an additional 27 pregnancies were terminated for NTDs, giving a total of 51 NTD-affected pregnancies. This equates to a prevalence of about 3.0 live births/10,000 total births, and 8.6 pregnancies/10,000 total births. The estimated NTD-affected rate of pregnancies in 2008–2015 is 10.3/10,000 births. Rates for pregnancies are likely to be underestimates as miscarriages are not a notifiable event in New Zealand. There has been a decline in NTD birth rates since 2000. Rates of NTD-affected live births are higher in Māori women than in NZEO women, but may also be declining.

The most recent available data, from 2011, suggest that there is a low level of both awareness of the importance of folate before and during pregnancy, and practice of periconceptional folic acid supplementation among New Zealand WCBA. This is of concern, and the underlying reasons need to be better understood and addressed where possible.

Analyses from 2012 suggest that moving from a voluntary (50% fortification coverage) to a mandatory (100% coverage) bread fortification program in New Zealand would prevent approximately 5–15 extra NTD pregnancies annually, with major benefits to individuals, families, and society in general. A critical consideration is prevention of a considerable degree of emotional trauma for women and their families in considering termination of an NTD-affected fetus, or dealing with stillbirth or a child who grows up with a significant disability.

4.2 Health benefits and risks

This report concludes that mandatory folic acid fortification of food will unequivocally reduce the prevalence of NTDs. There is only limited and weak evidence that it might conceivably have adverse effects. This conclusion is supported by other international authoritative reviews. The report also concludes that there is no evidence of harmful health effects of folic acid supplementation at low doses in adults. It finds that traditional epidemiological studies (interventional and observational) show no consistent evidence of increased cancer risks, and that ecological studies comparing trends in cancer rates with the introduction of mandatory fortification are also reassuring. However, limited and weak evidence from genetic studies—which has not been universally endorsed—suggests that higher blood folate levels might be associated with increased risk of prostate and colorectal cancer, but decreased risk of breast and total cancer.

The nature of science is that it cannot prove a negative—that is, there is no experimental design or methodology that can prove with 100% certainty that folic acid is completely 'safe'. However, on balance and considering all the current evidence, the Panel concludes that the benefits of mandatory fortification will outweigh any possible adverse effects on the health of the New Zealand population.

4.3 Areas for further consideration

Further research by those with expertise in interpreting the genetic (or Mendelian Randomisation) studies—a relatively new and complex type of analysis—is essential for a better understanding of their strengths and limitations.[18] This would most usefully focus on three questions:

a. What is the current assessment of the strengths and biases of genetic studies in general that would be especially relevant to the studies reviewed in this report?
b. Genetic studies investigate the variation between people with and without genetic mutations that influence blood folate levels. How reflective is this situation of mandatory fortification, which is expected to result in an increase in blood folate levels, particularly in terms of qualitative and quantitative differences in blood folate levels?
c. Given the different metabolic fate in the body of folate and folic acid, and the complexity of biochemical pathways involving folate, are there any features of genetic studies that may introduce as-yet unidentified biases?

These research areas would be most usefully examined in conjunction with close monitoring and evaluation of ongoing international science and policy developments in fortification.

The Panel unanimously agreed that WCBA need to achieve adequate folic acid intakes via supplementation and fortification. Indeed, the low proportion of folate-sufficient New Zealand WCBA is of particular concern, and indicates that there is an opportunity for substantially increasing the folate status of this target population to further reduce NTD risks. This may be achieved in part by greater public health and nutritional education, increasing the availability of fortified food, and improving package labelling so that WCBA are better informed on choices of fortified food. Improved labelling also assists other consumers who may wish to limit their folic acid intake to make appropriate decisions. It was also agreed that it is imperative that considerations of potential

[18] See Footnote 15.

interventions take into account a Treaty of Waitangi perspective. Finally, the Panel supports the continued use of folic acid and other micronutrient tablets for pregnant women as recommended by their healthcare professionals.

5 Appendices

5.1 Methodology

Recent comprehensive systematic reviews and reports, including those from recognised international scientific committees and taskforces, were synthesised to form the basis of this report. Additional literature searches were undertaken, without date restrictions, in Scopus, Web of Science (including Medline), the Cochrane Library (including the Cochrane Database of Systematic Reviews and the Cochrane Central Register of Controlled Trials), EMBASE, AGRICOLA, and BIOSIS. Greater focus was given to more recent publications and systematic reviews/meta-analyses.

Epidemiological literature comprising interventional (clinical), observational, ecological, and genetic studies were examined. Where possible, studies directly relating to voluntary or mandatory folic acid fortification were included. In keeping with the report's remit, focus has been placed on human studies, and animal experimental and *in vitro* studies have been excluded except where there have been compelling scientific reasons for their inclusion. Relevant reports from reputable organisations in New Zealand and other countries were also referenced. Social science literature and philosophical issues were considered where appropriate but with a secondary focus. Implementation of mandatory fortification is outside the scope of this report.

5.2 Background on folic acid fortification in New Zealand

5.2.1 History of folic acid fortification in New Zealand

Food fortification refers to the practice of adding essential micronutrients to a food, with the purpose of improving its nutritional quality, and conferring demonstrable and plausible public health benefits based on new and evolving scientific evidence [86].

In New Zealand, fortification is implemented in accordance with appropriate regulations governed by the Australia New Zealand Food Standards Code. A range of food products has been permitted to be voluntarily fortified with folic acid since 1996, including breakfast cereals, yeast-based spreads, fruit and vegetable juices, and milk alternatives such as those from soya and rice. The addition of other vitamins and minerals such as iron and vitamin C to breakfast cereals, juices, and other food products is also subject to voluntary fortification standards.

In 2007, New Zealand and Australia agreed to a mandatory folic acid fortification standard. In Australia, breadmaking flour was chosen as the vehicle for fortification, while in New Zealand bread was to be directly fortified instead due to potential commercial impact on third country trade for the small number of flour millers.

The requirement, as outlined in a 2007 food Standard[19], was to come into effect in late 2009. However, following concerns raised by the baking industry and consumers, implementation of the Standard in New Zealand was deferred to May 2012, and instead an amendment was made to enable the adoption of voluntary fortification of bread in the interim. Under this regime, practised from 2009–2012, manufacturers aimed to achieve a target range of 80–180 µg/100 g bread [87]. In May 2012, implementation of the Standard was further postponed to September 2012 to allow for

[19] Known in full as the New Zealand (Mandatory Fortification of Bread with Folic acid) Food Standard 2007

a full research review and public consultation to be undertaken prior to a decision on mandatory fortification.

Following these processes, the then-Minister for Food Safety revoked the 2007 Standard in favour of a new Standard[20] allowing for continued voluntary fortification of bread, which remains in place today. This Standard permits fortification at up to 250 µg/100 g bread. In 2014 the NZ Association of Bakers established a voluntary Code of Practice in conjunction with the Ministry for Primary Industries (MPI). This set an "aspirational" goal of 25–50% of bread by production volume being fortified at a higher target level (200 µg folic acid/100 g bread) than that proposed in the 2007 Standard (80–180 µg/100 g). Although subject to audit, these targets are non-legally binding.

Meanwhile, Australia has proceeded with mandatory fortification of wheat flour for bread-making purposes since 2009, and folate intake, folate levels, and population health outcomes before and after mandatory fortification have been closely monitored. In 2016 the Australian Institute of Health and Welfare released a report reviewing the health outcomes of the mandatory programme. Among the key findings was a 14.4% decrease in overall rate of NTDs, with particularly dramatic decreases seen among Indigenous women and teenagers (74% and 55%, respectively) [33] (Appendix 5.7.4.1). Mandatory fortification also did not lead to an appreciable proportion of adults exceeding the UL for folic acid.

Food Standards Australia New Zealand, which introduced the mandatory fortification scheme, commissioned a meta-analysis to assess the most up-to-date scientific literature on folic acid intake and cancer incidence [88]. The analysis found no significant[21] differences in relative risk for total incident cancer, various site-specific cancers, colorectal adenoma recurrence, or all-cause mortality.

Collectively, the health outcome data from Australia encompassing reduced NTD rates, low levels of excess intake, and lack of evidence linking high-dose folic acid to cancer suggested that (1) mandatory fortification has been effective in achieving its health objective; (2) public health safety does not appear to have been compromised; and (3) health equity may have improved. Therefore, the health benefits of mandatory fortification may outweigh the real and perceived risks. Other reports on food supply and industry compliance have also been published [42, 89], and a formal evaluation of the overall effectiveness of fortification found that it was "effective, equitable and efficient", having achieved its policy objective of NTD reduction and providing value for money [31].

Consequently, this has raised questions about whether the voluntary fortification scheme undertaken in New Zealand has been adequate to confer equivalent levels of efficacy, and whether implementation of mandatory fortification in New Zealand would provide additional health benefits to the population at minimal risk, to a similar extent as in Australia. In December 2016, the Ministry of Health's Acting Director of Public Health decided that a review of the health benefits and risks of folic acid fortification should be undertaken.

5.2.2 NTDs in New Zealand

Figure 1 shows the latest available New Zealand data for NTD-affected live births, live and stillbirths, and pregnancies. There is a downward trend in both NTD-affected live births and live-and-stillbirths

[20] Known in full as the New Zealand (Permitted Fortification of Bread with Folic Acid) Food Standard 2012 [14].
[21] In the Appendix, the term 'significant' is used as a statistical descriptor.

between 2000 and 2013/16 (Figure 1, orange and blue lines, respectively). These declines are statistically significant [16].[22] It is difficult to ascertain any changes in trend following increased voluntary fortification efforts since 2009 owing to data fluctuations, the relatively short post-intervention time frame, and the lag time of a potential response (that is, the time required for any changes in folic acid intake to be reflected in blood folate levels and thence NTD rates). In addition, bread fortification coverage did not increase appreciably until about 2015 (see Section 5.2.4). Bearing these limitations in mind, there does not appear to be an obvious change in trends after 2009.

The termination of pregnancies due to NTDs has been recorded since 2008, and data are currently available until 2015. These data provide a more complete picture of the true incidence (occurrence of new cases) of NTDs, compared to rates of live and/or stillbirths. Although there is also some indication of a downward trend, large fluctuations and the short time frame again pose a challenge in drawing any meaningful conclusions (Figure 1, purple line).

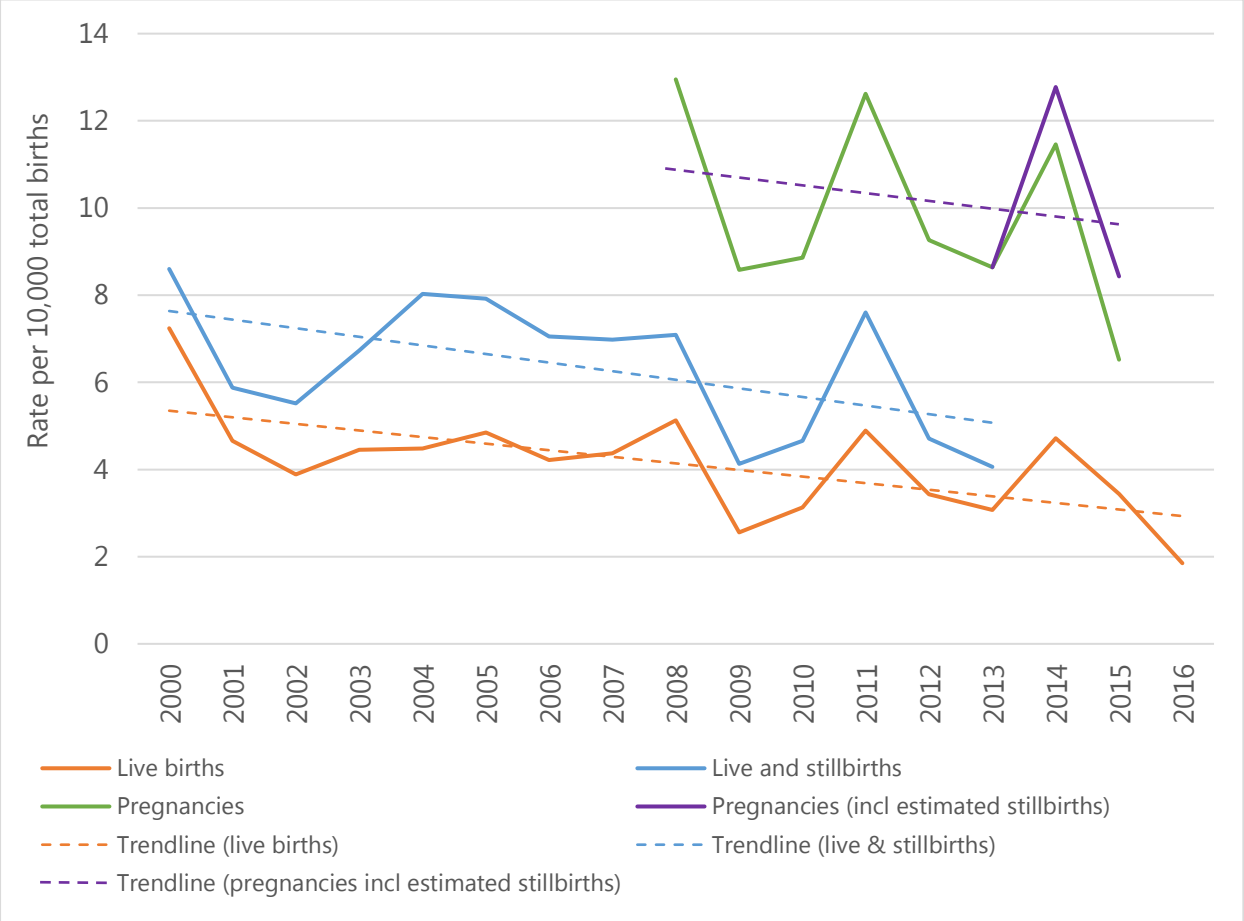


Figure 1: Rates of NTD-affected live births, live and stillbirths, and pregnancies in New Zealand. Data for total pregnancies in 2014–2015 (green line) are underestimates as they exclude NTD stillbirths. Adding an estimated 11 NTD-related stillbirths to 2014–2015 data (based on a mean of 10.7 between 2008 and 2013) results in an adjusted rate shown in purple. Although there is likely to be a floor (baseline) rate of NTDs, trendlines are derived from an assumed linear relationship for reasons of simplicity, and are presented for indicative purposes only. Rates for live births are per 10,000 live births

[22] 2000 to 2015 data were used for statistical analysis of live and stillbirths.

(not total births) due to incomplete data; however these data are likely to be minimally different if total births were used instead. Data plotted from [12].

The average rate of NTD pregnancies from 2008 to 2015 was estimated from the total numbers of NTD live births, stillbirths, and terminated pregnancies, and taking as the denominator the total number of NTD and non-NTD live and stillbirths. As NTD stillbirth data for 2014 and 2015 were unavailable, these were estimated by taking the average number of NTD stillbirths from 2008–2013 (10.7 cases/year). This yielded an estimate of 10.3/10,000 births. It should be noted that this figure is lower than a previous estimate by MPI of 12.9 pregnancies/10,000 births between 2005–2009 [15]. It is also lower than in Australia both pre- and post-fortification (12.8 and 11.2/10,000 births, respectively) [34].

Figure 2 shows rates of NTD live births by maternal ethnicity. Stillbirth and termination data by ethnicity are unavailable.

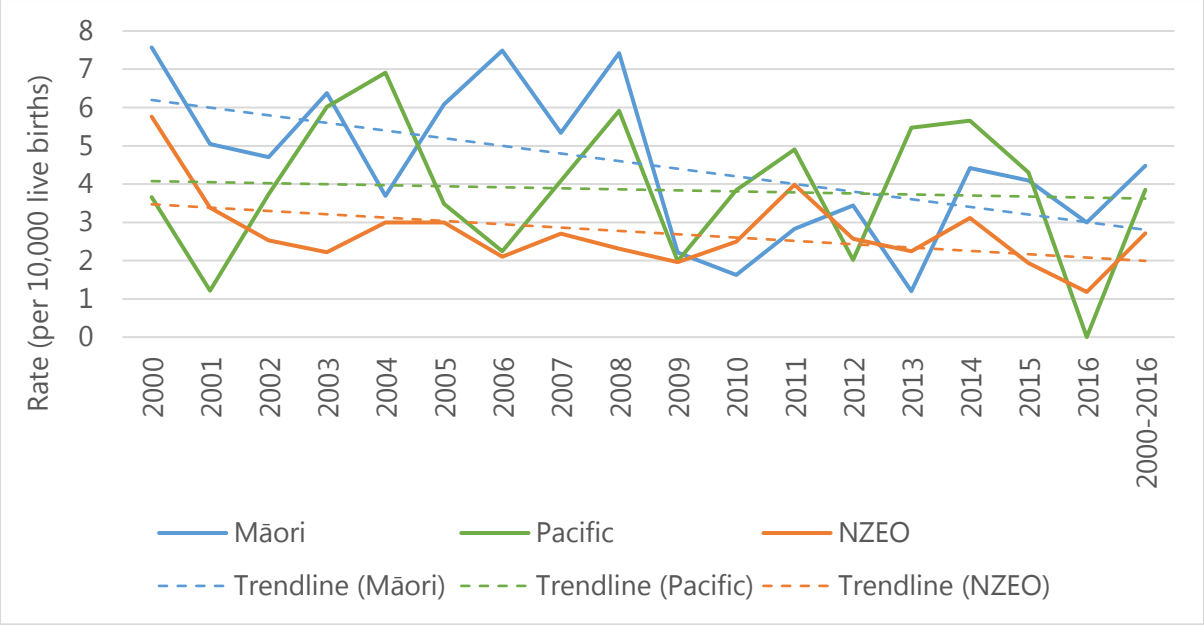


Figure 2: Rates of NTD-affected live births in New Zealand by maternal ethnicity. Although there is likely to be a floor (baseline) rate of NTDs, trendlines are derived from an assumed linear relationship for reasons of simplicity, and are presented for indicative purposes only. Note that the statistical analysis of combined data by MPI as described in Section 1.1.1 excludes 2016 figures. Rates are expressed per 10,000 live births (not total births) due to incomplete data; however these data are likely to be minimally different if total births were used instead. Data plotted from [12].

There is a notable drop in rates for Māori and Pacific women in 2009 compared to 2008, but this is unlikely to be due to increased voluntary fortification efforts given the absence of a lag time (Figure 1).

5.2.3 Folate status of New Zealand women of childbearing age

It is difficult to assess the impact of increased voluntary fortification since 2009 by the baking industry on the proportion of New Zealand WCBA who are folate sufficient. This is because it is not generally possible to directly compare blood folate levels across different surveys due to assay and other methodological variability. Earlier surveys used to discuss the prevalence of folate sufficiency

among New Zealand WCBA include the population-based 2008/9 New Zealand Adult Nutrition Survey [90], undertaken before increased voluntary fortification efforts, and the 2011 Folate and Women's Health Survey, which was commissioned by the Ministry of Agriculture and Forestry to monitor the efficacy of voluntary fortification on folate status in WCBA [13, 91, 92]. The 2008/9 survey found that 26% of WCBA were folate sufficient, while the 2011 survey reported a prevalence of 59%. This suggests a general improvement in folate status[23], and differing participant characteristics in these surveys may have introduced biases that underestimate the difference. For example, the 2008/9 data were obtained from women who were more likely to be regular breakfast consumers, have consumed fortified yeast extract, or taken folic acid supplements daily [72], while the 2011 survey was not nationally representative, having fewer Māori/Pacific women and more women of higher socioeconomic status.

It is important to note that no direct comparisons can be made between these surveys and the most recent 2014/15 New Zealand Health Survey reported in Section 1.1.2, as a different assay calibrator was used for the latter survey that is known to produce lower values. Nevertheless, a small proportion of the 2008/09 samples from WCBA were re-measured using the 2014/15 assay calibrator, and no statistically significant changes between the two time periods were found [16]. It is also difficult to ascertain the potential impact of the baking industry's 2014 Code of Practice on folate status, given that it was introduced just a few months before data for the 2014/2015 New Zealand Health Survey began to be collected.

Modelling work has shown that folate sufficiency in New Zealand WCBA could be achieved with either 12 weeks of using the pregnancy supplement, or 36 weeks of consuming mandatorily fortified bread [93, 94]. Hence, given continuous intake over a long enough time, a relatively low level of fortification may improve rates of folate sufficiency among WCBA to a similar extent as periconceptional supplements.

A 2008 dietary survey showed that New Zealand WCBA were consuming low amounts of total folate, with more than one-third unable to meet their EAR of 320 µg/d. Median folic acid intake was just 46 µg/d [95]. Simulated mandatory fortification of most bread at 130 µg folic acid/100 g bread would nearly triple median folic acid intake among WCBA to 135 µg/d, reduce the proportion of women not meeting their EAR for folate from 37% to 5%, and yet, importantly, not place any individual over the UL [95].

5.2.4 Bread fortification

In 2008, manufacturers' data for fortified foods based on label information showed that six breads and 70 varieties of breakfast cereal contained folic acid [96]. For bread, the mean folic acid level was 212 µg folic acid/100 g bread (min 200 µg, max 286 µg); for breakfast cereals, the mean level was 191 µg/100 g (min 53 µg, max 333 µg). No information was available on consumption levels of each product.

[23] RBC folate levels increased from 720 nmol/L to 996 nmol/L between the two surveys. Data expressed as the geometric mean (a measurement of the average that takes into account skewed data and smaller sample sizes). The level of increase in serum folate from 2008/9 to 2011 was predicted to reduce NTD rates by 18% [75, 92], but NTD data variability make this difficult to discern (Figure 1).

During the voluntary fortification regime undertaken by the baking industry between 2009 and 2012, a level of 80–180 µg/100 g was targeted. In 2012, 34 fortified packaged breads were available, comprising just 12.5% of total bread production [97].

The 2014 baking industry Code of Practice has set an "aspirational" target of 25–50% of packaged breads by production volume, stipulating a minimum rate of 25% fortification by 2014. This target was met in 2015, and coverage further increased in 2016 (Figure 3). The New Zealand Association of Bakers are working closely with a retail partner to increase fortification coverage to 50% (P. Rewi, NZAB, pers. comm., 30 October 2017) The currently fortified bread products span the lower to higher price range [21] and are therefore likely to be consumed by WCBA independent of their socioeconomic status.

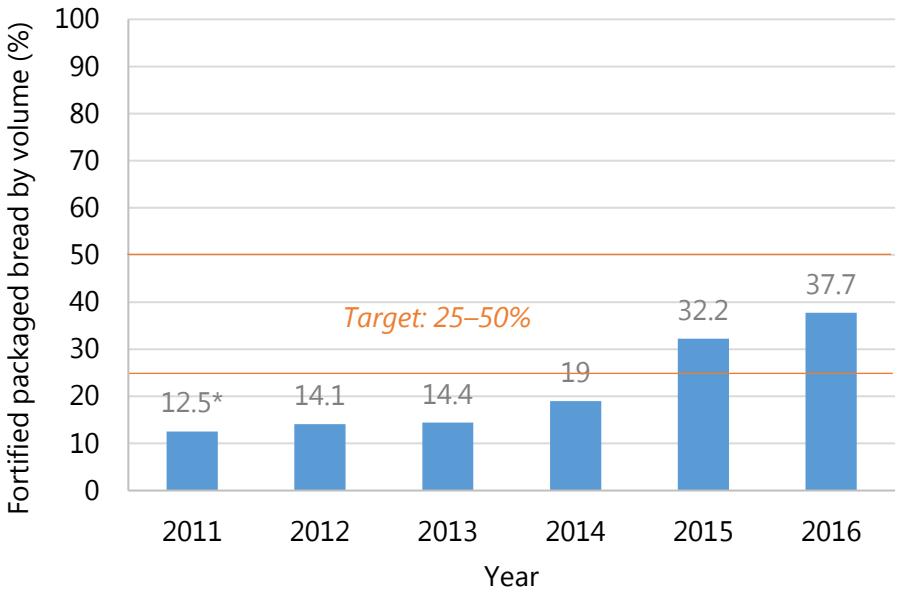


Figure 3: Proportion of folic acid-fortified packaged bread for sale in New Zealand. The baking industry voluntary target is 25–50%. Data for 2011 from [97]; data for 2012–2016 plotted from [21].

The 2011 Folate and Women's Health survey found that the 17 top-selling fortified breads contained highly variable levels of folic acid, and overall did not reach the target of 200 µg/100 g bread (median 144 µg; interquartile range 41, 189 µg) [91]. Industry audits found that median levels consistently rose over subsequent years (from 153 µg to 191 µg/100g bread over 2013–2016), but substantial variability was still seen (Figure 4). An analysis of fortified bread using different survey methodology, commissioned by MPI in 2016, found that the median folic acid concentration was just 130 µg/100 g bread, and that the median difference between the declared label concentration and the measured value was 91 µg/100 g bread (range of 5–192 µg/100 g) [16].

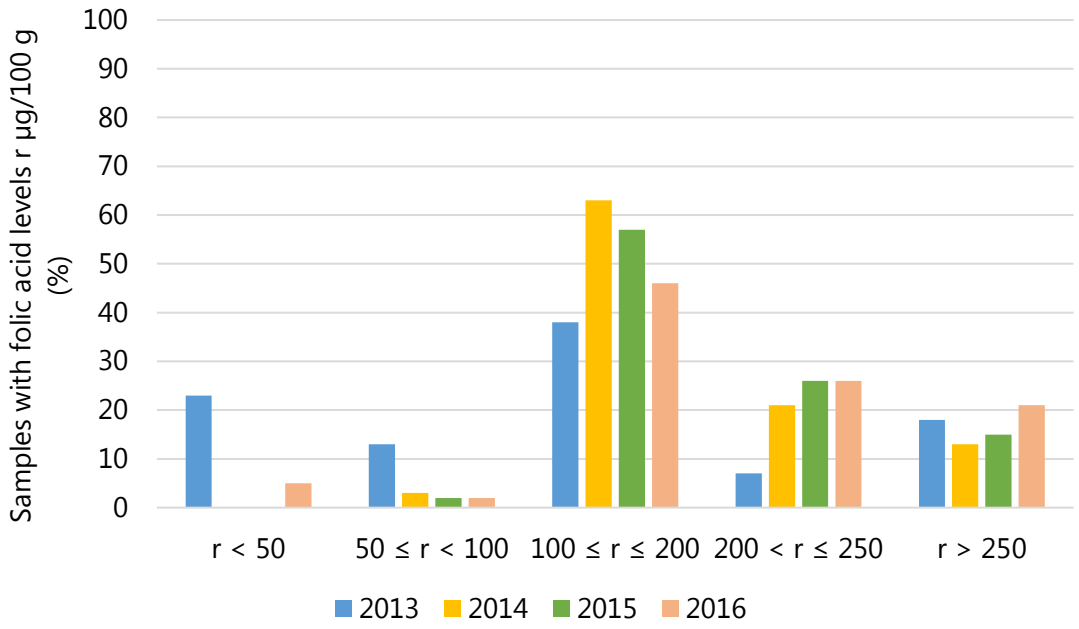


Figure 4: Folic acid levels in bread samples by year. Data plotted from [21].

There are ongoing efforts to address the issue of high variability in fortification levels. Adding folic acid to bread is technically challenging; as had been the case in Australia, this degree of variability likely reflects initial shorter-term manufacturing complexities involved in blending small amounts of particulate matter in a much larger volume of food product. While it is likely the average dose may even out in the long term, this can only be verified with blood folate measurements.

5.2.5 Dietary supplements

Supplementation refers to oral ingestion of dietary products containing specific ingredients to supplement that already obtained from the diet. Depending on the dose of the nutritive substance, dietary supplements can provide an individual with his/her entire recommended intake, subject to compliance with regular consumption.

In New Zealand, dietary supplements do not require pre-approval, although they are regulated under the Dietary Supplements Regulation 1985 which is administered by Medsafe (a business unit of the Ministry of Health). For supplements containing folic acid, the Regulations stipulate a maximum content of 300 µg per daily dose, or 500 µg if the supplement has been verified to be manufactured in accordance with the Code of Good Manufacturing Practice for Manufacture and Distribution of Therapeutic Goods [98]. Although Good Manufacturing Practice declarations are subject to audit, there is no specific ongoing monitoring programme to test for compliance with the Regulations; instead, the onus is on the sponsor (the individual legally responsible for making the product available to the market) to ensure the product's quality and safety [99].

The Ministry of Health recommends that 800 µg of folic acid (or 5 mg, for women who specifically require higher doses) is taken daily during the periconceptional period. It should be noted that folic acid-only tablets at these doses, which are subsidised on prescription, are classed as a registered medicine and not a dietary supplement.

5.3 Folate and folic acid

5.3.1 What are folate and folic acid?

Folate is the generic term for a group of chemically related B vitamins. Also known as vitamin B_9, folate is an essential nutrient (not synthesised by the body) that is required for cell function and tissue growth. Humans and other mammals depend solely on dietary sources to achieve adequate folate intake. It is naturally found in a wide variety of foods such as leafy green vegetables, legumes, grains, dairy products, meat and poultry, and seafood, with some of the highest levels being found in spinach, yeast, and liver.

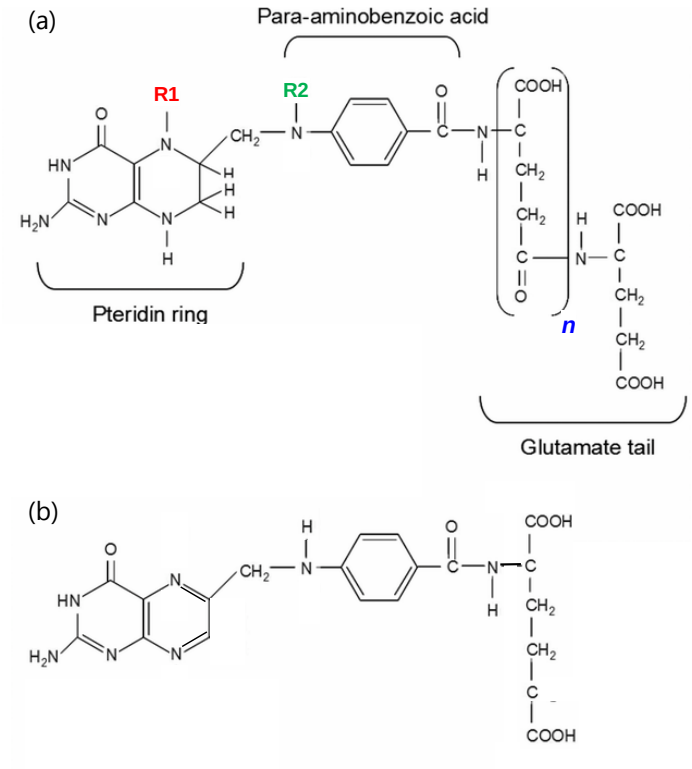
The primary determinant of an individual's folate status is dietary intake from food and supplements, although genetic variation (e.g. having a mutation in a folate metabolism enzyme) impacts on efficiency of folate metabolism. Age and sex may also play a role. Folate requirements differ among individuals depending on physiological status (e.g. age, pregnancy), disease (e.g. cancer, anaemia, coeliac disease), or use of certain medications (e.g. anticonvulsants, antifolate chemotherapeutic agents; Appendix 5.7.11).

Folic acid (pteroylmonoglutamic acid) is the synthetic and most chemically stable form of folate. It is the main component in tablets taken by people to increase their folate levels. Because it does not occur naturally, it is technically considered a pharmaceutical rather than a supplement, although it is commonly referred to as the latter. Similarly, although folic acid is strictly not an essential micronutrient as it is not naturally found in food, its addition to food is usually referred to as fortification.

5.3.2 Chemistry and pharmacokinetics

Folate is chemically unstable and easily degrades during the harvesting, storage, preparation, and cooking of food [100]. The degradation products have no known biological function.

Chemically, folate is a mixture of reduced polyglutamates (having multiple glutamic tails attached) (Figure 5), while folic acid exists as an oxidised monoglutamate form (conjugated to one glutamate residue). The bioavailability of folate—that is, the proportion of ingested folate that is absorbed and is available for metabolic processes or for storage—is about 50%, while that of folic acid is around 85% [25, 101]. The low bioavailability of folate compared to folic acid has been attributed to the need for polyglutamate removal during absorption, incomplete release from plant cellular structures, and digestive processes.



R1	R2	Folate species
H	H	THF
CH_3	H	5-methyl THF
$-CH_2-$		5,10-methylene THF

Figure 5: Chemical structure of (a) folate (as several of the natural derivatives) and (b) folic acid. R1 and R2 denote various substitutions to give rise to different derivatives. “n” indicates a varying number (1–8) of glutamate residues. THF=tetrahydrofolate. Figure 5a adapted from [100], with permission.

Folate and folic acid are metabolised differently by the body. During intestinal absorption, the glutamate tails on folate are first removed. The resultant form is then actively transported into intestinal mucosal cells, where further conversions involving reduction and methylation occur. This yields 5-methyltetrahydrofolic acid (5-MTHF), which is the primary circulating form of folate [102]. 5-MTHF is taken up by cells and metabolised to tetrahydrofolate (THF), a key one-carbon donor (Figure 6).

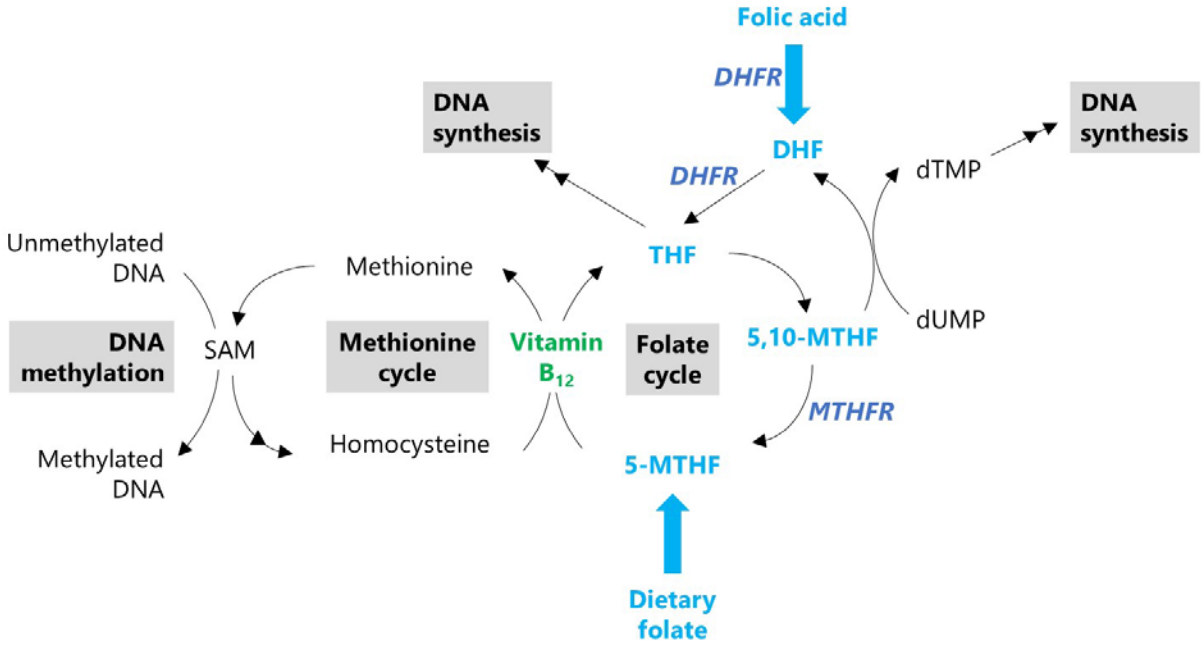


Figure 6: Simplified schematic of the metabolic fate of folate and folic acid. Only key substrates and (co)enzymes are shown. Folate serves as a substrate, and vitamin B_{12} as a coenzyme, in the synthesis of methionine. It is also used for synthesis of DNA nucleotides via two different pathways. 5-MTHF=5-methyltetrahydrofolic acid; THF=tetrahydrofolate; 5,10-MTHF=5,10-methylene-tetrahydrofolate; DHF=dihydrofolate; DHFR=dihydrofolate reductase; MTHFR= methylenetetrahydrofolate reductase.

In contrast, folic acid enters the folate pool by being sequentially reduced—first to dihydrofolate, and then to THF. The levels of the enzyme that catalyses these steps, dihydrofolate reductase, in the human gut mucosa and liver is low [103, 104]. This may account for the occasional detection of unmetabolised folic acid in the blood following intake of relatively high doses of folic acid (Section 2.7).

Folate and low (100–200 µg) doses of folic acid are mostly reabsorbed in the kidney. At higher (2.5–5 mg) doses, the kidney's reabsorption capacity becomes saturated and about half the dose is excreted in urine [29]. This limits its accumulation and storage in the body, unlike some fat-soluble vitamins such as vitamins A and D, of which excessive consumption may lead to accumulation in fat tissue with potential toxic effects.

Folate metabolism is intimately linked to that of the amino acid methionine via vitamin B_{12}. A low folate status leads to elevated levels of homocysteine, which is in turn linked to increased cardiovascular disease risk. Low folate may also lead to anaemia. The World Health Organization (WHO) defines folate deficiency based on serum/plasma folate levels at which homocysteine levels begin to rise, as this metabolic indicator correlates more strongly with folate levels than do haematological indices [105].

Through the methionine cycle, folate also serves as the primary carbon donor for DNA methylation, one of the fundamental epigenetic mechanisms that modulate gene expression independent of the DNA sequence. Folate deficiency or excess may therefore lead to altered epigenetic patterns that are transmitted through to subsequent cell generations, as seen in animal studies, but the implications of this for human health are not yet clear [106, 107].

An important enzyme in folate metabolism is methylenetetrahydrofolate reductase (MTHFR). Some individuals have a mutation (denoted C677T, or rs1801133) in the gene that codes for this enzyme, and as a result, they have lower levels of red blood cell (RBC) folate. Compared to those without the mutation, individuals with two mutated copies of the gene are estimated to have a 70% reduction in MTHFR activity and 16% lower RBC folate levels [82, 108]. These individuals not only have increased dietary folate needs, but are also less responsive to supplementation [109]. Having one copy of the mutation also has an impact on folate levels, albeit to a lesser extent. The prevalence of the *MTHFR* mutation differs substantially across ethnicities and regions; it has not been detectable in African populations but yet is found in up to 62% of an Amerindian subpopulation [110]. Asian and Caucasian women who have the mutation are at particular risk of having an NTD pregnancy [111].

There have been some discordances between findings from experimental and epidemiological studies in folate research, such as the inability of many rodent models to recapitulate NTD phenotypes seen in humans [27, 101, 112]. This may be at least partially explained by differences in folate metabolism between humans and animal models. For example, there is a marked difference in the activity of intestinal enzymes involved in folate digestion between rats—an exceedingly common animal model—and humans [113]. There are also substantially different profiles of various folate derivatives in plasma of humans and other animals such as rat, mouse, rabbit, and pig [114]. The normal range of serum folate in humans is about 6–8 times lower than average values found in rats and mice [115], suggesting that studies on folate depletion in rodents are dealing with a normative range in humans. Although these caveats must be borne in mind when determining the applicability of experimental findings to humans, animal models are still invaluable for elucidating folate-phenotype relationships [116].

5.3.3 Functions

Folate is an integral component of cellular processes involving one-carbon metabolism (the transfer of one unit of carbon between various molecules). These include the synthesis of the building blocks of DNA (the nucleotides adenine, guanine, and thymidine) and RNA, amino acid metabolism, methyl-transfer reactions, and metabolism of homocysteine.

Due to folate's role in cell growth and replication, its deficiency has an adverse impact on highly proliferative tissues such as the bone marrow and the gastrointestinal tract. This means that RBC synthesis and nutrient digestion are affected. Initially, folate deficiency is biochemically detected by decreased plasma folate, elevated plasma homocysteine, and inadequate supply of the methyl donor S-adenosylmethionine. After a few months, a condition known as megaloblastic anaemia[24] develops: RBC numbers in circulation begin to decrease as they reach the end of their 120-day lifespan, the cells become enlarged and show remnants of nucleic fragments, and white blood cells become abnormally shaped. This produces symptoms such as fatigue, heart palpitations, shortness of breath, and headaches. In addition, abnormalities in cells lining the gastrointestinal tract affect folate absorption, exacerbating the severity of the deficiency.

Adequate folate levels during pregnancy not only reduce the risk of having an NTD-affected fetus, but also protect against other birth defects such as orofacial (lip/palate) clefts, defects in the heart

[24] Anaemia is a condition characterised by low levels of circulating RBCs. It is classified as megaloblastic when it arises from inhibition of DNA synthesis.

and urinary tract, and Down Syndrome. Risks of adverse pregnancy and birth outcomes such as pre-eclampsia, detachment of placenta from the uterine wall, spontaneous abortion, pre-term delivery, stillbirth, and having a small baby are reduced [117, 118]. Having an optimal folate status confers benefits through the life course; it has been linked to protection against diseases in ageing such as strokes, cardiovascular diseases, certain cancers, osteoporosis, and cognitive dysfunction [28, 62, 119, 120].

An individual's folate status can be monitored in serum or RBCs. Serum folate is influenced by recent consumption of a high-folate meal or folic acid supplements, and by some medicines. RBCs accumulate folate only during erythropoiesis (the formation of mature RBCs), and so provide a better reflection of long-term folate status [121].

5.3.4 Recommended intakes

The National Health and Medical Research Council of Australia (NHMRC) publishes continually updated Nutrient Reference Values (NRVs) for Australia and New Zealand [122]. These levels indicate the recommended intakes of macronutrients, vitamins, and minerals for nutritional adequacy and for avoidance of adverse risks from excessive intake within the general population. They have been determined based on US/Canadian dietary reference intake levels and other supporting data in the literature.

The NRVs for folate are the same as those set by the US Institute of Medicine (IOM; now the National Academy of Medicine) in 1998 [101] (Table 7). The Estimated Average Requirement (EAR) specifies the average daily intake that is sufficient for nutritional sustenance or avoiding deficiency in half of all healthy individuals in a population. The EAR for folate is expressed in dietary folate equivalents (DFEs)[25].

Table 7: Nutrient Reference Values for folate and folic acid. Note that EAR refers to both folate and folic acid intake, while the UL relates to folic acid only. Data from [122].

	Estimated Average Requirement for folate (µg DFE/d)			**Upper Level for folic acid (µg/d)**	
Age	**Males and females**	**Pregnancy†**	**Lactation**	**Males and females**	**Pregnancy and lactation**
0–6 months	65*			Not possible to establish‡	
7–12 months	80*				
1–3 years	120			300	
4–8 years	160			400	
9–13 years	250			600	
14–18 years	330	520	450	800	800
19– >70 years	320	520	450	1,000	1,000

[25] A conversion factor to account for the bioavailability of food-derived folate being about 60% of that of synthetic folic acid. Thus, 1 µg DFE = 1 µg folate = 0.6 µg folic acid (taken with food). On an equivalent basis, 1 µg folic acid (taken with food) = 1.7 µg folate = 1.7 DFE.

† These values are exclusive of pregnancy-recommended folic acid supplements.
‡ Breast milk, formula, or food are recommended as the sole source of folate for infants.
* Values are the Adequate Intake, which gives the average daily intake that is assumed to be adequate in healthy individuals.

The EAR of folate for adults is 320 µg DFEs, which is equivalent to 320 µg natural folate or ~190 µg folic acid per day. Obtaining adequate amounts of folate can be achieved through a diet rich in high-folate food. Pregnant women have a much higher EAR of 520 µg DFE/d throughout the entire duration of pregnancy due to progressive increases in the rate of folate breakdown in accord with fetal growth [123], and it becomes difficult for many women to meet their EAR solely through folate-rich food sources (Table 8). This higher requirement is in addition to the medically recommended periconceptional folic acid supplement.

Table 8: Typical folate levels in folate-rich food. Data taken from [124].

Food	**Weight/measure**	**Approximate total folate (µg DFE)**	**Quantity required to achieve EAR during pregnancy‡ (520 µg DFE/d)**
Asparagus, boiled	250 ml/1 cup	130	4 cups
Beans, navy (baked beans), in tomato sauce)	250 ml/1 cup	100	5 cups
Beef liver	68 g/1 slice	172	3 slices
Broccoli, boiled	250 ml/1 cup	51	10 cups
Egg, hardboiled, medium	60 g/each	35	15
Orange, US	149 g	40	13
Spinach, boiled, English	250 ml/1 cup	200	3 cups
Bread fortified at 200 µg/100 g†	30 g/1 slice	100	5 slices

† This example is based on the voluntary (and proposed mandatory) target level of fortification in New Zealand.
‡ Exclusive of pregnancy-recommended folic acid supplements.

The WHO and many countries have set out guidelines on recommended dietary and supplement intake values for folic acid pertaining to women of childbearing age and pregnancy; the most commonly recommended supplement dosage is 400 µg/d folic acid [1, 125]. This intake increases blood folate by about 85% after 30 weeks [126]. The US Preventive Services Task Force undertook a recent systematic review on the safety and efficacy of folic acid supplementation in women of childbearing age, and reaffirmed its 2009 recommendation of a daily supplement of 400–800 µg [57, 127]. In New Zealand, the Ministry of Health recommends taking 800 µg[26] folic acid per day from at least 4 weeks before conceiving through to 12 weeks of pregnancy, in addition to having a diet inclusive of foods rich in folate or fortified with folate [130].

[26] The Ministry notes that 400 µg folic acid is sufficient to reduce NTD risk; 800 µg is recommended as it is the dose currently available as a registered medicine in New Zealand [128, 129].

Women with specific medical conditions such as obesity, diabetes, and epilepsy are at higher risk of an NTD pregnancy, and are generally advised to consume higher doses (4–5 mg/d or as medically recommended[27]).

5.3.5 Tolerable upper intake level

A central principle of toxicology is that excessively high doses of *any* substance, including essential vitamins and minerals, can have adverse health consequences. The risk posed depends on dose, an individual's sensitivity, and duration of exposure.

High intake of dietary folate has not been reported to cause any adverse effects. This is likely due in part to its low bioavailability and hence need for unrealistically high consumption of folate-rich foods to reach unsafe doses.

A Tolerable Upper Intake Level (UL) was established for folic acid by the IOM in 1998 (recent arguments questioning its validity are discussed later in this section). The UL indicates the maximum daily nutrient intake that is "likely to pose no risk of adverse effects" to nearly all individuals within the general population [101 p. 2]. It does not indicate a 'safe' level, nor is it a recommended level of intake. It was set at 1,000 µg (1 mg) per day of folic acid from fortified food or supplements, exclusive of naturally occurring food folate, for adults. Due to the lack of clinical evidence for potential adverse effects in children consuming very high levels of folic acid, the ULs for younger persons were based on the adult UL and adjusted according to relative body weight. These values have been adopted in NHMRC's guidelines on the Upper Level of intake (also known as UL; Table 7).

The UL for adults was derived from studies of patients deficient in vitamin B_{12} who were treated with high (1–30 mg/d) doses of folic acid (see Section 5.7.6). According to the IOM analysis, neurological complications almost always emerged at doses >5 mg/d, so this dose was taken as the Lowest Observed Adverse Effect Level. An uncertainty factor was then applied to provide a five-fold safety margin, giving a UL of 1 mg/d[28]. This uncertainty factor is conservative and the safety margin is considered to be large.

Both the European Commission's Scientific Committee on Food, and the UK Expert Group on Vitamins and Minerals, have since adopted the same dose in their guidelines for upper intake levels [131, 132]. The UK Group further noted the low likelihood of adverse effects arising from a total dose of 1.5 mg/d. In 2014 the EFSA performed an updated safety assessment and reaffirmed the validity of a UL of 1 mg/d [25]. Due to inadequate data, no reference values have yet been established to indicate the safe (rather than 'tolerable') upper level for daily consumption of folic acid over a lifetime.

A recent re-analysis suggests that the data originally used to set the UL were incorrectly interpreted, and that the UL therefore lacks a scientific basis [133]. Wald *et al.* found that in determining the Lowest Observed Adverse Effect Level, the IOM failed to take into account the numbers of patients in each folic acid dose group. When this factor was included, higher doses were no longer

[27] The Tolerable Upper Intake Level (UL) for folic acid of 1 mg/d (see Appendix 5.3.5) was based on effects on elderly vitamin B_{12}-deficient individuals. Given the low probability of this deficiency among women of childbearing age, the recommended dose of 5 mg/d for high-risk pregnant women is considered safe [129].
[28] Five mg/d is divided by a factor of 5 to give the UL of 1 mg/d.

associated with higher rates of neuropathological progression, and no dose-response relationship could be observed. The authors also argued that at the time that most of the studies were conducted, it was not yet recognised that the type of anaemia arising from folate deficiency could also be caused by vitamin B_{12} deficiency. Consequently, the neurological damage developed by vitamin B_{12}-deficient patients who were treated with folic acid instead of B_{12} became erroneously attributed to folic acid toxicity.

Abolition of the UL (as proposed by the authors), or its re-evaluation, is likely to have major implications for health risk assessments of folic acid, particularly in terms of the measuring or modelling UL exceedances across a population exposed to mandatory fortification. Nevertheless, because the UL relates to neurological deterioration as an adverse effect, these findings do not extend to the potential effects on cancer risk (Sections 2.2 and 3.2).

With respect to RBC folate levels, no formally accepted threshold has been established for upper levels at which there may be health consequences. References to "elevated" RBC folate levels in WHO documents are based on the assay's upper functional limits [105].

5.3.6 Neural tube defects

5.3.6.1 Types of NTDs

NTDs encompass a range of severe congenital anomalies that arise early in pregnancy when the neural tube—the embryonic structure that gives rise to the central nervous system comprising the brain and spinal cord—fails to close completely [134]. Prolonged exposure of the developing brain and spinal cord to the surrounding amniotic fluid causes tissue degeneration. The major forms of NTDs are spina bifida, anencephaly, and encephalocele (Figure 7).

Spina bifida

Spina bifida, the most common type of NTD, is caused by an opening in the spine [135]. A type of spina bifida known as meningomyelocele constitutes about 90% of all spina bifida cases. It arises when the spinal canal and backbone do not close properly, resulting in protrusion of a sac of fluid and the spinal cord through the opening in the vertebral column. The constituents of the sac are damaged, causing moderate to severe physical disabilities. The degree of severity can depend on the site of the spinal cord opening—upper (cranial/cervical/thoracic) defects are more severe than lower (lumbar/sacral) defects.

Anencephaly

Anencephaly arises when improper closure occurs at the upper end of the neural tube. It is characterised by absence of substantial parts of the brain, and abnormal skull bone formation. Anencephaly is fatal—most affected pregnancies end in miscarriage so live birth prevalence is relatively low; otherwise, it results in stillbirth or death shortly after birth.

Encephalocele

Encephalocele occurs when there is an opening in the centre of the skull. This results in protrusion of brain tissue with a sac, which may be observed from the nose, to the top of the head, to the back of the neck. This condition leads to neurological, intellectual, and physical disabilities.

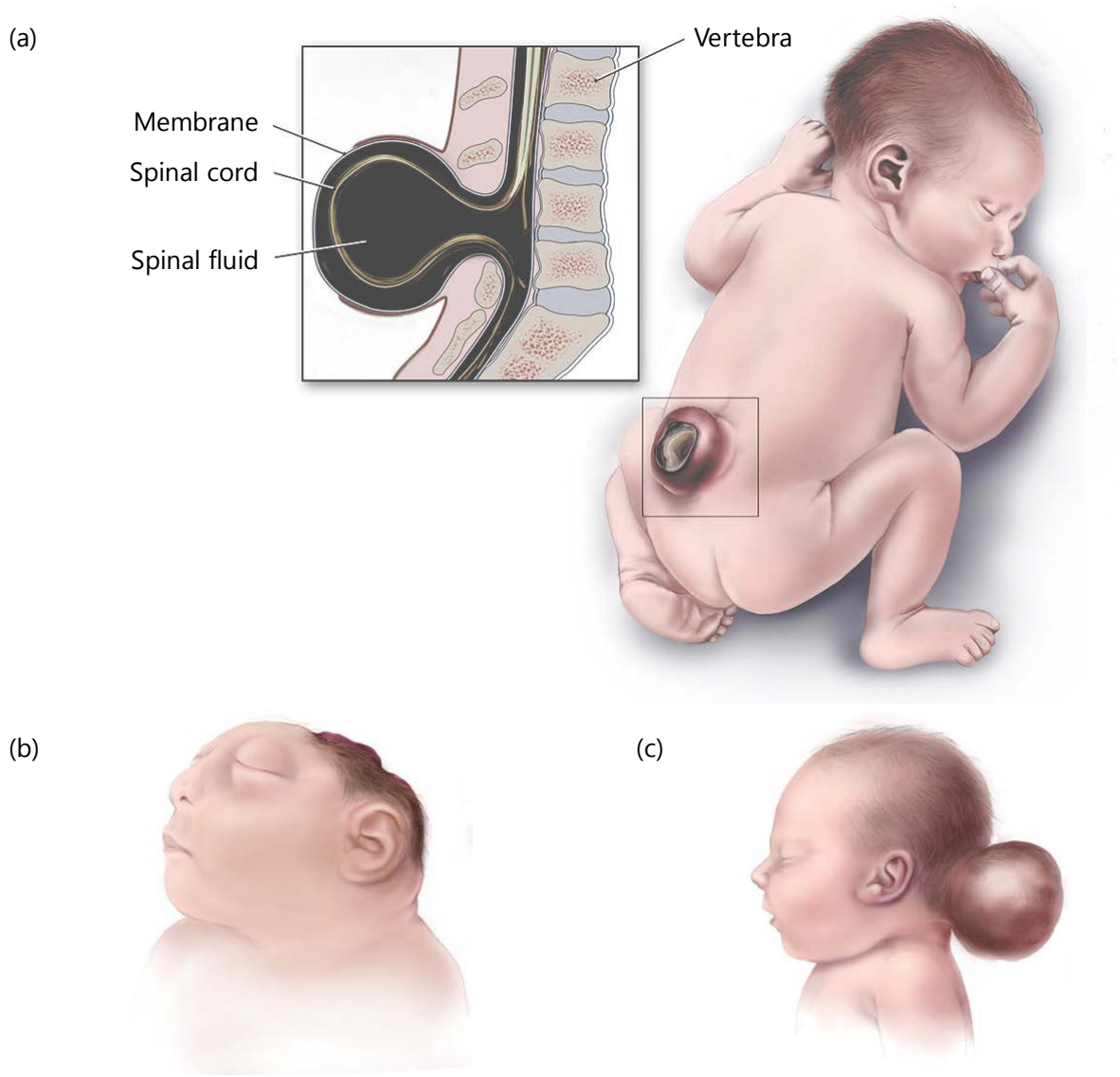


Figure 7: Clinical presentation of the major neural tube defects. (a) Spina bifida (open defect); inset: mid-sagittal section of the spine showing the protrusion containing the spinal cord and its covering membranes; (b) anencephaly; (c) encephalocele. Images courtesy of the Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities.

Fetuses affected by NTDs can be identified by an ultrasonography anatomy scan at 18–20 weeks gestation [136], and the pregnancy may be electively terminated[29]. In addition, miscarriages that can be attributed to NTDs are not notifiable events. Therefore, NTD prevalence based on observed cases among live births are underestimates. In New Zealand, terminations due to NTDs have been recorded since 2008, allowing reliable estimates of the prevalence of total NTD-affected pregnancies. Data estimates for 2008 and earlier rely on termination rates in Australia [17].

5.3.6.2 Global NTD prevalence

There is a lack of surveillance mechanisms or standardised reporting systems for NTDs in many countries. Recent systematic reviews have suggested that the prevalence of NTDs worldwide varies

[29] The termination rate in Australia in 2007–2011 was 45% [34]; the rate for the UK in 2015 was 80%, and that across the EU from 2011–2015 was 76% [137].

considerably among countries, even within the same geographical region and similar income levels [138, 139] (Table 9).

Table 9: Global prevalence of NTDs by WHO region. Reviewed studies for each country cover different cities/regions and span different time periods. Data from [138]. Recent Australia/NZ data are given for comparative purposes.

	Prevalence (per 10,000 births)†	
Region	**Range**	**Median**
Africa	5.2–75.4	11.7
Eastern Mediterranean	2.1–124.1	21.9
Europe	1.3–35.9	9.0‡
Americas	3.3–27.9	11.5
South-East Asia	1.9–66.2	15.8
Western Pacific	0.3–199.4	6.9
Australia	11.2 (post-fortification) [33]	
New Zealand	8.6 (2013 data)	

† Some data are likely to be underestimates due to exclusion of terminated pregnancies, and reasons for termination are not routinely available in health statistics.
‡ The prevalence estimate from another study—9.2/10,000 births over 2000–2010—equates to a total of 8,400 cases [140].

5.3.6.3 Folate and NTDs

The potential protective effect of folic acid in the development of NTDs has been noted since the 1960s [141]. In the early 1990s, rigorous clinical trials demonstrated a substantial reduction in NTD rates among women who adopted a periconceptional regimen of folic acid supplementation for preventing its recurrence [142] or first occurrence [143]. The strength of this evidence contributed to the 1992 US Public Health Service recommendation for folic acid supplementation to reduce NTD risk, and the New Zealand Ministry of Health has promoted this strategy since 1993. There is now also strong evidence that folic acid is effective in women who are at particular risk of NTDs [144, 145]. Because the neural tube closes by the 28th day post-conception, usually before the woman is aware that she is pregnant, and due to a high proportion of pregnancies being unplanned, all WCBA who are capable of pregnancy are encouraged to take folic acid supplements. These supplements raise blood folate levels gradually, which underscores the importance of starting this practice well before conception [146]. The folate derivative 5-MTHF has also been used to increase folate status in women [147], but evidence for its efficacy in preventing NTD pregnancies is lacking at present.

Despite its clinical efficacy, the molecular mechanism by which folate reduces the risk of NTDs are still not well understood. It has been postulated to involve folate's roles in nucleotide synthesis, methylation, one-carbon supply, and homocysteine homeostasis [148]. The relative importance of folate stores in the developing embryo versus folate availability in serum during neural tube closure also remains unknown, as does the developmental stage during which folate exposure is most crucial [149].

In addition to folate insufficiency in the periconceptional period, there is a range of other known biological risk factors for NTD pregnancies, including maternal age, family history, type 1 diabetes, obesity, vitamin B_{12} deficiency, and mutation in the *MTHFR* gene. The identification of other genetic

factors involved in folate metabolism that predispose to NTDs has met with relatively little success [148]. Behavioural and contextual factors such as smoking, socio-economic status, and area of residence have also been linked to higher risk of an NTD-affected pregnancy.

5.3.6.4 Optimal RBC folate in WCBA

Cut-off blood folate levels can indicate if an individual is folate *deficient* and is therefore at greater risk of developing megaloblastic anaemia and its associated symptoms. Cut-offs have also been established to determine the proportion of WCBA who are folate *insufficient*—that is, having folate levels that are not high enough to confer minimal risk of having an NTD pregnancy. The cut-offs for folate insufficiency are higher than that for deficiency, so women can still be folate insufficient without being folate deficient.

An RBC folate of >906 nmol/L (400 ng/mL) is the widely accepted benchmark for indicating adequate folate status in women of reproductive age (but see further in this section). This figure is derived from a 1995 study by Daly *et al.* of NTD births in Ireland, which concluded that RBC folate levels lower than 340 nmol/L conferred the greatest risk of NTDs, while levels greater than 906 nmol/L dramatically reduced the risk from 66 to 8.0 per 10,000 live births [20]. This figure was validated in a recent study in Chinese women which determined that an RBC folate level of about 1,000 nmol/L could confer minimal NTD risk (about 6 cases/10,000 births at 1,180 nmol/L) [150].

However, as noted in a 2015 WHO review of guidelines for folate sufficiency, there is still an overall dearth of data examining the link between folic acid intake, blood folate levels, and NTD rates [151]. Each of these variables may in turn be influenced by genetic and epigenetic factors, physiological status (e.g. age, pregnancy), ethnicity, and other cultural/contextual factors. The WHO has noted the need for stronger overall evidence linking RBC folate and NTD risk, but continues to strongly recommend that RBC folate concentrations among WCBA should be greater than 906 nmol/L to minimise the risk of NTD pregnancies [151]. As the cut-off is based on epidemiological data, it can only be used for assessments at the population level, and not the individual.

There are three main methods for analysing folate in food and biological tissues: microbiological, protein-binding, and chromatography. Each of these varies in specificity and sensitivity, and intra-assay variability has also been documented for microbiological assays owing to use of different calibrators [152]. While these factors are generally an accepted complication in analysis of nutritional biomarkers, to date no reference measurement procedure for RBC folate exists.

It has been recently recognised that the use of 906 nmol/L as a cut-off for determining relative NTD risk was only appropriate if the folate assay used in a particular survey was the same as that used in the Daly *et al.* study [20] from which the cut-off was derived. Indeed, there were substantial corrections to previous prevalence estimates of folate deficiency or insufficiency when data were adjusted to match the assays [153]. To this end, assay-adjusted cut-offs have been established for studies that use different methodologies; the cut-off for folate sufficiency for studies relying on datasets such as the large US National Health and Nutrition Examination Survey (NHANES) program, which routinely collects folate status data[30], is 748 nmol/L [153, 154]. This cut-off was used for the 2014/15 New Zealand Health Survey that measured WCBA blood folate status (Section 1.1.2).

[30] Employs the same microbiological assay but a different calibrator from Daly *et al.* [20]. The adjusted values are standardised to the microbiologic assay with chloramphenicol-resistant strain and 5-MTHF calibrator.

This report gives attention to whether previously published studies have used the correct cut-offs to determine prevalences of folate deficiency and insufficiency.

5.4 Folic acid fortification of food

Food fortification is generally considered one of the most effective nutritional interventions in terms of cost and reach of end users, and mandatory fortification with folic acid to mitigate the risk of NTDs has been cited as one of the most cost-effective and successful public health initiatives [155]. Mandatory folic acid fortification is not intended as a replacement for the practice of periconceptional supplementation; it is implemented as part of a multi-pronged approach that includes educational efforts to increase awareness of the importance of folic acid for WCBA, and maximising periconceptional access to folic acid supplements, to reduce NTDs across the population. Folic acid, rather than other natural forms of folate, is used in food fortification and supplements because it is more stable during food processing and more bioavailable upon ingestion.

5.4.1 Global folic acid fortification practices

Currently, 81 countries have instituted mandatory folic acid fortification of industrially milled wheat or maize flour, or rice [156] (Table 10). New Zealand and most European countries have adopted voluntary fortification programs (the exceptions are Moldova and Kosovo). While the UK does not mandate folic acid fortification, it does require wheat flour to be fortified with calcium and enriched[31] with iron, thiamine, and niacin [157]. Ireland and Scotland currently are exploring the possibility of implementing mandatory fortification of folic acid [28, 30].

Table 10: Countries mandating folic acid fortification of wheat flour, maize flour, or rice [156]

Antigua and Barbuda	Ghana	Nicaragua†
Argentina	Grenada	Niger
Australia	Guatemala†	Nigeria†
Bahamas	Guinea	Oman
Bahrain	Guyana	Palestine Occupied Territory
Barbados	Haiti	Panama†
Belize	Honduras	Paraguay
Benin	Indonesia	Peru
Bolivia	Iran	Saint Kitts and Nevis
Brazil†	Iraq	Saint Lucia
Burkina Faso	Jamaica	Saint Vincent and the Grenadines
Burundi†	Jordan	Saudi Arabia
Cameroon	Kazakhstan	Senegal
Canada	Kenya†	Sierra Leone
Cape Verde	Kiribati	Solomon Islands
Chile	Kosovo	South Africa†
Colombia	Kuwait	Suriname
Costa Rica†	Kyrgyzstan	Tanzania†

[31] 'Enriched' refers to the restoration of vitamins and minerals to the levels originally found in wheat prior to the milling process.

Cote d'Ivoire	Liberia	Togo
Cuba	Malawi†	Trinidad and Tobago
Djibouti	Mali	Turkmenistan
Dominica	Mauritania	Uganda†
Dominican Republic	Mexico†	United States of America†
Ecuador	Moldova	Uruguay
Egypt	Morocco	Uzbekistan
El Salvador†	Mozambique†	Yemen
Fiji	Nepal	Zimbabwe†

† Mandates folic acid fortification of multiple food vehicles.

Most of the countries mandating fortification are of low or middle income and have done so to address nutritional deficiencies in addition to reducing NTD rates. Countries that have adopted mandatory fortification for the primary purpose of NTD reduction include the United States, Canada, and Australia (Table 11). The US also mandates folic acid fortification of rice and enriched cereal grain products such as breads, macaroni, and noodles [158]. Fortification levels are set depending on multiple factors including levels of food vehicle consumption, fortification of other food, prevalences of folate deficiency or insufficiency, and rates of periconceptional folic acid supplement intake [159].

Table 11: Mandated levels of folic acid fortification in several high-income countries

Country	**Fortification level**	**Notes**	**References**
United States	140 µg/100 g cereal grain products	• Expected to provide lower level consumers of cereal grains with 320 µg/d folic acid from this food vehicle alone • Estimates of *increase* in folic acid intake have ranged from 100–200 µg/d	[158, 160]
Canada	150 µg/100 g flour		
Australia	200–300 µg/100 g wheat flour, for target of 120 µg/100 g bread (~3 slices)	To increase folic acid intake in WCBA by 100 µg/d (to 208 µg/d)	[33]
New Zealand (voluntary)	2007 Food Standard mandate (not implemented): 80–180 µg/100 g bread. 2014 industry Code of Practice target: 200 µg/100 g bread†	Fortification of 50% of bread at 200 µg/100 g is estimated to prevent ~11 NTD pregnancies a year to give a rate of 11.1/10,000 births (based on 2005–2009 NTD rates).	[15, 161]

† In New Zealand, folic acid is permitted to be added to a maximum of 250 µg/100 g bread to allow for losses during production [162].

A clinical trial has demonstrated that fortification to provide an additional 100 µg, 200 µg, or 400 µg/d would reduce NTD incidence by 22%, 41%, and 47% respectively [163]. In accord with this,

the US and Canada have found that increasing folic acid intake by 200 µg/d has had substantial beneficial effects on folate status and NTD prevalence [86].

5.4.2 Fortification approaches: voluntary *vs* mandatory

While voluntary fortification permits food manufacturers to add vitamins and minerals to food, mandatory fortification is a legal requirement and is undertaken when there is a compelling public health need that justifies intervention. Mandatory fortification of micronutrients is generally implemented to address a public health problem. This may be a demonstrable deficiency in the general population as measured by dietary intake and/or biochemical means, or, in the absence of observed deficiency, to a likely benefit conferred [86]. An example of the latter in New Zealand is the mandatory use of iodised salt in bread and bread products.

The levels of a fortified component in food may vary substantially depending on which regime is implemented. Mandatory fortification provides for a specific amount of the vitamin/mineral in a specific food category and has population-wide reach, while voluntary fortification can lead to widespread variability in a wide range of foods, and even within the same food category and brand, and may not necessarily reach target population groups [28]. In this regard, voluntary fortification of folic acid presents added complexities not only for monitoring programs, but also for consumers attempting to choose food products for optimal levels of micronutrient [164, 165].

The increasing use of dietary supplements can introduce further difficulties in assessing exposure amongst different demographic groups, particularly with the variations in exposure from voluntarily fortified foods [166]. An additional factor to be considered is overage, which is the practice of adding extra amounts of fortificant to offset losses during production and storage. The less restrictive levels of enforcement and regulation involved in voluntary fortification may therefore warrant continuous monitoring of food and supplement intakes, and fortification levels, to prevent exposure to excessive levels of fortificant.

In New Zealand, only one mandatory fortification standard is in place—the introduction in September 2009 of the requirement to use iodised salt instead of non-iodised salt in the manufacture of bread [167]. Although introduced at the same time as the intended start date for folic acid fortification, there was comparatively little public and political disquiet surrounding this mandatory programme. This has been attributed in part to the whole New Zealand population being at risk of iodine deficiency and therefore benefiting from the intervention.

5.5 Impact of mandatory folic acid fortification

This section summarises several issues that have been commonly raised in arguments supporting mandatory folic acid fortification of food; further comprehensive discussion can be found elsewhere (e.g. [9, 86, 168]).

5.5.1 Efficacy of behaviour change

Surveys of pregnant or post-partum women in different countries have found low rates of adherence to public health recommendations on folic acid supplementation prior to and during early pregnancy. Multiple reasons have been cited: lack of awareness of its importance; the practicalities of daily supplementation for more than 30 years, particularly if no pregnancy is

intended; non-widespread adherence even in planned pregnancies; a high rate of unplanned pregnancies; and the inconvenience and cumulative cost of purchasing folic acid tablets. Notably, studies across several countries including the US, UK, and Australia have found poor adherence to supplementation guidelines among women planning to conceive [169, 170], including those who had been previously affected by an NTD pregnancy [171, 172]. In Italy, surveys have shown that fewer than one in four women take preconceptional folic acid despite it being available at no cost [173].

In New Zealand, several studies have found that 40–64% of pregnancies are unplanned, and that a relatively low proportion of women consume periconceptional folic acid supplements even if their pregnancies were planned (Table 12). A survey of New Zealand women undergoing assisted reproduction, who therefore had much greater motivation to achieve pregnancy, found that despite high rates of supplement intake, 17% were still receiving levels lower than that recommended [174]. A 2014/15 population-wide survey of New Zealand women who have previously been pregnant showed that 48% took folic acid supplements before pregnancy, but rates were much lower among 16–24 year olds (21%) and Māori/Pacific women (31–34%) [175].

Table 12: Pregnancy planning and periconceptional folic acid supplement intake among pregnant New Zealand women

Planned pregnancies (%)	**Folic acid supplement use (%)**		**Reference**
	Planned pregnancy	**Unplanned pregnancy**	
36	53	11	[176]
44	35	2.8	[177]
56	54–56	3.3–3.6†	[178, 179]
60	58	9‡	[180]

† Of all recorded pregnancies, 67% did not practice periconceptional supplementation.
‡ Odds ratio of starting supplementation before pregnancy was 0.11 (95% CI 0.09–0.13) [181]. Of all recorded pregnancies, 16% of women took no folic acid supplements before or during pregnancy, and 61% did not take supplements before pregnancy [180, 181]. This study was designed to be ethnically and socioeconomically representative of New Zealand pregnant women.

Prescribed folic acid tablets are subsidised in New Zealand and cost $5 for a three-month supply. A 2016/17 survey of mothers, who were mostly well-educated, found that 80% had filled a prescription for folic acid and/or iodine, yet 50% still received <400 µg/d folic acid (which although is the recommended intake in some countries, is still lower than Ministry of Health guidelines of 800 µg/d) [182]. The most recent nationwide data show that, in 2015, 8.8% of all women who had a live or stillbirth used these tablets before pregnancy [183]. This is likely an underestimate of overall preconceptional folic acid use as it excludes privately purchased over-the-counter supplements such as Elevit.

A 2011 survey of postpartum New Zealand women found that 5% had specifically chosen fortified bread during the periconceptional period, and that this was not substantially higher (8%) among those who were aware of bread fortification and the preventive role of folate in NTDs [179]. Another survey of New Zealand WCBA reported that among women who were well-informed on folate and pregnancy, only half took supplements before pregnancy, and 3% chose food products for their folic acid content [22]. Hence, knowledge did not appear to translate to behavioural change.

In general, public health campaigns promoting the periconceptional use of folic acid appear to have relatively limited impact on effecting behavioural change [184]. It has therefore been argued that, by avoiding the reliance on behavioural change in a sizeable proportion of the population, mandatory fortification directly benefits women who have not planned their pregnancies, have lower accessibility to folic acid supplements, or for various reasons do not adhere to guidelines for intake. At the same time, it still provides a foundational level of folate to women who are consuming supplements.

5.5.2 Health inequities

Behavioural patterns of pregnancy planning, consumption of voluntarily fortified food, pre-pregnancy use of folic acid supplements, and use of folic acid-containing supplements for general health purposes are known to be influenced by sociodemographic variables such as age, ethnicity, and income level. Voluntary fortification is considered to have a weaker impact on disadvantaged groups who may receive less exposure to educational campaigns or delayed medical advice on the importance of periconceptional folic acid, and be less likely to consume multivitamin folic acid-containing supplements for general health purposes. Because of this, mandatory fortification may reduce social inequities in NTD-related health outcomes [185].

New Zealand research supports this premise: a 2010 nationally-representative survey of 1,000 WCBA found that those who were less informed on the importance of folate for pregnancy tended to be younger, less educated, and not of European ethnicity [22]. A separate survey of postpartum women found that belonging to less advantaged demographic groups, including being young, single, of Māori and Pacific ethnicity, having lower income and education, and having an unplanned pregnancy were predictors of both low awareness and low practice of periconceptional folic acid supplementation [178, 179]. However, these groups were also more likely to consume at least 3 slices of bread/day. Modelling suggested that mandatory fortification of bread at a modest level of 135 µg/100 g would essentially abolish disparities in levels of periconceptional folic acid intake [178].

A review of Australian data prior to mandatory fortification found that increases in folic acid supplement use and decreases in NTD prevalence favoured those of higher educational/socioeconomic status, and of non-Aboriginal heritage, respectively [186]. However, mandatory fortification has dramatically reduced the NTD burden on Aboriginal women in particular. A 2006 report from the UK SACN concluded that relying on voluntary fortification of certain foods and individuals' supplement use has further exacerbated existing variation in folic acid intake across the population, with lowest intakes in younger, socioeconomically deprived women, and highest intakes in consumers of high dose supplements [74]. In comparison, mandatory folic acid fortification of flour and avoidance of high dose supplements would have reduced the risks of excessive intake by offering greater control of dosage and being consumed more consistently across the population.

5.5.3 Societal, familial, and individual costs

Individuals affected by NTDs usually have greatly reduced life expectancy and severe, lifelong physical and cognitive impairments [187]. Each individual bears a high burden of living that has significant physical, social, emotional, and financial costs. These costs have flow-on effects on their families that further impose an economic burden on society. Women (and their families) who are

carrying an NTD-affected fetus face the difficult decision of whether to terminate their pregnancy; choosing to do so can exact a high toll on psychosocial and mental health. Similar consequences may be experienced by women whose fetuses miscarry or are stillborn. A 2012 estimate by MPI puts the lifetime economic burden of NTDs (discounted to the year of birth) at >$3.7 million for each live birth and >$5 million for each stillbirth or terminated pregnancy [17]. Therefore, although relatively rare, the cumulative psychic and financial costs of NTDs can be considerable. These costs could be dramatically reduced by employing what is already a well-established public health strategy to achieve primary prevention of many to most cases of NTDs.

5.6 Contemporary issues surrounding mandatory fortification

This section summarises several issues that have been commonly raised in arguments opposing mandatory folic acid fortification of food; further comprehensive discussion can be found elsewhere (e.g. [84, 188, 189]).

In New Zealand, a 2017 consumer survey reported a clear preference for voluntary folic acid fortification among the general population, with more than half (56%) of respondents (61% of WCBA) in support of voluntary practice and 23% (19% of WCBA) favouring a mandatory program [190]. In 2011, the respective numbers for all adults were 54% and 29% [191].

5.6.1 Safety

There have been longheld concerns about the safety of folic acid fortification relating to off-target health effects. There is ongoing debate on whether folate has a preventive or promotional effect on cancer onset and progression, in particular cancer of the colorectum, prostate, and breast (Appendix 5.7.5.1; [192-196]). Although this issue is far from resolved, removal of the element of choice by mandatory fortification may pose challenges for individuals who may wish to consume unfortified bread.

Concerns have also been raised about folic acid exacerbating neurological and cognitive impairment, or obscuring undiagnosed vitamin B_{12} deficiency. There has also been debate about the health impact of excess folic acid in the circulation in populations exposed to fortification, although no toxicological issues have been identified. Other recent studies have suggested that hypersensitivity-related outcomes (e.g. allergy, asthma, and atopic disease) and insulin resistance, relating to maternal folate status/folic acid exposure during pregnancy, warrant further attention.

In addition, there are multiple population subgroups that may be at greater risk with exposure to higher levels of folic acid such as the elderly, children, vegetarians or vegans in whom vitamin B_{12} inadequacy is more common, or even those who practise carbohydrate loading for endurance events. Regular consumers of folic acid-containing supplements are a potentially important at-risk group (see Section 2.8).

5.6.2 Philosophical

Some opponents to mandatory fortification perceive it as a form of ‘mass medication’ of food supply [197]. Others view such legislation as an impingement of rights with respect to the individual consumer’s right to choose [22]. In New Zealand, there tends to be a greater perception of some public health measures as political interference by a ‘nanny state’ [198]. In a 2017 consumer survey

of New Zealand adults, a major reason cited by those preferring voluntary over mandatory fortification was the preservation of choice [16].

5.6.3 Commercial

The deferment and revocation of mandatory folic acid fortification in New Zealand was, in part, due to concerns raised by the baking industry [199]. These included setup, operational, and compliance costs associated with ensuring compliance with dosage, adequate record keeping, and lost sales in an already declining market, although the latter has not been supported by an independent survey conducted by MPI [22] or published in the scientific literature [179].

5.7 Health outcomes of folic acid exposure

5.7.1 Recent comprehensive reports on the safety of folate and folic acid

In recent years, several reports from committees and organisations based in different countries have been released to provide an up-to-date overview of the health benefits and risks of folic acid. This section describes the specific purpose of each report and their methodologies and coverage.

In 2015, the US ***National Toxicology Program (NTP)***[32] released a monograph arising from a rigorous systematic literature search identifying research areas of high priority for evaluating the safe use of high intakes of folic acid [27, 200]. These areas were prioritised based on literature reports of adverse effects arising from folate intake exceeding 400 µg/day or from biomarkers of blood folate levels above that deemed deficient. Size and quality of the studies were also taken into account. A steering committee with expertise in folic acid and health provided input. The reviewed health outcomes encompassed cancer, cognition, hypersensitivity, and thyroid/diabetes-related disorders; UMFA was not considered. Subpanel expert groups for each research area were then charged with identifying in the literature both areas of consistency and research gaps at the pre-clinical (*in vitro* and animal) and clinical (human) levels, and proposing specific approaches to address uncertainties within the current evidence base. Literature search updates were conducted up to 1 December 2014, and data extraction was performed to consolidate the information for cross-study comparison and ease of interpretation. The information has been summarised in the Health Assessment Workplace Collaborative (HAWC; https://hawcproject.org), a web-based content management system for risk assessments relating to human health.

The ***Norwegian Scientific Committee for Food Safety (VKM)***'s Panel on Nutrition, Dietetic Products, Novel Food and Allergy undertook a risk assessment of folic acid in supplements, with a focus on cancer and UMFA, to evaluate if the UL of folic acid required amendment. Its systematic search included studies from 2009 to October 2014, and its report was published in 2015 [26].

In 2017, the UK ***Scientific Advisory Committee on Nutrition (SACN)*** published a report providing a comprehensive review of new evidence on potential adverse health impact of folic acid [30]. It was undertaken to inform an update to its 2006 and 2009 risk assessments and recommendations regarding the introduction of mandatory folic acid fortification [74, 194], and its evidence reviews are considerably more detailed than other organisations' reports. The health effects were chosen

[32] An interagency program, part of the National Institutes of Health (NIH)'s National Institute of Environmental Health Sciences, that tests and evaluates substances in the environment of public health concern.

based on previous identification in earlier risk assessments, in other reports, and from public submissions. These were masking of vitamin B_{12} deficiency, cognitive decline in elderly, cancer, and UMFA. Literature searches were conducted up to 28 June 2016, covering publications from 2005–2016.

Analysis was restricted to healthy populations and not those receiving folic acid treatment for an existing condition. Except for UMFA, where studies were individually assessed due to the smaller evidence base, only systematic reviews and meta-analyses were considered, with the most recent meta-analysis given greater weight for providing the most updated and reliable overview of the evidence. Studies examining the effect of folic acid in combination with other B vitamins (primarily B_{12}) were included to avoid excluding many important meta-analyses.

Four further publications, presented as narrative reviews, have independently reassessed the safety of folic acid with respect to:

1. Establishment of Dietary Reference Values for folate (by the ***European Food Safety Authority* (*EFSA*)**'s Panel on Dietetic Products, Nutrition and Allergies in 2014) [25];
2. Consideration of permitting the voluntary fortification of corn masa flour (by the US ***Food and Drug Administration* (*FDA*)** in 2016)[33] [29];
3. Review of fortification strategies to prevent birth defects (by the ***Food Safety Authority of Ireland* (*FSAI*)** in 2016 [28]; and,
4. Monitoring the effectiveness of mandatory fortification in Australia (by the ***Australian Health Ministers' Advisory Council* (*AHMAC*)** in 2017 [31].

In its Scientific Opinion, the EFSA evaluated cardiovascular disease, cancer and all-cause mortality, and cognition. The FDA safety review conducted a literature search covering scientific publications from 1998–2015. FSAI performed a limited summary of the evidence on undiagnosed vitamin B_{12} deficiency, UMFA, and cancer, drawing its conclusions largely from NTP, earlier SACN reports [74, 194], and other recent literature reviews. AHMAC conducted an updated literature review in 2017 to determine the range of identified potential adverse effects; it identified cancer, cognitive/neurological dysfunction, interaction with medication, UMFA, reproductive impacts, offspring impacts, and non-prenatally induced hypersensitivity.

At the time of writing, a Cochrane systematic review was underway that aimed to examine the benefits and possible risks of folic acid supplementation in women of childbearing age. The primary outcome measures were blood status of folate, vitamin B_{12}, homocysteine, and haemoglobin, and all types of cancers. The protocol for this review was published in 2015 [201].

A recent technical report published in November 2017 has reviewed the analyses by the NTP and SACN, as well as subsequently published studies. It concluded that the overall evidence did not establish risks of adverse effects arising from mandatory fortification [202].

[33] The FDA document reviewed in the 2017 SACN report (*FDA Memorandum for food additive petition: Folic acid in corn masa flour (2016)*) is not available online or elsewhere (FDA, pers. comm., 16 August 2017) and was therefore not reviewed in this Report.

5.7.2 Epidemiological study designs for long-term health outcomes

The four most common epidemiological study designs for assessing the health effects of an exposure—interventional, observational, genetic, and ecological—differ in the strength of evidence for causal effects.

5.7.2.1 Interventional studies

Interventional trials of participants randomised to consume folic acid-containing supplements comprise much of the scientific evidence on the safety of folic acid. These types of studies have long been considered the 'gold standard' of rigorous, minimally biased evidence as they can avoid confounding factors and reveal causal effects [203].

However, they are subject to several limitations. Ethical, logistical, and expense considerations tend to place limits on study size, trial duration, and subsequent follow-up, which may miss the typically long lag time between initiation of a tumour and its progression to clinically present as cancer. Further, with respect to fortification, trials generally do not mimic fortification scenarios as they often involve doses of folic acid higher than estimated increases in intake from fortified food. It may be difficult to untangle the specific effect of folic acid as it is often combined with other vitamins such as B_{12} in trials, in which cancer is often a secondary or exploratory outcome. There is also uncertainty about whether ingestion of folic acid as a single bolus dose or spread over several meals of fortified foods leads to differential absorption and metabolism patterns, such as in relation to UMFA.

Clinical trials may instead indicate an upper level of risk at various exposure levels, and pinpoint areas for further monitoring/research should there be suggestion of any adverse effects. They may also identify thresholds at which an effect is observed. However, depending on the populations being studied, it is not always possible to completely distinguish between exposure to supplements or fortification.

5.7.2.2 Observational studies

Observational studies, specifically cohort studies of 'free-living' populations, measure dietary folate consumption or blood folate levels, and then monitor health outcomes such as cancer. Their potential advantages include large study size and long follow-up duration, making them an appropriate complement to RCTs. The key disadvantage is confounding of results by other factors that correlate with folate consumption/levels, such as smoking and socioeconomic status, that are in turn associated with risk of an adverse health outcome. Thus, any observed association of folate with a negative health effect may be spurious. Although study data can be adjusted to reduce confounding, this requires that the factors are known and measured, and in reality confounding can never be completely eliminated in observational studies. A further disadvantage is that the measurement of folate may often be made a considerable time after the actual period of exposure that has mechanistically initiated an altered health outcome.

In general, despite inherent limitations in assigning causality, observational studies can give insights to associated health effects that warrant deeper investigation. Dietary folate/folic acid intake, although an indirect proxy for underlying folate status, was recently validated as a good marker in those with higher folate status [204]. Differentiating between folate intake from food, and folic acid intake from fortified food and supplements, and ascertaining the relative contribution of each to total folate intake, can be particularly informative given their cumulative biological effect.

5.7.2.3 Genetic studies

Genetic association (or Mendelian Randomisation; MR) studies exploit variations in folate levels in the population due to mutations in the genes responsible for folate metabolism (or that are robustly associated with circulating folate levels). MR is based on Mendel's law of random assortment where individuals inherit their genes (and corresponding mutations) from parents by chance [205, 206]. This means that folate levels are essentially randomised at conception, which minimises both confounding and reverse causality. MR is a type of 'instrumental variable' analysis that allows estimates to be made of the folate-disease association. These studies have had great influence on clinical practice [207], such as by demonstrating that high density lipoprotein did not actually cause coronary heart disease [208], resulting in the withdrawal of high density lipoprotein target drugs from the market.

In the context of folate, an extensively studied mutation (polymorphism) in the gene coding for the enzyme MTHFR, often referred to as C677T[34], is robustly associated with lower folate status. This effect is graded, such that having two, one, or no copies of the mutated gene leads to, respectively, relatively lower to higher blood folate levels. Comparisons in health outcome among the individuals with different copy numbers of the mutated gene allows testing for causality—and effect size—between folate exposure and disease risk [209]. This approach has been validated in relation to NTDs, where mothers or newborns with the TT genotype are more likely to be affected by NTDs [111, 210]. *MTHFR* mutations have been used to study the relationship between homocysteine and coronary heart disease [211], and the evidence base from MR studies for the folate-cancer relationship is still emerging.

Compared to RCTs, MR studies can be performed in much larger populations in which duration of exposure is essentially lifelong (since the genome is set from conception and is immutable through life). They are also generally less likely to be affected by confounding or by reverse causation, two biases pervasive in observational studies. However MR studies are still subject to several limitations[35]. For example, it is not known whether the gene encoding MTHFR is pleiotropic (i.e. the mutation also affects other biological processes unrelated to folate metabolism that in turn affect cancer risk), or whether it is another co-inherited allele, perhaps even in another gene, that may be the causative factor [30, 83].

Secondly, results may be biased by a type of confounding factor known as population stratification; this occurs if the mutation is highly variable within various population subgroups, and disease rates differ for these groups for reasons other than prevalence of the mutation [213]. Population stratification can be adjusted for, but, as with observational studies, the variables need to be identified and measured.

Nonetheless, MR studies potentially provide evidence for stronger causal inferences between nutritional exposures and health outcomes. The NTP has recommended further research to identify genes other than *MTHFR* that could be used in MR studies to assess the potential health effects of folic acid.

[34] The mutation, which is one of the most extensively studied of *MTHFR*, involves replacement of cytosine with thymine at position 677. Another mutation is of adenosine to cytosine at position 1298.

[35] The limitations and assumptions of MR studies, together with strategies for strengthening causal inference, are discussed in detail elsewhere [48, 212].

5.7.2.4 Ecological studies

These broadly refer to population-level studies that compare rates of disease before and after the introduction of fortification (i.e. so-called 'natural experiments'). This assumes that fortification has resulted in a large enough change in folate intake for the population as a whole, and that the lag time between the change in intake and putative changes in disease rates is short. Some diseases, such as many cancers, have a longer lag time of up to decades. Furthermore, concurrent changes in other factors such as diet, improved diagnostics, and introduction of disease screening programmes can confound interpretations and lead to false associations.

Thus, while able to inform on health trends, ecological studies have very limited use in monitoring the safety of fortification programmes because it is impossible from such studies to demonstrate a causal effect, and because of the multifactorial nature of cancer and many other non-communicable diseases. Ecological studies should generally be considered in the context of meta-analyses that review higher quality RCTs, genetic studies, and cohort studies.

5.7.3 Assessing the health effects directly attributable to food fortification

Although there is a large evidence base for the health risks of folic acid supplements or of high dietary folic acid/circulatory folate, there is comparatively less data on the effects that can be specifically attributed to food fortification due to numerous potential confounding factors. In populations exposed to mandatory fortification, intervention studies that can control for these factors are inherently precluded; observational and ecological studies therefore serve as the main study tools, but are limited by lack of a control group within the same population. In meta-analyses, quality assessments to determine risk of bias in included studies cannot be reliably applied to ecological studies, which may introduce biases relating to measurement of the effect and its latency [38].

In situations of voluntary fortification, interventional studies to determine a causal relationship between fortification and health outcomes of interest require longitudinal analysis of large numbers of participants, in whom folic acid intake will need to be tightly controlled, folate levels closely monitored, and health status needs to be assessed over the life course at least until disease onset. This poses obvious logistical challenges.

In the absence of robust data, some alternative approaches are available. Firstly, comparisons may be made between countries adopting different policies regarding fortification—also a natural experiment—but the reliability of insights gained is dependent on the assumption that both populations share similar characteristics such as diet, culture, and genetics. On this basis, New Zealand data may be compared with Australian data to infer the potential differences in impact of voluntary versus mandatory fortification. Secondly, an Interrupted Time Series analysis can evaluate health interventions at the level of the population by continuous observations over a duration that spans the pre- and post-intervention period [214]. This approach, which involves modelling and statistical analysis, underpins the monitoring programmes in many countries implementing mandatory fortification, as there is a clearly delineated intervention point. However, it is most appropriate for monitoring health outcomes with a shorter lag time such as blood folate levels or NTD rates, and not cancer.

At the time of writing, a Cochrane systematic review is underway to assess the health benefits and safety of folic acid fortification of wheat and maize flour on folate status and population health

outcomes, with a focus on at-risk subgroups [215]. There is otherwise an absence of prior Cochrane and other publications on the health outcomes specifically from folic acid fortification of food.

5.7.4 Neural tube defects

5.7.4.1 Mandatory fortification

Global

A 2013 systematic review of studies from nine countries pre- and post-fortification unequivocally demonstrated that mandatory fortification is associated with reductions in spina bifida, anencephaly, and overall NTD prevalence [5] (Figure 8). Mean reductions for the outcomes ranged from 31–36%. An updated 2017 synthesis covering studies from 13 countries has reinforced these findings [6, 216]; decreases in NTD occurrence following fortification ranged from 1–78%, with greater declines seen in countries with a higher baseline prevalence.

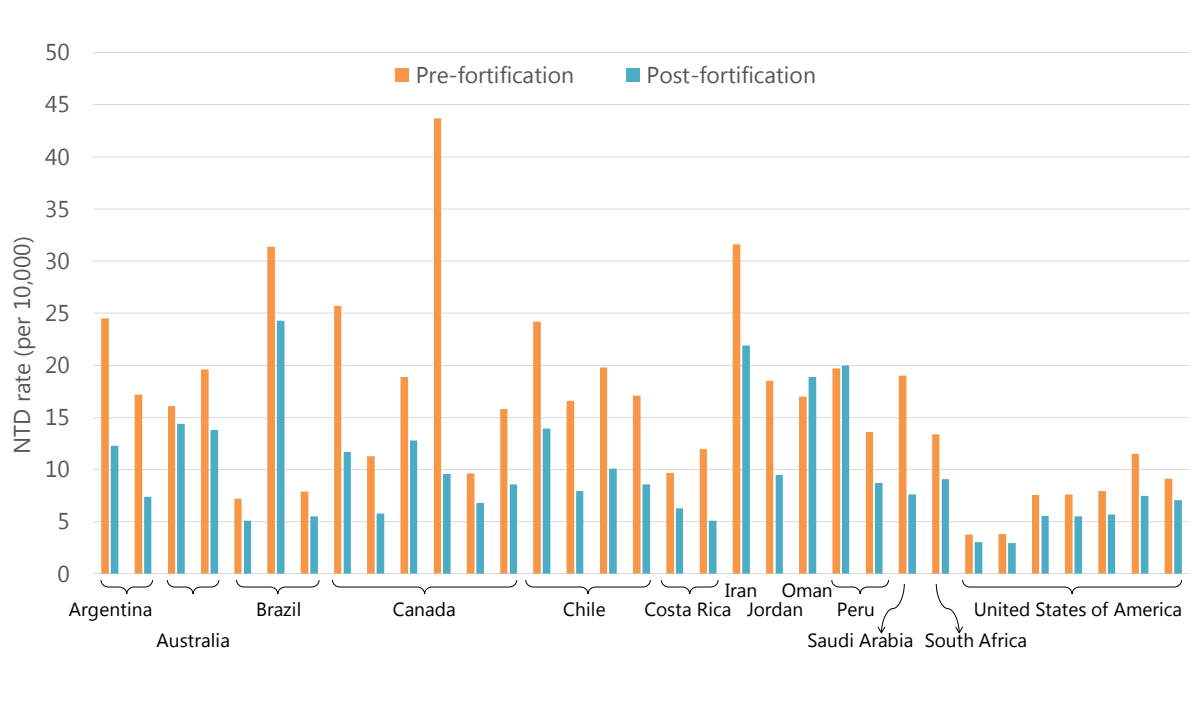


Figure 8: Declines in prevalence of NTDs in countries mandating folic acid fortification of flour. Variability in prevalence decreased after fortification, from a range of 3.8–43.7/10,000 births to 3.0–24.3/10,000 births, and the declines in most studies were statistically significant [6]. Each pair of bars represents data from one study; most of the countries shown have datasets from more than one study. Figure modified from [216], with permission.

A systematic review and meta-analysis has analysed the global prevalence of spina bifida by fortification status, geographic region, and pregnancy outcome (live/stillbirth or termination) [217]. It showed that regions with mandatory fortification had lower prevalence of spina bifida compared to those with voluntary fortification, and this finding held whether measured by live births or pregnancies. Regional differences were found that could be attributed, at least in part, to different practices in fortification.

United States

In the US, NTD prevalence decreased after the introduction of mandatory fortification and then stabilised for at least 13 years (Figure 9). Recent birth defects data from California corroborated the trend, with overall NTD prevalence lower than that pre-fortification, but showing a slowed decline after mandatory fortification [218]. These observations may be indicative of a 'floor' level being reached, but it was also postulated that rising rates of maternal obesity, as seen in the Californian women with NTD pregnancies, may be a contributing factor [218].

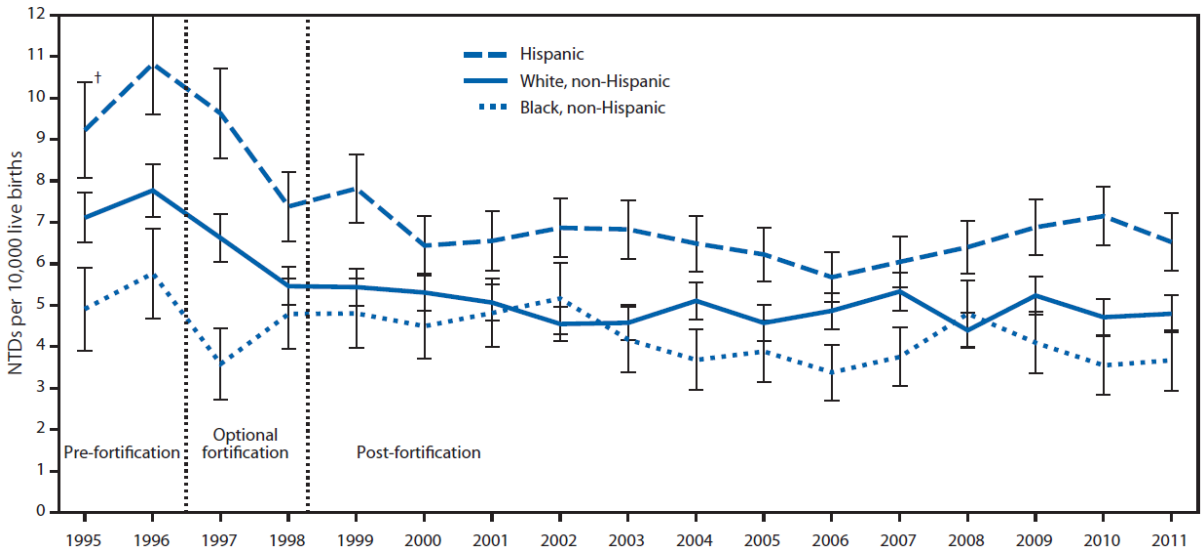


Figure 9: Trends in NTD prevalence in the US by maternal ethnicity. Image courtesy of Centers for Disease Control and Prevention, reference [32].

Several reasons have been suggested to account for the higher NTD prevalence among Hispanic women, even as rates have fallen in the post-fortification era. These include dietary differences such as lower consumption of bread in favour of corn masa-based diet, lower rates of folic acid intake via fortified food or supplements, and higher occurrence of the *MTHFR* gene mutation that promotes folate insufficiency [127, 219]. Modelling studies have been performed that estimated increases in folic acid intake and reductions in NTD prevalence among Hispanics if corn masa flour were fortified in addition to wheat flour [220, 221]. These factors, together with an updated evidence review of folic acid safety, underpin the 2016 FDA decision to permit the voluntary fortification of corn masa flour with folic acid [29].

A recent evidence report and systematic review for the US Preventive Services Task Force (USPSTF) found clear evidence of the beneficial effects of supplementation in studies conducted pre-fortification, but those conducted post-fortification were inconsistent in demonstrating a protective association [56, 57]. Interpretation of this finding is partially limited by differences in the quality of studies undertaken pre- and post-fortification—none of the studies published post-fortification were RCTs or prospective studies using cohorts; only case-control studies that have attendant biases in case ascertainment and recall that may lead to underestimation of a potential effect were available. The USPSTF findings suggest that mandatory fortification may be preventing the majority of folate-responsive NTDs and thus attenuating the benefits of supplementation [149]. However on the balance of all evidence of benefits and harms, and the quality of studies reviewed,

the USPSTF has continued to recommend that all women planning or capable of pregnancy take 400–800 µg folic acid per day [127].

Several modelling analyses have quantified the health impact of mandatory fortification: a 2015 estimate has placed the total number of NTD-affected births prevented annually at 1,326 [32], while another analysis has estimated the annual number of prevented live-born spina bifida cases at 767, for a conservative annual cost saving of USD607 million [222].

Australia

Australia has implemented voluntary folic acid fortification of bread and other food products since 1995 [223]. This was coincident with a decline in NTD rates from 1995–1998 [96]; the decline subsequently continued at a lower but steady rate of 0.2/10,000 births per year from 1998–2008 [224]. The Australian Institute of Health and Welfare (AIHW) monitoring report found that NTD rates over the pre-mandatory fortification period (2006–2008), the transition period during which the milling and baking industry began fortification, and the post-mandatory fortification period showed a graded decline of 12.8, 11.8, and 11.2 pregnancies/10,000 births[36] [33]. Overall, the post-fortification fall in NTD prevalence of 14.4% (RR 0.86; 95% CI 0.74, 0.99) was in accord with previously projected declines of 4–16%. The predicted number of prevented NTD pregnancies per year was 26 (range of 14–49); post-fortification modelling work estimated that 32 cases were prevented, but given the already declining NTD rates before the intervention, the actual number directly attributable to mandatory fortification was estimated at 14 [31].

Age and indigenous status appeared to be critical modifiers of NTD risk. There was a progressive increase in protection with younger maternal age. Pre-fortification, the rate among Indigenous women was 111% higher than for non-Indigenous women; fortification led to a 74.2% decrease for the former group (RR 0.26; 95% CI 0.12, 0.55), while the decrease for the latter, at 9.1% (RR 0.91; 95% CI 0.78, 1.1), was not statistically significant [33]. Post-fortification, the NTD rate among Indigenous women was at least 40% lower than among non-Indigenous women. It is not clear why there are ethnic differences in the protective effect of folic acid fortification. However, these observations are similar to those of the US population, where Hispanic women were at greater risk of NTD pregnancies before fortification, but experienced the largest relative protective effect with mandatory fortification [32].

NTD severity

There are emerging suggestions from ecological studies of mandatory fortification in the US and Canada that folate may have a role in reducing the severity of NTDs (Section 2.1). Recent data from the Netherlands support this premise, showing that preconceptional supplementation markedly shifts the proportion of spina bifida cases from the more to the less severe types [225]; of all NTD pregnancies, supplementation was associated with 6% of more severe cases and 51% of less severe cases, while the respective proportion of cases correlating with lack of supplementation was 26% and 30%.

[36] Statistics from New South Wales are underestimates due to under-detection and missing data. Therefore the figures omitting NSW data better reflect absolute measures of NTD risk, and those including NSW data are more reliable for determining proportional differences [33]. Figures cited in this Report include NSW data except for these three data points reporting absolute risk.

5.7.4.2 Voluntary fortification

This section considers both actual health outcomes and those modelled from the proposed implementation of mandatory fortification in other countries. Because voluntary fortification is not a specific intervention, there is usually no clearly defined period marking changes in folic acid intake in the population. It is therefore difficult to make pre- and post- voluntary fortification comparisons except by use of modelling data.

Global

Modelling work using global data has estimated that in 2015, just 13.2% of cases of folate-responsive spina bifida and anencephaly were prevented by mandatory fortification [41]. That is, nearly 87% of all folic acid-preventable NTD cases worldwide, or an estimated 233,233 affected births, occurred due to the lack of mandatory fortification. These numbers are likely to be substantial underestimates as they were derived from data that excluded stillbirths and elective terminations of affected pregnancies.

Europe

Nearly all countries in Europe do not mandate folic acid fortification. A study using the comprehensive European Surveillance of Congenital Anomalies (EUROCAT) database collated birth registry data for about 12.5 million births in 19 of these countries, finding that the prevalence of total NTDs (comprising live births, late miscarriages, and terminations) had not decreased from 1990–2011 [39]. However, live birth prevalence of NTD showed marked decreases over the two decades, likely due to antenatal diagnosis and increased terminations of pregnancy.

United Kingdom

Across Scotland, Northern Ireland, and Wales, the prevalence of NTD-affected pregnancies showed no decrease from 1998–2012 [40]. It was conservatively estimated that the implementation of mandatory fortification over that timeframe could have reduced the prevalence of NTD-affected pregnancies by 21% (or 2,014 pregnancies) [40]. Recent modelling data from Food Standards Scotland show that a combination of mandatory flour fortification scenarios, such as limiting the level of folic acid permitted in voluntarily fortified food, can reduce NTD pregnancy risk without placing greater numbers of people over the UL [226].

Ireland

In Ireland, where NTD prevalence had been amongst the world's highest, estimated rates of NTD-affected pregnancies fell from 12.5–18.8 to 9.3 per 10,000 births between 2001 and 2006 [164]. This was attributed to increased amounts of folic acid in the food supply. Plans for the mandatory folic acid fortification of staple foods were postponed in 2006 in favour of continuation of a liberal voluntary fortification policy. However, the latest data suggest an increase—albeit not statistically significant—in NTD prevalence, from 9.2/10,000 in 2009 to 11.7/10,000 in 2011[37] [28, 227], concurrent with decreased fortification levels in some foods (see Section 5.7.4.3).

[37] Reliable data from more recent years is unavailable due to tightening data protection regulations [28].

5.7.4.3 Dietary intake/blood folate status

Australia

The AIHW monitoring report found that mandatory fortification led to a marked increase in bread folic acid levels from 20–29 µg/100 g bread to 134–200 µg/100 g, which exceeded the target level of 120 µg/100 g [33]. Population-wide folic acid intakes were estimated post-fortification to determine exceedances of the UL. Among adults, UL exceedance increased from <1% to 1%, although estimations were based on a 1995 nutrition survey and did not consider supplement use. The proportion of children who exceeded their UL increased, and to a greater extent than projected—among children aged 2–3, the proportion increased from 5% pre-fortification to 21% post-fortification (projected increase of 9%), and among 4–8 year olds, the proportion increased from 3% to 15% (projected increase of 4%). The report considered that this was unlikely to pose a health risk because (1) the UL's built-in uncertainty factor allows for a five-fold safety margin; (2) the UL is derived from high-dose intakes in elderly adults with vitamin B_{12} deficiency, which is rarely seen in young children; and (3) children are exposed to levels of relative excess over a relatively short period over the whole life course [33].

The proportion of WCBA with inadequate folate intake decreased from 11 to 1%, or from 64 to 22% if measured against pregnancy intake guidelines. These data were determined using the EAR; additional folic acid supplements are still recommended to minimise NTD risk. It was not anticipated that inadequate folate intake would be entirely abolished given that folic acid consumption via fortification is not intended as a substitute for supplementation during pregnancy.

To measure serum and RBC folate levels, the Australian folate status survey used a specific assay for which no appropriate cut-off values for deficiency and insufficiency have been determined (Appendix 5.3.6.4). Therefore, the proportion of women with (in)sufficient folate status with respect to NTD-pregnancy risk could not be determined. Given that no pre-fortification samples were taken from this cohort, the AIHW has used serum folate levels from a separate survey undertaken prior to fortification as a proxy for baseline levels. The mean serum folate among women in their mid-twenties to mid-thirties was 27 nmol/L and 33 nmol/L pre- and post-fortification, respectively, although interpretation is limited by differences in assays used and participation bias [33].

Data were also available from a hospital laboratory that measured serum and RBC folate in the months preceding and following fortification. The trends in serum and RBC folate levels were suggestive of improved folate status with fortification, and decreases in prevalence of low serum and RBC folate (from 9.3 to 2.1%, and 3.4% to 0.5% respectively) were found [228]. The distribution of serum folate levels within the population showed a distinct shift to the right, suggesting an effect on the population as a whole. Although the blood samples were not representative as they were obtained for testing of possible folate deficiency, the relative changes suggest an effect of mandatory fortification.

Data on the folate status of Indigenous women and of teenagers pre- and post-fortification, that can be directly compared to the rest of the population, is not available.

United States

Within the United States general population, the introduction of mandatory fortification was associated with sharp increases in RBC and serum folate levels (1.5 times and 2.5 times, respectively)

[229]. Levels then decreased by 12–17% over a further 12 years. Prevalence of folate deficiency, which affected some demographic subgroups more than others pre-fortification, dropped to ≤1% throughout the whole population, and folate-deficiency anaemia among elderly individuals was almost completely eliminated [230].

Before mandatory fortification was in place, 59% of WCBA did not meet the optimal RBC folate levels for minimising NTD risk. Following fortification, this dropped substantially to 15%, but rose to about 23% in 2007–2012 [153, 154]. Risk factors for being folate insufficient included not only well-established ones such as smoking, obesity, poverty, and not using folic acid supplements, but also obtaining folic acid solely from cereal grain products (to the exclusion of cereals and supplements) [231].

Post-fortification data on dietary intake of folic acid from fortified foods and supplements showed that ≤3% of individuals aged 14+ exceeded the UL [44]. However, infants and children up to age 8 were more likely to exceed their UL especially if they consumed supplements. Rates of exceedance in this group ranged from 39%–58% depending on age and sex.

United Kingdom

Population-wide blood folate data from England, Scotland, Wales, and Northern Ireland were collected between 2008 and 2013 [45]. The mean RBC folate among all WCBA was 614 nmol/L (compared with the cut-off of >748 nmol/L for folate sufficiency), with just 25% of all women meeting the threshold. Younger women tended to have lower folate levels than older WCBA; the proportion of women who were folate sufficient was 19% among those aged 16–24 years, 25% for those aged 25–34 years, and 28% for those aged 35–49 years [45]. The proportion of folate-sufficient WCBA was 17% in Northern Ireland, 19% in Scotland, and 21% in Wales.

Ireland

In Ireland, following the 2006 deferral of mandatory fortification, the range and consumption of folic acid-fortified foods further increased, contributing to increased folic acid intake among WCBA [232]. Those who consumed the highest levels (95th percentile) reached just 44% of the UL. However, although increased consumption of fortified foods was linked to improved folate status in the general population, 66% of all WCBA still did not meet RBC folate levels for maximal protection against NTDs. Among women consuming moderate amounts of fortified foods, only one-third were able to reach the cut-off for optimal RBC folate [28, 233].

More recent data have shown that both coverage and levels of fortification have since decreased in several food staples, in particular fat/dairy spreads and some breads [28, 234]. Blood samples taken during 2008–2012 showed that 83% of WCBA were folate insufficient—a similar proportion to New Zealand WCBA [45]. Recent surveys of pregnant Irish women at their first antenatal visit found that 33–62% were folate insufficient [235, 236].

Germany

A large German survey found that just 2% of WCBA took folic acid supplements at the recommended dose of ≥400 µg/d. Modelling of food consumption data showed that, without fortification or supplementation, 4.8% of WCBA reached their recommended intake of folate [237]. Fortification of a variety of food products could raise this to 52.9%, with a low proportion of women (1.3%) exceeding the UL. Fortification could also enable 95.4% of WCBA supplement users to meet

the recommended intake, with 5% exceeding the UL. No recent data on German women's RBC folate levels are available[38].

5.7.5 Cancer

The development of cancer involves several sequential steps: first is tumour initiation, where a normal cell is changed to become capable of forming a tumour; then tumour promotion, where the cell survives and replicates; and finally tumour progression, where growth and invasiveness is greatly enhanced. Progression through the latter stages can take many years and therefore remain undetected in short-term studies.

5.7.5.1 Role of folate in carcinogenesis

Due to the role of folate as a carbon donor in the synthesis of nucleotides for DNA replication (Figure 6), the growth of tumours—being highly proliferative tissues with increased requirements for DNA—may be promoted in the presence of high levels of folate. Indeed, it is this principle that underlies the anti-tumour chemotherapeutic activity of compounds known as antifolates; these agents inhibit key enzymes in the folate metabolic pathways and so disrupt the ability of the tumour to grow [239]. Historic human studies from the 1940s, on which the development of antifolate agents was based, showed that very high folate supplementation increased progression and relapse rates in different types of leukaemia [196]. In accord with this, animal studies have shown that folate deficiency suppresses the progression—and induces regression—of pre-existing tumours [193].

Yet at the same time, there is experimental, clinical, and epidemiological evidence showing that in normal tissues and healthy individuals, cancer development is suppressed by folic acid supplementation and promoted by a diet deficient in folate [240-242]. There is also observational epidemiological evidence linking higher (but not excessive) folate status to lower risk of cancer at numerous sites, especially the colorectum, and to a lesser extent, the breast [196, 242]. These apparently paradoxical findings are in fact still consistent with the role of folate in DNA replication and repair. A deficiency in folate leads to incorporation of incorrect building blocks in newly synthesised or repaired DNA, which in turn leads to gene mutations and chromosomal breakage. Folate administration reverses these effects [243].

The proposed dual role of folate in cancer development suggests that under most circumstances, higher folate intake is protective against cancer, except for those who have pre-existing tumours or their precursors and are consuming extremely high levels of folic acid in supplements [242]. It also suggests that, even among individuals who do not have pre-existing tumours, the dose-response relationship between folate and cancer risk is unlikely to be linear. That is, higher risk is seen with both folate deficiency and excessive exposure from supplement use [244]. The key question that remains unanswered is what the size of the effect for each of folate's opposing roles.

Folate is also a key participant in DNA methylation, one of the fundamental cellular mechanisms that control gene activity. Folate is required for the generation of S-adenosylmethionine, the universal methyl donor for DNA methylation. Aberrant DNA methylation is found in all forms of cancer, although current understanding of the role of folate in epigenetic dysregulation leading to

[38] A recently published study of nearly 200 German WCBA has reported on the prevalence of folate inadequacy [238], but these data cannot be interpreted as an incorrect cut-off was used (see Appendix 5.3.6.4).

tumour formation remains incomplete [245]. It is thought that the lower availability of methyl groups leads to a build-up of S-adenosylhomocysteine, which inhibits methylation. Decreased methylation has been linked to chromosomal instability. It has also been observed in lymphocytes of folate-deficient humans, but can be reversed when adequate folate levels are restored [246]. On the other hand, increased methylation at tumour suppressor genes leads to their inactivation and drives tumour formation [247].

5.7.5.2 Limitations of the evidence base

The ***SACN*** found that the pattern of inconsistency across the evidence base for all cancer types was partially attributed to the differences in study types (interventional, observational, and genetic), data heterogeneity within a study type (for example due to wide variations in folate intake), and small numbers in the study groups (and as a consequence the study may lack statistical power—the ability to be confident that the findings are not due to chance).

The available RCTs provided limited information as they were predominantly not designed to detect associations between folic acid and cancer risk. Most did not have large enough numbers of participants, or involve a sufficiently long treatment duration and follow-up time to accommodate the long latency period of cancer. Indeed, the SACN observed that newer evidence from RCTs and observational studies did not yield any firmer conclusions than their previous two literature assessments.

The ***NTP*** literature review yielded a raft of recommendations of research areas at the pre-clinical and clinical levels that needed more elucidation. Clinical research needs included:

- Clarifying whether the increased risk suggested in some studies was contributed by certain subgroups, such as those of a particular age range, having certain genetic characteristics, or having pre-existing tumours;
- The need to follow-up on participants in previous trials to investigate longer-term outcomes;
- The need for new observational studies to identify subgroups at particular risk for tumour progression (as distinct from tumour initiation);
- Determining if the chemical form being ingested (folic acid or other folate derivatives) plays a role in cancer progression; and
- Determining if other nutrients involved in similar metabolic pathways to folate may influence the potential for folic acid to cause tumours. This is due to trials that suggested an adverse effect when folic acid was supplemented with other nutrients.

It was also agreed that experimental studies using more appropriate animal models were indispensable for following up on suggestive data of adverse effects in humans, and better understanding the dual beneficial/adverse effects that folic acid may exert.

The ***VKM*** found that only one meta-analysis considered the use of folic acid as the sole supplement component. However it has recognised the same limitations that preclude a conclusive assessment of the safety of supplementation, namely the inclusion of other vitamins with folic acid in RCTs; cancer not being the primary endpoint for most supplementation studies; and the inability of most studies to account for the long latency of cancer.

The ***EFSA*** similarly remarked that a long follow-up duration was imperative for clarifying the relationship between folic acid and cancer risk, particularly with respect to the dual effect in cancer progression.

5.7.5.3 Selected recent studies

A case-control study has examined the potential dual effect of folate by comparing serum folate levels in individuals with colorectal cancer with those of healthy individuals, and with individuals harbouring colorectal adenomatous polyps (AP; a precursor of colorectal cancer) [248]. Having higher serum folate levels was associated with a marked increase in colorectal cancer risk, but only for those with AP and not healthy subjects. This supports the dual nature of folate's role in colorectal cancer development, and can be reconciled with SACN's evidence summary that there is no clear effect.

A study investigated the link between serum folate and risk of mortality from overall cancer, cardiovascular disease, and all-causes six years later [249]. It found increased risk of mortality from all three causes only at the lower levels of serum folate, and that mortality was no greater at the highest folate levels, except for those aged <60 years. Of note, increased mortality risk was seen even at folate levels above clinically-defined levels of deficiency.

A very large study pooling data from 10 European countries investigated the relationship between dietary folate intake and risk of breast cancer, and found a marginally significant protective effect [250]. A limitation of this analysis was the absence of data on folic acid supplement intake, although it was known that supplement intake in this study group was low. The results are somewhat consistent with SACN's evidence summary.

Data from the same cohort also showed that plasma folate levels were not associated with breast cancer risk [251]. There was also no association between having the *MTHFR* mutation and breast cancer risk. The latter finding does not agree with the SACN summary, which found that the mutation was a breast cancer risk factor, but could be explained by the difference in folate levels in the study population: the highest levels of folate were still substantially lower than that in a US cohort for which higher plasma folate had a protective association with breast cancer [252].

An RCT showed that folic acid had no effect on risk of total cancer, of specific cancer types including colorectal and breast, or on cancer mortality [253]. Instead, it had a significant protective effect on cancer incidence in individuals with both low folate status and the *MTHFR* gene mutation. Overall, the findings are in accord with the SACN evidence summary.

5.7.5.4 Selected notable studies

This section discusses several studies that have elicited debate within the scientific community, and played a role in government or other organisations' risk assessments of folic acid.

In 2007, ***Cole et al.*** [79] published the results of the first large RCT investigating the effect of 1 mg/day folic acid supplementation on the risk of colorectal adenoma[39] among patients with a

[39] An adenoma is a benign (non-cancerous) tumour of glandular tissue; it is distinct from a carcinoma which is malignant (cancerous) and arises from the epithelial cells that line the body's cavities, blood vessels, and organs. Adenomas occasionally transform towards malignancy, and become known as adenocarcinomas. Adenomas in the colon, also known as adenomatous polyps, are a frequent occurrence; they are precursors to colorectal cancer and may be removed as a preventive measure.

history of colorectal cancer, over a concurrent treatment and follow-up period of up to eight years. This dose is about 5–10 times the estimated increase in folic acid intake from mandatory fortification (Table 10). No benefit of folic acid versus placebo was seen, with participants at similar risk of developing at least one adenoma after 3 years (RR 1.04; 95% CI 0.90–1.20) or at the second follow-up a further 3–5 years later (RR 1.13; 95% CI 0.93–1.37). Instead, the data indicated that participants who were receiving folic acid were, at the second follow-up, at increased risk of advanced lesions (RR 1.67; 95% CI 1.00–2.80), with a more than two-fold risk of developing at least three adenomas (RR 2.32; 95% CI 1.23–4.35). Further, subgroup analysis suggested a higher incidence of non-colorectal cancers—in particular of the prostate—among supplement consumers. ***Figueiredo et al.*** [254] subsequently estimated that the risk of prostate cancer diagnosis during a 10-year follow-up was greater than two-fold with folic acid treatment versus placebo (multivariable adjusted HR 2.58; 95% CI 1.14–5.86). However, this was based on a very small number of cases (n=34), and consideration of confounding by aspirin co-supplementation was deemed inadequate [194].

Several aspects of the Cole *et al.* study [79] require specific attention. First, for methodological reasons, the study recruited only participants who have previously had an adenoma, and excluded those with no similar prior history. Thus, the trial examined the capacity of folic acid to reduce rates of adenoma recurrence but not first adenomas—that is, secondary, but not primary, prevention. It did not test for cancer-protective effects of folic acid when taken as a primary preventive measure [46]. Second, trial participants had had a complete colonoscopy, with all detected polyps removed, and were therefore unlikely to be representative of the general population. Consequently the study cannot illuminate on the effects of folic acid on existing polyps, and indeed this important issue remains unanswered to date [255]. Nevertheless, the Cole *et al.* study has been recognised as robust, and has prompted greater caution on the overall assessment of folic acid and cancer risk. Examples include FSAI's 2008 recommendation to defer the introduction of mandatory fortification pending updated safety data [164], as well as SACN's 2009 recommendation for appropriate guidance on supplement use and population monitoring should mandatory folic acid fortification be undertaken in the UK [194].

A 2009 report from ***Ebbing et al.*** [80] described cancer and mortality outcomes from two RCTs of Norwegian ischaemic heart disease patients treated with 800 µg/d folic acid and vitamin B_{12}. This population was not exposed to folic acid food fortification, and vitamin supplement use was relatively low (23%). Treatment over a median of 39 months was associated with higher risk of cancer diagnosis (HR 1.21, 95% CI 1.03–1.41), cancer mortality (HR 1.38, 95% CI 1.07–1.79), and all-cause mortality (1.18, 95% CI 1.04–1.33), at a median follow-up duration of 38 months.

A closer examination of the data has shown that the total cancer incidence and pattern of cancers were reflective of the general Norwegian population, with a 25% higher incidence of lung cancer observed, likely due to the high proportion of former and current smokers [80]. Omission of lung cancer data decreased the relative risk of other cancer incidence (HR 1.16, 95% CI 0.98–1.37). The apparent impact on lung cancer was unexpected given the absence of prior—and subsequent [256]—evidence implicating an effect of folic acid. Of note, risk of colorectal cancer incidence and mortality were unaffected, and the 99% CIs for other cancer subtypes included the value of 1.

Vollset et al. [257] performed a meta-analysis, published in 2013, of 13 randomised trials that reported incidence of cancer in patients supplemented with folic acid for prevention of cardiovascular disease or for colorectal adenoma. The median daily dose was 2.0 mg, and mean

weighted treatment duration was 5.2 years. No statistically significant effects were found on overall incidence of cancer of any kind (excluding non-melanoma skin cancer; rate ratio 1.06), or of site-specific cancers including colorectal, prostate, breast, or lung. Notably, treatment duration bore no correlation with effect size.

Concerns have been raised about some results of this analysis. Although not statistically significant by the conventional definition of $p<0.05$, there was modest increase of 6% in overall cancer incidence, and the lower bound of the confidence interval (95% CI 0.99–1.13, $p=0.10$) prompted suggestions that the analysis lacked sufficient power to detect significance at that difference [258]. In addition, the estimate for prostate cancer—1.15 (99% CI 0.94–1.41, $p=0.07$)—was consistent with up to a 6% decrease in prostate cancer or a 41% increase in prostate cancer with folic acid supplementation.

Vollset *et al.*'s analysis [257] found that the slight increase in the point estimate for overall cancer incidence did not correlate with treatment duration or folic acid dose. With respect to follow-up duration, cancer risk was lowest after 1 year (RR 0.89; 95% CI 0.74–1.07), highest after 2 years (RR 1.16; 95% CI 0.96–1.40), and then progressively decreased through to at least 6 years' follow-up. It is not clear if this represents statistical noise (trend: $\chi^2_1=0.54$; $p=0.46$) or is a true reflection of cancer initiation and progression [259]. However, the doses used in the studies (median 2.0 mg/d) were greater than that typically found in vitamin supplements (0.1–0.8 mg), and nearly 17 times the estimated increase in daily intake from fortified bread (~120 µg)[40] at the New Zealand target fortification level of 200 µg/100 g bread. Thus, the slight increase in risk, if real, may not affect members of the general population but those who ingest folic acid from both fortification and high dose supplementation. It then becomes imperative to determine the fraction of population belonging to the latter group.

Vollset *et al.*'s conclusions were largely corroborated by a 2014 meta-analysis by ***Mackerras et al.*** of cancer and all-cause mortality from 26 folic acid supplementation RCTs [88]. This study was used by the Australian Institute of Health and Welfare to assess potential adverse health effects in the Australian population following mandatory fortification. It found no direct association between folic acid intakes in adults and risk of cancers of the colorectum, prostate, breast, lung, or overall cancer, or of all-cause mortality. In addition, there was little evidence of an association with recurrence of advanced (RR 1.11, 95% CI 0.87–1.42) or any (RR 0.97, 95% CI 0.83–1.14) colorectal adenoma.

It is also of note that there was no evidence in both meta-analyses that supplementation conferred *protection* against any site-specific cancers.

However, it must be noted that the follow-up durations of the RCTs reviewed by Mackerras *et al.* mostly ranged from 1–3 years (maximum of 7.3 years). Longer treatment times and follow-up durations are needed to determine if folic acid supplementation increases risk of cancer during and beyond the later phases of cancer development (that is, tumour promotion and progression). This may explain the findings of elevated risk of cancer from folic acid supplementation by Ebbing *et al.* [80], which allowed for a median total follow-up of about 6.5 years.

[40] Calculated assuming average consumption of about 60 g bread per day, as used in MPI modelling estimates [14 p. 7].

Thus, the current analyses of randomized trials both provide some reassurance (null findings from meta-analyses of shorter trials) and raise some concerns (a longer duration study finding some elevated risk). The challenges of relying on shorter duration RCTs to investigate cancer outcomes are well established [260].

Due to the potentially dual nature of the relationship between folate and cancer, it has been suggested that analyses involving very large populations (e.g. [250, 257]) may be underestimating the number of cancer cases attributable to folic acid intake [261]. The basis of this argument is that the (putative) increased risk for the subgroup of patients in whom folic acid has caused proliferation of pre-neoplastic cells, is masked by the larger population that is protected from cancer, leading to an overall null hazard ratio [250]. This thesis has received weak epidemiological support. In a study of a Chinese population largely unexposed to fortification or supplementation, there was a suggestion that pre-diagnostic plasma folate levels were positively associated with colorectal cancer risk (OR 1.33; 95% CI 0.90–1.98 for highest vs. lowest tertile of folate levels) [262]. This association was stronger for the subgroup of men with late-stage colorectal cancer (OR 2.66, 95% CI 1.03–6.86 for highest vs. lowest tertile of folate levels). A Swedish study found a protective role for low plasma folate against colorectal cancer risk among patients with a shorter follow-up period (less than the median of 10.8 years)—that is, who were undiagnosed or potentially had pre-neoplastic lesions [263].

Since it is not possible in epidemiological studies to determine and control for the timing of tumour initiation, studies on folic acid-induced tumour progression have mostly been performed in rodent models, but these can differ from human cancer in several respects. For example, a widely used rat model relies on the chemical 7,12-dimethylbenza[a]anthracene to induce tumours, and unlike humans, rats do not exhibit mutations in the tumour suppressor genes *p53* and *Brca* [264].

5.7.5.5 Ecological studies

Concerns about the potentially adverse effect of mandatory folic acid fortification on cancer have in large part stemmed from ecological studies that purported to find a link between increased cancer incidence and the start of fortification.

A 2007 study investigated secular trends in colorectal cancer incidence in the US and Canada, and related these to commencement of mandatory fortification [265]. Colorectal cancer incidence in both countries had been undergoing a steady decrease, but showed a rebound that was coincident with the start of fortification, leading the authors to propose a causal link between the two phenomena. The increase in incidence was, however, subsequently followed by a decline while fortification levels remained the same, in keeping with pre-fortification trends (Figure 10a, Appendix 5.7.5.6).

It was speculated that the temporary rebound in colorectal cancer incidence observed by Mason *et al.* [265] may be a manifestation of folate's dual effect on cancer development [261]. That is, in individuals with pre-existing preclinical neoplastic lesions, higher folic acid intake promoted lesion growth and proliferation, advancing them to present clinically and elevating the apparent incidence of colorectal cancer. In contrast, individuals without such lesions could have been protected from neoplastic initiation, and this was eventually reflected in the later decline in incidence.

However, others have suggested that the near absence of lag time in reversal of the trend lacked biological plausibility. No significant effects on incidence could be detected in several meta-

analyses of folic acid supplementation trials on colorectal cancer using greater doses and lasting longer than 3 years [88, 257]. Further, a subset of data examining overall cancer incidence by year of trial follow-up did not show any temporal coincidence between risk and duration of exposure to high-dose folic acid [257]. In addition, trends in mortality from colorectal cancer showed no deviations for at least 12–14 years post-mandatory fortification [257, 266], suggesting that the temporary increase in incidence may have been an artifact of increased detection and diagnosis. A SACN Working Group noted that improved screening could give rise to an immediate but temporary increase as incidence rates return to baseline levels after all cases become identified, and that a 1995 US policy endorsement of newer screening methods may provide an explanation [194]. However, no conclusions could be drawn given the lack of sufficient data on screening rates over time. Empirical data on the effect of high folate levels on neoplastic growth in humans is essential to resolve this debate [259].

It has since been recognised that the conclusions drawn from the ecological studies have failed to account for several factors [267]. Firstly, molecular and epidemiological data show that there is a long induction period between improvements in folate status and potential protection against colorectal cancer, with estimations that a minimum induction period of 12–16 years is necessary to uncover any protective (or adverse) effect. Secondly, it is unknown if a 1973 FDA regulatory change permitting an increase in the daily maximum folic acid dose in supplements and food from 100 to 400 μg, has impacted on colorectal cancer rates particularly in an ethnicity-dependent manner. Finally, changes in colorectal cancer screening rates over time may influence mortality and cancer incidence trends in ways that are only becoming understood. Taking consideration of these factors, Keum and Giovannucci [267] have reexamined secular trends in colorectal cancer incidence and mortality rates in the US by stratifying data by age, ethnicity, and sex. They observed a paradoxical phenomenon of decreasing mortality despite a temporary rise in incidence rates, which was proposed to reflect an effect of screening rather than that of risk factors that would instead increase both mortality and incidence rates. They concluded that the rebound reported by Mason *et al.* [265] was unlikely to be attributable to folic acid fortification, given the absence of a similar pattern among supplement users following the 1973 FDA regulatory change, but also the presence of a similar bump among white elderly men for whom screening was likely to have a larger effect.

Ecological studies are limited by an absence of data on folic acid intake in individuals. A very large US study tracking folate and folic acid intake pre- and post-fortification found that higher intake—whether from food or from supplements—had a protective effect on risk of colorectal cancer [268]. Adjustments to control for increased colorectal cancer screening and consequent detection and removal or precancerous lesions did not alter this conclusion. Furthermore, no link between total folate intake and colorectal cancer risk was seen among individuals with a history of colorectal polyps. However, it was not known if these individuals had polyps that were benign or capable of progressing into colorectal cancer, and if under-reporting had occurred [269]. The post-fortification follow-up period of 8–8.5 years may also have been unable to take into account the latency of colorectal cancer development.

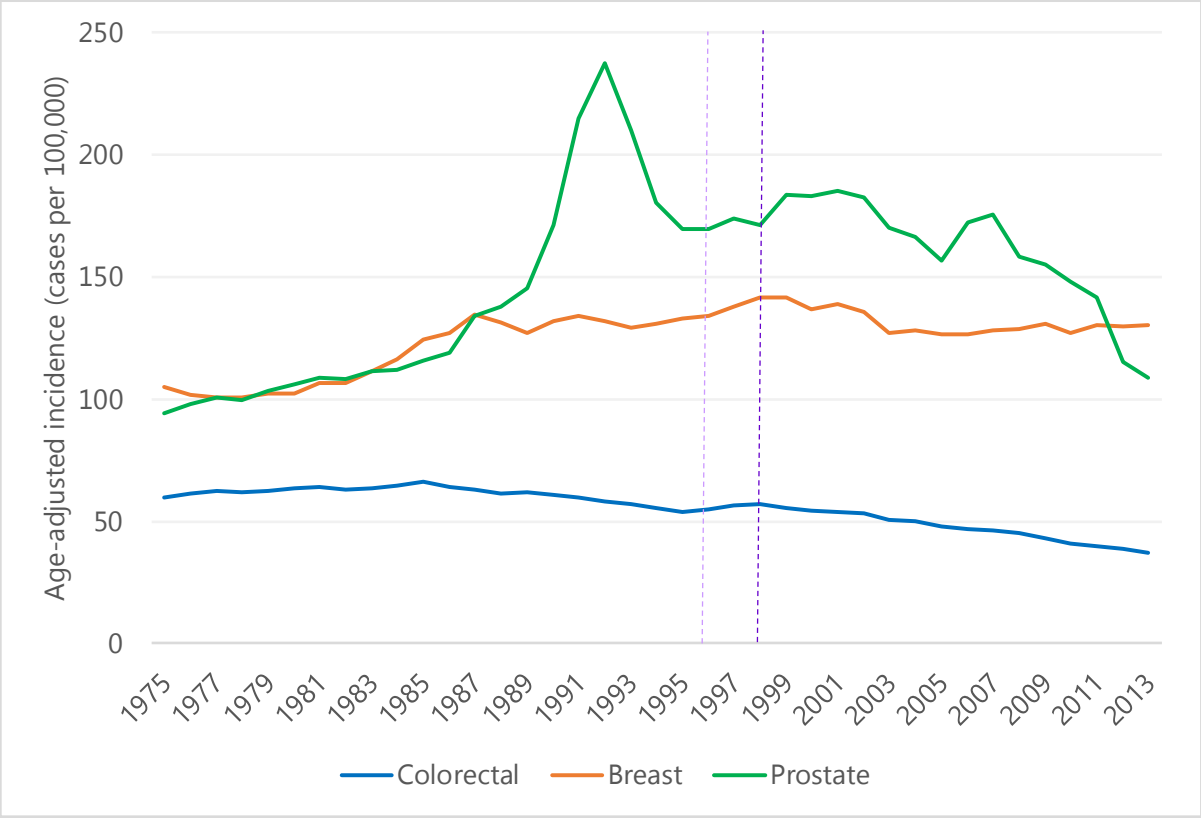
It has been about two decades since mandatory folic acid fortification was first introduced in the US and Canada. Longer term cancer incidence data show that the rebound was temporary, supporting the non-causal nature of the association (or, at least, that a new equilibrium has been established).

A study in 2009 reported trends in hospital discharge rates from colon cancer in Chile, where mandatory fortification of flour has been implemented since 2000 [270]. Discharge rates were used as a proxy for cancer incidence due to absence of a national cancer registry. Marked increases were found among those aged 45–79 years. These data have been difficult to interpret owing to poor study quality. Data are missing between 1996 and 2002, which encompasses the period immediately pre- and post-fortification, and the time scales were not corrected for the omission. The methodological flaws in this report do not permit meaningful conclusions to be drawn.

5.7.5.6 Cancer trends

Figure 10 shows the trends in rates for several common cancers over the pre- and post-mandatory fortification period in the US.

(a)



(b)

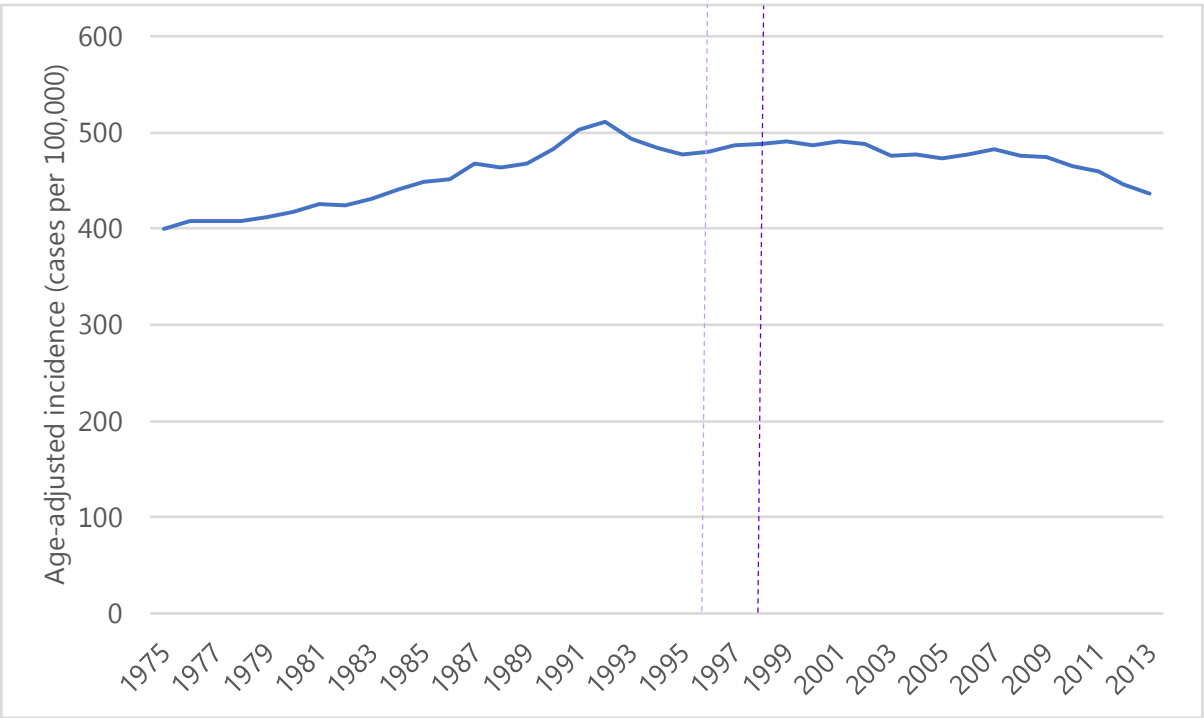
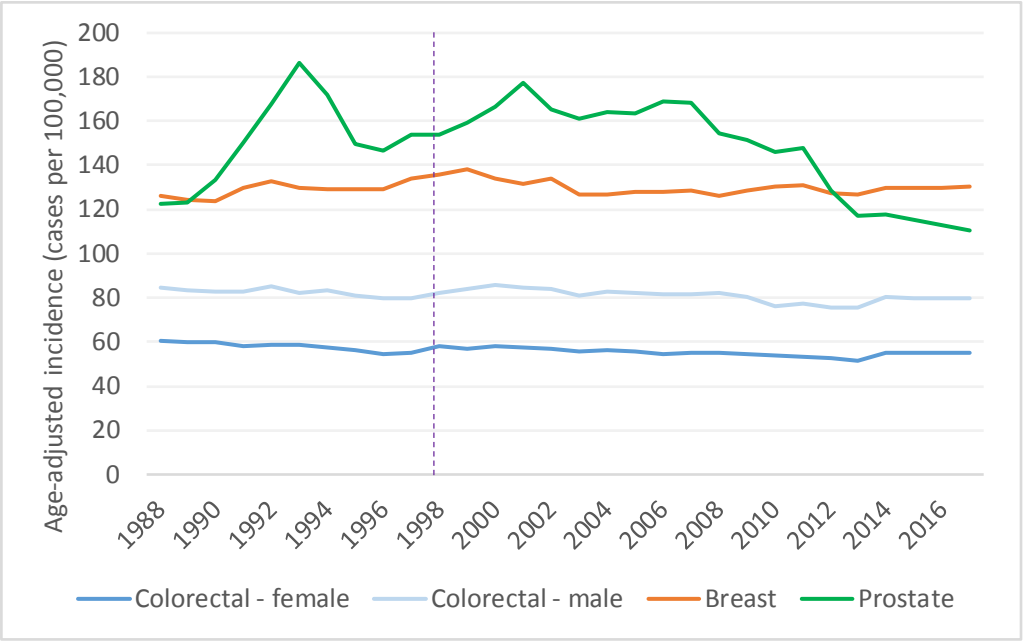


Figure 10: Rates of new cases of several common cancers in the US over the pre- and post-fortification period. (a) Colorectal, breast (female), and prostate cancer. The temporary 'rebound' at around 1996–1998 for colorectal cancer (Section 5.7.5.5) becomes less discernible in the context of a longer period; (b) all cancer. The light and dark purple dotted lines indicate the pre- and post-mandatory fortification period, respectively. The two-year interim period allowed for optional fortification. Data plotted from [51].

Canadian Cancer Society statistics show similar trends to the US, albeit less pronounced (Figure 11) [52].

(a)



(b)

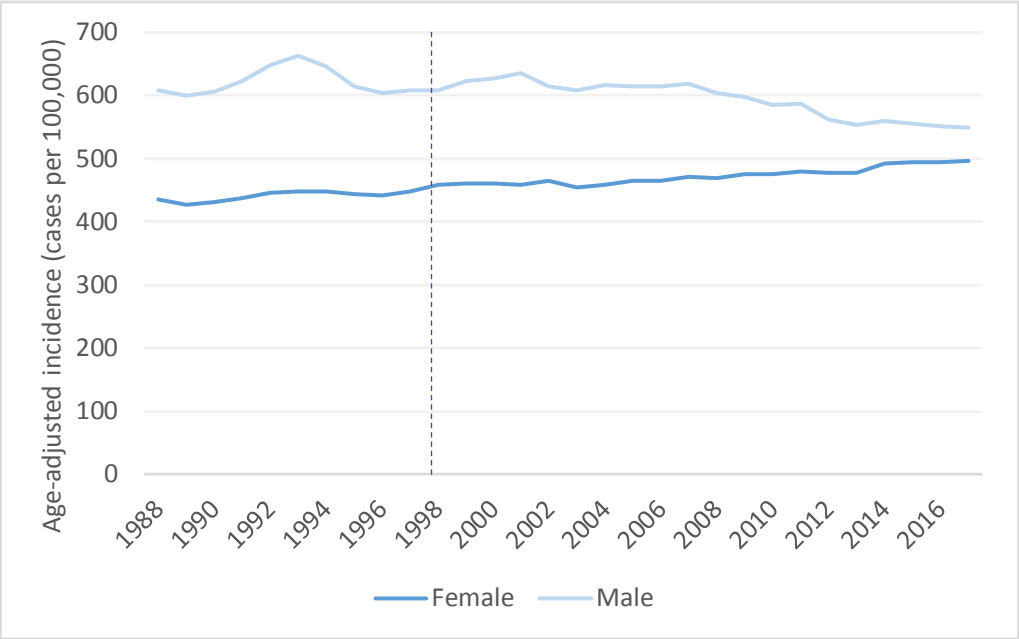
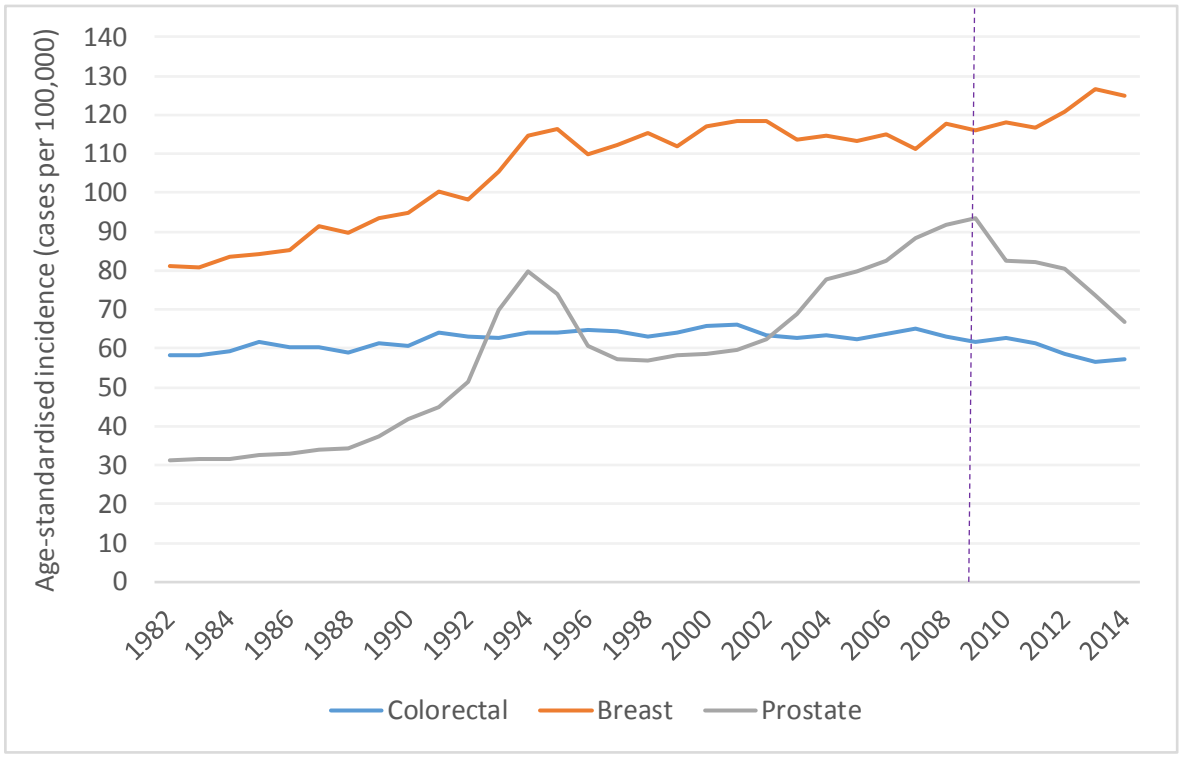


Figure 11: Rates of new cases of common cancers in Canada over the pre- and post-fortification period by sex. (a) Colorectal, breast (female), and prostate cancer; (b) all cancer. Purple dotted lines indicate the year in which mandatory fortification was introduced. Data plotted from [52].

Incidence of colorectal cancer has shown annual decreases of 1.6% since 2008 for males, and 0.8% since 2000 for females; prostate cancer incidence has decreased annually by 1.8% since 1992, and by 5.3% since 2007; and female breast cancer has shown no statistically significant changes since 1992. For all cancers in males, annual decreases of 0.8% were seen since 1992, with greater decreases of 1.7% since 2007; females showed a slight annual increases of 0.4% since 1992. Notably, the temporary rebound reported by Mason *et al.* [265] has been abolished with longer term trend data now available. There also appears to be no correlation between the commencement of mandatory fortification and the year in which cancer trends have changed. The Society noted that despite the multifactorial nature of cancer, peaks seen in incidence trends for each cancer type could be partially explained by increased waves of screening.

Figure 12 shows rates of common cancers in Australia. Longer-term data are needed to better discern post-fortification trends.

(a)



(b)

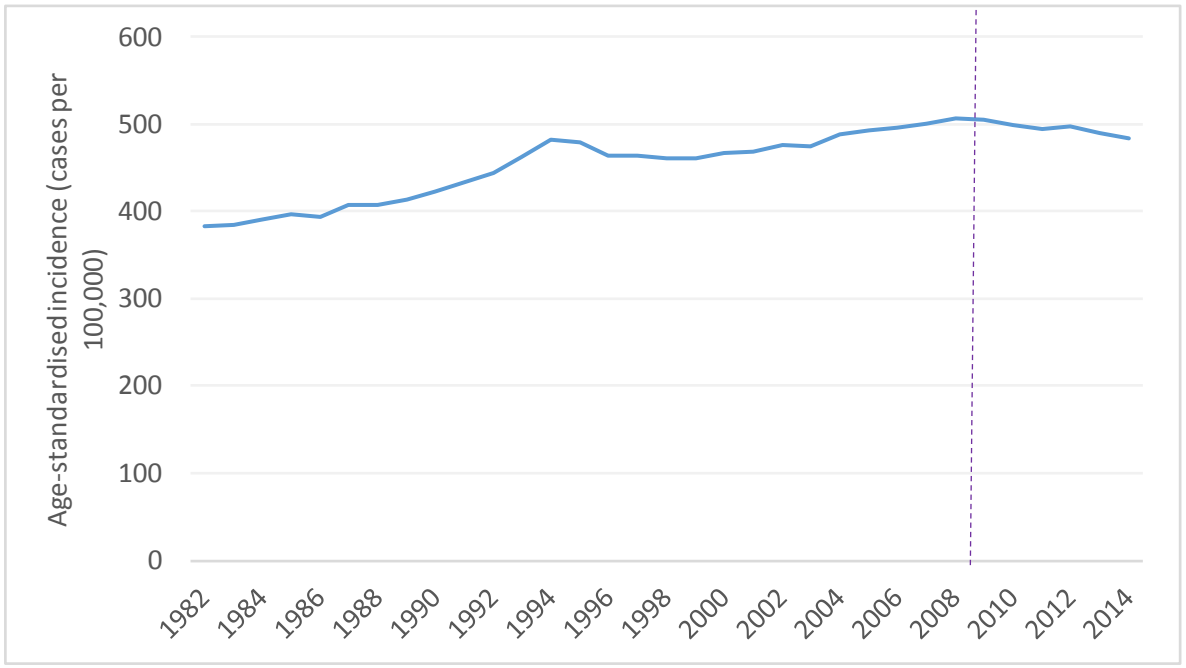
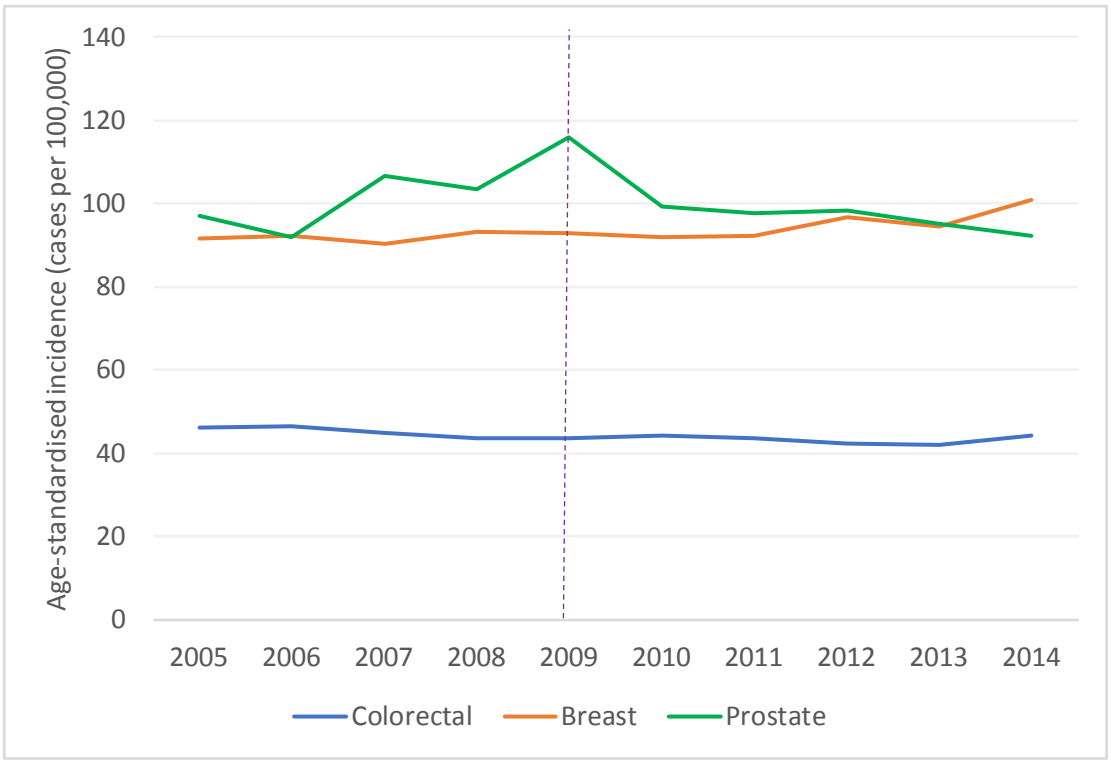


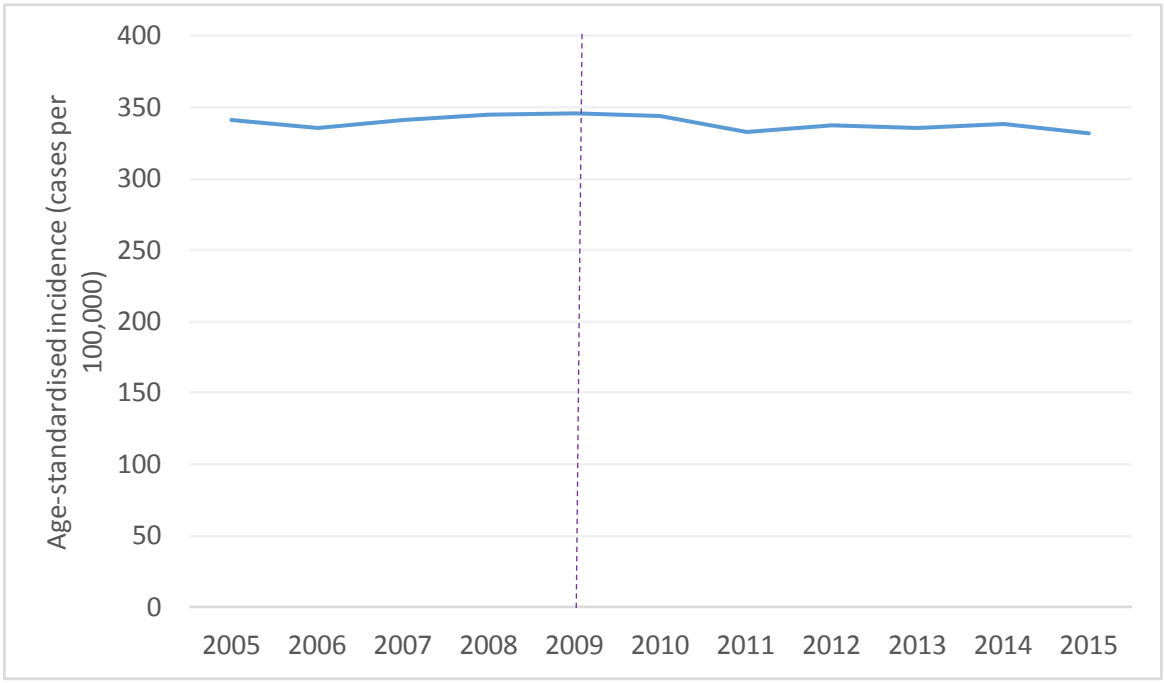
Figure 12: Rates of new cases of several common cancers in Australia over the pre- and post-fortification period. (a) Colorectal, breast (female), and prostate cancer; (b) all cancer. Purple dotted lines indicate the year in which mandatory fortification was introduced. Data plotted from [53].

New Zealand data from 1994–2008 show that annual changes in age-standardised incidence or mortality from colorectal cancer, prostate, and breast cancer were very modest, and ranged from a decrease of 2.3% in female colorectal cancer to an increase in prostate cancer of 0.7% [13]. More

recent data continue to suggest that no substantial changes in trends have occurred [54] (Figure 13).



(a)



(b)

Figure 13: Rates of new cases of several common cancers in New Zealand. (a) Colorectal, breast (female), and prostate cancer; (b) all cancer. Purple dotted lines indicate year in which increased voluntary fortification of bread was encouraged. Data plotted from [54, 55].

5.7.6 Neurological/cognitive impairment

Deficiency in vitamin B_{12} can have several causes, including having a diet low or absent in meat, fish, and dairy products, and conditions that impair B_{12} gastrointestinal absorption such as Crohn's disease or the autoimmune condition pernicious anaemia.

Due to the closely linked metabolic interactions between vitamin B_{12} and folate (Figure 6), individuals who are deficient in vitamin B_{12} may also develop anaemia and present with similar physical symptoms and haematological abnormalities as those deficient in folate. Treatment of B_{12}-deficient patients presenting with anaemia with high doses of folic acid will ameliorate the haematological abnormalities. However, this entails a risk of 'masking' the B_{12} deficiency which, left undiagnosed, can eventually lead to cognitive impairment and irreversible neurological damage.

A separate but related issue is the possibility that high levels of folic acid, in the presence of vitamin B_{12} deficiency, can itself lead to neurological deterioration. In this regard, there has been a longstanding concern about the potential effects of folic acid fortification, particularly on older individuals who are more vulnerable to vitamin B_{12} deficiency due to poorer absorption [131].

The UL of 1 mg/d for folic acid (Appendix 5.3.5) was determined based on various case reports and small-scale ($n \leq 48$) case series studies of vitamin B_{12}-deficient patients, with only one published after 1960 [101, Table 8.12]. It was recently argued that the UL had been established on the basis of erroneous data interpretation, and that a UL for folic acid is in fact unnecessary ([133]; Section 5.3.5).

The ***NTP*** found that relatively few studies exploring the effect of folic acid on human cognitive function also considered vitamin B_{12} status in subjects. There were difficulties in drawing meaningful conclusions given the wide variability in study parameters such as threshold of vitamin B_{12} deficiency and measured cognitive outcomes. There was also a lack of mechanistic evidence for how folic acid could exacerbate vitamin B_{12} deficiency. Genetic association studies (Appendix) were proposed to aid in clarifying the causal nature of potential associations.

The ***SACN*** did not identify any systematic reviews or meta-analyses on masking or exacerbation of B_{12} deficiency. It found that interpretation of other available evidence was hampered by the short (<1 year) duration of intervention studies, and having insufficient participants to obtain meaningful data specifically on low B_{12} participants. Where adverse outcomes had been reported, doses greater than the UL were involved, and limited evidence was available for lower doses.

The ***FDA*** noted inconsistent findings from epidemiological studies on dietary folate, but agreed with the conclusions from safety reviews by other regulatory authorities that folic acid intakes not exceeding the UL of 1 mg/d were unlikely to pose a risk of masking. Regarding the direct effects on neurological/cognitive decline, the FDA found no new evidence since the Institute of Medicine's 1998 evaluation of folic acid for determining its UL. In view of the typical inclusion of vitamin B_{12} with folic acid-containing supplements, it concluded that those >50 years whose folic acid intake exceeded the UL would nonetheless have adequate B_{12} status and therefore not be at risk.

A population-based study using US NHANES data has directly addressed concerns about higher exposure to folic acid adversely affecting the clinical presentation of B_{12} deficiency and delaying its diagnosis, by using participants without vitamin B_{12} deficiency-related haematological abnormalities. Adults aged >50 years who were asymptomatic for anaemia or for macrocytosis (having enlarged RBCs) were surveyed for prevalence of vitamin B_{12} deficiency [271]. Cross-

sectional data comparisons between pre- and post-mandatory fortification periods showed no significant differences in prevalence of overt B_{12} deficiency (<148 pmol/L). Indeed, prevalence of marginal deficiency (148–258 pmol/L) decreased post-fortification, and higher folic acid intake via fortified foods or supplements was linked to lower prevalence of both marginal and overt B_{12} deficiency in a graded manner.

There is still no satisfactory mechanistic explanation for how high folate levels may exacerbate vitamin B_{12} deficiency to result in adverse neurological effects. The widespread use of B_{12}-containing folic acid supplements among seniors suggests that those deficient in B_{12} may be unable to absorb the vitamin [272, 273]. Thus, the low B_{12}/high folate combination may tend to be found in individuals who are merely the most severely B_{12}-deficient subgroup of supplement users, and who consequently demonstrate poorest cognitive function. In other words, cognitive impairment may be due to low vitamin B_{12} status, and not indicative of a biological effect of folic acid [274].

Modelling work based on mandatory fortification of bread or flour at various levels was recently undertaken for FSAI to simulate the impact on risks of NTD-affected pregnancies and masking of vitamin B_{12} deficiency. It showed that bread fortification at 225 µg/100 g (a level similar to the target range in New Zealand), including current intakes from voluntarily fortified foods and supplements and an additional 25% as overage, could reduce NTD pregnancies by 31% while keeping folic acid intake for 99% of the population well under the UL [28, 275]. It was therefore likely to be a very small proportion of older adults who fulfil the multiple criteria for being at risk of masking: that is, having undiagnosed B_{12} deficiency, presenting with megaloblastic anaemia, continually exceeding the UL, and being particularly sensitive to folic acid given the five-fold margin of safety taken into account by the UL. On this basis, the risk posed to older adults by mandatory fortification was deemed negligible [28].

There still remains debate on the benefits and risks of folic acid fortification in the context of vitamin B_{12} deficiency [276]. However, in modern day medical practice, the risk of masking of vitamin B_{12} deficiency is considered minimal. Previously, B_{12} deficiency was diagnosed by the indirect haematologic index of enlarged RBCs, which is not observed when folate status is adequate. Today levels of vitamin B_{12} and related metabolites are directly measured as a first-line test, and in New Zealand, diagnostic testing of B_{12} deficiency among the elderly is routinely undertaken in general health checks irrespective of symptomatic presentation [15].

5.7.7 Diabetes

The literature base for this group of health outcomes is less extensive than that for cancer or cognitive impairment, and the majority of the available studies were unable to ascertain causality. A recent large observational study, while subject to the standard limitations of interpreting causal effects, found that higher serum folate was strongly associated with lower insulin resistance among nondiabetic adults after adjusting for multiple confounding factors [277].

The NTP also examined thyroid disorders due to the common underlying pathology with type 2 diabetes, and found that the existing studies were not designed with folic acid as an exposure and thyroid disease as an outcome, so no conclusions could be drawn. However, the NTP surmised that given the adequate knowledge base on thyroid disease and folate metabolism, this health outcome did not require prioritisation.

5.7.8 Folate in pregnancy – effects on offspring

There is overwhelming evidence that an individual's risk of certain diseases in later life is dependent on experiences early in the life course, such as maternal nutrition during gestation [278]. On this basis, questions have been raised about the potential effects of maternal folic acid supplementation during pregnancy on offspring health.

The ***NTP*** found interpretation of the literature base for hypersensitivity-related outcomes was greatly limited by quality of the available literature, and effects that were found were small and potentially arose from chance. There was also a lack of studies focusing on high exposures. It was concluded that much greater research focus was needed on asthma and sensitisation; available data on eczema/atopic dermatitis and respiratory infection suggested that this group no longer needed to be regarded as outcomes of high priority.

Recently, a very large prospective cohort in Norway—where virtually no folic acid fortification occurs—was studied for associations between maternal folate intake during pregnancy and asthma in offspring at age 7 [58]. Children born to mothers whose total folate intake from food and supplements fell between the first and fourth quintiles did not appear to be at increased risk of asthma. However there was a slight increase in risk (adjusted $RR_{Q5–Q1}$ 1.23; 95% CI 1.06–1.44) for the highest quintile (≥578 µg/d folic acid equivalents[41]), suggesting that an effect may only be seen beyond a certain threshold. This level of intake could be achieved by consuming the commonly recommended dose of 400 µg/d for pregnancy, together with 300 µg/d food folate or another folic acid-containing supplement. Women belonging to the highest quintile consumed a median of 500 µg/d folic acid supplements and a folate-rich diet.

Human and animal studies have suggested that maternal intake of folic acid may elevate diabetes risk and promote body fatness in children, especially with co-presence of vitamin B_{12} deficiency [279]. The ***NTP*** found an RCT reporting an association between supplementation and reduced risk of metabolic syndrome, and an observational study finding a small effect of high maternal folate status on greater insulin resistance and body fat in adolescents. The latter study was in an Indian population that had blood folate levels considerably higher than those seen in Western populations, potentially due to a predominantly vegetarian diet and prenatal use of high-dose folic acid supplements. The lack of sufficient data and conflicting evidence has prompted a recommendation for further research.

5.7.9 Other effects

The protection against CVD reported in a recent meta-analysis [62] (Section 2.6) was related to decreased levels of homocysteine, a metabolite closely involved in folate metabolism. Stratifying the studies by exposure of the population to mandatory fortification revealed an absence of effect for stroke, CVD, and coronary heart disease. However, the contribution of fortified food may be obscured in the context of high dose supplement intake.

A very small study of DNA methylation patterns in sperm obtained from US men pre-fortification, and Canadian men exposed to mandatory fortification for at least 6 years was recently reported [280]. No significant differences in methylation were found at genes known to be epigenetically responsive to high-dose folic acid supplementation, infertility, and ageing. In addition, no

[41] Note that this is different from dietary folate equivalents (DFEs) discussed in Section 5.3.4.

methylation differences were observed in sperm of men who took 400 µg/d folic acid compared to placebo.

5.7.10 Unmetabolised folic acid

Unmetabolised folic acid (UMFA) has no known biological function; it has not been clinically associated with any adverse health outcomes, and no toxicological data are available. However, it can be detected in serum following consumption of fortified foods containing 200–400 µg folic acid [281, 282]. It has also been found at low (0–2 nmol/L) levels across the population in countries with mandatory or voluntary fortification [283-285]. Because it is neither naturally occurring nor a known component of plasma or other tissues, there has been persistent concern regarding long-term effects of prolonged high dose folic acid intake (e.g. [286]).

Concerns about the health effects of UMFA have been especially fuelled by a single study of post-menopausal women finding an inverse association between plasma UMFA, but not plasma folate, and levels of natural killer (NK) cell-mediated cytotoxicity [287]. NK cells provide immune-based surveillance against cancer and pathogen-infected cells, and low NK activity has been associated with increased risk of cancer in a long-term human study [288]. The finding linking UMFA with NK activity, reported in 2006, has not been replicated to date and is confounded by all participants being overweight or obese. Obesity is known to affect body distribution and metabolism of folate, and this may have influenced levels of folate-binding protein in the participants' plasma; given the high affinity of folic acid for folate-binding protein, the measurements of UMFA may not have been meaningful [289]. A 2012 study has since shown a lack of association between serum folate levels and NK activity in healthy adults, even in participants with very high folate levels (>45 nmol/L) [290]. Further *in vitro* work indicated that human NK cytotoxicity was unaffected by both lack, and an excess, of folic acid [291].

The SACN review has summarised results from newer observational studies and intervention studies with folic acid supplementation, and national monitoring programmes in the US and UK. It found that 33 to 97% of participants in supplementation studies (mostly at 400 µg/d) had detectable UMFA, which comprised 1–3% of total serum folate. A small (n=38) study of women of childbearing age receiving 1,100 or 5,000 µg folic acid daily found neither significant increases in UMFA from each group's baseline, nor differences in UMFA at each dose [292]. A larger trial randomising post-first trimester pregnant women to folic acid or placebo supplementation showed that supplementation raised maternal folate status without concordant increase in UMFA [293].

UMFA has been found in umbilical cord blood and in breast milk, but at levels unrelated to maternal folic acid supplementation up to ~400 µg/d [294, 295]. It does not appear to accumulate in the fetus [296, 297]. Higher-dose supplement use in lactating women raises breast milk UMFA levels by ~150% [295], but little is known about the bioavailability of breast milk UMFA to infants compared to reduced folates[42].

The lack of a consistent dose-response association between folic acid and UMFA levels raises the possibility of homeostatic (balancing) mechanisms that are acting to limit exposure to circulating folic acid. An alternative but not mutually exclusive postulate is that UMFA is not a biomarker of

[42] An excess of milk folate over folate-binding proteins may lead to enhanced bioavailability [298]; yet, the higher affinity of folate-binding proteins for folic acid than for reduced folate could conversely suggest interference with infant folate absorption [299].

an overloaded folate metabolic capacity, but instead may be produced by oxidation of folate either within the body [296], or during cooking and storage of food [300]. This could account for (1) the consistent detection of UMFA in fasting individuals, at similar proportions of total folate across age groups and intake levels; and (2) the strong positive correlations between UMFA levels and those of 5-MTHF and of total folate [284, 301]. In line with this, a preliminary study has described the detection of UMFA in the remote Tsimane population in Bolivia at levels higher than in European samples, despite absence of folic acid exposure [300].

It has been pointed out that UMFA is unlikely to be a new phenomenon. Dietary supplement use has increased in US, while pregnant women have been prescribed folic acid tablets for nearly half a century, suggesting millions of person-years exposure to UMFA [272]. The appearance of UMFA with a 200 µg dose suggests that prior to fortification, any user of folic acid supplements would already have measurable UMFA, and any potential adverse effects would have been experienced.

5.7.11 At-risk subgroups

The NTP has noted the current lack of a unified approach to identify subgroups most at risk of exposure to high folic acid, as subgroups may differ in their susceptibility to various health outcomes. This section discusses specific groups within the general, healthy population that have been signalled as potentially being at greater risk from high folic acid intakes.

A well-recognised group whose intake of folic acid may exceed the UL if exposed to fortification are users of dietary supplements containing high doses of folic acid. Canadian data have found that fortification was essential to enable the population to achieve an adequate intake of folate, but supplement use was the key factor in UL exceedance [70]. There is no established RBC folate cut-off threshold above which levels are considered elevated beyond the normal range or likely to have adverse health effects. The study therefore used NHANES RBC folate concentrations at the 95th percentile to derive a cut-off of >1,800 nmol/L for elevated RBC folate. This placed 7–12% of supplement users in that category, compared to 1–2.5% of non-supplement users.

In general, older age is a risk factor not only for neurological/cognitive decline, but also for prostate and colorectal cancer. Tumour precursors in the colorectum and prostate have a long progression time—usually over many years—and frequently occur in older, apparently healthy adults [242]. Given the potential for high folate exposure to facilitate the establishment and progression of the precursors into tumours, and the greater use of supplements among elderly individuals, this group may be placed at greater risk within a background of mandatory folic acid fortification.

A recent analysis has found that age may play a role in serum folate levels in males irrespective of folate consumption [302]. A comparison between dietary folate intake and blood folate levels in males found that although folate intake did not change appreciably with age, men who consumed the highest amounts of folate (≥1,000 µg/d) tended to have higher serum folate levels as they aged. As this observation persisted after correcting for supplement usage, it suggests that there are other factors at play such as age-related changes in metabolism. This may have implications for studies that have used dietary folate intake to seek associations with risk of prostate cancer (which is more prevalent among older men), as these rely on the assumption that consuming a certain amount of DFEs would impact on blood folate levels in a known, predictable manner, and therefore relate to clinical outcomes in an equivalent way [302].

Children's exceedance of the UL is most likely to result from supplement use, although the AIHW has also made note of the contribution of food fortification given children's greater levels of food intake by body weight than adults [33]. A recent Irish risk assessment to estimate the safe maximum levels at which folic acid can be added to fortified foods and supplements has commented that establishing a level for children is unlikely to be necessary [303]. This is because there have been no reported adverse effects at high intakes, and also because the UL for children had been derived from that for adults based on adverse neurological effects not applicable to children.

This report does not focus on subgroups with certain pathologies that, as part of their medically supervised treatment, are either required to take high-dose folic acid supplements or avoid excess folic acid intake to prevent attenuation of their medications' effects due to folate-drug interactions. The former group includes women at greater risk of NTD pregnancies due to prior affected pregnancy, obesity, or diabetes [304, 305]. The latter includes patients undergoing antifolate chemotherapy for cancer, on certain anticonvulsant medications for epilepsy, or taking the antifolate methotrexate for certain autoimmune diseases such as rheumatoid arthritis; these individuals may need extra monitoring as folic acid supplements may alleviate side-effects at low doses, but interfere with drug efficacy at higher levels [239]. As all these groups are receiving specific medical advice and not considered part of the general healthy population, the UL does not apply [101]. The UL also does not apply to folate-drug interactions. However, it is recognised that these individuals, and their treating clinicians, will need to be cognisant of their cumulative folic acid intake from fortified foods, whether derived from voluntary or mandatory manufacturing practices.

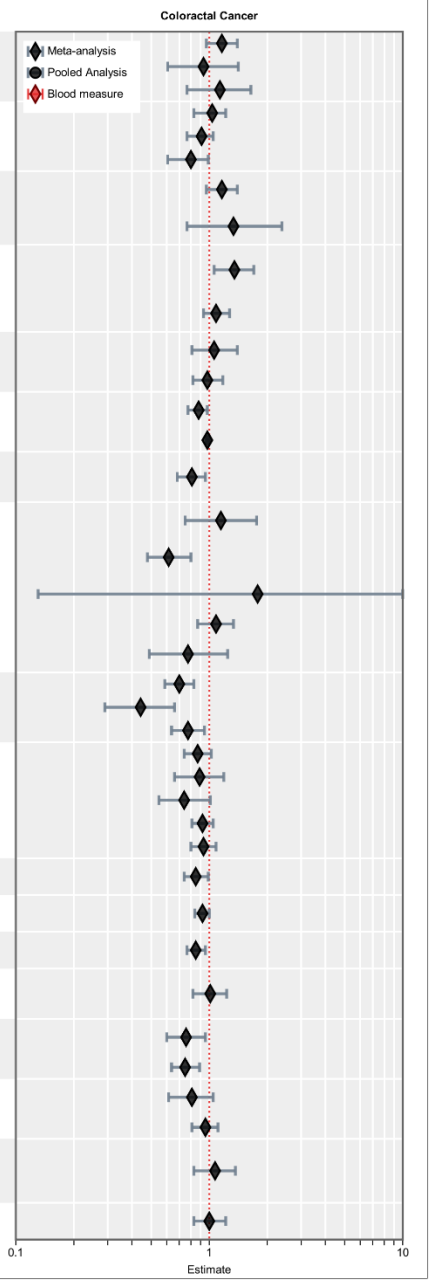
5.8 Other limitations in the literature

Limitations in the evidence base with respect to cancer are discussed in Section 5.7.5.2. The recent reports assessing folic acid safety have identified several more general areas in which there is insufficient research that is critical for making a fully informed risk assessment. These include information about at-risk groups that are most likely to exceed the UL such as the elderly, supplement users, and children. There is also an over reliance on observational studies, and inconsistencies in methods used to determine folate/folic acid exposure [31]. The lack of information on ethnicity is another issue with a literature based on predominantly Caucasian populations [26]. The SACN has also discussed in detail the problems with confounding in the reviewed studies [30].

5.9 NTP Health Assessment Workplace Collaborative cancer data

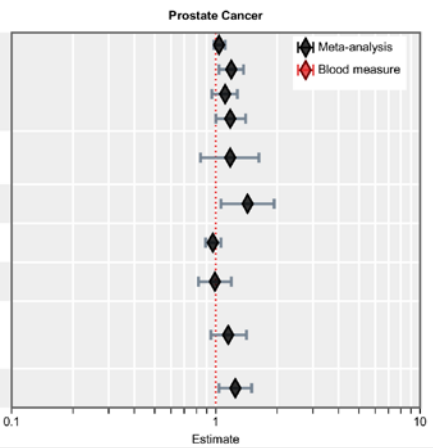
(a)

Study	Protocol	Exposure	Result Label	N (subjects)
Carroll, 2010	Folic acid and prevention of colorectal cancer	folic acid supplementation	Adenoma recurrence, Folic acid vs. placebo (2 studies)	749
			Adenoma recurrence, Folic acid vs. placebo (3 studies)	840
			Colorectal cancer incidence, plus antioxidants	11,062
Chuang, 2013	Circulating folate and colorectal cancer	Circulating folate	Colorectal cancer, circulating folate, Microbiological assay	5,831
			Colorectal cancer, circulating folate, Overall	10,516
			Colorectal cancer, circulating folate, Radioimmunoassay	4,685
Cooper, 2010	Folic acid and colorectal adenomas	Folic acid	Recurrence of any adenoma, Folic acid alone vs. placebo alone	749
			Incidence of advanced adenoma, Folic acid alone vs. placebo alone	749
Fife, 2011	RCTs for Folic Acid Supplementation and Colorectal Cancer Risk	folic acid supplementation	adenoma and advanced adenoma, folate supplementation, longer follow-up, >3 years	6,736
			adenoma and advanced adenoma, folate supplementation, shorter follow up, <4 years	3,686
Figueiredo, 2011	AFPPS, NHS/HPFS, and ukCAP, 1994-2001	Folic acid	Advanced adenoma within 42 months, Folic acid	1,922
			Any adenoma within 42 months, Folic acid	1,957
Heine-Br&#xfffd;ring, 2015	Dietary supplement use and colorectal cancer risk	folic acid	colorectal cancer, folic acid, highest-lowest supplement use	291,006
			colorectal cancer, intake of supplemental folic acid	291,006
Hutter, 2012	Colorectal cancer and gene-environment inteactions	Dietary folate	Colorectal cancer, Folate	16,843
Ibrahim, 2010	Folic acid and colorectal adenoma recurrence	Folic acid supplementation	Colorectal adenomas, 0.5mg/day folic acid supplementation, random effects	419
			Colorectal adenomas, 1mg/day folic acid supplementation, random effects	1,041
			Colorectal adenomas, 5mg/day folic acid supplementation, random effects	20
			Colorectal adenomas, folic acid supplementation, fixed effects	1,486
			Colorectal adenomas, folic acid supplementation, random effects	1,480
Kantor, 2014	GECCO and CCFR studies	dietary folate	colorectal cancer, dietary folate	18,440
			colorectal cancer, dietary folate, case–control studies	10,798
			colorectal cancer, dietary folate, nested case–control studies	7,642
Kennedy, 2011	Folate intake and the risk of colorectal cancer	dietary folate	colorectal cancer, dietary folate, case control	3,276
			colorectal cancer, dietary folate, case control, men only	695
			colorectal cancer, dietary folate, case control, women only	691
			colorectal cancer, dietary folate, cohort	472,531
			colorectal cancer, dietary folate, cohort, women only	291,720
Kennedy, 2011	Folate intake and the risk of colorectal cancer	total folate	colorectal cancer, total folate, case control	1,679
Kim, 2010	Pooled analyses of folate intake and colon cancer	dietary folate intake	colon cancer, folate intake, dietary	725,134
Kim, 2010	Pooled analyses of folate intake and colon cancer	total folate intake	colon cancer, folate intake, total	526,166
Qin, 2013	Folic acid supplementation and cancer risk	folic acid treatment	colorectal cancer, folic acid	33,824
Sanjoaquin, 2005	Folate intake and colorectal cancer	dietary folate intake	colorectal cancer, dietary folate, case-control	15,842
			colorectal cancer, dietary folate, cohort	2,394
Sanjoaquin, 2005	Folate intake and colorectal cancer	total folate intake	colorectal cancer, total folate, case-control	2,467
			colorectal cancer, total folate, cohort	2,689
Vollset, 2013	Folic acid supplementation and site-specific cancer incidence	folic acid	cancer incidence; colorectal; folic acid	49,621
Wien, 2012	Cancer risk with folic acid supplements	folic acid intervention	colon and rectum cancer incidence, RCTs, folic acid >=400ug/day vs. placebo/control	32,639



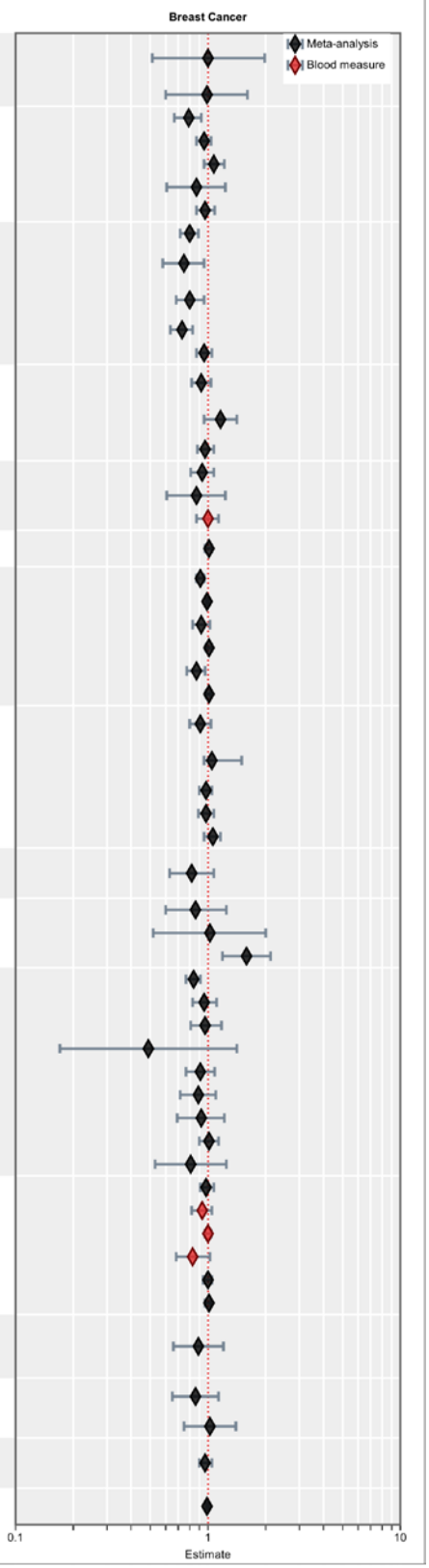
(b)

Study	Protocol	Exposure	Result Label	N (subjects)
Collin, 2010	Circulating folate and risk of prostate cancer: with ProtecT study	blood (serum or plasma) folate	Fixed effects pooled estimate, including ProtecT	144,234
			Fixed effects pooled estimate, prospective cohort studies	141,266
			Random effects pooled estimate, including ProtecT	144,234
			Random effects pooled estimate, prospective cohort studies	141,266
Qin, 2013	Folic acid supplementation and cancer risk	folic acid treatment	prostate cancer, folic acid	27,065
Tio, 2014b	Folate intake and risk of prostate cancer	blood folate	prostate cancer, blood folate	13,232
Tio, 2014b	Folate intake and risk of prostate cancer	dietary folate	prostate cancer, dietary folate	146,782
Tio, 2014b	Folate intake and risk of prostate cancer	total folate	prostate cancer, total folate	93,781
Vollset, 2013	Folic acid supplementation and site-specific cancer incidence	folic acid	cancer incidence; prostate; folic acid	49,621
Wien, 2012	Cancer risk with folic acid supplements	folic acid intervention	prostate cancer incidence, RCTs, folic acid >=400ug/day vs. placebo/control	25,738



(c)

Study	Protocol	Exposure	Result Label	N (subjects)
Chan, 2011	Multivitamin supplement use and risk of breast cancer	multivitamins	case-control, breast cancer in women and multivitamin use/no use	6,970
			cohort, breast cancer in women and multivitamin use/no use	56,294
Chen, 2014	Folate and risk of breast cancer	Folate	Breast cancer, Folate Intake, Dietary, Case-Control Studies	39,806
			Breast cancer, Folate Intake, Dietary, Prospective Studies	655,249
			Breast cancer, Folate Intake, Supplement, Prospective Studies	86,647
			Breast cancer, Folate Intake, Total, Case-Control Studies	6,403
			Breast cancer, Folate Intake, Total, Prospective Studies	476,625
Larsson, 2007	Folate and risk of breast cancer	dietary folate	breast cancer, dietary folate increments, case-control	19,370
			breast cancer, dietary folate increments, postmenopausal, case-control	11,667
			breast cancer, dietary folate increments, premenopausal, case-control	13,084
			breast cancer, dietary folate, case-control	19,370
			breast cancer, dietary folate, prospective	302,959
Larsson, 2007	Folate and risk of breast cancer	dietary folate increments	breast cancer, dietary folate increments, postmenopausal, prospective	194,857
			breast cancer, dietary folate increments, premenopausal, prospective	96,037
			breast cancer, dietary folate increments, prospective	302,959
Larsson, 2007	Folate and risk of breast cancer	total folate	breast cancer, total folate increments, case-control	5,417
			breast cancer, total folate, case-control	5,417
			breast cancer, total folate, prospective	306,209
Larsson, 2007	Folate and risk of breast cancer	total folate increments	breast cancer, total folate increments, prospective	306,209
Lewis, 2006	Folate intakes or levels and breast cancer	dietary folate	breast cancer, dietary folate, case-control	19,400
			breast cancer, dietary folate, cohort	335,066
			breast cancer, dietary folate, postmenopausal, case-control	6,970
			breast cancer, dietary folate, postmenopausal, cohort	223,351
			breast cancer, dietary folate, premenopausal, case-control	10,379
			breast cancer, dietary folate, premenopausal, cohort	186,201
Liu, 2014	Dietary folate intake and breast cancer	dietary folate intake	breast cancer, dietary folate intake, estrogen receptor - / progesterone receptor -	0
			breast cancer, dietary folate intake, estrogen receptor + / progesterone receptor +	0
			breast cancer, dietary folate intake, overall	1,836,566
			breast cancer, dietary folate intake, postmenopausal	0
			breast cancer, dietary folate intake, premenopausal	0
Qin, 2013	Folic acid supplementation and cancer risk	folic acid treatment	breast cancer, folic acid	19,800
Tio, 2014c	Folate intake and breast cancer	blood folate level	breast cancer, blood folate level	5,226
			breast cancer, blood folate level, ER-	2,100
			breast cancer, blood folate level, ER+	2,100
Tio, 2014c	Folate intake and breast cancer	dietary folate	breast cancer, dietary folate	607,625
			breast cancer, dietary folate , ER-	379,382
			breast cancer, dietary folate , ER-/PR-	183,322
			breast cancer, dietary folate , ER-/PR+	27,100
			breast cancer, dietary folate , ER+	214,606
			breast cancer, dietary folate , ER+/PR-	89,329
			breast cancer, dietary folate , ER+/PR+	183,322
			breast cancer, dietary folate , PR-	212,466
			breast cancer, dietary folate , PR+	150,237
Tio, 2014c	Folate intake and breast cancer	total folate intake	breast cancer, total folate intake	544,460
			breast cancer, total folate intake, ER-	279,897
			breast cancer, total folate intake, ER+	189,234
			breast cancer, total folate intake, ER+/PR-	87,657
			breast cancer, total folate intake, PR-	149,361
			breast cancer, total folate intake, PR+	149,361
Vollset, 2013	Folic acid supplementation and site-specific cancer incidence	folic acid	cancer incidence; breast; folic acid	49,621
Wien, 2012	Cancer risk with folic acid supplements	folic acid intervention	breast cancer incidence, RCTs, folic acid >=400ug/day vs. placebo/control	11,636
			breast cancer incidence, cohorts, folic acid >=400ug/day	60,423
Zhang, 2014a	Folate intake and risk of breast cancer	folate intake, highest versus lowest	breast cancer, folate intake, highest versus lowest	677,858
Zhang, 2014a	Folate intake and risk of breast cancer	folate intake, per 100 ug/day	breast cancer, folate intake, per 100 ug/day	677,858



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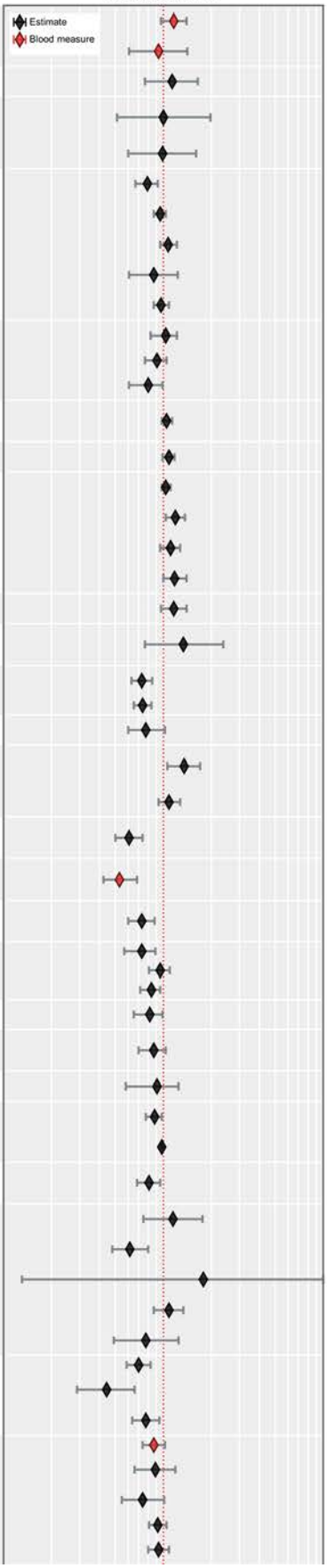
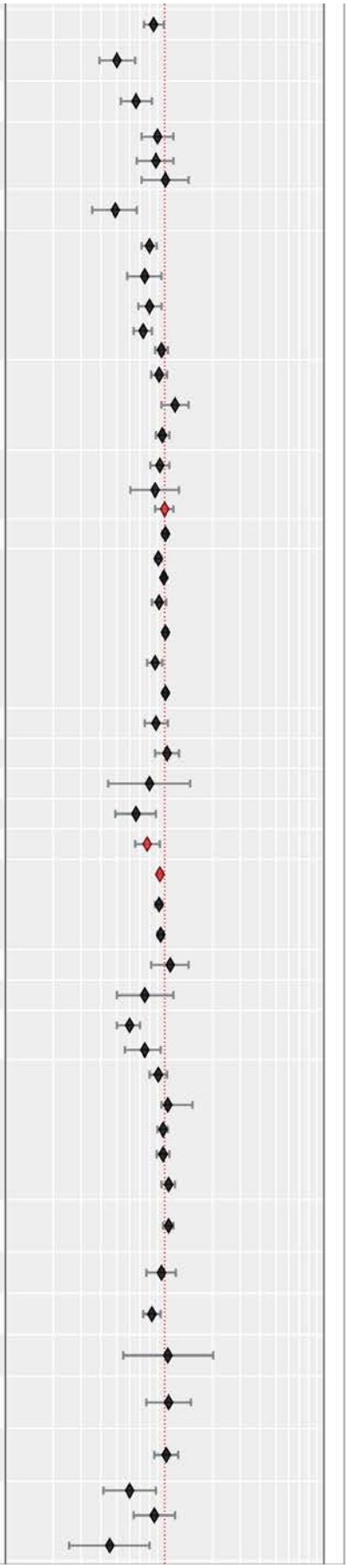
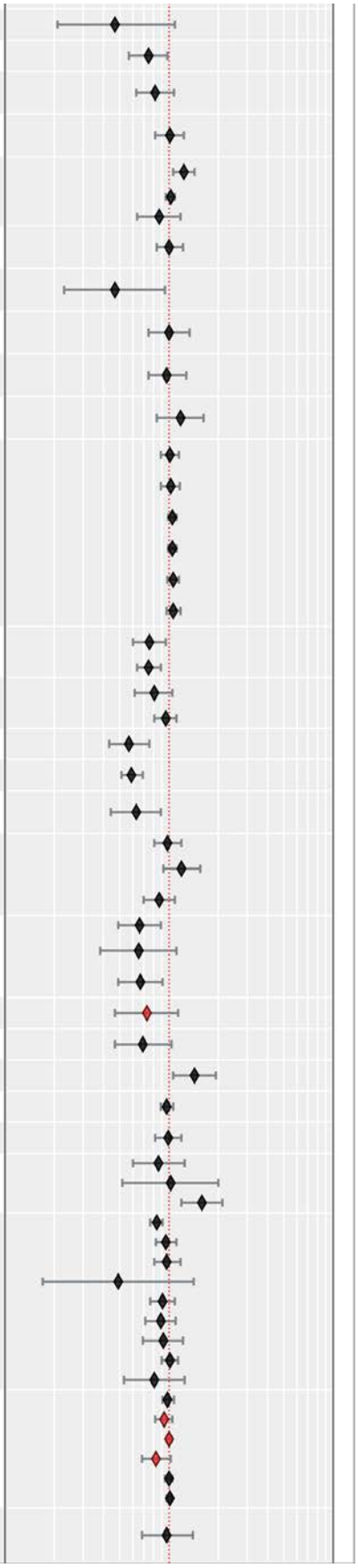
Study	Protocol	Outcome	Exposure	Result Label	N (subjects)
Carroll, 2010	Folic acid and prevention of colorectal cancer	Adenoma recurrence	folic acid supplementation	Adenoma recurrence, Folic acid vs. placebo (2 studies)	749
				Adenoma recurrence, Folic acid vs. placebo (3 studies)	840
Carroll, 2010	Folic acid and prevention of colorectal cancer	Colorectal cancer incidence	folic acid supplementation	Colorectal cancer incidence, plus antioxidants	11,062
Chan, 2011	Multivitamin supplement use and risk of breast cancer	breast cancer	multivitamins	case-control, breast cancer in women and multivitamin use/no use	6,970
				cohort, breast cancer in women and multivitamin use/no use	56,294
Chen, 2014	Folate and risk of breast cancer	Breast cancer	Folate	Breast cancer, Folate Intake, Dietary, Case-Control Studies	39,806
				Breast cancer, Folate Intake, Dietary, Prospective Studies	655,249
				Breast cancer, Folate Intake, Supplement, Prospective Studies	86,647
				Breast cancer, Folate Intake, Total, Case-Control Studies	6,403
				Breast cancer, Folate Intake, Total, Prospective Studies	476,625
Chuang, 2013	Circulating folate and colorectal cancer	Colorectal cancer	Circulating folate	Colorectal cancer, circulating folate, Microbiological assay	5,831
				Colorectal cancer, circulating folate, Overall	10,516
				Colorectal cancer, circulating folate, Radioimmunoassay	4,685
Clarke, 2010	Folic acid supplementation and cancer incidence	cancer incidence	folic acid	cancer incidence, folic acid	35,603
Clarke, 2011	B vitamins and risk of cancer	cancer	B-vitamin supplements	cancer events, B vitamins	29,867
Collin, 2010	Circulating folate and risk of prostate cancer: with ProtecT study	prostate cancer	blood (serum or plasma) folate	Fixed effects pooled estimate, including ProtecT	144,234
				Fixed effects pooled estimate, prospective cohort studies	141,266
				Random effects pooled estimate, including ProtecT	144,234
				Random effects pooled estimate, prospective cohort studies	141,266
Cooper, 2010	Folic acid and colorectal adenomas	Any adenoma	Folic acid	Recurrence of any adenoma, Folic acid alone vs. placebo alone	749
Cooper, 2010	Folic acid and colorectal adenomas	Incidence of advanced adenoma	Folic acid	Incidence of advanced adenoma, Folic acid alone vs. placebo alone	749
Dai, 2013	Folate intake and lung cancer risk	lung cancer	folate intake	lung cancer, folate intake, dietary	9,275
				lung cancer, folate intake, overall	10,528
Dai, 2013	Folate intake and lung cancer risk	lung cancer	serum folate levels	lung cancer, serum folate levels, overall	6,008
Fife, 2011	RCTs for Folic Acid Supplementation and Colorectal Cancer Risk	Colorectal Cancer	folic acid supplementation	adenoma and advanced adenoma, folate supplementation, longer follow-up, >3 years	6,736
				adenoma and advanced adenoma, folate supplementation, shorter follow up, <4 years	3,686
Goh, 2007	Prenatal multivitamin supplementation and rates of pediatric cancers	Acute Lymphoblastic Leukemia	maternal multivitamin consumption	Acute Lymphoblastic Leukemia, maternal multivitamin consumption	1,995
Goh, 2007	Prenatal multivitamin supplementation and rates of pediatric cancers	Neuroblastoma	maternal multivitamin consumption	Neuroblastoma, maternal multivitamin consumption	585
Goh, 2007	Prenatal multivitamin supplementation and rates of pediatric cancers	Pediatric brain tumors	maternal multivitamin consumption	Pediatric brain tumors, maternal multivitamin consumption	933
He, 2014	Folate intake and risk of bladder cancer	bladder cancer	folate intake	bladder cancer, folate intake, case-control	8,752
				bladder cancer, folate intake, cohort	489,620
				bladder cancer, folate intake, overall	498,372
He, 2014	Folate intake and risk of bladder cancer	bladder cancer	folate intake from diet	bladder cancer, folate intake from diet	0
He, 2014	Folate intake and risk of bladder cancer	bladder cancer	folate intake from diet and supplement	bladder cancer, folate intake from diet and supplement	0
He, 2014	Folate intake and risk of bladder cancer	bladder cancer	folate intake from supplement	bladder cancer, folate intake from supplement	0
Heine-Bröring, 2015	Dietary supplement use and colorectal cancer risk	colorectal cancer	folic acid	colorectal cancer, folic acid, highest-lowest supplement use	291,006
				colorectal cancer, intake of supplemental folic acid	291,006
Hutter, 2012	Colorectal cancer and gene-environment inteactions	Colorectal cancer	Dietary folate	Colorectal cancer, Folate	16,843
Ibrahim, 2010	Folic acid and colorectal adenoma recurrence	Recurrence of colorectal adenomas	Folic acid supplementation	Colorectal adenomas, 0.5mg/day folic acid supplementation, random effects	419
				Colorectal adenomas, 1mg/day folic acid supplementation, random effects	1,041
				Colorectal adenomas, 5mg/day folic acid supplementation, random effects	20
				Colorectal adenomas, folic acid supplementation, fixed effects	1,486
				Colorectal adenomas, folic acid supplementation, random effects	1,480
Kantor, 2014	GECCO and CCFR studies	colorectal cancer	dietary folate	colorectal cancer, dietary folate	18,440
				colorectal cancer, dietary folate, case–control studies	10,798
				colorectal cancer, dietary folate, nested case–control studies	7,642
Kennedy, 2011	Folate intake and the risk of colorectal cancer	colorectal cancer	dietary folate	colorectal cancer, dietary folate, case control	3,276
				colorectal cancer, dietary folate, case control, men only	695
				colorectal cancer, dietary folate, case control, women only	691
				colorectal cancer, dietary folate, cohort	472,531
				colorectal cancer, dietary folate, cohort, women only	291,720



Kennedy, 2011	Folate intake and the risk of colorectal cancer	colorectal cancer	total folate	colorectal cancer, total folate, case control	1,679
Larsson, 2006	Folate intake and esophageal, gastric, and pancreatic cancer	esophageal cancer, adenocarcinoma	folate intake	esophageal cancer, adenocarcinoma, folate intake	1,769
Larsson, 2006	Folate intake and esophageal, gastric, and pancreatic cancer	esophageal cancer, squamous cell carcinoma	folate intake	esophageal cancer, squamous cell carcinoma, folate intake	3,408
Larsson, 2006	Folate intake and esophageal, gastric, and pancreatic cancer	gastric cancer	folate intake	gastric cancer, folate intake, all studies	73,335
				gastric cancer, folate intake, case-control	8,341
				gastric cancer, folate intake, cohort	64,994
Larsson, 2006	Folate intake and esophageal, gastric, and pancreatic cancer	pancreatic cancer	folate intake	pancreatic cancer, folate intake, all studies	237,510
Larsson, 2007	Folate and risk of breast cancer	breast cancer	dietary folate	breast cancer, dietary folate increments, case-control	19,370
				breast cancer, dietary folate increments, postmenopausal, case-control	11,667
				breast cancer, dietary folate increments, premenopausal, case-control	13,084
				breast cancer, dietary folate, case-control	19,370
				breast cancer, dietary folate, prospective	302,959
Larsson, 2007	Folate and risk of breast cancer	breast cancer	dietary folate increments	breast cancer, dietary folate increments, postmenopausal, prospective	194,857
				breast cancer, dietary folate increments, premenopausal, prospective	96,037
				breast cancer, dietary folate increments, prospective	302,959
Larsson, 2007	Folate and risk of breast cancer	breast cancer	total folate	breast cancer, total folate increments, case-control	5,417
				breast cancer, total folate, case-control	5,417
				breast cancer, total folate, prospective	306,209
Larsson, 2007	Folate and risk of breast cancer	breast cancer	total folate increments	breast cancer, total folate increments, prospective	306,209
Lewis, 2006	Folate intakes or levels and breast cancer	breast cancer	dietary folate	breast cancer, dietary folate, case-control	19,400
				breast cancer, dietary folate, cohort	335,066
				breast cancer, dietary folate, postmenopausal, case-control	6,970
				breast cancer, dietary folate, postmenopausal, cohort	223,351
				breast cancer, dietary folate, premenopausal, case-control	10,379
				breast cancer, dietary folate, premenopausal, cohort	186,201
Li, 2013	Folate intake and ovarian cancer	ovarian cancer	dietary folate intake	ovarian cancer, dietary folate intake	227,859
Li, 2013	Folate intake and ovarian cancer	ovarian cancer	total folate intake	ovarian cancer, total folate intake	114,135
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	blood folate level	pancreatic cancer, blood folate level	0
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	dietary folate intake	pancreatic cancer, dietary folate intake	0
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	folate intake overall	pancreatic cancer, folate intake overall	0
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	incremental dietary folate intake	pancreatic cancer, incremental dietary folate intake, all studies	0
				pancreatic cancer, incremental dietary folate intake, case-control	0
				pancreatic cancer, incremental dietary folate intake, cohort	0
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	supplemental folate intake	pancreatic cancer, supplemental folate intake	0
Lin, 2013	Folate intake and pancreatic cancer risk	pancreatic cancer	total folate intake	pancreatic cancer, total folate intake	0
Liu, 2011	Folate intake and esophageal cancer	esophageal cancer	folate intake	esophageal cancer, folate intake	9,495
				esophageal cancer, folate intake, smokers	0
Liu, 2014	Dietary folate intake and breast cancer	breast cancer	dietary folate intake	breast cancer, dietary folate intake, estrogen receptor - / progesterone receptor -	0
				breast cancer, dietary folate intake, estrogen receptor + / progesterone receptor +	0
				breast cancer, dietary folate intake, overall	1,836,566
				breast cancer, dietary folate intake, postmenopausal	0
				breast cancer, dietary folate intake, premenopausal	0
Martí-Carvajal, 2013	Cochrane review of homocysteine-lowering interventions for preventing cancer	cancer	homocysteine-lowering treatment	cancer, homocysteine-lowering treatment	32,869
Milne, 2010	Maternal folate and risk of ALL, with Aus-ALL Participants 2003-2007	Acute Lymphoblastic Leukemia	Vitamins before pregnancy	Acute Lymphoblastic Leukemia, Folate supplementation, Vitamins before pregnancy	1,918
Milne, 2010	Maternal folate and risk of ALL, with Aus-ALL Participants 2003-2007	Acute Lymphoblastic Leukemia	Vitamins during pregnancy	Acute Lymphoblastic Leukemia, Folate supplementation, Vitamins during pregnancy	8,839
Milne, 2010	Maternal folate and risk of ALL, with Aus-ALL Participants 2003-2007	Acute Lymphoblastic Leukemia	Vitamins only before pregnancy	Acute Lymphoblastic Leukemia, Folate supplementation, Vitamins only before pregnancy	5,470
Milne, 2010	Maternal folate and risk of ALL, with Aus-ALL Participants 2003-2007	Acute Lymphoblastic Leukemia	Vitamins with folate versus no folate during pregnancy	Acute Lymphoblastic Leukemia, Folate supplementation, Vitamins with folate vs no folate during pregnancy	2,042
Milne, 2010	Maternal folate and risk of ALL, with Aus-ALL Participants 2003-2007	Acute Lymphoblastic Leukemia	Vitamins with folate versus no vitamins during pregnancy	Acute Lymphoblastic Leukemia, Folate supplementation, Vitamins with folate vs no vitamins during pregnancy	3,220
Myung, 2011	Vitamin intake and risk of cervical neoplasm	cervical neoplasm	folate intake	cervical neoplasm, folate intake	5,203
				cervical neoplasm, folate intake, studies adjusted for HPV	2,692
				cervical neoplasm, folate intake, studies unadjusted for HPV	2,511



Myung, 2011	Vitamin intake and risk of cervical neoplasm	cervical neoplasm, carcinoma in situ	folate intake	cervical neoplasm, carcinoma in situ, folate intake	2,775
Myung, 2011	Vitamin intake and risk of cervical neoplasm	cervical neoplasm, invasive cancer	folate intake	cervical neoplasm, invasive cancer, folate intake	2,428
Qin, 2013	Folic acid supplementation and cancer risk	breast cancer incidence	folic acid treatment	breast cancer, folic acid	19,800
Qin, 2013	Folic acid supplementation and cancer risk	colorectal cancer incidence	folic acid treatment	colorectal cancer, folic acid	33,824
Qin, 2013	Folic acid supplementation and cancer risk	hematological malignancy	folic acid treatment	cancer, folic acid, <= 1 mg/day, low median	11,972
				cancer, folic acid, > 1 mg/day, high median	37,434
				hematological malignancy, folic acid	25,670
Qin, 2013	Folic acid supplementation and cancer risk	lung cancer incidence	folic acid treatment	lung cancer, folic acid	31,864
Qin, 2013	Folic acid supplementation and cancer risk	melanoma incidence	folic acid treatment	melanoma, folic acid	19,128
Qin, 2013	Folic acid supplementation and cancer risk	other gastrointestinal cancer incidence	folic acid treatment	other gastrointestinal cancer, folic acid	20,228
Qin, 2013	Folic acid supplementation and cancer risk	other genitourinary cancer incidence	folic acid treatment	other genitourinary cancer, folic acid	20,228
Qin, 2013	Folic acid supplementation and cancer risk	prostate cancer incidence	folic acid treatment	prostate cancer, folic acid	27,065
Qin, 2013	Folic acid supplementation and cancer risk	total cancer incidence	folic acid treatment	total cancer incidence, folic acid, fortified, fixed effects	13,377
				total cancer incidence, folic acid, fortified, random effects	13,377
				total cancer incidence, folic acid, overall, fixed effects	49,406
				total cancer incidence, folic acid, overall, random effects	49,406
				total cancer incidence, folic acid, unfortified, fixed effects	30,507
				total cancer incidence, folic acid, unfortified, random effects	30,507
Sanjoaquin, 2005	Folate intake and colorectal cancer	colorectal cancer	dietary folate intake	colorectal cancer, dietary folate, case-control	15,842
				colorectal cancer, dietary folate, cohort	2,394
Sanjoaquin, 2005	Folate intake and colorectal cancer	colorectal cancer	total folate intake	colorectal cancer, total folate, case-control	2,467
				colorectal cancer, total folate, cohort	2,689
Tio, 2014a	Folate intake and upper gastrointestinal cancers	esophageal adenocarcinoma	dietary folate intake	esophageal adenocarcinoma, retrospective, dietary folate intake	3,546
Tio, 2014a	Folate intake and upper gastrointestinal cancers	esophageal cancer	dietary folate intake	esophageal cancer, dietary folate intake	11,537
Tio, 2014a	Folate intake and upper gastrointestinal cancers	esophageal squamous cell carcinoma	dietary folate intake	esophageal squamous cell carcinoma, retrospective, dietary folate intake	3,977
Tio, 2014a	Folate intake and upper gastrointestinal cancers	gastric cancer	dietary folate intake	gastric cancer, dietary folate intake	209,689
				gastric cancer, prospective, dietary folate intake	197,159
				gastric cancer, retrospective, dietary folate intake	12,530
Tio, 2014a	Folate intake and upper gastrointestinal cancers	pancreatic cancer	dietary folate intake	pancreatic cancer, dietary folate intake	295,526
				pancreatic cancer, prospective, dietary folate intake	291,958
				pancreatic cancer, retrospective, dietary folate intake	3,568
Tio, 2014a	Folate intake and upper gastrointestinal cancers	pancreatic cancer	plasma folate	pancreatic cancer, prospective and retrospective, plasma folate	2,215
Tio, 2014a	Folate intake and upper gastrointestinal cancers	pancreatic cancer	total folate intake	pancreatic cancer, prospective and retrospective, total folate intake	261,727
Tio, 2014b	Folate intake and risk of prostate cancer	prostate cancer	blood folate	prostate cancer, blood folate	13,232
Tio, 2014b	Folate intake and risk of prostate cancer	prostate cancer	dietary folate	prostate cancer, dietary folate	146,782
Tio, 2014b	Folate intake and risk of prostate cancer	prostate cancer	total folate	prostate cancer, total folate	93,781
Tio, 2014c	Folate intake and breast cancer	breast cancer	blood folate level	breast cancer, blood folate level	5,226
				breast cancer, blood folate level, ER-	2,100
				breast cancer, blood folate level, ER+	2,100
Tio, 2014c	Folate intake and breast cancer	breast cancer	dietary folate	breast cancer, dietary folate	607,625
				breast cancer, dietary folate , ER-	379,382
				breast cancer, dietary folate , ER-/PR-	183,322
				breast cancer, dietary folate , ER-/PR+	27,100
				breast cancer, dietary folate , ER+	214,606
				breast cancer, dietary folate , ER+/PR-	89,329
				breast cancer, dietary folate , ER+/PR+	183,322
				breast cancer, dietary folate , PR-	212,466
				breast cancer, dietary folate , PR+	150,237
Tio, 2014c	Folate intake and breast cancer	breast cancer	total folate intake	breast cancer, total folate intake	544,460
				breast cancer, total folate intake, ER-	279,897
				breast cancer, total folate intake, ER+	189,234
				breast cancer, total folate intake, ER+/PR-	87,657
				breast cancer, total folate intake, PR-	149,361
				breast cancer, total folate intake, PR+	149,361
Vollset, 2013	Folic acid supplementation and site-specific cancer incidence	bladder cancer incidence	folic acid	cancer incidence; bladder; folic acid	49,621



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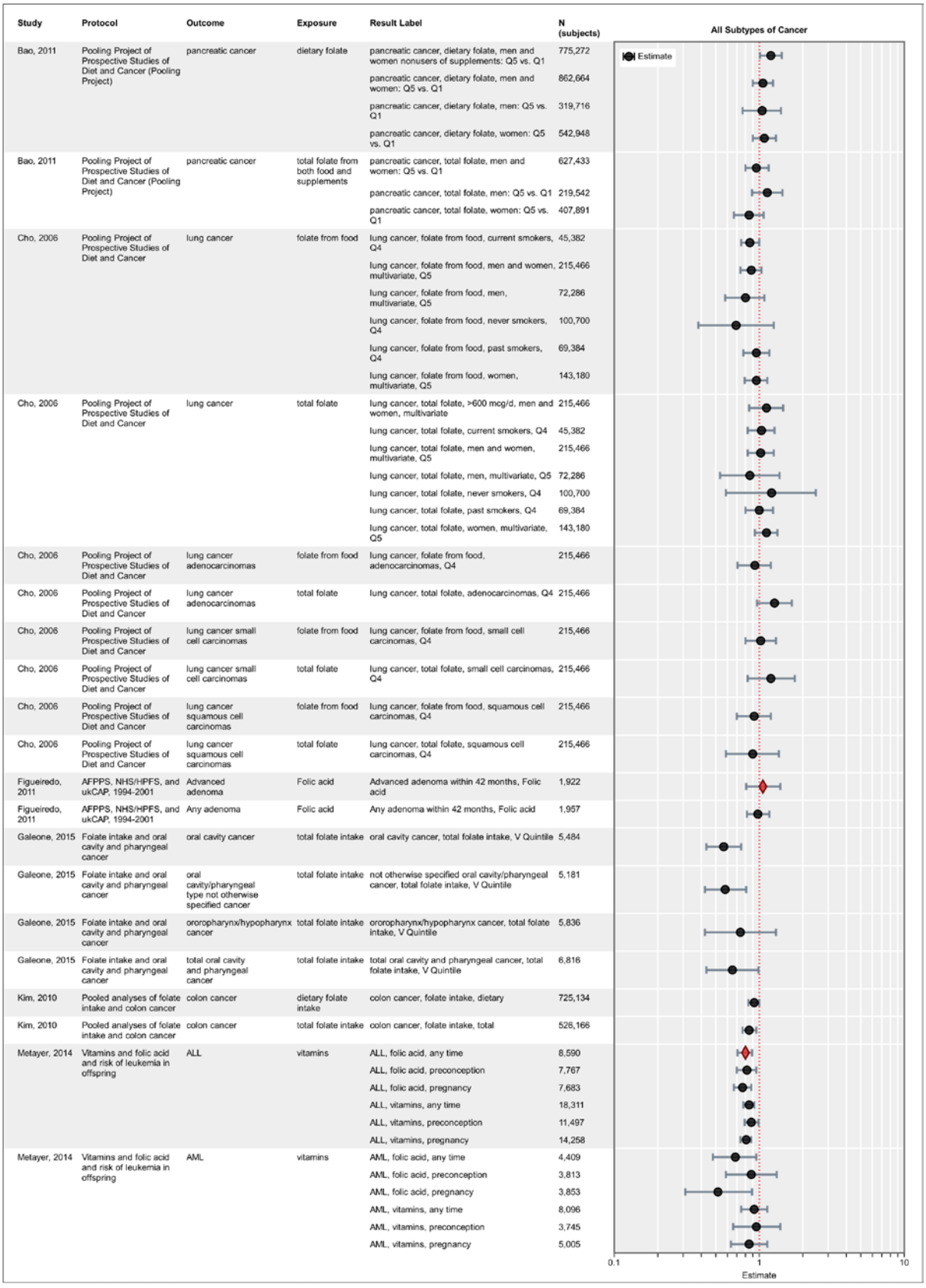

Study	Protocol	Outcome	Exposure	Result Label	N (subjects)
Bao, 2011	Pooling Project of Prospective Studies of Diet and Cancer (Pooling Project)	pancreatic cancer	dietary folate	pancreatic cancer, dietary folate, men and women nonusers of supplements: Q5 vs. Q1	775,272
				pancreatic cancer, dietary folate, men and women: Q5 vs. Q1	862,664
				pancreatic cancer, dietary folate, men: Q5 vs. Q1	319,716
				pancreatic cancer, dietary folate, women: Q5 vs. Q1	542,948
Bao, 2011	Pooling Project of Prospective Studies of Diet and Cancer (Pooling Project)	pancreatic cancer	total folate from both food and supplements	pancreatic cancer, total folate, men and women: Q5 vs. Q1	627,433
				pancreatic cancer, total folate, men: Q5 vs. Q1	219,542
				pancreatic cancer, total folate, women: Q5 vs. Q1	407,891
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer	folate from food	lung cancer, folate from food, current smokers, Q4	45,382
				lung cancer, folate from food, men and women, multivariate, Q5	215,466
				lung cancer, folate from food, men, multivariate, Q5	72,286
				lung cancer, folate from food, never smokers, Q4	100,700
				lung cancer, folate from food, past smokers, Q4	69,384
				lung cancer, folate from food, women, multivariate, Q5	143,180
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer	total folate	lung cancer, total folate, >600 mcg/d, men and women, multivariate	215,466
				lung cancer, total folate, current smokers, Q4	45,382
				lung cancer, total folate, men and women, multivariate, Q5	215,466
				lung cancer, total folate, men, multivariate, Q5	72,286
				lung cancer, total folate, never smokers, Q4	100,700
				lung cancer, total folate, past smokers, Q4	69,384
				lung cancer, total folate, women, multivariate, Q5	143,180
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer adenocarcinomas	folate from food	lung cancer, folate from food, adenocarcinomas, Q4	215,466
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer adenocarcinomas	total folate	lung cancer, total folate, adenocarcinomas, Q4	215,466
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer small cell carcinomas	folate from food	lung cancer, folate from food, small cell carcinomas, Q4	215,466
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer small cell carcinomas	total folate	lung cancer, total folate, small cell carcinomas, Q4	215,466
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer squamous cell carcinomas	folate from food	lung cancer, folate from food, squamous cell carcinomas, Q4	215,466
Cho, 2006	Pooling Project of Prospective Studies of Diet and Cancer	lung cancer squamous cell carcinomas	total folate	lung cancer, total folate, squamous cell carcinomas, Q4	215,466
Figueiredo, 2011	AFPPS, NHS/HPFS, and ukCAP, 1994-2001	Advanced adenoma	Folic acid	Advanced adenoma within 42 months, Folic acid	1,922
Figueiredo, 2011	AFPPS, NHS/HPFS, and ukCAP, 1994-2001	Any adenoma	Folic acid	Any adenoma within 42 months, Folic acid	1,957
Galeone, 2015	Folate intake and oral cavity and pharyngeal cancer	oral cavity cancer	total folate intake	oral cavity cancer, total folate intake, V Quintile	5,484
Galeone, 2015	Folate intake and oral cavity and pharyngeal cancer	oral cavity/pharyngeal type not otherwise specified cancer	total folate intake	not otherwise specified oral cavity/pharyngeal cancer, total folate intake, V Quintile	5,181
Galeone, 2015	Folate intake and oral cavity and pharyngeal cancer	oropharynx/hypopharynx cancer	total folate intake	oropharynx/hypopharynx cancer, total folate intake, V Quintile	5,836
Galeone, 2015	Folate intake and oral cavity and pharyngeal cancer	total oral cavity and pharyngeal cancer	total folate intake	total oral cavity and pharyngeal cancer, total folate intake, V Quintile	6,816
Kim, 2010	Pooled analyses of folate intake and colon cancer	colon cancer	dietary folate intake	colon cancer, folate intake, dietary	725,134
Kim, 2010	Pooled analyses of folate intake and colon cancer	colon cancer	total folate intake	colon cancer, folate intake, total	526,166
Metayer, 2014	Vitamins and folic acid and risk of leukemia in offspring	ALL	vitamins	ALL, folic acid, any time	8,590
				ALL, folic acid, preconception	7,767
				ALL, folic acid, pregnancy	7,683
				ALL, vitamins, any time	18,311
				ALL, vitamins, preconception	11,497
				ALL, vitamins, pregnancy	14,258
Metayer, 2014	Vitamins and folic acid and risk of leukemia in offspring	AML	vitamins	AML, folic acid, any time	4,409
				AML, folic acid, preconception	3,813
				AML, folic acid, pregnancy	3,853
				AML, vitamins, any time	8,096
				AML, vitamins, preconception	3,745
				AML, vitamins, pregnancy	5,005

Figure 14: Forest plots of the associations between folate/folic acid and (a) colorectal adenoma/cancer, (b) prostate cancer, (c) breast cancer, (d) total cancer (meta-analyses), and (e) total cancer (pooled analyses). Estimates >1 suggest an adverse effect of folate/folic acid on cancer risk. Figures from NTP

Health Assessment Workplace Collaborative, available online at https://hawcproject.org/summary/assessment/94/visuals/, with permission.

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