# An update on folic acid fortification: benefits and risks 2012

A. David Smith and Helga Refsum

Prepared for the

New Zealand Baking Industry Research Trust

and

New Zealand Food and Grocery Council

February 2012

# Contents

# Executive Summary

## 1. How big is the problem of neural tube defects in New Zealand?

The current prevalence of neural tube defects (NTDs) in New Zealand is not precisely known and so there is no baseline from which estimates of the effects of folic acid fortification can be made. All that can be said is that the prevalence had been falling for several years until 2003 and, if this decline has continued, the prevalence may by now have reached about 7 per 10,000 births per year. This is likely to be a floor level, where folic acid may have no further effect. The lack of baseline data is a serious problem because it is government policy to monitor closely the effects of fortification, but in the current situation such monitoring will not be possible.

## 2. What impact would folic acid fortification have on the prevalence of neural tube defects?

The general folate status of New Zealand women of child-bearing age is very good. It is the same as, or better than, that of women in the USA after the introduction of mandatory fortification. Notably, in the USA the folate status appears to be sufficient to prevent all folate-sensitive NTDs. It is very difficult to estimate the effect of fortification on the prevalence of NTDs in New Zealand because there is no reliable data on the current prevalence of NTD pregnancies. Based on the best evidence available, we suggest that fortification may prevent no more than 6 NTD pregnancies per year, but quite possibly none.

## 3. Are there any other potential benefits of fortification?

### a) Population studies

There are rather few population studies on other possible beneficial effects of folic acid fortification. The data on other congenital defects is inconsistent and this may in part be due to changes in diagnostic practice over time; some defects appeared to be more common after fortification, while others (e.g. atrial septal defect) may have decreased. There were significant and important decreases in stroke mortality, and also in stroke incidence, following fortification in USA and Canada. Any associations with the incidence of myocardial infarction are much less clear and, if they occurred, were delayed. Causal links cannot be assumed, but the observations are of a similar kind to those used to support the effect of fortification on the incidence of NTDs.

### b) Randomized clinical trials

It should be recognised that the doses of folic acid used in randomized trials are much in excess of the amounts that could be obtained from food after fortification. Furthermore, many of the trials have used a combinations of vitamins, not only folic acid. Randomized trials, together with some observational studies, indicate that the periconceptual consumption of vitamin supplements containing folic acid prevent a high proportion (about 70%) of NTDs. The evidence is less secure concerning the prevention of other birth defects, but some cardiac malformations may be partly prevented. The

evidence that the supplements prevent other unwanted pregnancy outcomes is inconsistent. Trials in patients with cardiovascular disease are inconsistent and there is no evidence that ischaemic heart disease is prevented, but some studies appear to show a preventive effect on stroke. Certain subgroups might benefit from B vitamin treatment, but more studies are needed on this question. Trials of B vitamin mixtures that include folic acid have shown a protective effect against age-related macular degeneration, age-related hearing loss, and age-related cognitive decline. Furthermore, the accelerated brain atrophy associated with cognitive impairment in the elderly can be slowed by B vitamins. The effect of B vitamins on brain atrophy and cognitive decline is limited to a subgroup that had high baseline levels of plasma homocysteine. The trials showing beneficial effects of vitamin supplements are few and need to be confirmed.

## 4. Are there any possible harmful effects of fortification?

There is no doubt that consumption of folic acid can cause harm. In the elderly the most concerning effect is the increased risk of anaemia and of cognitive deficit in those with high folate status and low vitamin B12 status that has been shown in USA following fortification. The ability of folic acid to interfere with the action of the widely-used anti-folate drugs has not been thoroughly studied, but evidence that folic acid supplements lead to a higher death rate of children treated with anti-malarial drugs in countries with endemic malaria is of great concern. Women who are exposed to folic acid fortification have a higher risk of twin pregnancy if they use artificial reproductive technology, but there is no convincing evidence that normal pregnancy is affected. Unmetabolized folic acid is present in the blood of about 40% of Americans and the levels vary greatly due to differences between individuals in their metabolism. In elderly women, blood levels of unmetabolized folic acid are related to decreased natural killer cell cytotoxicity, with possible consequences for how the body deals with infections and cancer. Epigenetic effects of folic acid, through which intake by the mother can affect the future health of the child are possible. In India, high folate status in the mother was associated with an increased risk of type 2 diabetes in the 6-year old child. Other studies indicate that some allergic reactions may be enhanced in the children of mothers who were exposed to folic acid late in pregnancy. It is nearly impossible to provide solid evidence in terms of trial data for most of the suspected side effects. Such studies will always rejected by ethical review boards.

## 5. Folate and cancer

The relationship of folate and cancer is complex. Folate has a dual role in cancer, depending on the stage. For normal tissues folate is protective against factors that tend to make the cells neoplastic. For pre-neoplastic cells and cancer cells, however, folate can be harmful because it stimulates cell multiplication. Whether or not fortification with folic acid will reduce, or increase, the risk of cancer depends upon the timing of the exposure in an individual, the sensitivity of particular cancers to folate, the prevalence of particular subgroups of the population at greater risk, and the baseline level of folate in the population. It is quite possible for the overall effect of fortification to be neutral for cancer risk in the whole population, but for certain subgroups, such as women with particular classes of hormone receptors in breast tissue, or people with the TT

polymorphism of the gene for MTHFR, to show an increased risk. Population studies in countries that have already fortified are difficult to interpret, but a recent meta-analysis on 10 trials (38,000 people) in which folic acid (0.4 – 1 mg per day) was administered show an overall 7% increased risk of new cancers, with a 24% increased risk in prostate cancer.

## 6. What is the likely overall balance in public health benefit or harm if fortification is introduced?

**6.1** Possible benefits to public health of folic acid fortification are difficult to quantify because a lot of factors remain unknown. The most likely benefits are a small reduction in NTDs of up to 6 pregnancies per year and, perhaps, a reduction in cardiac birth malformations amounting to approximately 20 births per year. Due to the already very good folate status in New Zealand, any additional beneficial effect by fortification is probably limited.

**6.2** The possible harms to public health in New Zealand if fortification is introduced are difficult to estimate with any precision. Quantitatively, the best documented concerns will include: i) about 20,000 elderly (over 65) who may be at risk of cognitive impairment and anaemia due to an imbalance between high folate and low vitamin B12 status; ii) subjects with very high folate, likely to occur in at least 10% of the population, who may have an increased risk of cancer, leading to up to an extra 1,000 cases a year; iii) prostate cancer is very sensitive to folate and raised levels of folate in men may increase the incidence of prostate cancer by about 300 cases a year. iv) certain subgroups of the population (e.g. those with a particular genotype, TT, of MTHFR) who, when exposed to high folate, suffer from increased risk of being diagnosed or dying from certain cancers.

**There is increasing evidence that high intake of folic acid may cause harm. On public health grounds, taking into account the precautionary principle, it is difficult to justify exposing the whole population of 4.4 million to folic acid. Although fortification might prevent up to 6 NTD pregnancies per year, thousands of people may possibly suffer harm. Folic acid is a drug and there can be few examples in the history of medicine where whole populations have been exposed to a drug with such little research into the balance between beneficial and harmful effects.**

**Appendix: Notes on recent official reports from other countries**

The current situation is that no European country has introduced mandatory folic acid fortification. In Ireland, a country originally strongly in favour of fortification, it is considered that the level of folate in the population is sufficient to prevent most folate-sensitive NTDs. In other European countries, there is concern about possible harmful effects of fortification, but insufficient data to decide if these harms are important. However, since the above reports were published some new evidence, notably on cancer, has been published and we consider it highly unlikely that the EU or any European government would proceed with mandatory fortification in 2012, since policy decisions in public health are usually made with the precautionary principle in mind.

## Tables



## Supplementary Tables (separate)



## Figures

## List of abbreviations used in the text

ART, assisted reproduction techniques
CVD, cardiovascular disease
FSANZ, Food Standards Australia New Zealand
HR, hazard ratio
MI, myocardial infarction
MTHFR, methylene tetrahydrofolate reductase
*MTHFR*, methylene tetrahydrofolate reductase gene
NTD, neural tube defect
RCT, randomized controlled trial
RR, relative risk
tHcy, plasma total homocysteine
TUL, tolerable upper limit

## Author addresses and disclosures

Professor A. David Smith
University Department of Pharmacology
Mansfield Rd.
Oxford OX1 3QT
UK
david.smith@pharm.ox.ac.uk
+44-1865-271617

Professor Helga Refsum
Department of Nutrition
Institute of Basic Medical Sciences
University of Oslo
0316 Oslo
Norway
helga.refsum@medisin.uio.no
+47-22-85317

David Smith declares that he is named as inventor on two patents held by the University of Oxford on the use of folic acid and B vitamins to treat Alzheimer's disease or mild cognitive impairment.

Helga Refsum declares that she is named as inventor on a patent held by the University of Oxford on the use of and B vitamins to treat mild cognitive impairment. Helga Refsum is Norwegian representative on the European Working Group on the "Analysis of risks and benefits of fortification of food with folic acid". The views she expresses here are her own and do not represent those of the Working Group.

This review was commissioned by the Baking Industry Research Trust, but the Trust had no influence on its content or the conclusions drawn, for which the authors are entirely responsible.

# An update on folic acid fortification: benefits and risks 2012

**A. David Smith and Helga Refsum**

*Rarely has there been a case where the science has been so unequivocal, uncontentious and universally accepted, yet the development and implementation of appropriate policy continues to be problematic.* New Zealand Ministry of Health, 2003 (Ministry of Health, 2003)

## Introduction

More than 60 countries have introduced mandatory fortification of flour with folic acid, with the aim of reducing the prevalence of neural tube defects (NTDs) (Flour Fortification Initiative, 2011). But some countries, notably most European countries, have not followed this path, although many of them permit voluntary fortification of foods with folic acid. Australia and New Zealand, on the advice of Food Standards Australia New Zealand (FSANZ) (FSANZ, 2006), planned to introduce fortification in September 2009. Although Australia went ahead, the New Zealand government decided to defer the process until May 2012, but to encourage voluntary fortification of certain foods.

The purpose of this report is to review scientific and clinical research on the public health and safety aspects of the addition of folic acid to food and/or consumption by humans that has been published in the period 2008-2011, with particular relevance to New Zealand. In the context of the statement from the Ministry of Health at the start of this review, the following questions will be discussed:

1. How big is the problem of NTDs in New Zealand?
2. What impact would folic acid fortification have on the prevalence of NTDs?
3. Are there any additional possible benefits of fortification?
4. Are there any possible harmful effects of fortification?
5. Folate and cancer
6. What is the likely overall balance in public health benefit or harm if fortification is introduced?

We feel that it is important to start by reminding the reader that folic acid occurs rarely in nature and that is not a significant component of our food. It is a synthetic compound, with different pharmacodynamic and pharmacokinetic properties from the natural folates. From that perspective, it would be better viewed as a drug with beneficial effects, but also with side effects. Like a drug, it will be important to identify the optimum dose that gives benefit without significant harm. We will examine the evidence for folic acid's beneficial effects and ask whether there are any important deleterious side effects.

**1. Neural tube defects in New Zealand**

The reported prevalence of NTDs varies greatly across the world. In countries without mandatory fortification the reported rate, including stillbirths and pregnancy terminations, varies from 139/10,000 births in the Shanxi province of China (Li *et al.*, 2006) to 2/10,000 births in south Portugal (EUROCAT, 2009). New Zealand has a fairly low rate of NTDs and this rate appears to have declined in recent years (Figure 1). Unfortunately, the data for New Zealand is incomplete after 2003 since from this date NTD pregnancy terminations were not recorded and there are also no published records of NTD stillbirths, but only records of live-birth NTDs. Live-birth NTDs are an unreliable indication of prevalence because the majority of NTDs are diagnosed ante-natally and are frequently aborted. Thus there is no accurate data for NTD prevalence in New Zealand after 2003. On scientific grounds, this situation is highly regrettable for three reasons. First, it makes it difficult to draw any conclusions about trends and so we cannot use the data to estimate future NTD prevalence in the absence of fortification. Second, it will also make it difficult to detect an effect on prevalence if fortification is introduced. Third, not knowing the precise rate of NTD pregnancies makes it difficult to estimate the benefits of fortification in relation to the risks to other sectors of the population.

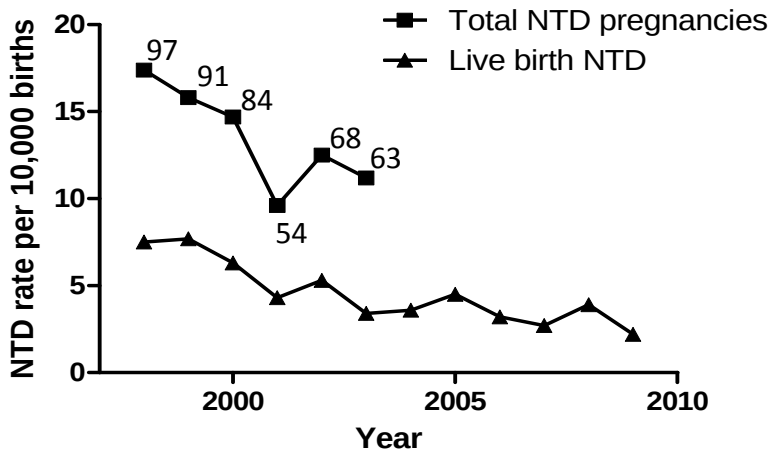



**Figure 1. Reported NTD prevalence in New Zealand since 1998**. Graph shows rate of NTDs per 10,000 births, with the actual number of NTD pregnancies up to 2003 beside each point. Data up to 2003 from Baseline Report (Australian Institute of Health and Welfare, 2011). Data after 2003 from New Zealand Birth Defects Registry (New Zealand Birth Defects Registry, 2011) and from Statistics New Zealand (Statistics, 2011).

The decrease of 1.3 cases/10,000 births per year in the rate of total NTD pregnancies between 1998 and 2003 is statistically significant ($r = -0.83$, $P = 0.04$) and similar trends have occurred in other countries without, or prior to, mandatory fortification, e.g. Australia (Australian Institute of Health and Welfare, 2011), California (Chen *et al.*, 2008), and several parts of Europe (EUROCAT, 2009). If the declining trend in New Zealand has continued, the total number of NTD pregnancies is likely to have reached a 'floor level' of about 7 NTDs per 10,000 births per year by 2010 at which level folic acid is unlikely to have any further effect (see below).

***Conclusion. The current prevalence of neural tube defects (NTDs) in New Zealand is not precisely known and so there is no baseline from which estimates of the effects of folic acid fortification can be made. All that can be said is that the prevalence had been falling for several years until 2003 and, if this decline has continued, the prevalence may by now have reached about 7 per 10,000 births per year. This is likely to be a floor level, where folic acid may have no further effect. The lack of baseline data is a serious problem because it is government policy to monitor closely the effects of fortification, but in the current situation such monitoring will not be possible***.

**2. What impact would folic acid fortification have on the prevalence of NTDs?**

It is established that fortification of flour with folic acid has reduced the prevalence of NTDs in many countries. Two recent meta-analyses have found a similar average degree of reduction in NTD prevalence of 46% (Blencowe *et al.*, 2010) and 41% (Imdad *et al.*, 2011). However, it should be noted that, in the latter report, 5 out of the 11 studies analysed were done in the same country (Canada). Furthermore, the reductions in prevalence ranged from 0% in California to 78% in Newfoundland, Canada (Imdad *et al.*, 2011). Some of the differences in the degree of reduction can be explained by the fact that the effect of fortification depends upon the baseline prevalence of NTDs in a population. This was strikingly shown for the different parts of China in a large study on folic acid supplementation (Berry *et al.*, 1999). In northern China, the baseline prevalence of NTDs was 48/10,000 births while in southern China, the prevalence was 10/10,000. Women who took 0.4 mg of folic acid per day periconceptually showed an 85% reduction in NTD pregnancies in northern China and a 41% reduction in southern China. A similar strong effect of baseline prevalence has been reported on the response to fortification in Canada, where the eastern provinces had a high rate of NTDs, while the western provinces had a lower rate before mandatory fortification (De Wals *et al.*, 2007). Between 2 and 4 years after fortification the NTD rates had fallen in all parts of Canada, but the percentage decrease was greater in the eastern provinces than in the west (Figure 2).

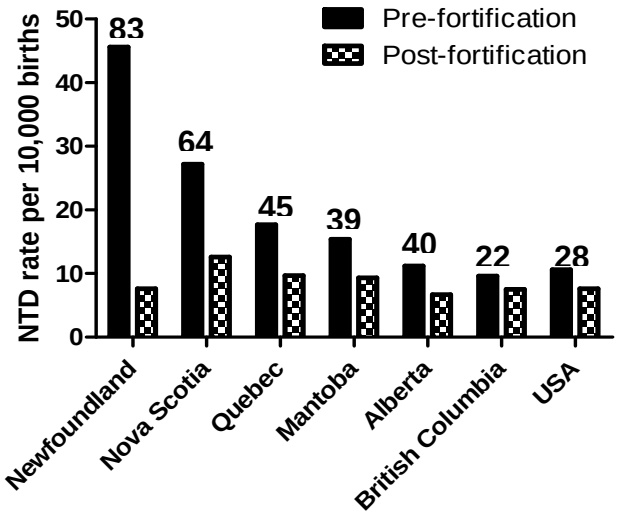


**Figure 2. Reported NTD rates in Canadian provinces and in USA before and after fortification.** Plot shows rate of NTDs per 10,000 births, with the % decrease after fortification indicated. For Canada based on Table 4 in De Wals (De Wals *et al.*, 2007) and for USA based on a CDC report on 8 districts that recorded NTD pregnancy terminations as well as stillbirths and live births (CDC, 2004).

The figure also includes the data for the USA, which started with a rather low NTD prevalence and shows a correspondingly small decline after fortification.

These results raise the question whether there is floor effect below which the prevalence of NTDs can no longer be reduced by folic acid. This floor effect could simply be because larger amounts of folic acid are required than are obtained from fortified foods in Canada and USA, both of which added 140 μg/100g flour, estimated to provide an additional intake of 100 μg per day on average. However, it has been pointed out that a floor effect is also apparent in clinical trials in which much higher doses of folic acid have been given (Heseker *et al.*, 2009). The latter authors concluded that a floor effect for folic acid occurs at a prevalence of about 7 cases of NTD per 10,000 births, depending to some extent on the racial mix of a population (Williams *et al.*, 2005). The existence of a floor effect has been acknowledged by authors from the Centers for Disease Control and Prevention, USA, who wrote "The data suggest that a prevalence of 5-6 cases per 10,000 pregnancies represents the lowest prevalence that is achievable through current folic acid fortification practices…" (Crider *et al.*, 2011). An important report from the National Birth Defects Prevention Study in the USA is consistent with a floor effect. A comparison of mothers of 564 cases of NTDs and 3,963 controls in the period after the introduction of mandatory fortification (1998-2003) found that additional periconceptional folic acid supplement use did not reduce the risk of having a pregnancy affected by an NTD (Mosley *et al.*, 2009). Furthermore, maternal intake of dietary folic acid and natural folates was not significantly associated with NTDs. One explanation for these findings suggested by the authors was that "Folate intake among the US child-bearing population may have reached levels where nearly all folate-sensitive neural tube defects have been prevented." A similar result was reported from the Slone Birth Defects Study (Ahrens *et al.*, 2011) on mothers of 205 cases of NTDs compared with 6357 control mothers: there was no reduction in the incidence of spina bifida in mothers who took folic acid-containing supplements periconceptually. However, in contrast to the National Birth Defects Prevention Study, there was a dose-related association of dietary folate intake with a reduced risk of spina bifida: for each 0.1 mg of dietary folate equivalents consumed, there was a 13% reduction in risk of spina bifida. The authors' conclusion was "In the era of dietary folic acid fortification, our study findings raise the possibility that supplementation with folic acid during the months immediately preceding neural tube closure does not offer further benefit in reducing the risk of a spina-bifida-affected pregnancy." (Ahrens *et al.*, 2011).

A floor effect for folic acid could arise if other factors are needed to prevent some NTDs. There is observational evidence of the possible role of many other factors, but so far no clinical trials to test these. Table 1 shows some of the factors, in addition to folate status, which have been reported to influence the occurrence of NTDs.

**Table 1. Other non-genetic factors associated with NTDs**

| Maternal risk factor for NTD | Relative risk or Odds ratio (95% C.I.) | Reference |
|---|---|---|
| Low vitamin B12 status | 2.41 (1.9-3.06) | (Molloy *et al.*, 2009, Wang *et al.*, 2011) |
| Obesity | 1.87 (1.62-2.15) | (Stothard *et al.*, 2009) |
| Influenza or fever | 3.93 (2.48-6.23) | (Acs *et al.*, 2005, Li *et al.*, 2007) |
| Use of antipyretic drugs | 4.86 (1.33-17.78) | (Li *et al.*, 2007) |
| Influenza AND anti-pyretics | 13.91 (3.04-63.55) | (Li *et al.*, 2007) |
| Hyperthermia | 1.92 (1.61-2.29) | (Moretti *et al.*, 2005) |
| Tea consumption | 3.4 (1.4-8.3) | (Ye *et al.*, 2011) |
| Low serum choline concentration | 2.4 (1.3-4.7) | (Shaw *et al.*, 2009) |

It is clear that low folate status is not the only factor that causes NTDs and so it will not be possible to prevent all NTDs by administering folic acid, in spite of a claim to the contrary (Wald *et al.*, 2001). Thus, the proportion of NTDs that is folate-dependent will depend not only upon the existing NTD prevalence and upon the folate status of the population (Bell and Oakley, 2009, Wald *et al.*, 2001), but also upon the population exposure to the other risk factors, which is largely unknown. Estimates have been made that up to 85% (Wald *et al.*, 2001), or up to 75% (Bell and Oakley, 2006) of NTDs worldwide are preventable by folic acid. It would be a mistake to apply these percentages to estimate the likely reduction in NTDs in New Zealand after fortification since they do not take account of the baseline folate status of women of child-bearing age, or of how close the baseline NTD prevalence is to the floor level, discussed above.

### *2.1 Folate status in New Zealand women*

Folate intake of a sample from the New Zealand population has been reported in a survey carried out in 1997 (Ministry of Health, 1999) but in this report no measurements of blood folate were given. There is one report of folate measurements in 114 women of child-bearing age recruited in 2000 into a clinical trial at the University of Otago (Norsworthy *et al.*, 2004). Plasma folate concentrations between 18 and 21 nmol/L and red blood cell concentrations between 608 and 637 nmol/L were found.

In the report on the most recent national nutritional survey in 2008 it was stated “Reliable data on intake of naturally occurring folate and of folic acid (from fortified foods) are not available due to limitations in analytical techniques” (Ministry of Health, 2011). However, the latter survey did report blood levels of folate in a sample of > 4,000 people, which is particularly

relevant to our discussion. In women, the mean red blood cell concentration was 901 nmol/L and the mean serum folate was 31 nmol/L. The extract in Table 2 shows red blood cell and serum folate concentrations for different ages in all the women in the study group

**Table 2. Folate status in NZ women in the 2008/9 nutrition survey** (Ministry of Health, 2011)

| | | Red blood cell folate (nmol/L) | | Serum folate (nmol/L) | |
|---|---|---|---|---|---|
| | | Mean (95% CI) | % low[1] (95% CI) | Mean (95% CI) | % low[2] (95% CI) |
| Females | 15–18 | 758 (708–808) | 3.3 (0.8–5.7) | 24.4 (22.4–26.4) | 1.8 (0.1–3.5) |
| | 19–30 | 763 (692–834) | 5.3 (1.1–9.6) | 29.2 (25.2–33.2) | 1.8 (0.4–5.0) |
| | 31–50 | 868 (821–915) | 2.4 (0.7–4.1) | 29.1 (26.2–32.0) | 1.8 (0.4–3.1) |
| | 51–70 | 1017 (952–1083) | 1.0 (0.0–2.0) | 33.8 (30.8–36.8) | 1.3 (0.0–2.6) |
| | 71+ | 1064 (999–1128) | 1.1 (0.1–2.1) | 39.7 (34.2–45.1) | 0.8 (0.1–1.5) |
| | Total | 901 (870–932) | 2.5 (1.4–3.6) | 31.1 (29.4–32.8) | 1.6 (0.8–2.3) |

Although the folate values given above for women in 2000 are probably not representative of the population, it can be seen from Table 2 that there appears to have been a significant improvement in folate status in New Zealand women of child-bearing age in the period up to 2008/9. A further improvement in folate status had occurred by 2011, as shown in the Folate and Women's Health Survey (Bradbury *et al.*, 2011). In the latter study, 300 women of child-bearing age from the cities of Dunedin and Wellington had mean red blood cell folate levels of 1096 nmol/L and mean serum levels of 35.6 nmol/L. These improvements in folate status may be one of the reasons for the fall in prevalence of NTDs found since 1998 (Figure 1). The folate status of NZ women is now the same as, or better than, that of women in the USA several years after mandatory folic acid fortification. The most recent survey in the USA (2005-2006) showed that women aged 20 to 59 years-old had a median serum folate concentration of 27 nmol/L and a median red blood cell folate of 614 nmol/L (McDowell *et al.*, 2008), compared with median values of 29 nmol/L and 989 nmol/L , respectively, for NZ women in 2011 (Bradbury et al., 2011).

In the report 'Focus on Nutrition' it was stated "Among women of child-bearing age, 27% had [folate] levels associated with a low risk of NTDs ($\geq$ 906 nmol/L) and 4% had red blood cell folate levels associated with a high risk of NTDs ($\leq$ 339 nmol/L)." (Ministry of Health, 2011). These proportions had changed by 2011: in the Folate and Women's Health Survey (Bradbury *et al.*, 2011) 59% had red blood cell folate levels of $\geq$ 906 nmol/L and 1% had levels of $\leq$ 339 nmol/L. Thus, a very small proportion of NZ women seem to be at high risk of a folate-dependent NTD pregnancy in 2011. In contrast, almost 60% of women have red blood cell folate levels ($\geq$ 906 nmol/L) associated with a very low risk of NTD, the same as obtained by

taking a daily 400 μg folic acid supplement, which is considered to be "highly protective" (Daly *et al.*, 1997).

### *2.2 Estimating the effect of fortification on NTDs.*

We can use the latest folate data to estimate the percentage reduction in NTD pregnancies that might occur in NZ after fortification, by means of the Wald model that was used by FSANZ in Attachment 9 of their Proposal P295 (FSANZ, 2006). The Wald model relates folate intake to blood folate levels and thus to NTD prevention. It was concluded that "the relation between changes in serum folate and the risk of NTD is almost reciprocal; doubling serum folate roughly halves the risk of NTDs." (Wald *et al.*, 2001). Applying the equation introduced by Wald:

Relative odds = (new serum folate/old serum folate)$^{-0.81}$

to the 2011 folate level of 15.7 ng/mL (35.6 nmol/L) in New Zealand women of child-bearing age, we find that a 0.2 mg per day increase in folic acid intake, which would lead to about a 2 ng/mL increase (from 15.7 ng/mL to 17.7 ng/mL) in serum folate, would result in about a 9% reduction in the risk of NTDs. This is likely to be a maximum figure since we have assumed that fortification will increase folate intake on average by 0.2 mg per day, although FSANZ (FSANZ, 2006) estimated that the average increase due to mandatory fortification would be less, at 0.14 mg/day. It is not possible reliably to estimate what a 9% reduction in NTDs would mean in actual numbers since there are no baseline prevalence figures for total NTDs later than 2003, as pointed out in Section 1. Let us consider two scenarios. First, that the total number of NTDs will be the same in 2012 as it was in 2003, i.e. 63. In this case, a 9% reduction would mean that there would be about 6 fewer NTDs per year. The second scenario would use our estimate above that the number of NTDs per year might have reached a floor level of 7 per 10,000 births by 2010, corresponding to about 40 cases per year. In that case, a 9% reduction would theoretically mean about 4 fewer NTD cases per year. But, in view of the floor effect for folic acid, there would in fact probably not be any reduction in the number of cases. We can conclude that the effect of fortification would be to decrease the total number of NTD pregnancies by between nil and 6 per year.

Our estimates can be compared with those made by FSANZ (FSANZ, 2006) also using the model proposed by Wald (Wald *et al.*, 2001). The P295 report estimated a reduction in NTD prevalence in New Zealand of between 5 and 20% (page 33), corresponding to a decrease in total NTD cases of 8 (95% C.I. 4-14) and one less live birth NTD each year (Table 5, page 34). These estimates differ from ours in part because P295 used a higher baseline prevalence of 72 NTDs per year and also because the authors did not have access to the latest data for the folate status of

the NZ population, using instead the data reported earlier by Norsworthy (Norsworthy *et al.*, 2004).

***Conclusion. The general folate status of New Zealand women of child-bearing age is very good. It is the same as, or better than, that of women in the USA after the introduction of mandatory fortification. Notably, in the USA the folate status appears to be sufficient to prevent all folate-sensitive NTDs. It is very difficult to estimate the effect of fortification on the prevalence of NTDs in New Zealand because there is no reliable data on the current prevalence of NTD pregnancies. Based on the best evidence available, we suggest that fortification may prevent no more than 6 NTD pregnancies per year, but quite possibly none.***

## 3. Are there any additional possible benefits of fortification?

Folates play a key role in metabolism, mainly as carriers of one-carbon groups for methylation and for synthesis of nucleic acids. It is thus possible that extra folic acid in the diet, which is converted to folates in the body, could have health benefits beyond the prevention of NTDs. There is considerable evidence that raised concentrations of plasma total homocysteine (tHcy) are associated with several different disease outcomes (Refsum *et al.*, 2006) (Selhub, 2006) and, since folic acid fortification has lowered tHcy in the population (Pfeiffer *et al.*, 2005), it is natural to ask whether fortification has had any impact on the diseases related to tHcy. In this section we will briefly review recent evidence that fortification may have additional effects on health outcomes. Because population studies are often difficult to interpret, we will also consider randomized controlled trials in which folic acid has been administered.

### *3.1 Population studies in countries that already have mandatory fortification*

Overall, there have been relatively few good quality studies at the population level on outcomes other than NTDs in the countries that have introduced mandatory fortification. It is to be regretted that not many countries have established proper monitoring systems in which the accurate prevalence of a disease that might be linked to folate is established before the introduction of fortification, and then monitored afterwards (Abeywardana *et al.*, 2010, Rosenberg, 2005).

With the successful prevention of one class of congenital defect by fortification, it was natural to ask if any ***other birth defects*** might be prevented, especially as animal experiments have suggested a role for folic acid in many developmental defects. We summarise the results from the largest population studies in Table 3, from which it can be seen that there is relatively little consistency in the findings. It should be noted that the studies from USA, South America and Alberta were very comprehensive and so if they did not report an effect on any particular defect they did not find one. Two studies reported reductions in atrial septal defects after

fortification and these would have been included in a third study, from Quebec, which reported a decreased prevalence of severe congenital heart defects (Figure 3). It is noteworthy that some defects appeared to increase in prevalence after fortification, especially in the Alberta study. The increase in obstructive genitourinary defects in Alberta was also noted in the USA study. The poor overall agreement in these studies may in part be related to differences in diagnostic practice and coding but also to the lack of power some studies may have had to detect small changes in the prevalence of rare defects. In an editorial comment on the paper from Quebec (Ionescu-Ittu *et al.*, 2009), Gardiner and Fouron (Gardiner and Fouron, 2009) pointed out that prenatal detection rates for congenital heart defects increased over the fortification period and so some decrease in the prevalence of live births-defects may have occurred for this reason. These considerations underline the difficulties in interpreting population studies, but of course the same difficulties apply to the study of NTDs. We discuss below evidence from clinical trials.

**Table 3. Folic acid fortification and birth defects other than NTDs: population studies**

| **Study and size** | **Birth defect** | **Significant % change** | **Reference** |
|---|---|---|---|
| National Birth Defects Prevention Network USA<br>ca. 6 million births | Transposition of great arteries<br>Cleft palate only<br>Pyloric stenosis<br>Renal agenesis<br>Obstructive genitourinary<br>Upper limb reduction<br>Omphalocele<br>Down's syndrome | -12<br>-12<br>-5<br>-28<br>**+12**<br>-11<br>-21<br>**+6** | (Canfield *et al.*, 2005) |
| Alberta Congenital Anomalies Surveillance System<br>ca. 400,000 births | Atrial septal defect<br>Obstructive genitourinary<br>Abdominal wall<br>Gastroschisis<br>Pyloric stenosis | -20<br>**+45**<br>**+40**<br>**+91**<br>**+49** | (Godwin *et al.*, 2008) |
| Administrative data-bases from Quebec<br>1, 324,440 births | Severe congenital heart defects | -6% per year | (Ionescu-Ittu *et al.*, 2009) |
| Estudio Colaborativo Latino Americano de Malformaciones Congénitas (ECLAMC)<br>3,347,559 births in Chile, Argentina & Brazil | 52 defects checked; only spina bifida, anencephaly and hip-subluxation changed in all 3 countries.<br>Hip-subluxation<br>Artial septal defect (2 countries) | <br><br><br><br>-29, -58, -60<br>-31 and -44 | (Lopez-Camelo *et al.*, 2010) |

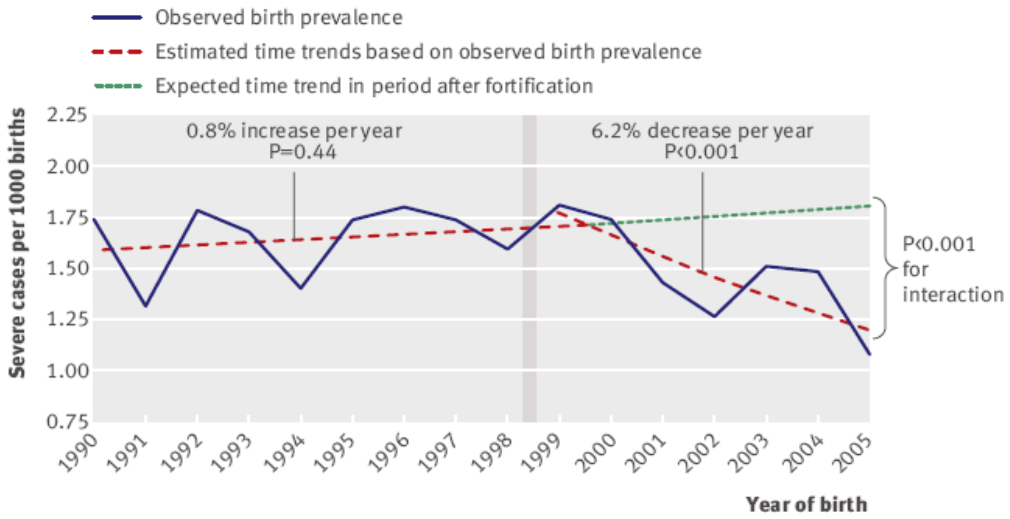



**Figure 3. Changes in prevalence of severe congenital heart defects in Quebec**
From (Ionescu-Ittu *et al.*, 2009)

Raised concentrations of plasma total homocysteine (tHcy) are associated with several ***adverse pregnancy outcomes*** (Murphy and Fernandez-Ballart, 2011) and so the question arises: have pregnancy complications decreased in prevalence after fortification? There appear to have been few studies of this question, but a Canadian report showed no change in the prevalence of placental abruption after fortification was introduced (McDonald *et al.*, 2005). On the other hand, a large population-based observational Norwegian study suggested that the higher doses of folic acid present in supplements taken in pregnancy appear to reduce the prevalence of placental abruption (Nilsen *et al.*, 2008).

Raised tHcy is a risk factor for ***stroke*** (Spence, 2007) and mortality from stroke appears to have declined more rapidly after fortification in both USA and Canada (Yang *et al.*, 2006). The comparison with England and Wales, where fortification is only voluntary, is striking (Figure 4), and makes it unlikely that the differences in USA and Canada are only due to improved treatment methods.


**Figure 4. Actual and estimated age-adjusted stroke mortality per 100,000 women in Canada and in England and Wales, 1990-2002.** The estimates are based on simple segmented log-linear regression analysis of the observed data. From (Yang *et al.*, 2006)

The difference after mandatory fortification is important in public health terms, with about 12,900 fewer deaths from stroke per year in the USA, and about 2,800 fewer in Canada, than if the trend before fortification had continued. The decline in mortality could have been due to a reduced incidence of stroke, or to a reduced case-fatality rate, or both. A recent report indicates that there may have been a decrease in stroke incidence in Canada following fortification, using hospital admissions for acute stroke as a measure (Figure 5)

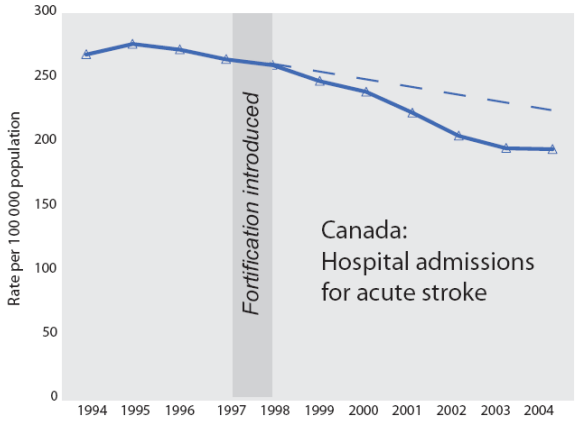



**Figure 5. Age and sex-standardized rates of hospital admissions because of acute stroke per 100,000 population over 20 y.** Modified from (Tu *et al.*, 2009). The dashed line and the shaded area were not on the original figure.

There has also been an overall fall in hospitalizations for stroke in USA (see Figure 7, below), although not all age groups showed this trend for ischaemic stroke (Lee *et al.*, 2011). Population studies such as these are open to many different interpretations, but, in view of the potential public health implications, it is important that similar data are collected for other countries that have introduced mandatory fortification to see if the association is consistent. If it is, then prevention of stroke would be an argument in favour of fortification. We will discuss below evidence from randomized clinical trials in stroke.

Raised tHcy is also an established risk factor for ***myocardial infarction*** (MI) and so it is of interest to ask if there have been any changes in population statistics for MI in countries that have fortified. Interpreting mortality data is difficult because changes in treatment practice can have big effects. But the data for hospital admissions is a closer, though not perfect, reflection of incidence. The same Canadian study described above reported trends in hospital admissions for MI and these are shown in Figure 6. There is a fall in admissions that starts about 4 years after fortification was introduced.

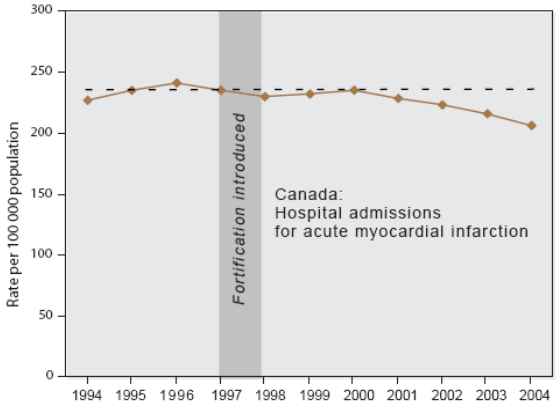



**Figure 6. Age and sex-standardized rates of hospital admissions in Canada because of acute myocardial infarction, per 100,000 population over 20 y.** Modified from (Tu *et al.*, 2009). The dashed line and the shaded area were not on the original figure.

The pattern of hospitalization rates for acute myocardial infarction the USA is shown for comparison in Figure 7.

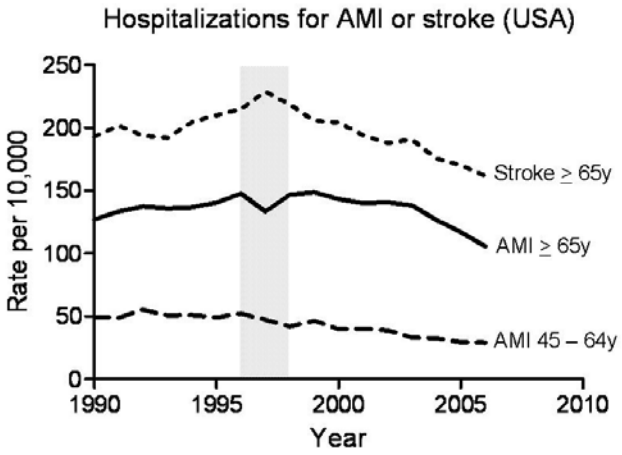



**Figure 7. Hospitalization rates for stroke and acute myocardial infarction in USA**.
Data plotted from Charts 3-21 and 3-52 of (National Heart, 2009). The shaded area shows the period during which fortification with folic acid was introduced.

These data are presented for information. Since many other factors could influence hospital admissions for a disease, it is not possible to draw the conclusion that any (delayed) tendency for rates of hospital admissions for MI to decline was a consequence of fortification, but similar data should certainly be collected for other countries that have introduced, or plan to introduce, fortification.

Studies on possible beneficial effects on *cancer incidence* will be discussed in the section below on possible harmful effects of fortification.

***Conclusions. There are rather few population studies on other possible beneficial effects of folic acid fortification. The data on other congenital defects is inconsistent, and this may in part be due to changes in diagnostic practice over time; some defects appeared to be more common after fortification, while others (e.g. atrial septal defect) may have decreased. There were significant and important decreases in stroke mortality, and also in stroke incidence, following fortification in USA and Canada. Any associations with the incidence of myocardial infarction are much less clear and, if they occurred, were delayed. Causal links cannot be assumed, but the observations are of a similar kind to those used to support the effect of fortification on the incidence of NTDs.***

### *3.2 Randomized controlled trials of folic acid supplements showing evidence of benefit*

*Randomized controlled trials* (RCTs) are widely considered to be the 'gold-standard' for establishing causality for an intervention. It was the UK MRC trial in 1991 that led a change in public health policy and to the eventual introduction of folic acid fortification. In considering other possible benefits, or harms, of fortification, it is natural to ask what RCTs show for the different outcomes. We will be summarising this evidence below, but would like to point out some caveats that should be born in mind. First, the dose of folic acid used in all trials is considerably higher (800 – 2500 μg per day) than would be obtained from food in a country with mandatory fortification (100 – 200 μg/day). Thus, the results of RCTs cannot be directly extrapolated to a population but, if the outcome is clear, they indicate an effect of the intervention at a particular dose. Further dose-effect studies would be necessary before any conclusions could be drawn about causality in the population context. However, such RCTs generate hypotheses that can be tested by looking retrospectively for population effects of fortification over time. Second, many RCTs are carried out on people selected for a particular reason and so the results can only be applied to people with these characteristics. An example is the trials of folic acid-containing supplements in people with cardiovascular disease, where the aim was to see whether the vitamins prevented a second cardiovascular event; this is called a secondary prevention trial. For a population, what is needed are primary prevention trials in people who do not have the disease in question, but these are very difficult to carry out. Third, for practical reasons, most RCTs are of relatively short duration, a few years, which may not be long enough for an effect of the treatment to become statistically significant. This situation contrasts with fortification, where the exposure is permanent. Fourth, many of the trials included several other vitamins/nutrients, and sometimes other drugs, thereby complicating the interpretation in terms of folic acid.

Following the initial trail-blazing RCTs in the UK on folic acid and the recurrence of NTDs, and in Hungary on the first occurrence of NTD (reviewed (Botto *et al.*, 1999, Pitkin, 2007), (Czeizel *et al.*, 2011), and (Wald, 2011)), there have been few other trials on ***NTD prevention*** for ethical reasons. A recent Cochrane review only found 5 RCTs, all conducted before 2001, in 6,105 women and concluded that daily consumption of a folic acid-containing supplement reduced the risk of an NTD pregnancy by 72% (De-Regil *et al.*, 2010). A striking large-scale study is a community-based intervention carried out in almost 250,000 women in China, where reductions in NTD pregnancies of 79% in the high-prevalence area of North China and 41% in the low-prevalence area of South China were obtained with daily supplements of 0.4 mg folic acid (Berry *et al.*, 1999). A review of observational studies on the use of folate supplements and NTDs revealed 4 articles of satisfactory quality, all of which found a reduction in NTD prevalence in women who reported taking supplements containing folic acid (Wolff *et al.*, 2009).

Since ethically it is not possible to withhold folic acid from women planning to become pregnant, all subsequent studies on possible preventive effects of folic acid on ***other pregnancy outcomes*** have had to be observational and population based. Such studies have reported beneficial effects of vitamin supplements containing folic acid, usually during the second trimester, on the incidence of facial clefts, pre-eclampsia, placental abruption, spontaneous abortion and gestational hypertension (for references see (Johnson and Little, 2008), (Nilsen *et al.*, 2008) and (Nilsen *et al.*, 2010)). A population cohort study provided evidence that some of the adverse effects of smoking in pregnancy, notably low birth-weight, may be partly prevented by folic acid-containing supplements (Bakker *et al.*, 2011). The use of folic acid containing supplements, often including other vitamins, during pregnancy has been reported to be associated with a reduction in the proportion of pre-term births in Hungary (Czeizel *et al.*, 2010, Czeizel and Banhidy, 2011), and in USA after fortification (Bukowski *et al.*, 2009), but not in Norway (Nilsen *et al.*, 2010). Although the Norwegian study also found no association of blood folate levels with infant birth size (Nilsen *et al.*, 2010), a study from the Netherlands has reported that periconceptual use of folic acid supplements reduced the risk of low birth weight (Timmermans *et al.*, 2009). As suggested in an editorial, the definitive answer to the role of folate in pre-term birth requires a clinical trial, but this is unlikely to be done on ethical grounds (Callaway *et al.*, 2009).

A comprehensive meta-analysis of case-control or cohort studies and early randomized trials has suggested a protective effect of multi-vitamin preparations containing folic acid against several ***congenital anomalies***, including NTDs, cardiovascular defects, and limb defects (Goh *et*

*al.*, 2006). The latter report is really only hypothesis-generating since there are many potential confounders relating to the use of vitamins and because it is not possible to determine if it is the folic acid and/or the other vitamins that are important. In the original Hungarian randomized trial of a B vitamin supplement containing folic acid (0.8 mg), vitamins B2, B6 and B12, a reduction of almost half in other major congenital abnormalities was observed, reviewed by (Czeizel and Banhidy, 2011). The main abnormalities prevented were ***cardiovascular defects*** and this result was confirmed in a subsequent cohort controlled trial in Hungary, where the biggest effect was a reduction of 74% in ventricular septal defects in the B vitamin group (Czeizel and Banhidy, 2011). This finding was confirmed in a large population-based case-control study in the Netherlands, which reported an overall reduction in risk of 18% for all congenital heart disease with the largest reduction (38%) for isolated septal defects (van Beynum *et al.*, 2010). Although it is likely that the majority of women in the Dutch study who consumed vitamin supplements took folic acid alone, the authors were not able to test whether the protective effect was associated with folic acid and/or with the other vitamins in the multivitamin preparation. Thus, it is difficult to use the studies described in this paragraph to predict possible effects of folic acid fortification. Circumstantial evidence is consistent with the protective effect being due largely to folic acid since women who take folate antagonists have a higher incidence of babies with heart defects (Hernandez-Diaz *et al.*, 2000), and animal experiments have shown that severe folate deficiency in the mother is associated with heart defects in the progeny (Zhu *et al.*, 2007). *On balance, these population studies, together with the studies described above of small but significant decreases in the occurrence of some congenital heart defects in countries following mandatory fortification, are consistent with a protective effect of folic acid against cardiac malformations in infants. But it must be pointed out that the most recent Cochrane review of randomized clinical trials (all performed before 2001) concluded that there was no effect of folic acid supplementation on birth defects apart from NTDs (De-Regil et al., 2010).*

Folate intake has been implicated in ***depression*** and a few RCTs have investigated the effect of adding folic acid supplements to existing drug treatments; there is a hint of benefit, but more trials are needed (Taylor *et al.*, 2004). A prospective study in nearly 7,000 pregnant women has found that use of folic acid during pregnancy did not seem to influence depression during pregnancy or 8 months afterwards, but that it did reduce the risk of depression 21 months post-partum (Lewis *et al.*, 2011). It is noteworthy that the association was only found in women who had the TT genotype of the *MTHFR* gene, an example of a subgroup effect.

A number of observational studies, but no RCTs, have looked at the possible association of maternal intake of folic acid during pregnancy and ***outcomes in the child***. Such studies are

particularly susceptible to multiple confounding and so the results must be treated with great caution. We will mention a few of these studies (for others see references cited by (Roth *et al.*, 2011)). Low intake (< 0.4 mg/day) of folate by 253 mothers in the first trimester of pregnancy was associated with a lower mental development index in their one-year old infants, but only in the mothers who had the TT genotype of the *MTHFR* gene (del Rio Garcia *et al.*, 2009). Use of supplements containing folic acid was associated with fewer behavioural problems in infants at 18 months (Roza *et al.*, 2010) and in better verbal, motor, cognitive and behavioural scores at 4 years (Julvez *et al.*, 2009). A large Norwegian study in 38,954 children found that severe language delay at age 3 was less common in those whose mothers reported taking folic acid supplements, or supplements containing folic acid, between 4 and 8 weeks gestation (Roth *et al.*, 2011). Studies in which mothers are questioned about their use of supplements are at risk of bias, but two studies have assessed folate status by blood measurements: lower maternal red cell folate levels at 14 weeks gestation were associated with hyperactivity and peer problems in 8-year old children (Schlotz *et al.*, 2010). A study in India on 536 women showed a concentration-dependent association between maternal serum folate levels, measured at 30 weeks gestation, and cognitive measures of learning ability and long-term storage and retrieval, visuo-spatial ability, and attention and concentration in 9 – 10 year-old children (Veena *et al.*, 2010). *It seems that maternal folate status in pregnancy is important for the development of the child.*

As mentioned above, plasma total homocysteine (tHcy) is an established risk factor for ***cardiovascular disease*** (CVD) and, since administration of folic acid and two other B vitamins (B12 and B6) can lower tHcy levels it is natural to ask if taking these vitamins will reduce the incidence of CVD. This question has been extensively studied in a series of RCTs to see if B vitamin mixtures including folic acid will prevent the recurrence of CVD in patients who have either had a stroke or a myocardial infarction. A meta-analysis of trials on the effects of B vitamins on *stroke* concluded that taking folic acid-containing supplements reduces the risk of a first stroke by 18%, but that there was no effect on second strokes (Wang *et al.*, 2007). However, the most recent meta-analysis of 8 trials on a total of 37,485 patients concluded that folic acid-containing supplements had no significant effects on cardiovascular events (stroke or myocardial infarction) in a 5-year period (Clarke *et al.*, 2010). In contrast to the meta-analysis by Wang et al (Wang *et al.*, 2007), that by Clarke et al. did not find any protective effect against first stroke. A key question for any meta-analysis that claims lack of an effect of a treatment is: did the study have sufficient power to detect an effect? The authors stated that their analysis had a more than 99% power to detect a 10%, or greater, reduction in cardiovascular events (Clarke *et al.*, 2010). There is an important difference between these RCTs and a population who would be exposed to fortified food: the participants in the trials had all suffered a cardiovascular event and were all

being treated with a host of drugs to protect them against further events. It is thus not correct to conclude from the RCTs that folic acid supplements will also have no effect in a relatively drug-free population. A multi-centre RCT on patients with CVD that included countries with, and countries without, mandatory fortification (HOPE-2) gave some important results. Overall, the B vitamin treatment reduced the risk of a stroke by 25%, but examination of subgroups showed that the treatment effect was greater in patients younger than 69 years, from regions without folic acid fortification, with higher baseline cholesterol and homocysteine levels, and those not receiving anti-platelet or lipid-lowering drugs at enrolment (Saposnik *et al.*, 2009). The greater effect in those not receiving anti-platelet drugs, like aspirin, is interesting in relation to an analysis by Wald et al. A comparison of trials on *heart disease* patients in which the majority of patients were receiving aspirin with trials in which a smaller proportion took aspirin, suggested that aspirin use interfered with an apparent protective effect of folic acid against heart disease (Wald *et al.*, 2011). The authors suggested that folic acid might therefore have a role in the primary prevention of heart disease, but not in secondary prevention where the use of aspirin is routine. Such a conclusion is consistent with the apparent beneficial effects of fortification in Canada and USA on hospital admissions for heart disease, described above. *In conclusion, we cannot exclude a protective effect of folic acid against CVD, but the evidence from RCTs is largely negative, with the possible exception of stroke.* It will be important to analyse the trial results again by looking at subgroups according to whether or not they were receiving aspirin and any future trials will need to consider more detailed subgroup analysis involving not only aspirin but other factors, including polymorphisms in the *MTHFR* gene and those listed above for the HOPE-2 trial.

Raised concentrations of tHcy have been associated in several studies with ***age-related macular degeneration*** and an RCT in which daily folic acid, vitamins B6 and B12 were administered for an average of 7.3 years to over 5,000 women showed a 34% reduction in risk in the B vitamin group (Christen *et al.*, 2009). The dose of folic acid was 2.5 mg per day and so this result should not be extrapolated to populations with folic acid fortification.

Low folate status has been associated with ***hearing loss***, especially in the elderly, and a trial in the Netherlands of folic acid (0.8 mg per day) on over 700 elderly slowed down the decline in hearing of the speech frequencies associated with aging (Durga *et al.*, 2007).

Raised concentrations of tHcy have been associated in many studies with ***age-related cognitive decline and dementia*** (vascular dementia and Alzheimer's disease) (Smith, 2008) and low folate levels (Ravaglia *et al.*, 2005) and low folate intake (Luchsinger *et al.*, 2007) have been

associated with a greater risk of developing Alzheimer's disease. There have been several RCTs to test whether lowering tHcy levels by administering supplements of B vitamins, including folic acid, will affect cognition. A meta-analysis of these trials concluded that there was 'no effect of folic acid, with or without other B vitamins, on cognitive function within 3 years of the start of treatment.' (Wald *et al.*, 2010) However, as the authors admitted, the median length of the trials reviewed was 6 months, which is probably too short to see any effect on cognition. Two trials of longer duration have shown a beneficial effect of folic acid-containing supplements, but this was related to the baseline concentration of tHcy. A trial of folic acid (0.8 mg per day) alone on over 800 volunteers who were recruited on the basis of a baseline tHcy level of $\geq$13 μmol/L was carried out in the Netherlands, a country that does not have mandatory fortification. The folic acid treatment lowered tHcy by 26% and slowed cognitive decline in those domains that tend to decline with age (Durga *et al.*, 2007). Elderly (168) with mild cognitive impairment, a precursor stage for dementia, were recruited into a trial in the UK in which daily B vitamins (folic acid, 0.8 mg; vitamin B12; 0.5 mg vitamin B6, 20 mg) were given. The B vitamin treatment lowered tHcy by 32% compared with placebo and slowed the rate of atrophy of the brain by an average of almost 30%. The effect of B vitamins on brain atrophy was strongly related to the baseline level of tHcy, such that those with $\geq$13 μmol/L showed a 53% slowing of brain atrophy (Smith *et al.*, 2010). In the same trial, the B vitamin treatment also slowed cognitive decline in several domains and improved the clinical status, but only in those whose baseline tHcy was $\geq$13 μmol/L (de Jager *et al.*, 2011). These trials illustrate the crucial importance of studying subgroups, in this case those with high baseline tHcy and/or those with cognitive impairment.

***Conclusion. Randomized trials, together with some observational studies, indicate that the periconceptual consumption of vitamin supplements containing folic acid prevent a high proportion (about 70%) of NTDs. The evidence is less secure concerning the prevention of other birth defects, but some cardiac malformations may be partly prevented. The evidence that the supplements prevent other unwanted pregnancy outcomes is inconsistent. Trials in patients with cardiovascular disease are inconsistent and there is no evidence that heart disease is prevented, but some studies appear to show a preventive effect on stroke. Certain subgroups might benefit from B vitamin treatment, but more studies are needed on this question. Trials of B vitamin mixtures that include folic acid have shown a protective effect against age-related macular degeneration, age-related hearing loss, and age-related cognitive decline. Furthermore, the accelerated brain atrophy associated with cognitive impairment in the elderly can be slowed by B vitamins. The effect of B vitamins on brain atrophy and cognitive decline is limited to a subgroup that had high baseline levels of plasma homocysteine. The trials showing beneficial effects of vitamin supplements are few and need to be confirmed.***

**4. Are there any possible harmful effects of fortification?**

We discussed this question in our Commentary in 2008 '*Is folic acid good for everyone?*' (Smith *et al.*, 2008) and refer the reader to this article for detailed comments. We will here outline the evidence that has been published since the review, under the following headings:

a) interactions with vitamin B12; b) interactions with anti-folate drugs and folate-dependent diseases; possible effects on twinning; d) possible epigenetic effects; e) possible harmful effects of free folic acid. The topic of folate and cancer will be dealt with in Section 5. As with the beneficial effects of fortification, we will discuss population studies in countries that have fortified separately from studies using randomized controlled trials.

### *4.1 Population studies in countries that already have mandatory fortification*

#### *a) Interactions with vitamin B12*

A long-standing concern has been that increasing the folate status of the population might mask the anaemia associated with vitamin B12 deficiency, which occurs in about 20% of the elderly and in some other subgroups, notably vegans and pregnant women and their infants. This could lead to irreversible damage to the nervous system since the B12 deficiency remains uncorrected. Studies in North America have provided some support for this concern. In Canada, the proportion of women with B12-deficiency and supraphysiological concentrations of folate (> 45 nmol/L) increased almost 7-fold after fortification (Ray *et al.*, 2003) and in the USA, the proportion of people with low serum B12 but not showing macrocytosis (a sign that alerts the physician to B12 deficiency) increased from 70% to 87% after fortification (Wyckoff and Ganji, 2007). Functional implications of these changes were reported in a study on nearly 1,500 elderly in USA, where it was found that after fortification those with high serum folate (> 59 nmol/L) and low B12 had a greater risk of anaemia and of cognitive impairment (Morris *et al.*, 2007). The results are illustrated in Figure 8, from which it can be seen that people with normal B12 and high folate had a reduced risk of cognitive impairment, while those with high folate and low

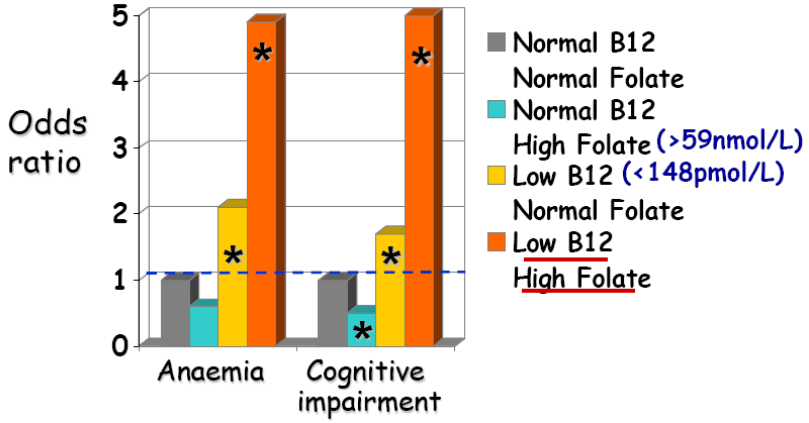


**Figure 8. Effect of fortification on anaemia and cognitive impairment in the elderly: the interaction between folate and vitamin B12 (plotted from (Morris *et al.*, 2007)).**

vitamin B12 had a 5-fold greater risk of cognitive impairment and anaemia. This result illustrates an important principle: **C*ertain subgroups may be harmed by the high folate levels that occur after fortification***, in this case those who have low B12 status. In the USA, this subgroup corresponds to about 4% of the elderly, close to 2 million people (Smith, 2007). A possible reason for the interaction between high folate and low B12 was suggested by the finding that markers of B12 insufficiency (methylmalonic acid, homocysteine and holotranscobalamin) were exacerbated in people with high folate (Miller *et al.*, 2009, Selhub *et al.*, 2007). Two studies in other countries claim to have failed to replicate the results obtained in the USA, but these studies took place in countries without mandatory fortification, and so the proportion with high folate was lower than in USA. A UK study found that the 17 elderly with low B12 and with folate > 60 nmol/L had an odds of cognitive impairment of 2.46 (0.9-6.7) which was not significant, probably due to the small numbers involved (Clarke *et al.*, 2008). A study from Ireland in young students (Mills *et al.*, 2011) found no evidence in this population that high folate (defined as level > 30 nmol/L) was associated with markers of B12 insufficiency – but it should be noted that not only were the subjects much younger, but the level of folate selected as a cut-off was half that used in the study by Morris et al. (Morris *et al.*, 2007). On balance, we think that the USA studies are sufficiently clear-cut to raise concerns about the effect of very high folate levels on the functioning of vitamin B12.

A common argument used in relation to these observations is that these high folate levels are only observed in people who also take folic acid containing supplements. Even if this is the case, it does not solve the problem in relation to fortification. If one has to start with large information campaigns warning about the potential harm of too much folic acid intake and tell people not to take supplements, but then add folic acid to flour by law the public will become very confused.

***b) Interactions with anti-folate drugs and folate-dependent diseases***

Drugs that interfere with the functions of folate in the cell are widely used in medicine as antibiotics, anti-malarial drugs, anti-cancer drugs, and to treat psoriasis and rheumatoid arthritis. Basic pharmacology would suggest that increasing the level of folate in the body may reduce the effectiveness of these drugs (Robien et al., 2005). Secondly, if these diseases can be treated by anti-folates then presumably they thrive on folate and so the question arises: will increasing the

folate level increase the prevalence and/or the severity of these diseases? These questions have not been addressed in any systematic manner in a population setting, with the exception of cancer, to be discussed below. The few reports in the literature were summarised in our earlier review (Smith *et al.*, 2008). There has since been a preliminary report based on case histories in one hospital, which found that the mean doses of methotrexate being used to treat rheumatoid arthritis in 36 patients rose by 34% from a period of 9 years prior to fortification compared with 3 years after fortification, implying reduced efficacy of the drug (Arabelovic *et al.*, 2007). A different conclusion was drawn by another group who studied the mortality rate for childhood from acute lymphoblastic leukemia in countries with and without fortification. A decreasing mortality trend was observed in all countries to a similar extent, and the authors concluded "folate fortification does not appear to have caused an increase in therapeutic failure…" (Kennedy *et al.*, 2011). However, that is by no means the only interpretation of the data. Good quality population-based studies are clearly needed on the questions raised above. Of particular interest would be studies on the prevalence of folate-sensitive diseases over time.

***c) Possible effect on twinning***

There has been much debate about whether periconceptual exposure to folic acid increases the frequency of twin pregnancies, which are an increased risk for mother and child. The consensus appears to be that, when pregnancies achieved by assisted reproduction techniques are allowed for (Vollset *et al.*, 2005), there is an insignificant increase in twins after exposure to folic acid. A maximum estimate from 5 retrospective studies in relation to fortification in the USA was an increase of 4.6%, although the authors commented on the methodological shortcomings of the primary studies (Muggli and Halliday, 2007). In contrast to natural conceived twin pregnancies, it has been noted that the incidence of multiple embryos in pregnancies achieved by assisted reproduction techniques increased by 11 – 13% after fortification in USA (Figure 9).

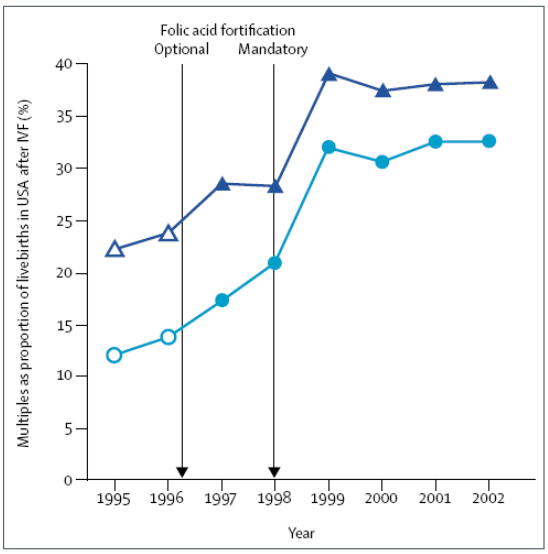


**Figure 9. Multiple births in USA in pregnancies from assisted reproduction techniques.** Circles show pregnancies with two embryos transferred; triangles show those with three. From web appendix in paper by Haggarty et al. (Haggarty et al., 2006).

The temporal relationship between the increase in multiple embryos and the introduction of folic acid fortification is striking. Implications will be discussed in Section 6.

***d) Possible epigenetic effects***

Since folate carries one-carbon units that are required for the methylation of DNA and histones, it has been suggested that modulating folate levels might cause epigenetic changes and that such effects might occur in the fetus *in utero* and so impact on the child's health. Direct evidence that fortification has such effects is not available, but there are a number of observations that are consistent with the idea of epigenetic effects of folic acid in humans, as has been shown in animals (for a short summary see our previous review (Smith *et al.*, 2008)). Most of the reports relate to increasing folate status by giving supplements and so will be discussed below. For a recent introductory review see Hussain (Hussain, 2012).

***e) Possible harmful effects of free folic acid***

Insignificant amounts of folic acid occur in our natural foods, which contain folate in the reduced form, coupled to several glutamate residues. Folic acid is in an oxidised state and has to be reduced by the enzyme dihydrofolate reductase in the liver (Wright *et al.*, 2007) before it can enter into metabolism in the body. Folic acid is a very poor substrate for the enzyme and furthermore, in man, the activity of this enzyme is very low compared with laboratory animals and varies about 5-fold between individuals (Bailey and Ayling, 2009). This finding may in part explain why unmetabolized folic acid is found in 40% of blood samples from the elderly in the USA (Bailey *et al.*, 2010) , and also occurs in human blood in countries where there is no mandatory fortification (Obeid *et al.*, 2010, Sweeney *et al.*, 2005, Sweeney *et al.*, 2009). Relatively small doses, around 400 μg, can lead to unmetabolized folic acid in serum (Sweeney *et al.*, 2007) and fasting blood levels went up after fortification in the USA even in those who did not take supplements (Kalmbach *et al.*, 2008). Some of the wide variation in levels of folic acid in blood may be related to a common polymorphism in dihydrofolate reductase (Kalmbach *et al.*, 2008) that reduces its activity and leads to higher levels of unmetabolized folic acid in plasma and to lower levels of red cell folate. The existence of subgroups of the population with different levels of unmetabolized folic acid in spite of similar exposure to dietary folic acid is likely to be important since it defines a subgroup that may respond differently to fortification. A strong hint

that unmetabolized folic acid matters was provided by the seminal study of Troen et al. (Troen *et al.*, 2006) who found a concentration-related inhibition of Natural Killer Cell cytotoxicity by folic acid in plasma in fasting samples taken after fortification. Natural Killer Cells are an important component of the non-specific immune response and are involved in killing virus-infected cells and tumour cells. Another study in the elderly found that the consumption of more than 400 μg of folic acid a day from supplements was associated with subsequent cognitive decline in the elderly (Morris et al., 2005). In a cohort where 33% of elderly Americans (mean age 70y) had detectable unmetabolized folic acid in their serum, it was found that folic acid levels were associated with an increase in anaemia and with lower cognitive test scores in those with poor vitamin B12 status (Morris et al., 2010). These authors speculated that circulating unmetabolized folic acid might harm the nervous system. More studies are needed on possible adverse effects of unmetabolized folic acid.

### *4.2 Randomized controlled trials of folic acid supplements, and some observational studies showing evidence of harm*

Apart from studies on cancer (see Section 5), there have been relatively few randomized trials in which the harmful effects of folic acid were studied. Hence, we will also consider here selected observational studies related to this question, in which supplements containing folic acid were administered.

#### *a) Interactions with anti-folate drugs*

Resistance to the therapeutic effects of anti-folate drugs is a serious problem, especially in cancer and antimalarial chemotherapy. Two randomized trials, one in Tanzania and one in Nepal, looked at outcomes after giving young children supplements containing folic acid. In Nepal, an area without malaria, there were no adverse effects, but in Tanzania, where children are routinely given anti-folates to protect against endemic malaria, there was a greater death rate in those given folic acid. An editorial stated "*The conclusion must be that the risks of death or severe illness of routine iron plus folic acid supplementation…. in young children exposed to high rates of malaria infection seem to outweigh any immediate benefits***."** (English and Snow, 2006). Methotrexate, an anti-folate, is one of the most widely used drugs in medicine and is used in therapy for cancer, rheumatoid arthritis, psoriasis and ectopic pregnancy. A post-hoc analysis of two randomized trials found that patients taking 1 – 2 mg of folic acid per day had a poorer clinical response to methotrexate (Dervieux et al., 2005). Evidence that folic acid can interfere with the therapeutic effect of methotrexate in psoriasis is proved by an open-label trial (Chladek et al., 2008) from which the authors concluded: "The antipsoriatic effect of methotrexate during

the remission-induction phase of treatment is influenced by folate status and may be significantly less if combined treatment with folic acid is used, irrespective of pre-treatment folate levels." Significantly, the reduced efficacy of methotrexate occurred at serum folate levels between 20 and 40 nmol/L, which include levels present in almost the entire US population after fortification (Pfeiffer et al., 2005). Further discussion of this question can be found in our earlier review (Smith et al., 2008) and in the following articles (Assaraf, 2007, Chattopadhyay *et al.*, 2007, Montaudie *et al.*, 2011, Porcelli *et al.*, 2011, Prey and Paul, 2009).

***b) Possible effect on twinning***

Since any effect of folic acid on the incidence of multiple births is thought be a result of its influence on pregnancies resulting from assisted reproduction techniques, it is important to establish how big this effect might be. A prospective cohort study (Haggarty et al., 2006) found that the mother's folate intake or plasma folate level was not related to the probability of a successful pregnancy, but that both were significantly related to an increased incidence of twins. A striking concentration-dependent effect was found between plasma folate and likelihood of twins, depending on the age of the mother (Figure 10).

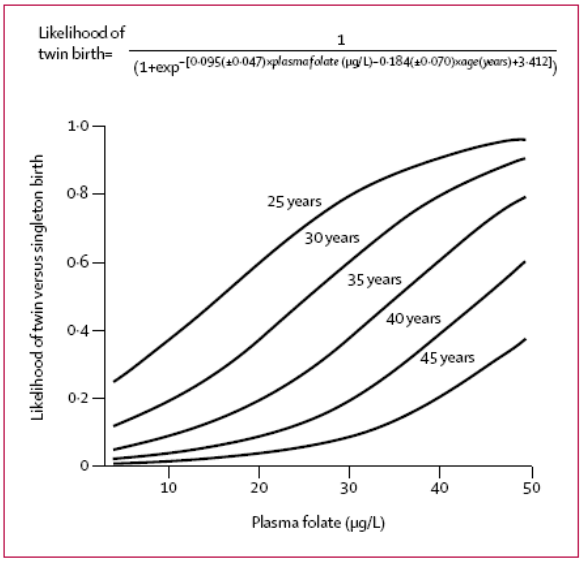



**Figure 10. Plasma folate and twin births in assisted reproduction in infertile women.**

Conditional effects plot, from (Haggarty et al., 2006)

***c) Possible epigenetic effects***

We are not aware of any randomized trials that concern possible epigenetic effects of folic acid, but there are several observational studies. We include in this discussion any effects of consumption of folic acid by the mother on the child.

One population study comes from India. High erythrocyte folate levels in mothers at 28 weeks gestation were predictive of increased *adiposity* and increased *insulin resistance* in their children at the age of 6 years (Yajnik *et al.*, 2008); the authors speculated that this might be mediated by epigenetic changes in the baby brought about by high maternal folate status. There was a concentration-dependent relationship between erythrocyte folate and increased risk of insulin resistance, starting from a level around 800 nmol/L, which is similar to the levels in women of childbearing age in New Zealand (see Table 2).

Allergic airways disease, of which *asthma* is the prime example, are increasingly common in children and it is therefore of interest that a leading researcher made this statement in 2008: "*One cannot ignore the observation that the increase in asthma prevalence over recent decades approximately coincides with worldwide campaigns that recommend periconceptional dietary folate supplementation*." (Miller, 2008) This comment was made in relation to an animal study in which it was shown that feeding the mother mouse a diet enriched in folic acid and several other methyl donors led to enhanced allergic inflammation in the progeny. This paralleled changes observed in promoter DNA methylation in many genes, some of which are known to be involved in the regulation of allergic responses (Hollingsworth et al., 2008). The levels of folic acid and other methyl donors were very high in this study and so it is of particular importance that a study from Norway on 32,077 children has found that the at 18 months of age the children of mothers who reported taking folic acid supplements in the first trimester of pregnancy showed increased risk for wheeze, lower respiratory tract infections and hospitalizations for respiratory tract infections (Haberg et al., 2009). The latter risk was increased by 24%. The authors suggested "that *methyl donors in the maternal diet during pregnancy may influence respiratory health in children, consistent with epigenetic mechanisms*." A prospective cohort study in Australia supported this hypothesis, reporting that children of mothers who took supplements containing folic acid late in pregnancy had a 26% greater risk of asthma at age 3.5 years (Whitrow et al., 2009). It is noteworthy that this association was only found for folic acid in supplement form and not for intake of natural folates from the diet. A Dutch study on 3,786 children has also reported that use of folic acid supplements by the mother is associated with an increased risk of wheeze at 1 year of age, but overall over 8 years there was no association with adverse allergic or respiratory problems (Bekkers et al., 2011). An valuable follow-up study to the earlier Norwegian report showed that maternal plasma folate levels measured at 18 weeks gestation were related to an increased risk of asthma in the child at age 3: children whose mothers' folate was in the top quintile; these children had a 66% increased risk of asthma compared with those in the bottom quintile of folate. There was a concentration

relationship between maternal plasma folate and risk. The top quintile of folate were levels above 18 nmol/L, which levels are present in more than 95% of New Zealand women of childbearing age (Table 2).

An Australian study found that intake of 500 μg/day of folic acid from supplements by the mother in the third trimester was associated with an 85% increased risk of eczema in the child at 1 year old, compared with mothers who consumed < 200 μg/day (Dunstan et al., 2011), but there was no relationship to folate intake from the diet. However, there was no association between maternal or cord blood serum folate and eczema. But there was a relationship between cord folate and the risk of allergic sensitization: at both low (<50) and high (>75 nmol/L) levels the child was at greater risk of allergic sensitization. These complex relationships indicate that the association between maternal folate status and the child's immune status is not straightforward. It seems that the timing of the exposure in pregnancy may be critical, as noted in the excellent review by Sharland et al. (Sharland et al., 2011), and two recent studies have failed to find any relationship of children's allergic status and folic acid use early in pregnancy (Magdelijns *et al.*, 2011, Martinussen *et al.*, 2011). It is noteworthy that, in these studies, it was only folic acid from supplements that was related to the outcomes in children, which might explain why the detailed study on the ALSPAC cohort found no association with maternal dietary folate intake (Granell et al., 2008).

Possible mechanisms of hypothetical epigenetic effects of folic acid in humans have been explored in a Dutch study (Steegers-Theunissen et al., 2009). Periconceptual consumption of 400 μg folic acid per day by the mother was associated with an increase in the methylation of the gene for IGF2, an embryonic growth factor, in the child aged 12 – 18 months. This study is consistent with the basic hypothesis, that folic acid in the mother can influence key genes in the child.

***d) Possible harmful effects of free folic acid***

We have discussed this matter in Section 4.1e above. We know of no intervention studies designed to compare the potential harmful effects of folic acid with that of reduced folates. We would like to draw attention, however, to reports that seem to show that the source of folate is important. In the study by Troen et al. on the effect of unmetabolized folic acid on natural killer cell activity it was found that the association was only found between plasma unmetabolized folic acid and the outcome, and not with plasma reduced folates (Troen et al., 2006). A prospective study on a large Danish cohort of more than 56,000 found a protective effect of reported dietary folate intake on colon cancer, but no protective effect of supplementary folic acid (Roswall *et al.*, 2010). A difference was also observed for lung cancer in the same cohort: in

this case, dietary folate *increased* the risk, while supplementary folic acid had no effect (Roswall *et al.*, 2010). In the studies on maternal folate and childhood allergy, two of the reports noticed that the association was only found for supplementary folic acid and not for dietary folate (Dunstan *et al.*, 2011, Whitrow *et al.*, 2009). A large observational study in USA on 25,400 women found that intake of folates from food was not associated with a risk of breast cancer, while women taking folic acid supplements (≥ 400 μg/day) did have an increased risk(Stolzenberg-Solomon et al., 2006). An Australian study reported that intake of food folates was protective against oesophageal cancer but that consumption of folic acid supplements increased the risk, especially of precancerous dysplasic Barrett's oesophagus (Ibiebele et al., 2011). A similar finding was reported in a trial on prostate cancer (Figueiredo et al., 2009). Baseline plasma folate levels in non-users of multivitamins were protective against the development of prostate cancer (Hazard Ratio 0.41 [0.18-0.96]) whereas those treated with 1 mg of folic acid per day had an increased risk of prostate cancer (HR 2.63 [1.23 – 5.65]). All these observations are consistent with important biological differences between folic acid and the natural folates, as is likely from their chemical properties (see Section 4.1e and our earlier review (Smith et al., 2008)). One way of looking at this complex picture was suggested by Ulrich (Ulrich, 2007), who argued that the relationship between folate status and risk of cancer was non-linear (Figure 11) with a higher risk in those taking folic acid supplements compared with natural folates. Another likely scenario is that folic acid has unique effects that differs from the natural folates. Indeed, based on the available data, both mechanisms appear probable.

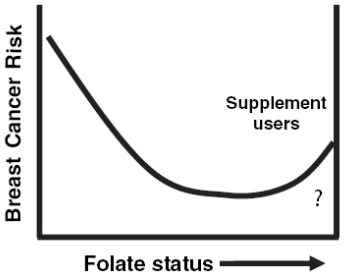



**Figure 11. Hypothesized non-linear relationship between folate status and breast cancer risk.** From (Ulrich, 2007)

***Conclusion. There is no doubt that consumption of folic acid can cause harm. In the elderly possibly the most concerning effect is the increased risk of anaemia and of cognitive deficit in those with high folate status and low vitamin B12 status that has been shown in USA following fortification. The ability of folic acid to interfere with the action of the widely-used anti-folate drugs has not been thoroughly studied, but evidence that folic acid supplements lead to a higher death rate of children treated with anti-malarial drugs in countries with endemic malaria is of concern. Women who are exposed to folic acid fortification have a higher risk of twin pregnancy if they use artificial reproductive technology, but there is no convincing evidence that normal pregnancy is affected. Unmetabolized folic acid is present in***

***the blood of about 40% of Americans and the levels vary greatly due to differences between individuals in their metabolism. In elderly women, blood levels of unmetabolized folic acid are related to decreased Natural Killer Cell cytotoxicity, with possible consequences for how the body deals with infections and cancer. Epigenetic effects of folic acid, through which intake by the mother can affect the future health of the child, are possible. In India, high folate status in the mother was associated with an increased risk of type 2 diabetes in the 6-year old child. Other studies indicate that some allergic reactions may be enhanced in the children of mothers who were exposed to folic acid late in pregnancy. It is nearly impossible to provide solid evidence in terms of trial data for most of the suspected side effect; such studies would always rejected by ethical review boards.***

## 5. Folate and cancer

### *5.1 Population studies*

A landmark article by Kim (Kim, 2004) was entitled "Will mandatory folic acid fortification prevent or promote cancer?". In this article Kim postulated that folate plays a dual role in cancer: a good folate status protects against the development of cancer by reducing the risk of neoplastic transformation of normal tissue, while at the same time high folate status stimulates the conversion of pre-neoplastic cells into cancer cells and promotes the growth of existing cancer cells. The different levels at which folate might act are shown in Figure 12.

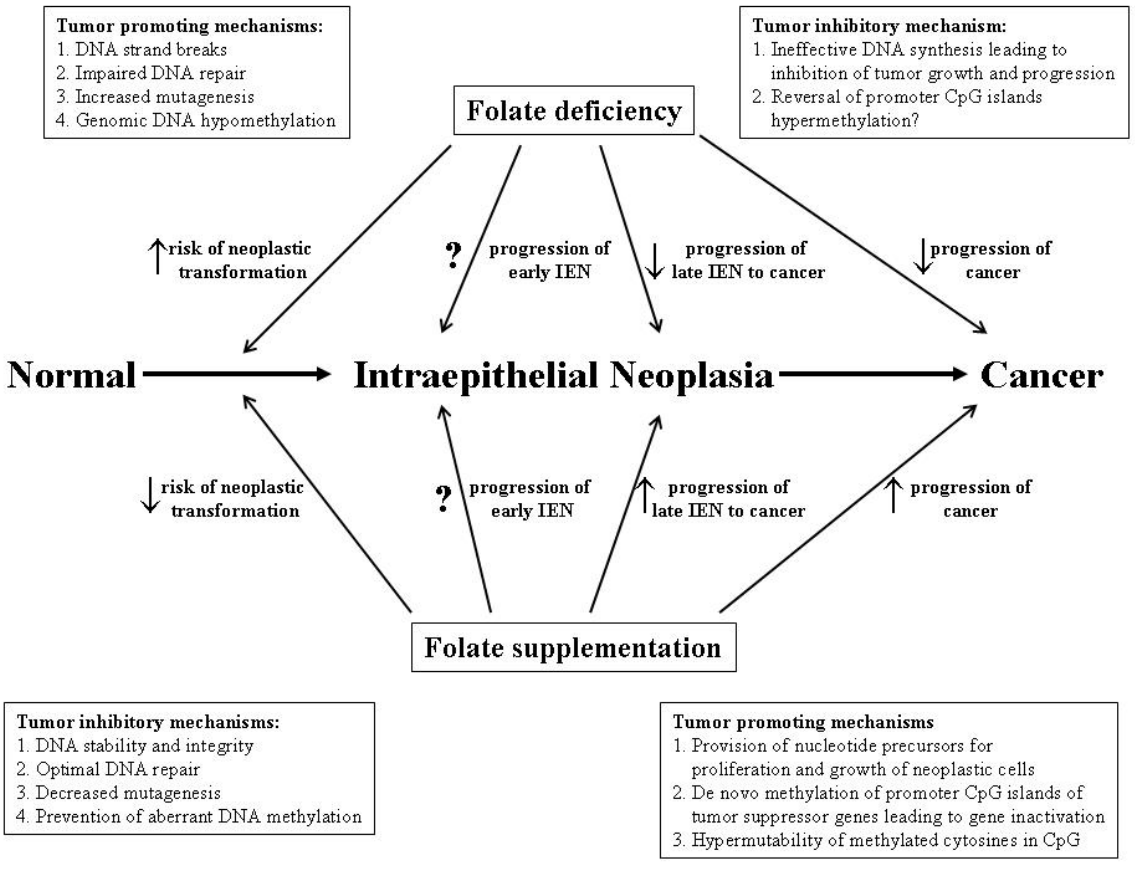



**Figure 12. Postulated dual modulatory role of folate in carcinogenesis.**
IEN, intraepithelial neoplasia. From (Smith *et al.*, 2008)

Kim's 'dual role hypothesis' is consistent with a great deal of evidence and it clarifies our understanding of the possible effects of folic acid fortification on cancer, while at the same time presenting us with a major dilemma. What the hypothesis predicts is that the effects of extra folate will depend upon the stage at which the cell transformations have reached. This is likely to vary according to the type of tissue, genetic polymorphisms and other environmental factors, of which the most common are smoking and alcohol consumption. The dilemma is that we cannot predict whether folic acid will be a 'good thing' or a 'bad thing' in a population. Rather, it is likely to be good for some people and bad for others. Thus, studies on the whole population may not tell us much if only a subset is affected by fortification. Nevertheless, let us look at the evidence from population studies to see if there are some hints.

In 2007 Mason et al. (Mason *et al.*, 2007) published a paper with the title "A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis." In this report they showed that the incidence rate of *colorectal cancer* in both the USA and Canada had significantly increased in the period immediately following the introduction of fortification. The data are shown in Figure 13. As can be seen, in both countries there was a trend for the incidence of colorectal cancer to

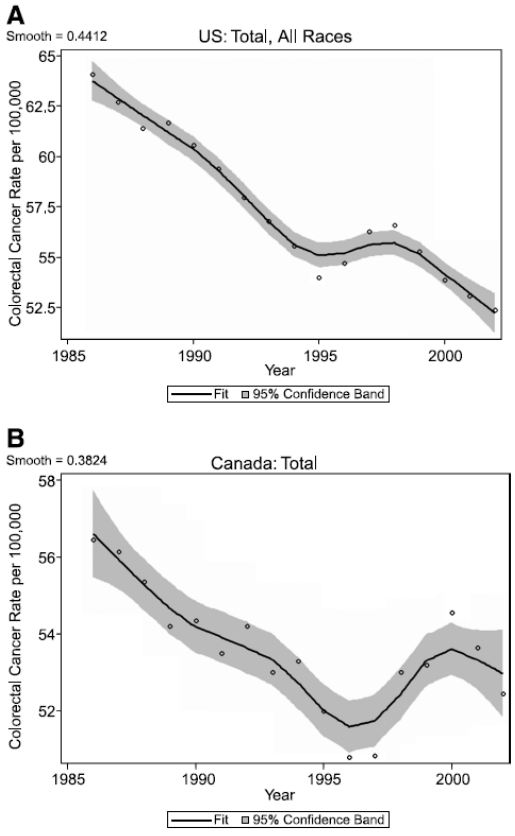
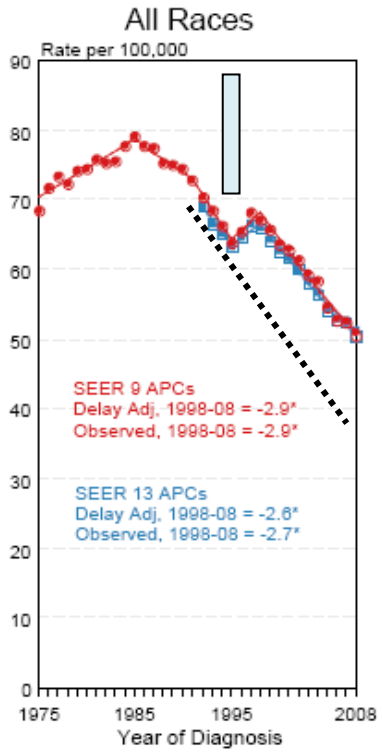



**Figure 13. Age-adjusted incidence of colorectal cancer in USA (A) and Canada (B).** From (Mason *et al.*, 2007)

decrease after 1985, but this trend was interrupted in about 1996 or 1997 by a sharp rise. The authors stated: “In each instance, the sudden increase in cancer incidence represents a highly statistically significant deviation from the pre-1996/1997 trend, resulting in an excess of about 4 to 6 additional cases per 100,000 individuals.” This would correspond to about an additional 15,000 cases per year in the USA. The authors’ hypothesis was that this change might have been related to the introduction of fortification, in view of the known effects of folic acid on the growth of cancer cells. The paper caused a lot of discussion, with arguments that the apparent changes in incidence may have been the consequence of new screening methods for diagnosis. The authors had considered this explanation and showed that an expansion in endoscopy screening came a few years later. A longer timespan can be seen from official statistics plotted in Figure 14A, from which it appears that the change was permanent so that the additional 15,000 cases per year continued. In comparison, we have plotted the data on the incidence of colorectal cancer in the UK over the same time period and using the same scale (Figure 14B), from which it can be seen that an apparent increase in the rate occurred at roughly the same time in the UK, but to a smaller extent; this needs to be examined statistically to see if it is significant. Two differences can be seen between the USA and UK data: the UK rate did not show an obvious steady decline before 1995 and the steep increase after 1995 was not maintained in the same way as in the USA.

**A.**




**B.**

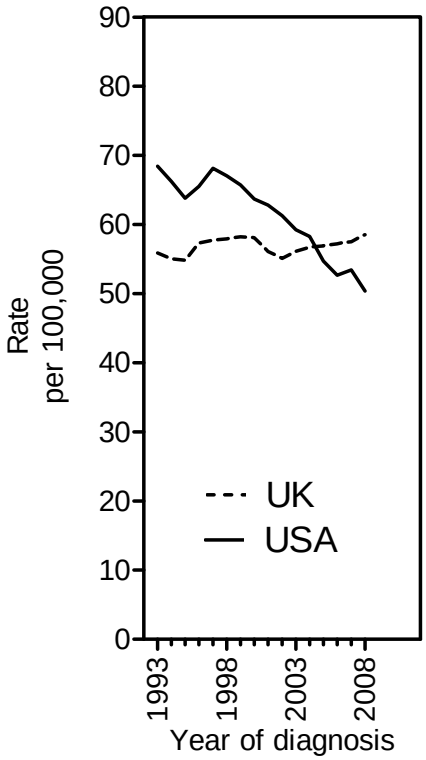


**Figure 14. Colorectal cancer incidence rates in men.**
**A. USA.** From the Surveillance, Epidemiology and End Results (SEER) database of the National Cancer Institute (http://seer.cancer.gov/). Red line shows the data for 9 SEER areas and blue line for 13 SEER areas. Blue bar was added to show period when fortification was introduced. The dashed line was added to show how the trend would have continued without the step increase in 1996-7. **B. USA (SEER) and UK.** UK data plotted from Cancer Research UK database: (http://info.cancerresearchuk.org/cancerstats/types/bowel/incidence/?vi)

These results make it very difficult to argue that the step increase in colorectal cancer incidence in USA and Canada around 1996 was solely related to folic acid fortification. The same caution should apply to a report from Chile which used hospital discharge rates as an indirect measure of incidence and found a sharp rise in rates for colorectal cancer a few years after mandatory fortification was introduced (Hirsch *et al.*, 2009).

Let us look at one other example of this approach: the incidence of *prostate cancer*. The data for the USA are shown in Figure 15. Dramatic changes in the incidence are shown after 1989, but these are most probably due to the introduction of the prostate-specific antigen (PSA) test. After 1992, the incidence fell sharply but stopped falling in 1996, when manufacturers started adding folic acid to flour products. This was followed by second increase in incidence that slowed around 2001. Very similar changes were found in Canada, notably the increase in incidence starting around 1997. But, as with colorectal cancer, we cannot conclude that the latter increase

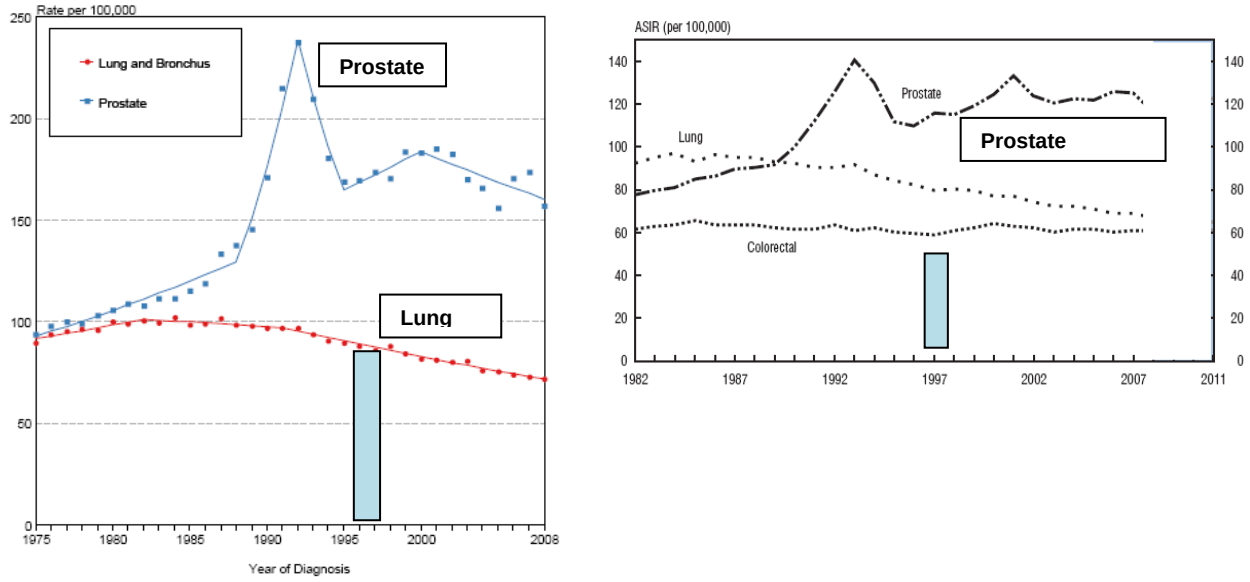



**Figure 15. Prostate cancer incidence rates in USA (left) and Canada (right).**

Left: prostate cancer in blue, lung cancer in red (from SEER reports, see Fig, 11). Right: the uppermost line shows the data for prostate cancer, the middle line for lung cancer and the lowest line for colorectal cancer (from Canadian Cancer Statistics 2011, Canadian Cancer Society http://www.cancer.ca). Blue bars added to show the period during which mandatory folic acid fortification was introduced.

is solely, or even at all, a consequence of mandatory fortification because a similar rise in prostate cancer incidence occurred in the UK, starting about 1999, as can be seen from the

Cancer Research UK database (http://info.cancerresearchuk.org/cancerstats/types/prostate/incidence/#trends). It is noteworthy that a similar temporary rise also occurred at this time period in New Zealand – See Figure 2 in (Ministry of Health, 2011).

These examples show how hazardous it is to draw inferences from population data of this type. Perhaps a similar degree of caution should be used in looking at the data on the incidence of NTDs in countries that have fortified? For instance, the modest decline in NTD incidence in the USA following introduction of fortification is difficult to isolate from the already declining NTD prevalence that occurred in the 1990's.

### *5.2 Randomized controlled trials*

The scientifically most rigorous way of finding out if increasing exposure to folic acid will increase the risk of developing cancer is to carry out randomized controlled trials. Such trials are ethically rather difficult to justify because the hypothesis being tested is one that may harm the participants. Hence, most trials have been based on answering other questions and the outcome of cancer has been a secondary aim. Most of the trials of homocysteine-lowering B vitamins mentioned in Section 3.2 had cancer incidence as a subsidiary endpoint, and 8 of these trials including 37,485 people have been reviewed in a meta-analysis (Clarke *et al.*, 2010). The authors concluded "folic acid allocation had no significant effect on the rate ratios (95% confidence intervals) for overall cancer incidence (1.05 [0.98-1.13]), or cancer mortality (1.00 [0.85-1.18])". In fact, the analysis showed a possible 5% increase in cancer that was not quite significant (the lower C.I. was 0.98) in the folic acid group. The authors did not give an estimate of the power of their analysis, i.e. how many subjects in each group would have been needed in order to obtain a significant effect of folic acid on cancer. We have done this estimate: in order to detect a significant increase of 5%, about 70,000 subjects would be needed in each arm. Thus, this meta-analysis cannot be used to conclude that there was no significant effect on cancer, as has been widely done in the media. Another short-coming of the meta-analysis is that the only certain pre-specified subgroups were examined, where no significant effect was found. As we will show below, further subgroup analysis may be critical.

An important study in Norway, in which the data from two trials were combined to give a total of 6,837 patients with heart disease, has shown that treatment for a median of 39 months with folic acid (0.8 mg) combined with vitamin B12 (0.4 mg) significantly increases the incidence of cancer and cancer mortality. The median follow-up period was a total of 77 months and the national registries were used to identify cases. There was a 21% increase in overall

cancer incidence and a 38% increase in deaths from cancer in the folic acid group (Ebbing *et al.*, 2009). The risk of developing cancer and of dying from cancer was related to the level of folate in the blood during the trial, with those having the highest folate level being most at risk. Notably, the serum folate level in those at highest risk was > 60 nmol/L, which is a level found in 10 – 15% of Americans after fortification (Pfeiffer et al., 2005). Although the trial was not powered to detect changes in the different subtypes of cancer, it is of interest that the estimated hazard ratio for colorectal cancer incidence was 1.00 while those for other major cancer types (lung, prostate, haematological, other) were all greater than 1, though not reaching significance. The increase in overall cancer incidence was mainly driven by increased lung cancer incidence. The results on overall cancer incidence are shown in Figure 16.

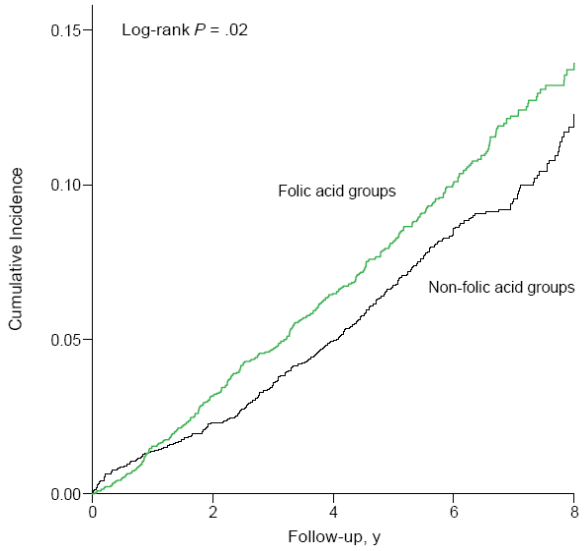



**Figure 16. Cumulative incidence of cancer in 6,837 heart disease patients treated with placebo or a combination of folic acid (0.8mg) and vitamin B12 (0.4mg).** Kaplan-Meier curves from Ebbing et al. (Ebbing *et al.*, 2009).

Analysis of subgroups gave an important result: patients with the TT genotype of the *MTHFR* gene were at the greatest risk of death from cancer, with a hazard ratio of 4.57 (1.55-13.9) for the folic acid groups compared with placebo. This result shows that other studies that do not look at such subgroups might miss a significant effect.

A recent meta-analysis of trials in which folic acid was administered and cancer was one of the outcomes, has confirmed and extended the findings of the Norwegian trials (Wien *et al.*, 2012). This meta-analysis was based on a systematic review and included 10 trials in a total of 38,233 people. There was a significant overall risk ratio for cancer incidence in the folic acid groups of 1.07 (1.00-1.14). In other words, there was a relative increase in risk of 7%. When an additional trial was included in which methyl-tetrahydrofolate was used instead of folic acid, the increased risk was 8% and the confidence intervals were 1.01-1.14. Subgroup analysis revealed

four studies in which folic acid consumption particularly increased the risk of cancer: studies in which the dose was between 0.4 and 1.0 mg per day; studies with >30% of smokers; studies with > 70% of men; and studies with follow-up greater than 5 years. In looking at the different types of cancer, the only one that reached significance was prostate cancer, where there was an increased risk of 24% in the folic acid groups (RR 1.24, CI 1.03,1.49). This meta-analysis did not look at *MTHFR* genotypes, probably because many trials did not report these results.

The significant increased overall risk of cancer of 7%, or 8% including the trial with methyl-THF, obtained in this meta-analysis is quite similar to the non-significant increased risk of 5% reported by Clarke (Clarke *et al.*, 2010), but the authors draw a very different conclusion. The 24% increased risk of prostate cancer is striking, and will be further discussed below. The possible public health significance of these findings will be discussed in Section 6.

***Some observational studies on folate and cancer***

There is a vast literature describing observational studies on folate and cancer in which folate intake is estimated from food frequency questionnaires or folate status is estimated from blood levels. Some selected recent reviews and observational studies are: on multiple types of cancer (Larsson *et al.*, 2006, Smith *et al.*, 2008, Wien *et al.*, 2012); colorectal (Gibson *et al.*, 2011, Hubner and Houlston, 2009, Kennedy *et al.*, 2011, Kim *et al.*, 2010, Kim, 2007) (Stevens et al., 2011) (Lee *et al.*, 2011); pancreatic (Bao *et al.*, 2011, Oaks *et al.*, 2010); prostate (Collin *et al.*, 2010); breast (Stevens *et al.*, 2010); ovarian (Webb *et al.*, 2011). There is no consistency in these studies, some reporting that high folate status is protective against cancer, while others report the opposite, and others report no association. We suggest that, apart from the possibility that there are genuine differences between different cancer types in their response to folate, these discrepancies probably reflect at least two main factors: first, the different stages of pre-cancer and cancer in the populations studied and, second, the different prevalence of particular subgroups at risk in the population studied.

A good example of the importance of subgroups is provided by the observational study on *breast cancer* in the Malmö Diet and Cancer Cohort of 11,699 women. In the first paper, entitled “High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmö Diet and Cancer cohort”, the authors reported that women in the highest quintile of total folate intake (including supplements) had a 44% lower risk of breast cancer than those in the bottom quintile (Ericson *et al.*, 2007). The Swedish media and public reacted with demands that mandatory folic acid fortification be introduced immediately (Jagerstad, 2007). Two years later, the authors reported that the association of high folate intake with protection

against breast cancer was limited to a subgroup of the women in the cohort, those who had the 677CT/1298AC genotype of the *MTHFR* gene. In contrast, women who had the 677TT genotype or the 677CT/1298AA actually showed an increased risk of breast cancer with higher folate intake (Ericson *et al.*, 2009). In the same year, the authors reported a nested case-control study in which they measured plasma folate: they found that the women with high plasma folate and the 677TT or 677CT genotypes had an increased risk of post-menopausal breast cancer, while the CC genotype showed no association with cancer and folate levels (Ericson *et al.*, 2009). Finally, to add another level of complexity, the authors reported that the association of high plasma folate with increased risk of breast cancer was limited to those whose breast cancer type was negative for estrogen receptor-beta (Ericson *et al.*, 2010). They concluded their last report with the statement "…the observations may be especially valuable in the on-going discussions, in many countries, concerning folic acid fortification". This important series of papers on the same cohort of women began by advocating increasing folate intake in order to prevent breast cancer, then found an adverse effect of folate on women with certain *MTHFR* genotypes, and finally concluded with a warning that women with breast tissue negative for estrogen receptor-beta might be at increased risk of cancer if they consume too much folic acid. *We have here, in a microcosm, the same situation that is faced by society as a whole: high folate status is good for the population as a whole, but subgroups within the population may be suffer serious harm.*

The importance of subgroups in analysing the biological effects of folic acid is being increasingly recognised. An excellent review includes this statement about genetic subtypes: "It is highly probable…..that studies seeking to define the relationship between folate intake and colorectal cancer risk that do not stratify subjects by genotype at two or three polymorphic loci at the very least will miss important disease–nutrient associations in subgroups of the population defined by these genetic variants." (Hubner and Houlston, 2009). We have collected some examples of how subgroups can influence the response to folic acid in Table 4.

**Table 4. The importance of subgroups in studies on the association of folate with disease**

| **Subgroup(s) involved** | **Outcome** | **Reference** |
|---|---|---|
| | | |
| *MTHFR* polymorphisms | Gastrointestinal cancers | (Larsson et al., 2006) |
| *ditto* | Colorectal adenomas | (van den Donk et al., 2007) |
| *ditto* | Colorectal cancer | (Hubner and Houlston, 2007)<br>(Kim et al., 2012) |
| *ditto* | Type of colorectal cancer | (Hubner *et al.*, 2007)<br>(Guerreiro et al., 2008)<br>(Hubner and Houlston, 2009) |
| *ditto* | Lung cancer | (Hung et al., 2007) |
| *ditto* | Breast cancer | (Ericson *et al.*, 2009, Ericson *et al.*, 2009) |

| | | |
|---|---|---|
| *ditto* | Overall cancer incidence & mortality | (Ebbing et al., 2009) |
| *ditto* | Stroke | (Holmes et al., 2011) |
| *ditto* | NTD | (Lacasana et al., 2012) |
| *MTHFR* genotype and gender | Rectal cancer | (Murtaugh et al., 2007) |
| Thymidylate synthase polymorphisms | Colorectal adenoma | (Hubner *et al.*, 2007) |
| Alcohol consumption | CVD, cancer | (Jiang et al., 2003) |
| *ditto* | Breast cancer | (Larsson et al., 2007) |
| *ditto* | Colorectal cancer | (Roswall *et al.*, 2010) |
| *ditto* | Oesophageal cancer | (Ibiebele et al., 2011) |
| *ditto* | Anaemia in elderly | (Morris et al., 2010) |
| Smoking | Overall cancer incidence & mortality | (Ebbing et al., 2009) |
| *ditto* | Lung cancer | (Johansson et al., 2010) |
| *ditto* | Overall cancer incidence | (Wien et al., 2012) |
| Estrogen receptor status | Breast cancer | (Ericson *et al.*, 2010, Harris *et al.*, 2011, Lin *et al.*, 2008, Roswall *et al.*, 2010) |
| Gender | Pancreatic cancer | (Oaks et al., 2010) |
| *ditto* | Rectal cancer | (Curtin et al., 2011) |
| Vitamin B12 | Cognition in elderly | (Morris *et al.*, 2007, Morris *et al.*, 2010) |
| *ditto* | Cervical cancer | (Piyathilake et al., 2009) |
| Plasma total homocysteine | Reduction in stroke | (Saposnik et al., 2009)Saposnik *et al.*, 2009) |
| *ditto* | Slowing of brain atrophy in MCI | (Smith et al., 2010) |
| *ditto* | Slowing cognitive decline in MCI | (de Jager et al., 2011) |
| Unknown genetic factors | Lowering tHcy | (Cotlarciuc et al., 2011) |

### *Prostate cancer and folic acid*

In view of the finding in the meta-analysis by Wien et al. (Wien et al., 2012) that men being treated with folic acid had a 24% increased risk of developing prostate cancer, we thought it would be of interest to look at some recent studies on this topic. One of the trials in the meta-analysis was that by Figueiredo et al. (Figueiredo et al., 2009). A total of 643 men were randomized to folic acid (1 mg per day) or placebo. The main result is shown in Figure 17.

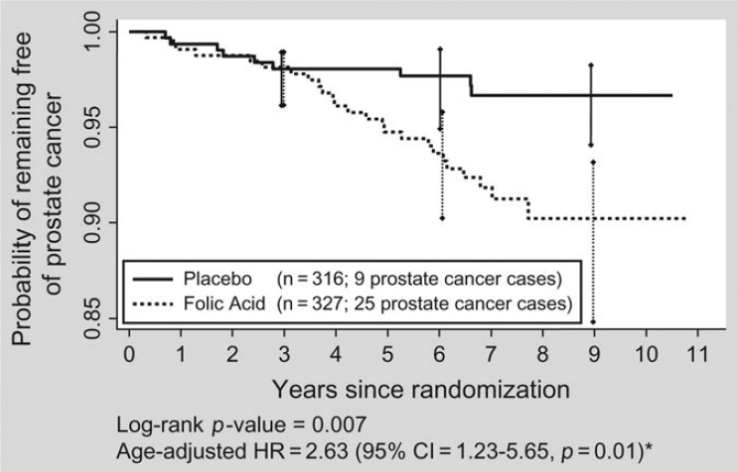



**Figure 17. Kaplan-Meier plot showing prostate-cancer free status over time.** The folic acid group received 1 mg per day. From (Figueiredo et al., 2009).

It can be seen that those receiving folic acid did not stay free of cancer for as long as those on placebo, so that after 10 years 25 of them had developed cancer compared with 9 in the placebo group. An unexpected feature of this trial is that while the patients receiving folic acid had an increased risk (HR 2.63), a high baseline plasma folate level in those who did not take multivitamin tablets was actually protective against prostate cancer (HR 0.41). The numbers developing cancer in this trial were small and so the risk ratios should be interpreted with caution. Nevertheless, a meta-analysis on 25,738 subjects in 6 trials, of whom 632 developed prostate cancer, confirmed that folic acid increases the risk of prostate cancer (RR 1.24 [1.03-1.49]) (Wien et al., 2012).

Recent observational population studies on folate and prostate cancer do not give a clear picture. The large EPIC case-control study in Europe found no association between plasma folate levels and risk of prostate cancer, but any association might have been lost in the large variation in plasma folate levels between participating countries, ranging from 5.6 nmol/L in Sweden to 15.3 nmol/L in Germany (Johansson et al., 2008). However, this result was confirmed in another large case-control study (ProtecT) in the UK, where there was no difference in folate levels between 1,507 controls and 1,461 cases (Collin *et al.*, 2010). However, in a meta-analysis of prospective studies omitting ProtecT a significant increased risk of high baseline folate was found, with and odds ratio of 1.19 [1.03-1.37] (Collin *et al.*, 2010). An indirect measure of the rate of progression of prostate cancer is the rate of change in the level of prostate-specific antigen (PSA) over time, called PSA velocity. This measure was weakly associated with plasma folate levels in the ProtecT study (Collin *et al.*, 2010). Striking evidence that folate may be related to prostate cancer and its progression was found in a small case-control study in USA (Tomaszewski et al., 2011): the mean fasting plasma folate level was 63 nmol/L in 87 cases and 17 nmol/L in 20 controls. Prostate tissue folate levels were also higher in cases than in controls. Using Ki67 staining, a tissue marker of cancer progression, there was much higher staining in cancer tissue from cases with high plasma folate than in those with lower folate, suggesting that high folate favours progression of the cancer. Surprisingly, only 55% of the cases with high serum folate (> 68 nmol/L) admitted taking supplements and so the authors suggested that the high levels may have been due to the sustained consumption of fortified food.

It is well known that high concentrations of folic acid in culture media will stimulate the growth of several different types of cancer cells, but a study by Petersen et al. (Petersen et al., 2011) asked the question whether concentrations of folic acid that are within the physiological range have any effect on prostate cancer cells. They found that folic acid in the range 4 – 100 nmol/L stimulated both the growth and the invasiveness of prostate cancer cells in culture in a concentration-dependent fashion. Experiments in mice are consistent with this study: a

transgenic mouse model called ‘transgenic model of the mouse prostate (TRAMP)’ shows very vigorous growth of prostate cancer, but it was found that feeding these mice on a diet with about one-seventh of the normal folate content almost completely stopped both the growth and the spread of the tumours (Bistulfi et al., 2011). The recent findings summarized here are consistent with prostate cancer being particularly sensitive to folate. Possible public health implications will be discussed in Section 6.

***The puzzle of folate and cancer***

Can we make any sense out of the different studies on folate and cancer? It helps if we move away from a simplistic ‘cause and effect’ relationship to consider the underlying biology, already highlighted in the ‘dual mechanisms’ hypothesis of Kim described above (see Figure XX). Some factors that need to be considered are: i) the timing of the exposure to folate in relation to the stage in the sequence of normal tissue to precancerous to cancer; ii) the likelihood that any relationship is not linearly related to folate intake, and so will be influenced by the baseline folate levels; iii) the sensitivity of the cancer to folate; iv) the prevalence of subgroups at particular risk in the population being studied; v) the possibility that the effect of folic acid is different from that of natural folates. There is not space to discuss these issues in detail, but some of the studies cited above illustrate these points. Further reports are: on the importance of timing (Lee *et al.*, 2011, Luebeck *et al.*, 2008) and on non-linearity (Aune *et al.*, 2011, Lee and Chan, 2011, Ulrich, 2007).

***Conclusions. The relationship of folate and cancer is complex and the evidence is consistent with a dual role: a good folate status protects against the development of cancer by reducing the risk of neoplastic transformation of normal tissue, while at the same time high folate status stimulates the conversion of pre-neoplastic cells into cancer cells and promotes the growth of existing cancer cells. Whether or not fortification with folic acid will reduce, or increase, the risk of cancer depends upon the timing of the exposure in an individual, the sensitivity of particular cancers to folate, the prevalence of particular subgroups of the population at greater risk, and the baseline level of folate in the population. It is quite possible for the overall effect of fortification to be neutral for cancer risk in the whole population, but for certain subgroups, such as women with particular classes of hormone receptors in breast tissue, or people with the TT polymorphism of the gene for MTHFR, to show an increased risk. Population studies in countries that have already fortified are difficult to interpret, but a recent meta-analysis on 10 trials (38,000 people) in which folic acid (0.4 – 1 mg per day) was administered shows an overall 7% increased risk of new cancers, with a 24% increased risk in prostate cancer.***

**6. What is the likely overall balance in public health benefit or harm if fortification is introduced?**

A simple approach to answer this question would be to compare the prevalence of various diseases before and after fortification has been introduced in those countries that introduced mandatory fortification several years ago. We have shown that this approach is not reliable, for many reasons. It is also limited because it fails to take into account that everyone is not at equal risk of a disease because genetic and environmental factors influence the risk. Accordingly, we have to look at evidence from randomized trials and, if possible, at particular subgroups within these trials. The problem with that approach is that the dose of folic acid used in most trials is considerably higher than the average intake of folic acid from fortified foods. Hence, we must compare, if possible, the folate levels in the blood during a trial with the levels that occur in populations where fortification has been introduced.

***Folate levels after fortification in USA***

Comprehensive studies on the folate status of the US population have been carried out for many years and soon after fortification serum and red blood cell folate levels more than doubled (Pfeiffer et al., 2007). The levels dropped a little in subsequent years, but the decrease was mainly in the upper values, as can be seen from Figure 18. (We have converted the units to nmol/L for ease of comparison with the rest of this review.)

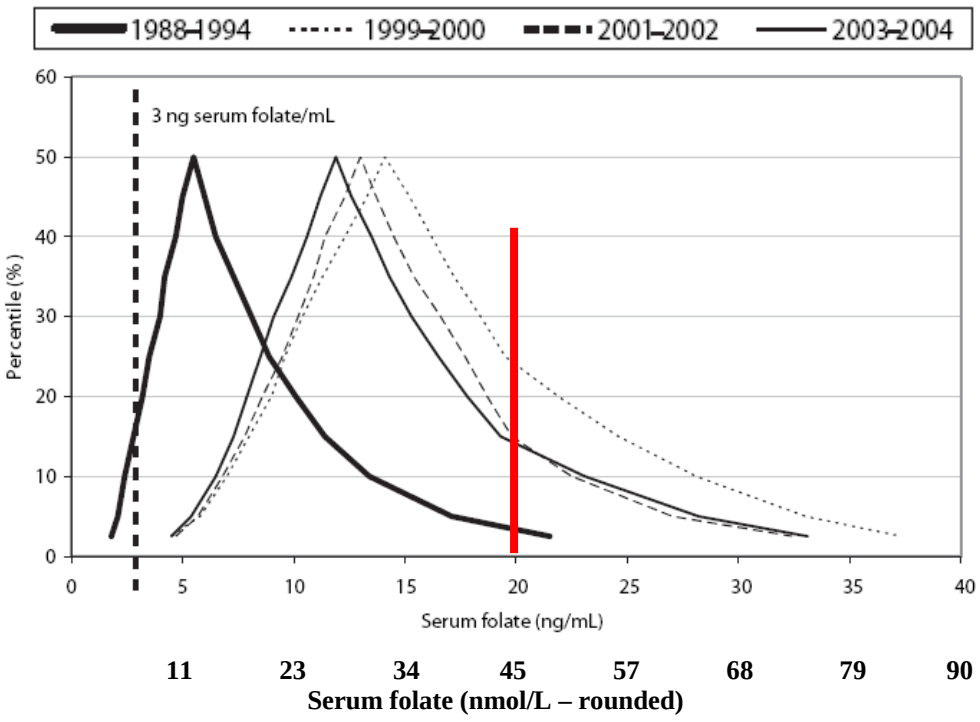



**Figure 18. Frequency distribution of serum folate among the entire population of the United States according to the National Health and Nutrition Examination Surveys.**

The vertical dashed line indicates the cut-off value for low folate (deficiency). We have added a solid red vertical line to show the value above which levels are considered to be supraphysiological. Fortification was introduced between 1996 and 1998. The surveys took samples from about 5,000 people each year. (Pfeiffer et al., 2007)

The graph above shows that in 1999-2000, soon after fortification, about 25% of the population have serum folate levels above 45 nmol/L, usually considered supraphysiological. This proportion subsequently fell but was still about 15% in 2003-4. The reports also give the distribution of folate values between different sectors of the population, according to race, gender and age (Pfeiffer *et al.*, 2005, Pfeiffer *et al.*, 2007) (McDowell et al., 2008). The most recent report showed the age-distribution for samples collected in 2005-2006 (Figure 19) from which it can be seen that two age groups had almost 50% higher serum folate levels than the rest: children aged 4 – 11 years and the elderly.

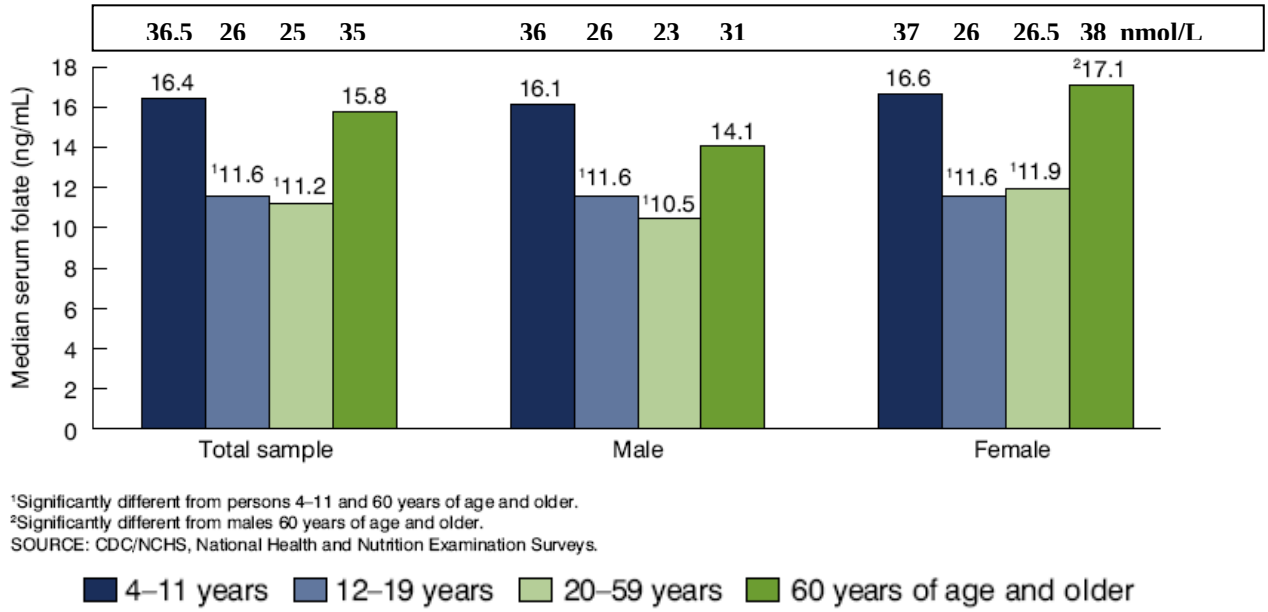



**Figure 19. Median serum folate levels by age and sex, USA 2005-6**
Note that values are given in ng/mL. In the box at the top we have converted the median values to nmol/L by multiplying by 2.266 and rounding. (McDowell et al., 2008).

Half of all small children have folate levels greater than 36.5 nmol/L. In 2010 one of us expressed concern that small children had such high folate levels, pointing out that after fortification 19% of children aged 4 - 11y had serum folate levels above a level considered non-physiological, i.e. 45 nmol/L, which probably meant that many were consuming more than the Tolerable Upper Limit (TUL) recommended for children of 300 – 400 μg per day (Smith, 2010). This turns out to be the case: a detailed study of folate and folic acid intake by children aged from 1 to 13 years by Bailey et al. found that many American children aged 1 to 8 exceeded the limit (Bailey *et al.*, 2010). Children aged 1 to 3 consumed on average 385 μg/day (TUL = 300) and children aged 4 to 8 consumed on average 534 μg/day (TUL = 400) from food. Of the 385 μg/day, 156 came from folic acid in fortified food; of the 534 μg/day, 236 came from folic acid

in fortified food. Thus, folic acid added during fortification comprised more than 40% of the total folate in the diet of children aged 1 to 8 y. We can conclude that fortified food was a major reason why so many of these children exceed the TUL. The situation is even worse if we add the folic acid from supplements. About 28% of children in the USA take supplements containing folic acid and 60% of those who took supplements exceeded the TUL when considering diet and supplements together (Bailey *et al.*, 2010). ***Two major points come from this analysis: first, children in a country that has fortified are being exposed to levels of folic acid from fortified food that cause many of them to consume more than the TUL for folate and, second, even higher levels of folic acid intake occur in those children who consume supplements.***

The other group with high levels of folate are those over 60 y-old. In the 2003-4 dataset, 32% of these had serum folate levels above 45 nmol/L (Pfeiffer et al., 2007). Levels of unmetabolized folic acid have been measured in another group of elderly from the 2001-2 NHANES cohort and 38% were found to have detectable free folic acid in their fasting serum (Bailey *et al.*, 2010). The mean serum folate in this cohort was 55 nmol/L and the unmetabolized folic acid level was 4.4 nmol/L, i.e. about 7% of total folates. 61% of those with detectable unmetabolized serum folic acid reported using supplements containing folic acid and it was estimated that they consumed an average of 235 μg/day from supplements and a further 202 μg/day of folic acid from fortified foods (Bailey *et al.*, 2010).

These figures provide a useful background to consider the possible impact of fortification in New Zealand. In considering possible benefits, or harm, of fortification, we have to remember that the folate status in New Zealand is already very good, with New Zealand women of child-bearing age having serum folate levels about the same as women today in the USA, and red blood cell levels considerably higher (Section 2.1). The estimated additional intake of folic acid from fortification is 0.14 mg/day (FSANZ, 2006), but in Section 2.2 we assumed a larger increase of 0.2 mg/day, as appears to have happened in the USA. ***This increased intake upon fortification is estimated to raise the average serum folate level in New Zealand from 35.6 nmol/L to 40.1 nmol/L, in other words by about 13%.*** Notably, following fortification in the USA with similar amounts of folic acid, serum folate increased from an from an average of 13 nmol/L in 1994 to 28 nmol/L in 2004, an increase of 115% (Pfeiffer et al., 2007). It is thus difficult to use changes in the USA after fortification to predict what might happen in New Zealand. Although an increase in the mean serum folate level of only 13% seems small, it should be realised that the overall effect will be to shift the distribution curve to the right, i.e. to higher concentrations (see Figure 18). A shift of the right-hand side of the curve further to the right could easily bring some people into a zone where harm occurs. A shift of the left-hand side of the curve to the right could bring some people out of a zone where they do not have enough

folate into a safe zone. It can be seen from Figure 18 that not only is the distribution of folate values shifted to the right (i.e. to higher values) but the whole distribution is broadened. This broadening probably reflects the broadening of total folate intakes in the population after fortification, as illustrated in Figure 20. Such a broadening of intakes has public health implications for it means that part of the population may be consuming amounts of folate greater than the recommended TUL, as shown above for children. It also means that a larger proportion of the population will have folate levels that might take them into the risk zone for harm.

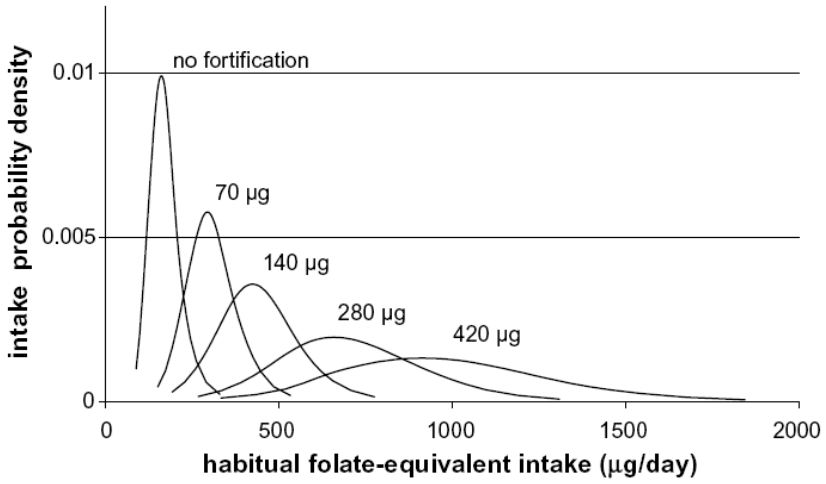



**Figure 20. Distribution of habitual intakes of folate equivalents (total intake of food folates and folic acid) comparing 'no fortification' with fortification scenarios of different levels of folic acid in bread For women aged 19-50 y.** The plots were derived from a mathematical model (Hoekstra *et al.*, 2008). In reality, the distribution is likely to be skewed.

### *6.1 Possible public health benefits of fortification in New Zealand*

*Decrease in the prevalence of NTDs.* This is the reason why fortification is being considered. Because the folate level in women of child-bearing age in New Zealand is so good, we believe that a floor may almost have been reached for the prevention of NTDs. We have estimated in Section 2.2 that between 0 and 6 NTD pregnancies each year out of a total of about 40 per year might be prevented by fortification. If we take the upper figure, then 6 NTD pregnancies may be prevented out of a total of 63,000 births in a population of 4.4 million. More likely, the effect will be much lower. Our conclusions are similar to those of a leading New Zealand expert (Professor C.M. Skeaff), who opposed the proposal to introduce mandatory fortification and wrote in his submission to the FSANZ ((FSANZ, 2006) page 160):

"Does not support the proposed approach for mandatory folic acid fortification of bread making flour for the following reasons: it will cause a negligible decrease in NTD rates; ….considers New Zealand women have high folate status and there is a low rate of NTD, which suggests that mandatory fortification will have a minimal effect on NTD rates; ….. the folate status of women of childbearing age in Dunedin, New Zealand, is as good as that of women in the US after fortification, and thus suggests there will be little further reduction in the rate of NTDs with mandatory folic acid fortification of bread flour."

*Other birth defects.* We could find no strong evidence that other birth defects might be prevented by fortification, with the possible exception of some cardiac defects. Congenital heart defects are the commonest form of congenital defect and the two most frequent are atrial and ventricular septal defects, averaging about 330 per year in New Zealand (New Zealand Birth Defects Registry http://www.nzbdmp.ac.nz/). If we take as a guide the Canadian study reporting a 6.2% fall in severe congenital heart disease after fortification (Ionescu-Ittu et al., 2009), then it is possible that up to 20 such births a year might be prevented in New Zealand. But it must be stressed that this is highly speculative (Section 3).

*Child development.* We reviewed evidence that a good maternal folate status was beneficial for cognitive and behavioural development in the child, but it is difficult to quantify this. In a study from India the maternal plasma folate levels over which improvement in the child's cognition was observed ranged from < 17 to > 50 nmol/L and so included the level of 36 found in New Zealand women (Veena et al., 2010). But it is doubtful that a change from 36 to 40 would have a significant effect on the child's development.

*Cardiovascular disease*. The decreases in incidence and mortality from stroke in USA and in Canada following fortification occurred without any parallel changes in England and Wales, and thus might not unreasonably be considered to be caused in part by fortification. However, randomized trials are only partly consistent with this interpretation, with only one trial giving a positive result (Saposnik *et al.*, 2009). In the latter trial, B vitamin treatment reduced the risk of stroke by about 25%, but only in those with high tHcy levels. We need up-to-date values for tHcy in the New Zealand population before we could consider predicting an effect of fortification on stroke incidence. There is no evidence that the incidence of heart disease would be reduced by fortification.

*Cognitive decline and dementia.* Randomized trials have shown that folic acid alone, or in combination with vitamins B12 and B6, can slow age-related cognitive decline and the rate of brain atrophy, but only in people with raised levels of tHcy. It is not possible to predict if fortification will have any benefit on people with cognitive impairment in New Zealand because the current levels of tHcy in the elderly population are not known.

***Conclusions. Possible benefits to public health of folic acid fortification are difficult to quantify because a lot of factors remain unknown. The most likely benefits are a small reduction in NTDs of up to 6 pregnancies per year and, perhaps, a reduction in cardiac birth malformations amounting to approximately 20 births per year. Due to the already very good folate status in New Zealand, any additional beneficial effect by fortification is probably limited.***

### *6.2 Possible harm to public health of fortification in New Zealand*

*Increase in prevalence of some congenital malformations.* Population studies after fortification have reported an increase in certain malformations, most commonly obstructive genitourinary defects, but since this has not been observed in clinical trials there may be causes other than folic acid.

*Increase in anaemia and cognitive decline in a subgroup of elderly with low vitamin B12 status.* Population studies in USA after fortification have shown that supraphysiological levels (>59 nmol/L) of serum folate are associated with a 5-fold increased risk of anaemia and of cognitive deficit in those elderly who have a poor vitamin B12 status. About 4% of the elderly in the USA had this combination of low vitamin B12 and high folate. If we assume that the proportion is the same in New Zealand then, since there are about 550,000 over 65, *approximately 22,000 elderly might be at risk of harm.*

*Increase in twin births in pregnancies from assisted reproduction techniques.* In the USA after fortification there were about 12% more twin births in ART pregnancies. The association with folic acid was confirmed in a UK prospective cohort study and the authors estimated that an additional 600 twin births would occur if the UK were to introduce fortification (Haggarty et al., 2006). It is not at present possible to apply the same approach to New Zealand because the data for ART pregnancies are pooled with those of Australia (http://www.aihw.gov.au/WorkArea/DownloadAsset.aspx?id=10737420484). However, from the latest statistics on this web site there were some 13,000 deliveries in 2009, of which 16% were multiple. An increase of 12% would man an additional 170 multiple deliveries for both Australia and New Zealand. If these are divided simply in relation to the populations, then about *an additional 30 twin births from ART pregnancies would result from fortification.*

*Possible adverse effects in children conceived after fortification.* There is just one observational study which showed a higher risk of insulin resistance in children of mothers with red blood cell folate levels above 800 nmol/L, which is close to the current level in women in New Zealand. Until more studies are done, we cannot extrapolate this finding to New Zealand. There are several studies suggesting that maternal consumption of folic acid may increase the risk of allergic responses in their children, and one of these related maternal plasma folate levels to the risk of asthma in their 3-year old children. The risk rose in relation to increased folate and became significant above 17 nmol/L (Haberg et al., 2011). Since this is well within the levels in women in New Zealand, it is possible that the increased folate associated with fortification might further amplify the effect and so lead to an increase in childhood asthma. It is difficult to quantify this effect without more data.

*Increase in incidence of certain cancers.* The population studies on cancer incidence in countries that have already fortified have not been very helpful. Although they suggested an increase in colorectal cancer and in prostate cancer after fortification, it turns out that similar, although smaller, increases occurred at about the same time in the UK and in New Zealand, which have not fortified. Observational studies in populations or in cohorts have provided inconsistent results, but one of them, the Malmö Diet and Cancer Cohort, has been very instructive. We have learnt from this study that overall a good folate status is protective against breast cancer, but that there are subgroups of women who are harmed by high folate status. These are, firstly, women with the TT genotype of the gene *MTHFR* who have an increased risk of cancer as folate levels rise. Secondly, women whose breast tissue does not contain estrogen receptor-beta were the only ones to show an increased risk as folate status rose. Since the frequency of the TT genotype in New Zealand is close to 10% (Jones et al., 2005), we can estimate the number of women at increased life-time risk of breast cancer as folate levels increase to be about 200,000.

The randomized clinical trials in which folic acid, with or without vitamin B12 and/or vitamin B6, were administered have been more informative because they are better controlled. The trials with the longest follow-up period (8 years) are the two cardiovascular trials done in Norway. Combining these two trials and assessing cancer outcomes using national registries gave clear evidence that those in the arm of the trials in which folic acid and vitamin B12 was administered had an overall 21% increased risk of cancer and of 38% increased risk of dying from cancer. The increased risk was directly related to the serum folate level, and those with levels > 60 nmol/L had the highest risk. Values as high as this occur in USA after fortification in about 10% of the population (see Figure 18) and it is quite likely that a proportion of the population in New Zealand will also have such high levels. As in the USA, older people in New Zealand have higher serum folate levels, as shown by the 2008/9 Adult Nutrition Survey: women aged 15-18 had a mean level of 24.4 nmol/L while in those over 71 y the mean level was 39.7 nmol/L (Ministry of Health, 2011). We cannot estimate the numbers involved since the reports do not give the data on folate levels in deciles, but fortification will shift these values up and will increase the proportion with values in the range above 60 nmol/L, where the Norwegian trial showed a significant increased risk of cancer. In view of the fact that the folate status of the New Zealand is already as good, or better than, that in the USA we can predict that more than 10% of the elderly population will fall into this risk category after fortification. An important finding in the Norwegian trial was that the subgroup who had the TT genotype of the gene *MTHFR* were at a 4-fold greater risk of dying from cancer if they consumed 800 μg of folic acid. As we pointed

out above, approximately 10% of the New Zealand population carry this genotype, i.e. about 400,000 people.

It is not justifiable to make predictions from the results of a single trial, but we now have the results of a meta-analysis of 10 trials in which folic acid was administered and where the outcome was cancer. This meta-analysis on more than 38,000 people found that folic acid containing supplements, with a dose of between 0.4 mg and 1 mg per day, caused an overall increase in cancer incidence of 7%. Of the individual cancer types, prostate cancer showed an 24% increase in the folic acid groups. These results have clear implications for public health and cannot be ignored. The question is, do they just reflect an increased risk of the high doses of folic acid used or will folate levels after fortification also increase the risk of cancer? As we have mentioned, the Norwegian trials, which used 0.8 mg folic acid, measured the serum folate and found that the risk of cancer was high in those with levels above 60 nmol/L. We consider it very likely that a proportion of the population will reach these levels after fortification, as they have in the USA. Thus, we consider that this meta-analysis can be used to predict a possible increase in cancer of up to 5% in New Zealand. There were 20,317 new cancer registrations in 2008 (Ministry of Health, 2011), so *we are predicting up to an additional 1,000 new cases of cancer per year after fortification has been introduced.*

Of particular concern are men who are at risk of prostate cancer, which is increasing world-wide. Incidence rates vary greatly across the world, with Australia and New Zealand having the second highest rates (Delongchamps et al., 2006). Prostate cancer was the most common cancer registered in New Zealand in 2008, accounting for 14% of all registrations (Ministry of Health, 2011). It is notable that the latest annual survey of cancer in the USA reported that overall cancer rates in adults have been decreasing, with the exception of prostate cancer, which has been increasing (Kohler et al., 2011). We showed some of the data in Figure 15 above. It is believed that there is a strong environmental influence on the incidence of prostate cancer because people who move from low-incidence regions to high incidence regions take on the high incidence (Delongchamps et al., 2006). Could folic acid be one of the environmental factors? The 24% increased incidence found in the meta-analysis described above in people who took folic acid in trials is a disturbing result. As we have discussed above, folic acid promotes the growth of prostate cancer cells and so could it be a factor that converts latent prostate cancer, which is very common in men as they age (Delongchamps et al., 2006), into clinically significant cancer? If these speculations have some validity, then it must be a concern that folic acid fortification might increase the incidence of prostate cancer. The population studies in USA that we discussed above cannot be used to answer this question because the changes in incidence over time were influenced by many factors. We consider that there is a significant risk that

fortification will increase the number of prostate cancer cases in New Zealand, perhaps by between 10 and 20%. There were 2,939 new cases in 2008 (Ministry of Health, 2011), *so we are predicting a further 300 or more cases of prostate cancer per year a few years after fortification is introduced* (these would be included in the above estimate of about 1,000 total new cancer cases).

***How can we assess the balance of benefit and harm?***

Modern approaches for assessing benefit and harm in public health use common measures of outcome such as Quality-adjusted life years (QALYs) and Disability-adjusted life years (DALYs), or they attempt to express the benefit in economic terms (EFSA, 2006). We are not qualified to use these methods, but refer to reader to an excellent summary of this approach and an attempt to model benefit and risk for folic acid fortification by Hoekstra et al. (Hoekstra *et al.*, 2008). Their study was based on earlier data and so their conclusions are not necessarily relevant today.

Is it correct to describe the current situation as in the statement by the New Zealand Ministry of Health, quoted at the beginning of our review: "*Rarely has there been a case where the science has been so unequivocal, uncontentious and universally accepted.*"? The answer must be 'No' for two reasons. First, the current scientific evidence that we have described above does not fit with this description. Second, the case for fortification never was 'universally accepted' nor was the evidence 'unequivocal', since there has never been any systematic large-scale, controlled, investigation of potential harmful effects in relation to long-term exposure with low-dose folic acid. Because much of the evidence available is difficult to apply in precise quantitative terms to the risk-benefit discussion, we will give our best estimates of the quantitative outcomes. Since these are just estimates, we suggest that the 'precautionary principle' (*Vorsorgeprinzip*), a strategy for dealing with uncertainty (Health Council of the Netherlands, 2008), should also be applied. In public health terms, this strategy can be defined as follows:

*The precautionary principle provides justification for public policy actions in situations of scientific complexity, uncertainty or ignorance, where there may be a need to act in order to avoid, or reduce, potentially serious or irreversible threats to health of members of the population.*

We have shown that there is sufficient evidence, albeit imprecise in quantitative terms, of potential harm if fortification is introduced today in New Zealand. Thus, on the basis of the precautionary principle, a policy of exposing the entire population to extra folic acid, without

individual choice or personal medical advice, cannot be justified on the grounds of potential benefit to a small number of the population.

***Conclusions. The possible harms to public health in New Zealand if fortification is introduced are difficult to estimate with any precision. Quantitatively, the best documented concerns will include: i) about 20,000 elderly (over 65) who may be at risk of cognitive impairment and anaemia due to an imbalance between high folate and low vitamin B12 status; ii) subjects with very high folate, likely to occur in at least 10% of the population, who may have an increased risk of cancer, leading to up to an extra 1,000 cases a year; iii) prostate cancer is very sensitive to folate and raised levels of folate in men may increase the incidence of prostate cancer by about 300 cases a year. iv) certain subgroups of the population (e.g. those with a particular genotype, TT, of MTHFR) who, when exposed to high folate, suffer from increased risk of being diagnosed or dying from certain cancers.***

***There is increasing evidence that high intake of folic acid may cause harm. On public health grounds, taking into account the precautionary principle, it is difficult to justify exposing the whole population of 4.4 million to folic acid. Although fortification may prevent up to 6 NTD pregnancies per year, thousands of people may possibly suffer harm. Folic acid is a drug and there can be few examples in the history of medicine where whole populations have been exposed to a drug with such little research into the balance between beneficial and harmful effects.***

---

## Appendix: Notes on recent official reports from other countries

### *United Kingdom*

Following the report of the Scientific Advisory Committee on Nutrition (SACN) in 2006, in which it was recommended that the UK should introduce mandatory folic acid fortification (Scientific Advisory Committee on Nutrition, 2006), a report appeared in the scientific literature linking fortification in the USA and Canada to an increase in colorectal cancer (Mason *et al.*, 2007). This paper, together with other papers, led the Chief Medical Officer for England to ask the Food Standards Agency (FSA) to review the evidence for a causal link between folic acid and cancer. The FSA convened an international advisory group and also referred the matter to SACN. The advisory group reported in 2007(Sanderson *et al.*, 2007) and concluded that: "The timing of folate exposure with respect to carcinogenesis, as well as the dose and form of folate, were considered key issues for future research. Also, the need to study further the influence of genetically defined subgroups was highlighted for future research."

The SACN set up a Working Group and used their report as the basis of the reply to the Chief Medical Officer in 2009 (Scientific Advisory Committee on Nutrition, 2009). We have selected some statements in the reply that are relevant to our discussion in Section 5:

- "…the possibility that folic acid might be associated with increased cancer risk cannot be excluded as the meta-analysis (Clarke *et al.*, 2010) had limited statistical power to detect an effect of folic acid on cancer risk."
- "At the full SACN meeting in June 2009, the Committee agreed that there were still uncertainties regarding folic acid and cancer risk."
- "The majority of Members supported the previous recommendation to introduce mandatory fortification alongside controls on voluntary fortification. However, it was agreed that the recommendation should be amended to include precautionary advice on consumption of supplements containing folic acid by those at greater risk of developing colorectal adenomas and those with existing premalignant adenomas. One SACN Member did not support mandatory folic acid fortification because of the uncertainties regarding folic acid and cancer risk."

We have been unable to find any official government response to this report to the Chief Medical Officer, but the fact is that the UK has not yet gone ahead with mandatory fortification.

### *Ireland*

In 2006 a National Committee on Folic Acid Food Fortification, established by the Food Safety Authority of Ireland, recommended that "all bread…. should be fortified on a mandatory basis with folic acid at a level which provides 120 μg/100g of bread as consumed." (National Committee on Folic Acid Food Fortification, 2006).The Minister for Health and Children established an Implementation Group and charged it with putting these recommendations into practice.

The Implementation Group became concerned about the reports linking cancer to folic acid and in 2009 recommended that mandatory fortification "*should be put on hold until definitive data are available on the safety of this initiative*." (Implementation Group on Folic Acid Food Fortification, 2009) During this period, the Implementation Group had been monitoring the incidence of NTDs and the blood levels of folate in Irish women. They reported that the incidence of NTDs had fallen from about 12 - 19 per 10,000 births down to about 9 per

10,000 births over five years (2001-6). The group considered that this decline was largely due to the increasing amount of folic acid added to foods on a voluntary basis. About 25% of bread was fortified and more than 200 fortified food products were identified in 2009. The group suggested that the voluntary fortification was the main reason why the blood folate levels in Ireland had increased, such that by 2007 93% of women had erythrocyte folate levels considered adequate to protect against NTDs.

The Press Release from the Food Safety Authority of Ireland that accompanied the publication of the report of the Implementation Group was entitled: "*Currently No Need for Mandatory Fortification – Increased Folate Status Negates Mandatory Folic Acid Fortification at This Time."* (Food Safety Authority of Ireland, 2009). The Chairman of the Implementation Group, Alan Reilly, was quoted as saying that the current NTD rate of 9.3 per 10,000 births "*is close to the lowest level that can be achieved through folic acid fortification of food, therefore any further steps in this area would not provide much additional protection and mandatory fortification is no longer necessary at this time*."

It is noteworthy that the Implementation Group expressed concern at their finding of very high folate levels in young children, with as many as 25% of children having serum levels above 45 nmol/L, even higher than found in the USA after fortification. The group concluded: "An unexpected finding in this study was the high proportions of children (a quarter) and older adults (over a third) with high circulating levels of folate (measured as plasma folate in this study). However, given the high levels found, it is likely a significant proportion will be in unmetabolised form, which may accelerate growth of existing cancerous tumours. Older adults are a high risk group for the existence of cancerous tumours."

It is indeed surprising that voluntary fortification has led to such high levels of folate in certain sectors of the Irish population (the very young and the old), the same sectors who show high levels in the USA after mandatory fortification. The concerns of the Implementation Group are the same as we expressed in Section 6.

***Netherlands***

Prior to 1996, fortification of any food with folic acid was not permitted by law in the Netherlands. But European free trade regulations caused this policy to be abandoned and after 1996 voluntary fortification was allowed up to a level of 100 μg of folic acid per 100 kcal of foodstuffs. In 2006 the Ministry for Health, Welfare and Sport asked the Netherlands Health Council for advice on the optimum level of folic acid in foods, including the use of mandatory fortification, taking into account risk-benefit analysis. The Council reported in 2008 (Health Council of the Netherlands, 2008).

The Council estimated that mandatory fortification of staple foodstuffs might prevent 15 NTD births a year, but could cause irreversible neurological symptoms in 33 elderly people with vitamin B12 deficiency. Nevertheless, calculations in terms of DALYs showed that the benefits of fewer NTDs "far outweighed the increased risk of masking B12 deficiency." In relation to cancer, the committee made the following statement:

"The committee recommends that doctors should advise patients with benign growths in the colon not to take dietary supplements containing folic acid. This is because it is impossible to exclude the possibility that excessively high folic acid intake may accelerate the transformation of a benign growth into a malignant tumour."

The committee emphasised that, when fortifying foods, it is essential to ensure that folic acid intake remains below the safe upper level of intake. The Council concluded that there was a risk that children would ingest too much folic acid and so they recommended that only staple foodstuffs, like bread, should be fortified and that the fortification of other specific foodstuffs should be discontinued. However, a policy of limiting or banning voluntary fortification would be counter to European legislation on free trade and so cannot be adopted at present.

The Health Council, in a later report (Health Council of the Netherlands, 2008), applied the precautionary principle to the fortification issue. They stated that, "given the lack of clarity over risks and benefits, it might be useful or even desirable to adopt a strategy based on the precautionary principle…" However, the strategy was not worked out in this document and the situation regarding fortification in the Netherlands appears to be unresolved at present, probably because the authorities await guidance from the EU.

It is likely that the policy in the Netherlands, described above, is confusing to the Dutch public. A rather similar proposal was originally made in the UK, where SACN recommended mandatory fortification of flour products but at the same time proposed restrictions on voluntary fortification and supplement availability (Scientific Advisory Committee on Nutrition, 2006).

***European Union***

Europe has a European Food Safety Authority (EFSA) and this body has established rules about adding substances to food that are applicable throughout the EU and associated countries, such as Switzerland and Norway. In 2008 the EFSA established a Working Group on the "Analysis of risks and benefits of fortification of food with folic acid" and the EFSA also called a meeting in 2009 on *Folic Acid: An Update on Scientific Developments.* The discussions at latter meeting have been summarised in a report (EFSA, 2009). The key conclusions on the cancer issue were:

> 73. There are currently insufficient data to allow a full quantitative risk assessment of folic acid and cancer or to determine whether there is a dose-response relationship or a threshold level of folic acid intake associated with potential colorectal cancer risk.
> 74. The current evidence does not show an association between high folic acid intakes and cancer risk but neither do they confidently exclude a risk. The uncertainties in relation to cancer risk highlight the importance of ensuring monitoring systems are set up for assessment of folic acid intake and status and NTD and cancer incidence in countries that decide to introduce mandatory fortification.

The report of the Working Group on the "Analysis of risks and benefits of fortification of food with folic acid" has also been published (EFSA, 2009). The report highlighted the lack of solid evidence upon which to base policy decisions regarding possible risks of fortification. Their final three conclusions were:

- Setting maximum safe levels for the amount of folic acid that can be added to foods voluntarily fortified and supplements will be important in ensuring that consumption of foods fortified with folic acid and folic acid supplements does not lead to intakes above the UL for any population subgroup, including young children.

- There is currently insufficient data to allow a full quantitative risk assessment of folic acid and cancer risk. Scientific developments within this area should be closely monitored.

- The targeted generation of additional data and knowledge, both epidemiological and animal/mechanistic, might be important in informing the risk/benefit assessment of folic acid in the future.

***Comment.*** An editorial in The Lancet at the time that these discussions were occurring was entitled *"Shall we put the world on folate?"* But the writers' conclusion was much more sobering: *"Because the safety of folate might depend on its chemical structure (natural folate or synthetic folic acid), its dose, and the time of intervention, several long-term follow-up intervention studies assessing the safety of a high folate intake are needed before any country decides on mandatory fortification of food with folic acid."* (Osterhues *et al.*, 2009). These studies remain to be carried out.

***Conclusions***

***The current situation is that no European country has introduced mandatory folic acid fortification. In Ireland, a country originally strongly in favour of fortification, it is considered that the level of folate in the population is sufficient to prevent most folate-sensitive NTDs. In other European countries, there is concern about possible harmful effects of fortification, but insufficient data to decide if these harms are important. However, since the above reports were published some new evidence, notably on cancer, has been published and we consider it highly unlikely that the EU or any European government would proceed with mandatory fortification in 2012, since policy decisions in public health are usually made with the precautionary principle in mind.***

## References


Abeywardana S, Bower C, Halliday J, Chan A, Sullivan EA. Prevalence of neural tube defects in Australia prior to mandatory fortification of bread-making flour with folic acid. *Aust N Z J Public Health* 2010; 34: 351-5.

Acs N, Banhidy F, Puho E, Czeizel AE. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. *Birth Defects Res A Clin Mol Teratol* 2005; 73: 989-96.

Ahrens K, Yazdy MM, Mitchell AA, Werler MM. Folic acid intake and spina bifida in the era of dietary folic acid fortification. *Epidemiology* 2011.

Arabelovic S, Sam G, Dallal GE, Jacques PF, Selhub J, Rosenberg IH, et al. Preliminary evidence shows that folic acid fortification of the food supply is associated with higher methotrexate dosing in patients with rheumatoid arthritis. *J Am Coll Nutr* 2007; 26: 453-5.

Assaraf YG. Molecular basis of antifolate resistance. *Cancer Metastasis Rev* 2007; 26: 153-81.

Aune D, Lau R, Chan DS, Vieira R, Greenwood DC, Kampman E, et al. Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies. *Gastroenterology* 2011; 141: 106-18.

Australian Institute of Health and Welfare. Mandatory folic acid and iodine fortification in Australia and New Zealand. 2011 [Accessed: 10 December 2011]; Available from: http://www.aihw.gov.au/publication-detail/?id=10737418875

Bailey RL, McDowell MA, Dodd KW, Gahche JJ, Dwyer JT, Picciano MF. Total folate and folic acid intakes from foods and dietary supplements of US children aged 1-13 y. *Am J Clin Nutr* 2010; 92: 353-8.

Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, et al. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged > or =60 y in the United States. *Am J Clin Nutr* 2010; 92: 383-9.

Bailey RL, Mills JL, Yetley EA, Gahche JJ, Pfeiffer CM, Dwyer JT, et al. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged >=60 y in the United States. *Am J Clin Nutr* 2010; 92: 383-9.

Bailey SW, Ayling JE. The extremely slow and variable activity of dihydrofolate reductase in human liver and its implications for high folic acid intake. *Proc Natl Acad Sci U S A* 2009; 106: 15424-9.

Bakker R, Timmermans S, Steegers EA, Hofman A, Jaddoe VW. Folic acid supplements modify the adverse effects of maternal smoking on fetal growth and neonatal complications. *J Nutr* 2011; 141: 2172-9.

Bao Y, Michaud DS, Spiegelman D, Albanes D, Anderson KE, Bernstein L, et al. Folate intake and risk of pancreatic cancer: pooled analysis of prospective cohort studies. *J Natl Cancer Inst* 2011.

Bekkers MB, Elstgeest LE, Scholtens S, Haveman A, de Jongste JC, Kerkhof M, et al. Maternal use of folic acid supplements during pregnancy and childhood respiratory health and atopy: the PIAMA birth cohort study. *Eur Respir J* 2011.

Bell KN, Oakley GP, Jr. Tracking the prevention of folic acid-preventable spina bifida and anencephaly. *Birth Defects Res A Clin Mol Teratol* 2006; 76: 654-7.

Bell KN, Oakley GP, Jr. Update on prevention of folic acid-preventable spina bifida and anencephaly. *Birth Defects Res A Clin Mol Teratol* 2009.

Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, et al. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* 1999; 341: 1485-90.

Bistulfi G, Foster BA, Karasik E, Gillard BM, Miecznikowski J, Dhiman VK, et al. Dietary folate deficiency blocks prostate cancer progression in the TRAMP model. *Cancer Prev Res (Phila)* 2011; 4: 1825-34.

Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. *Int J Epidemiol* 2010; 39 Suppl 1: i110-i21.

Botto LD, Moore CA, Khoury MJ, Erickson JD. Neural-tube defects. *N Engl J Med* 1999; 341: 1509-19.

Bradbury KE, Skeaff CM, Oey I, Williams SM, Mann J. Monitoring voluntary fortification of bread with folic acid. 2011 [Accessed: 23 December 2011]; Available from: http://www.maf.govt.nz/news-resources/publications

Bukowski R, Malone FD, Porter FT, Nyberg DA, Comstock CH, Hankins GD, et al. Preconceptional folate supplementation and the risk of spontaneous preterm birth: a cohort study. *PLoS Med* 2009; 6: e1000061.

Callaway L, Colditz PB, Fisk NM. Folic acid supplementation and spontaneous preterm birth: adding grist to the mill? *PLoS Med* 2009; 6: e1000077.

Canfield MA, Collins JS, Botto LD, Williams LJ, Mai CT, Kirby RS, et al. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. *Birth Defects Res A Clin Mol Teratol* 2005; 73: 679-89.

CDC. Spina bifida and anencephaly before and after folic acid mandate --- United States, 1995--1996 and 1999--2000. *MMWR Morb Mortal Wkly Rep* 2004; 53: 362-5.

Chattopadhyay S, Tamari R, Min SH, Zhao R, Tsai E, Goldman ID. Commentary: a case for minimizing folate supplementation in clinical regimens with pemetrexed based on the marked sensitivity of the drug to folate availability. *Oncologist* 2007; 12: 808-15.

Chen BH, Carmichael SL, Selvin S, Abrams B, Shaw GM. NTD prevalences in central California before and after folic acid fortification. *Birth Defects Res A Clin Mol Teratol* 2008.

Chladek J, Simkova M, Vaneckova J, Hroch M, Chladkova J, Martinkova J, et al. The effect of folic acid supplementation on the pharmacokinetics and pharmacodynamics of oral methotrexate during the remission-induction period of treatment for moderate-to-severe plaque psoriasis. *Eur J Clin Pharmacol* 2008; 64: 347-55.

Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the Women's Antioxidant and Folic Acid Cardiovascular Study. *Arch Intern Med* 2009; 169: 335-41.

Clarke R, Sherliker P, Hin H, Molloy AM, Nexo E, Ueland PM, et al. Folate and vitamin B12 status in relation to cognitive impairment and anaemia in the setting of voluntary fortification in the UK. *Br J Nutr* 2008; 100: 1054-9.

Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med* 2010; 170: 1622-31.

Collin SM, Metcalfe C, Refsum H, Lewis SJ, Smith GD, Cox A, et al. Associations of folate, vitamin B12, homocysteine, and folate-pathway polymorphisms with prostate-specific antigen velocity in men with localized prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 2833-8.

Collin SM, Metcalfe C, Refsum H, Lewis SJ, Zuccolo L, Smith GD, et al. Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010; 19: 1632-42.

Cotlarciuc I, Andrew T, Dew T, Clement G, Gill R, Surdulescu G, et al. The basis of differential responses to folic acid supplementation. *J Nutrigenet Nutrigenomics* 2011; 4: 99-109.

Crider KS, Bailey LB, Berry RJ. Folic acid fortification - its history, effect, concerns, and future directions. *Nutrients* 2011; 3: 370-84.

Curtin K, Samowitz WS, Ulrich CM, Wolff RK, Herrick JS, Caan BJ, et al. Nutrients in folate-mediated, one-carbon metabolism and the risk of rectal tumors in men and women. *Nutr Cancer* 2011; 63: 357-66.

Czeizel AE, Puho EH, Langmar Z, Acs N, Banhidy F. Possible association of folic acid supplementation during pregnancy with reduction of preterm birth: a population-based study. *Eur J Obstet Gynecol Reprod Biol* 2010; 148: 135-40.

Czeizel AE, Banhidy F. Folic acid supplementation and risk reduction in preterm birth. *Am J Clin Nutr* 2011; 94: 1651-2.

Czeizel AE, Banhidy F. Vitamin supply in pregnancy for prevention of congenital birth defects. *Curr Opin Clin Nutr Metab Care* 2011; 14: 291-6.

Czeizel AE, Dudas I, Paput L, Banhidy F. Prevention of neural-tube defects with periconceptional folic acid, methylfolate, or multivitamins? *Ann Nutr Metab* 2011; 58: 263-71.

Daly S, Mills JL, Molloy AM, Conley M, Lee YJ, Kirke PN, et al. Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* 1997; 350: 1666-9.

De-Regil LM, Fernandez-Gaxiola AC, Dowswell T, Pena-Rosas JP. Effects and safety of periconceptional folate supplementation for preventing birth defects. *Cochrane Database Syst Rev* 2010: CD007950.

de Jager CA, Oulhaj A, Jacoby R, Refsum H, Smith AD. Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial. *Int J Geriatr Psychiatry* 2011; (on-line ahead of publication).

De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, et al. Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med* 2007; 357: 135-42.

del Rio Garcia C, Torres-Sanchez L, Chen J, Schnaas L, Hernandez C, Osorio E, et al. Maternal MTHFR 677C>T genotype and dietary intake of folate and vitamin B(12): their impact on child neurodevelopment. *Nutr Neurosci* 2009; 12: 13-20.

Delongchamps NB, Singh A, Haas GP. The role of prevalence in the diagnosis of prostate cancer. *Cancer Control* 2006; 13: 158-68.

Dervieux T, Furst D, Lein DO, Capps R, Smith K, Caldwell J, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. *Ann Rheum Dis* 2005; 64: 1180-5.

Dunstan JA, West C, McCarthy S, Metcalfe J, Meldrum S, Oddy WH, et al. The relationship between maternal folate status in pregnancy, cord blood folate levels, and allergic outcomes in early childhood. *Allergy* 2011; on-line ahead of publication.

Durga J, van Boxtel MP, Schouten EG, Kok FJ, Jolles J, Katan MB, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet* 2007; 369: 208-16.

Durga J, Verhoef P, Anteunis LJ, Schouten E, Kok FJ. Effects of folic acid supplementation on hearing in older adults: a randomized, controlled trial. *Ann Intern Med* 2007; 146: 1-9.

Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, et al. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *Jama* 2009; 302: 2119-26.

EFSA. The EFSA's 6th Scientific Colloquium Report - Risk-benefit analysis of foods: methods and approaches 2006 [Accessed: 13 February 2012]; Available from: http://www.efsa.europa.eu/en/supporting/pub/116e.htm

EFSA. ESCO Report on Analysis of Risks and Benefits of Fortification of Food with Folic Acid. 2009 [Accessed: 14 February 2012]; Available from: http://www.elika.net/datos/articulos/Archivo453/ESCO%20folic_acid_report_en.pdf

EFSA. Folic acid: an update on scientific developments. 2009 [Accessed: 14 February 2012]; Available from: http://www.efsa.europa.eu/en/home/publication/efsafolicacid.pdf

English M, Snow RW. Iron and folic acid supplementation and malaria risk. *Lancet* 2006; 367: 90-1.

Ericson U, Sonestedt E, Gullberg B, Olsson H, Wirfalt E. High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmo Diet and Cancer cohort. *Am J Clin Nutr* 2007; 86: 434-43.

Ericson U, Sonestedt E, Ivarsson MI, Gullberg B, Carlson J, Olsson H, et al. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmö Diet and Cancer Cohort. *Cancer Epidemiol Biomarkers Prev* 2009; 18: 1101-10.

Ericson U, Borgquist S, Ivarsson MI, Sonestedt E, Gullberg B, Carlson J, et al. Plasma folate concentrations are positively associated with risk of estrogen receptor beta negative breast cancer in a Swedish nested case control study. *J Nutr* 2010; 140: 1661-8.

Ericson UC, Ivarsson MI, Sonestedt E, Gullberg B, Carlson J, Olsson H, et al. Increased breast cancer risk at high plasma folate concentrations among women with the MTHFR 677T allele. *Am J Clin Nutr* 2009; 90: 1380-9.

EUROCAT. Special Report: Prevention of Neural Tube Defects by Periconpceptional Folic Acid Supplementation in Europe - December 2009. 2009 [Accessed: 10 December 2011]; Available from: http://www.eurocat-network.eu/PREVENTIONAndRISKFACTORS/FolicAcid/FolicAcidSpecialReports

Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 2009; 101: 432-5.

Flour Fortification Initiative. Countries with mandatory fortification regulations. 2011 [Accessed: 10 December 2011]; Available from: http://www.sph.emory.edu/wheatflour//globalmap.php

Food Safety Authority of Ireland. Currently No Need for Mandatory Fortification – Increased Folate Status Negates Mandatory Folic Acid Fortification at This Time. 2009 [Accessed; Available from: http://www.fsai.ie/news_centre/press_releases/11032009.html

FSANZ. Final assessment report. Proposal P295. Consideration of mandatory fortification with folic acid. 2006 [Accessed: 10 December 2011]; Available from: http://www.foodstandards.gov.au/_srcfiles/FAR_P295_Folic_Acid_Fortification_%20Attachs_1_6.pdf

Gardiner HM, Fouron JC. Folic acid fortification and congenital heart disease. *Bmj* 2009; 338: b1144.

Gibson TM, Weinstein SJ, Pfeiffer RM, Hollenbeck AR, Subar AF, Schatzkin A, et al. Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr* 2011.

Godwin KA, Sibbald B, Bedard T, Kuzeljevic B, Lowry RB, Arbour L. Changes in frequencies of select congenital anomalies since the onset of folic acid fortification in a Canadian birth defect registry. *Can J Public Health* 2008; 99: 271-5.

Goh YI, Bollano E, Einarson TR, Koren G. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis. *J Obstet Gynaecol Can* 2006; 28: 680-9.

Granell R, Heron J, Lewis S, Davey Smith G, Sterne JA, Henderson J. The association between mother and child MTHFR C677T polymorphisms, dietary folate intake and childhood atopy in a population-based, longitudinal birth cohort. *Clin Exp Allergy* 2008; 38: 320-8.

Guerreiro CS, Carmona B, Goncalves S, Carolino E, Fidalgo P, Brito M, et al. Risk of colorectal cancer associated with the C677T polymorphism in 5,10-methylenetetrahydrofolate reductase in Portuguese patients depends on the intake of methyl-donor nutrients. *Am J Clin Nutr* 2008; 88: 1413-8.

Haberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child* 2009; 94: 180-4.

Haberg SE, London SJ, Nafstad P, Nilsen RM, Ueland PM, Vollset SE, et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. *J Allergy Clin Immunol* 2011; 127: 262-4, 4 e1.

Haggarty P, McCallum H, McBain H, Andrews K, Duthie S, McNeill G, et al. Effect of B vitamins and genetics on success of in-vitro fertilisation: prospective cohort study. *Lancet* 2006; 367: 1513-9.

Harris HR, Bergkvist L, Wolk A. Folate intake and breast cancer mortality in a cohort of Swedish women. *Breast Cancer Res Treat* 2011.

Health Council of the Netherlands. Towards an optimal use of folic acid. 2008 [Accessed; Available from: http://www.gezondheidsraad.nl/sites/default/files/200802E_0.pdf

Health Council of the Netherlands. Prudent precaution. Publication no 2008/18E 2008 [Accessed: 13 February 2012]; Available from: http://www.gezondheidsraad.nl/en/publications/prudent-precaution

Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 2000; 343: 1608-14.

Heseker HB, Mason JB, Selhub J, Rosenberg IH, Jacques PF. Not all cases of neural-tube defect can be prevented by increasing the intake of folic acid. *Br J Nutr* 2009; 102: 173-80.

Hirsch S, Sanchez H, Albala C, de la Maza MP, Barrera G, Leiva L, et al. Colon cancer in Chile before and after the start of the flour fortification program with folic acid. *Eur J Gastroenterol Hepatol* 2009; 21: 436-9.

Hoekstra J, Verkaik-Kloosterman J, Rompelberg C, van Kranen H, Zeilmaker M, Verhagen H, et al. Integrated risk-benefit analyses: method development with folic acid as example. *Food Chem Toxicol* 2008; 46: 893-909.

Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest* 2008; 118: 3462-9.

Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet* 2011.

Hubner RA, Houlston RS. MTHFR C677T and colorectal cancer risk: A meta-analysis of 25 populations. *Int J Cancer* 2007; 120: 1027-35.

Hubner RA, Liu JF, Sellick GS, Logan RF, Houlston RS, Muir KR. Thymidylate synthase polymorphisms, folate and B-vitamin intake, and risk of colorectal adenoma. *Br J Cancer* 2007; 97: 1449-56.

Hubner RA, Lubbe S, Chandler I, Houlston RS. MTHFR C677T has differential influence on risk of MSI and MSS colorectal cancer. *Hum Mol Genet* 2007; 16: 1072-7.

Hubner RA, Houlston RS. Folate and colorectal cancer prevention. *Br J Cancer* 2009; 100: 233-9.

Hung RJ, Hashibe M, McKay J, Gaborieau V, Szeszenia-Dabrowska N, Zaridze D, et al. Folate related genes and the risk of tobacco-related cancers in Central Europe. *Carcinogenesis* 2007; 28: 1334-40.

Hussain N. Epigenetic influences that modulate infant growth, development and disease. *Antioxid Redox Signal* 2012.

Ibiebele TI, Hughes MC, Pandeya N, Zhao ZT, Montgomery G, Hayward N, et al. High intake of folate from food sources is associated with reduced risk of esophageal cancer in an Australian population. *J Nutr* 2011; 141: 274-83.

Imdad A, Yakoob MY, Bhutta ZA. The effect of folic acid, protein energy and multiple micronutrient supplements in pregnancy on stillbirths. *BMC Public Health* 2011; 11 Suppl 3: S4.

Implementation Group on Folic Acid Food Fortification. Report of the Implementation Group on Folic Acid Food Fortification to the Department of Health and Children. 2009 [Accessed: 14 February 2012]; Available from: http://www.fsai.ie/assets/0/86/204/cc3c2261-7dc8-4225-bf79-9a47fbc2287b.pdf

Ionescu-Ittu R, Marelli AJ, Mackie AS, Pilote L. Prevalence of severe congenital heart disease after folic acid fortification of grain products: time trend analysis in Quebec, Canada. *Bmj* 2009; 338: b1673.

Jagerstad M. [Sweden should introduce compulsory folic acid supplementation--now. The number of cases with neural tube defects will decrease and protection against common diseases will increase] (in Swedish). *Lakartidningen* 2007; 104: 2667-9.

Jiang R, Hu FB, Giovannucci EL, Rimm EB, Stampfer MJ, Spiegelman D, et al. Joint association of alcohol and folate intake with risk of major chronic disease in women. *Am J Epidemiol* 2003; 158: 760-71.

Johansson M, Appleby PN, Allen NE, Travis RC, Roddam AW, Egevad L, et al. Circulating concentrations of folate and vitamin B12 in relation to prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition study. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 279-85.

Johansson M, Relton C, Ueland PM, Vollset SE, Midttun O, Nygard O, et al. Serum B vitamin levels and risk of lung cancer. *JAMA* 2010; 303: 2377-85.

Johnson CY, Little J. Folate intake, markers of folate status and oral clefts: is the evidence converging? *Int J Epidemiol* 2008; 37: 1041-58.

Jones GT, Harris EL, Phillips LV, van Rij AM. The methylenetetrahydrofolate reductase C677T polymorphism does not associate with susceptibility to abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2005; 30: 137-42.

Julvez J, Fortuny J, Mendez M, Torrent M, Ribas-Fito N, Sunyer J. Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. *Paediatr Perinat Epidemiol* 2009; 23: 199-206.

Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: relation to folic acid fortification. *Am J Clin Nutr* 2008; 88: 763-8.

Kalmbach RD, Choumenkovitch SF, Troen AP, Jacques PF, D'Agostino R, Selhub J. A 19-base pair deletion polymorphism in dihydrofolate reductase is associated with increased unmetabolized folic acid in plasma and decreased red blood cell folate. *J Nutr* 2008; 138: 2323-7.

Kennedy DA, Grupp S, Greenberg M, Koren G. Folate fortification and survival of children with acute lymphoblastic leukemia. *Paediatr Drugs* 2011; 13: 193-6.

Kennedy DA, Stern SJ, Moretti M, Matok I, Sarkar M, Nickel C, et al. Folate intake and the risk of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol* 2011; 35: 2-10.

Kim DH, Smith-Warner SA, Spiegelman D, Yaun SS, Colditz GA, Freudenheim JL, et al. Pooled analyses of 13 prospective cohort studies on folate intake and colon cancer. *Cancer Causes Control* 2010; 21: 1919-30.

Kim J, Cho YA, Kim DH, Lee BH, Hwang DY, Jeong J, et al. Dietary intake of folate and alcohol, MTHFR C677T polymorphism, and colorectal cancer risk in Korea. *Am J Clin Nutr* 2012.

Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr* 2004; 80: 1123-8.

Kim YI. Folate and colorectal cancer: An evidence-based critical review. *Mol Nutr Food Res* 2007; 51: 267-92.

Kohler BA, Ward E, McCarthy BJ, Schymura MJ, Ries LA, Eheman C, et al. Annual report to the nation on the status of cancer, 1975-2007, featuring tumors of the brain and other nervous system. *J Natl Cancer Inst* 2011; 103: 714-36.

Lacasana M, Blanco-Munoz J, Borja-Aburto VH, Aguilar-Garduno C, Rodriguez-Barranco M, Sierra-Ramirez JA, et al. Effect on risk of anencephaly of gene-nutrient interactions between methylenetetrahydrofolate reductase C677T polymorphism and maternal folate, vitamin B12 and homocysteine profile. *Public Health Nutr* 2012: 1-10.

Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. *Gastroenterology* 2006; 131: 1271-83.

Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. *J Natl Cancer Inst* 2007; 99: 64-76.

Lee JE, Chan AT. Fruit, vegetables, and folate: cultivating the evidence for cancer prevention. *Gastroenterology* 2011; 141: 16-20.

Lee JE, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, et al. Folate intake and risk of colorectal cancer and adenoma: modification by time. *Am J Clin Nutr* 2011.

Lee LK, Bateman BT, Wang S, Schumacher HC, Pile-Spellman J, Saposnik G. Trends in the hospitalization of ischemic stroke in the United States, 1998-2007. *Int J Stroke* 2011; on-line ahead of print.

Lewis SJ, Araya R, Leary S, Smith GD, Ness A. Folic acid supplementation during pregnancy may protect against depression 21 months after pregnancy, an effect modified by MTHFR C677T genotype. *Eur J Clin Nutr* 2011; in press.

Li Z, Ren A, Zhang L, Ye R, Li S, Zheng J, et al. Extremely high prevalence of neural tube defects in a 4-county area in Shanxi Province, China. *Birth Defects Res A Clin Mol Teratol* 2006; 76: 237-40.

Li Z, Ren A, Liu J, Pei L, Zhang L, Guo Z. Maternal flu or fever, medication use, and neural tube defects: a population-based case-control study in Northern China. *Birth Defects Res A Clin Mol Teratol* 2007; 79: 295-300.

Lin J, Lee IM, Cook NR, Selhub J, Manson JE, Buring JE, et al. Plasma folate, vitamin B-6, vitamin B-12, and risk of breast cancer in women. *Am J Clin Nutr* 2008; 87: 734-43.

Lopez-Camelo JS, Castilla EE, Orioli IM. Folic acid flour fortification: impact on the frequencies of 52 congenital anomaly types in three South American countries. *Am J Med Genet A* 2010; 152A: 2444-58.

Luchsinger JA, Tang MX, Miller J, Green R, Mayeux R. Relation of higher folate intake to lower risk of Alzheimer disease in the elderly. *Arch Neurol* 2007; 64: 86-92.

Luebeck EG, Moolgavkar SH, Liu AY, Boynton A, Ulrich CM. Does folic acid supplementation prevent or promote colorectal cancer? Results from model-based predictions. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 1360-7.

Magdelijns FJ, Mommers M, Penders J, Smits L, Thijs C. Folic Acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics* 2011; 128: e135-44.

Martinussen MP, Risnes KR, Jacobsen GW, Bracken MB. Folic acid supplementation in early pregnancy and asthma in children aged 6 years. *Am J Obstet Gynecol* 2011.

Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 1325-9.

McDonald SD, Vermeulen MJ, Ray JG. Folic acid and placental pathology. *Epidemiology* 2005; 16: 419-20.

McDowell MA, Lacher DA, Pfeiffer CM, Mulinare J, Picciano MF, Rader JI, et al. Blood folate levels: The latest NHANES results. *NCHS data briefs no 6*. Hyattsville, MD: National Center for Health Statistics; 2008. p. 1-8.

Miller JW, Garrod MG, Allen LH, Haan MN, Green R. Metabolic evidence of vitamin B12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. *Am J Clin Nutr* 2009; 90: 1586-92.

Miller RL. Prenatal maternal diet affects asthma risk in offspring. *J Clin Invest* 2008; 118: 3265-8.

Mills JL, Carter TC, Scott JM, Troendle JF, Gibney ER, Shane B, et al. Do high blood folate concentrations exacerbate metabolic abnormalities in people with low vitamin B-12 status? *Am J Clin Nutr* 2011.

Ministry of Health. NZ Food: NZ People Key results of the 1997 National Nutrition Survey. 1999 [Accessed: 10 December 2011]; Available from: http://www.moh.govt.nz/moh.nsf/0/8f1dbeb1e0e1c70c4c2567d80009b770/$FILE/nns.pdf

Ministry of Health. Improving Folate Intake in New Zealand: Policy implications. 2003 [Accessed: 10 December 2011]; Available from: http://www.moh.govt.nz/moh.nsf/0/fc14e853446559f1cc256d95007ecb59/$FILE/ImprovingFolate.pdf

Ministry of Health. Cancer: New registrations and deaths 2008. 2011 [Accessed; Available from: http://www.moh.govt.nz/moh.nsf/indexmh/cancer-reg-deaths-2008

Ministry of Health. A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition. 2011 [Accessed: 23 December 2011]; Available from: http://www.moh.govt.nz

Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC, et al. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. *Pediatrics* 2009; 123: 917-23.

Montaudie H, Sbidian E, Paul C, Maza A, Gallini A, Aractingi S, et al. Methotrexate in psoriasis: a systematic review of treatment modalities, incidence, risk factors and monitoring of liver toxicity. *J Eur Acad Dermatol Venereol* 2011; 25 Suppl 2: 12-8.

Moretti ME, Bar-Oz B, Fried S, Koren G. Maternal hyperthermia and the risk for neural tube defects in offspring: systematic review and meta-analysis. *Epidemiology* 2005; 16: 216-9.

Morris MC, Evans DA, Bienias JL, Tangney CC, Hebert LE, Scherr PA, et al. Dietary folate and vitamin B12 intake and cognitive decline among community-dwelling older persons. *Arch Neurol* 2005; 62: 641-5.

Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007; 85: 193-200.

Morris MS, Jacques PF, Rosenberg IH, Selhub J. Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. *Am J Clin Nutr* 2010; 91: 1733-44.

Mosley BS, Cleves MA, Siega-Riz AM, Shaw GM, Canfield MA, Waller DK, et al. Neural tube defects and maternal folate intake among pregnancies conceived after folic acid fortification in the United States. *Am J Epidemiol* 2009; 169: 9-17.

Muggli EE, Halliday JL. Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006. *Med J Aust* 2007; 186: 243-8.

Murphy MM, Fernandez-Ballart JD. Homocysteine in pregnancy. *Adv Clin Chem* 2011; 53: 105-37.

Murtaugh MA, Curtin K, Sweeney C, Wolff RK, Holubkov R, Caan BJ, et al. Dietary intake of folate and co-factors in folate metabolism, MTHFR polymorphisms, and reduced rectal cancer. *Cancer Causes Control* 2007; 18: 153-63.

National Committee on Folic Acid Food Fortification. Report of the National Committee on Folic Acid Food Fortification. 2006 [Accessed: 14 February 2012]; Available from: http://www.fsai.ie/uploadedFiles/folic_acid.pdf

National Heart, Lung and Blood Institute,. Morbidity and mortality: 2009 chart book on cardiovascular, lung, and blood diseases. 2009 [Accessed: 1 Februray 2012]; Available from: http://www.nhlbi.nih.gov/resources/docs/2009_ChartBook_508.pdf

New Zealand Birth Defects Registry. Liveborn cases of birth defects reported to the New Zealand Birth Defects Registry, 2000-2009. 2011 [Accessed: 10 December 2011]; Available from: http://www.nzbdmp.ac.nz/assets/FILES/Final%20published%20table%202000-2009.pdf

Nilsen RM, Vollset SE, Rasmussen SA, Ueland PM, Daltveit AK. Folic acid and multivitamin supplement use and risk of placental abruption: A population-based registry study. *Am J Epidemiol* 2008; 167: 867-74.

Nilsen RM, Vollset SE, Monsen AL, Ulvik A, Haugen M, Meltzer HM, et al. Infant birth size is not associated with maternal intake and status of folate during the second trimester in Norwegian pregnant women. *J Nutr* 2010; 140: 572-9.

Norsworthy B, Skeaff CM, Adank C, Green TJ. Effects of once-a-week or daily folic acid supplementation on red blood cell folate concentrations in women. *Eur J Clin Nutr* 2004; 58: 548-54.

Oaks BM, Dodd KW, Meinhold CL, Jiao L, Church TR, Stolzenberg-Solomon RZ. Folate intake, post-folic acid grain fortification, and pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2010; 91: 449-55.

Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. *Am J Clin Nutr* 2010; 92: 1416-22.

Osterhues A, Holzgreve W, Michels KB. Shall we put the world on folate? *Lancet* 2009; 374: 959-61.

Petersen LF, Brockton NT, Bakkar A, Liu S, Wen J, Weljie AM, et al. Elevated physiological levels of folic acid can increase in vitro growth and invasiveness of prostate cancer cells. *BJU Int* 2011.

Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. *Am J Clin Nutr* 2005; 82: 442-50.

Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, et al. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988 2004. *Am J Clin Nutr* 2007; 86: 718-27.

Pitkin RM. Folate and neural tube defects. *Am J Clin Nutr* 2007; 85: 285S-8S.

Piyathilake CJ, Macaluso M, Alvarez RD, Bell WC, Heimburger DC, Partridge EE. Lower risk of cervical intraepithelial neoplasia in women with high plasma folate and sufficient vitamin B12 in the post-folic acid fortification era. *Cancer Prev Res (Phila)* 2009; 2: 658-64.

Porcelli L, Assaraf YG, Azzariti A, Paradiso A, Jansen G, Peters GJ. The impact of folate status on the efficacy of colorectal cancer treatment. *Curr Drug Metab* 2011.

Prey S, Paul C. Effect of folic or folinic acid supplementation on methotrexate-associated safety and efficacy in inflammatory disease: a systematic review. *Br J Dermatol* 2009; 160: 622-8.

Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, et al. Homocysteine and folate as risk factors for dementia and Alzheimer disease. *Am J Clin Nutr* 2005; 82: 636-43.

Ray JG, Vermeulen MJ, Langman LJ, Boss SC, Cole DE. Persistence of vitamin B12 insufficiency among elderly women after folic acid food fortification. *Clin Biochem* 2003; 36: 387-91.

Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* 2006; 136: 1731S-40S.

Robien K, Boynton A, Ulrich CM. Pharmacogenetics of folate-related drug targets in cancer treatment. *Pharmacogenomics* 2005; 6: 673-89.

Rosenberg IH. Science-based micronutrient fortification: which nutrients, how much, and how to know? *Am J Clin Nutr* 2005; 82: 279-80.

Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Source-specific effects of micronutrients in lung cancer prevention. *Lung Cancer* 2010; 67: 275-81.

Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient intake and breast cancer characteristics among postmenopausal women. *Eur J Cancer Prev* 2010; 19: 360-5.

Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. Micronutrient intake and risk of colon and rectal cancer in a Danish cohort. *Cancer Epidemiol* 2010; 34: 40-6.

Roth C, Magnus P, Schjolberg S, Stoltenberg C, Suren P, McKeague IW, et al. Folic acid supplements in pregnancy and severe language delay in children. *JAMA* 2011; 306: 1566-73.

Roza SJ, van Batenburg-Eddes T, Steegers EA, Jaddoe VW, Mackenbach JP, Hofman A, et al. Maternal folic acid supplement use in early pregnancy and child behavioural problems: The Generation R Study. *Br J Nutr* 2010; 103: 445-52.

Sanderson P, Stone E, Kim YI, Mathers JC, Kampman E, Downes CS, et al. Folate and colo-rectal cancer risk. *Br J Nutr* 2007; 98: 1299-304.

Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. *Stroke* 2009; 40: 1365-72.

Schlotz W, Jones A, Phillips DI, Gale CR, Robinson SM, Godfrey KM. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. *J Child Psychol Psychiatry* 2010; 51: 594-602.

Scientific Advisory Committee on Nutrition. Folate and disease prevention. 2006 [Accessed: 12 February 2012]; Available from: http://www.sacn.gov.uk/reports/#

Scientific Advisory Committee on Nutrition. Folic acid and colorectal cancer risk: Review of recommendation for mandatory folic acid fortification. 2009 [Accessed: 12 February 2012]; Available from: http://www.sacn.gov.uk/pdfs/summary_of_sacn_report_to_cmo_19_october_2009.pdf

Selhub J. The many facets of hyperhomocysteinemia: studies from the Framingham cohorts. *J Nutr* 2006; 136: 1726S-30S.

Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. *PNAS* 2007; 104: 19995-20000.

Sharland E, Montgomery B, Granell R. Folic acid in pregnancy - is there a link with childhood asthma or wheeze? *Aust Fam Physician* 2011; 40: 421-4.

Shaw GM, Finnell RH, Blom HJ, Carmichael SL, Vollset SE, Yang W, et al. Choline and risk of neural tube defects in a folate-fortified population. *Epidemiology* 2009; 20: 714-9.

Smith AD. Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12. *Am J Clin Nutr* 2007; 85: 3-5.

Smith AD. The worldwide challenge of the dementias: A role for B vitamins and homocysteine? *Food Nutr Bull* 2008; 29: S143-72.

Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? *Am J Clin Nutr* 2008; 87: 517-33.

Smith AD. Folic acid nutrition: what about the little children? *Am J Clin Nutr* 2010; 91: 1408-9; author reply 9.

Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, Agacinski G, et al. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment. A randomized controlled trial. *PLoS ONE* 2010; 5: e12244.

Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol* 2007; 6: 830-8.

Statistics, New Zealand,. Births and deaths year ended September 2011. 2011 [Accessed: 10 December 2011]; Available from: http://www.stats.govt.nz/browse_for_stats/population/births/info-releases.aspx

Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, et al. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. *PLoS One* 2009; 4: e7845.

Stevens VL, McCullough ML, Sun J, Gapstur SM. Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer in the Cancer Prevention Study II Nutrition Cohort. *Am J Clin Nutr* 2010; 91: 1708-15.

Stevens VL, McCullough ML, Sun J, Jacobs EJ, Campbell PT, Gapstur SM. High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. *Gastroenterology* 2011; 141: 98-105, e1.

Stolzenberg-Solomon RZ, Chang S-C, Leitzmann MF, Johnson KA, Johnson C, Buys SS, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Clin Nutr* 2006; 83: 895-904.

Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *Jama* 2009; 301: 636-50.

Sweeney MR, McPartlin J, Weir DG, Daly S, Pentieva K, Daly L, et al. Evidence of unmetabolised folic acid in cord blood of newborn and serum of 4-day-old infants. *Br J Nutr* 2005; 94: 727-30.

Sweeney MR, McPartlin J, Scott J. Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. *BMC Public Health* 2007; 7: 41.

Sweeney MR, Staines A, Daly L, Traynor A, Daly S, Bailey SW, et al. Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? *BMC Public Health* 2009; 9: 295.

Taylor MJ, Carney SM, Goodwin GM, Geddes JR. Folate for depressive disorders: systematic review and meta-analysis of randomized controlled trials. *J Psychopharmacol* 2004; 18: 251-6.

Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. *Br J Nutr* 2009; 102: 777-85.

Tomaszewski JJ, Cummings JL, Parwani AV, Dhir R, Mason JB, Nelson JB, et al. Increased cancer cell proliferation in prostate cancer patients with high levels of serum folate. *Prostate* 2011.

Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J Nutr* 2006; 136: 189-94.

Tu JV, Nardi L, Fang J, Liu J, Khalid L, Johansen H. National trends in rates of death and hospital admissions related to acute myocardial infarction, heart failure and stroke, 1994-2004. *CMAJ* 2009; 180: E118-25.

Ulrich CM. Folate and cancer prevention: a closer look at a complex picture. *Am J Clin Nutr* 2007; 86: 271-3.

van Beynum IM, Kapusta L, Bakker MK, den Heijer M, Blom HJ, de Walle HE. Protective effect of periconceptional folic acid supplements on the risk of congenital heart defects: a registry-based case-control study in the northern Netherlands. *Eur Heart J* 2010; 31: 464-71.

van den Donk M, van Engeland M, Pellis L, Witteman BJ, Kok FJ, Keijer J, et al. Dietary folate intake in combination with MTHFR C677T genotype and promoter methylation of tumor suppressor and DNA repair genes in sporadic colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2007; 16: 327-33.

Veena SR, Krishnaveni GV, Srinivasan K, Wills AK, Muthayya S, Kurpad AV, et al. Higher maternal plasma folate but not vitamin B-12 concentrations during pregnancy are associated with better cognitive function scores in 9- to 10- year-old children in South India. *J Nutr* 2010; 140: 1014-22.

Vollset SE, Gjessing HK, Tandberg A, Ronning T, Irgens LM, Baste V, et al. Folate supplementation and twin pregnancies. *Epidemiology* 2005; 16: 201-5.

Wald DS, Kasturiratne A, Simmonds M. Effect of folic acid, with or without other B vitamins, on cognitive decline: meta-analysis of randomized trials. *Am J Med* 2010; 123: 522-7 e2.

Wald DS, Morris JK, Wald NJ. Reconciling the evidence on serum homocysteine and ischaemic heart disease: a meta-analysis. *PLoS One* 2011; 6: e16473.

Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. *Lancet* 2001; 358: 2069-73.

Wald NJ. Commentary: A brief history of folic acid in the prevention of neural tube defects. *Int J Epidemiol* 2011; 40: 1154-6.

Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007; 369: 1876-82.

Wang ZP, Shang XX, Zhao ZT. Low maternal vitamin B12 is a risk factor for neural tube defects: a meta-analysis. *J Matern Fetal Neonatal Med* 2011.

Webb PM, Ibiebele TI, Hughes MC, Beesley J, van der Pols JC, Chen X, et al. Folate and related micronutrients, folate-metabolising genes and risk of ovarian cancer. *Eur J Clin Nutr* 2011; 65: 1133-40.

Whitrow MJ, Moore VM, Rumbold AR, Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: A prospective birth cohort study. *Am J Epidemiol* 2009; 170: 1486-93.

Wien TN, Pike E, Wisloff T, Staff A, Smeland S, Klemp M. Cancer risk with folic acid supplements: a systematic review and meta-analysis. *BMJ Open* 2012; 2: e000653.

Williams LJ, Rasmussen SA, Flores A, Kirby RS, Edmonds LD. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995-2002. *Pediatrics* 2005; 116: 580-6.

Wolff T, Witkop CT, Miller T, Syed SB. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2009; 150: 632-9.

Wright AJ, Dainty JR, Finglas PM. Folic acid metabolism in human subjects revisited: potential implications for proposed mandatory folic acid fortification in the UK. *Br J Nutr* 2007; 98: 667-75.

Wyckoff KF, Ganji V. Proportion of individuals with low serum vitamin B-12 concentrations without macrocytosis is higher in the post folic acid fortification period than in the pre folic acid fortification period. *Am J Clin Nutr* 2007; 86: 1187-92.

Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 2008; 51: 29-38.

Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. *Circulation* 2006; 113: 1335-43.

Ye R, Ren A, Zhang L, Li Z, Liu J, Pei L, et al. Tea drinking as a risk factor for neural tube defects in northern China. *Epidemiology* 2011; 22: 491-6.

Zhu H, Wlodarczyk BJ, Scott M, Yu W, Merriweather M, Gelineau-van Waes J, et al. Cardiovascular abnormalities in Folr1 knockout mice and folate rescue. *Birth Defects Res A Clin Mol Teratol* 2007; 79: 257-68.