The relationship between dietary folate intake of women of child-bearing age and risk of neural tube defects in the foetus.

Diet-disease relationship review

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Background information

In December 2003, the Australia and New Zealand Food Regulation Ministerial Council (the Ministerial Council) agreed to a Policy Guideline on Nutrition, Health and Related Claims. The Policy Guideline provides the policy principles to underpin the regulation of nutrition, health and related claims. It aims to ensure that the health and safety of the public is protected, while allowing for food industry innovation and trade. It does this by incorporating a number of elements designed to ensure claims made on food or in advertising are true, scientifically substantiated and not misleading.

The Policy Guideline describes nutrition, health and related claims as "all claims referring to nutrient content, nutrient function, enhanced function, reduction of disease risk or maintenance of normal health". It outlines a claims classification framework, which distinguishes between two broad categories of claim: general level claims and high level claims. High-level claims are those claims that refer to a serious disease or a biomarker.

As part of the standard development process, FSANZ has undertaken to pre-approve a number of high-level claims for incorporation into the standard at the date of gazettal. One of these pre-approved claims may relate to dietary folate (see terminology below) and neural tube defects (NTD). Folate fortification for the prevention of NTD was first considered in Australia in 1994 by an expert committee convened by The National Health and Medical Research Council (NHMRC, 1994). The purpose of this review is to determine whether there is a substantiated relationship between dietary folate intake of women of childbearing age and risk of neural tube defects in the foetus, and if so, the degree of certainty with which it is substantiated. The findings of this review will be used to guide the development of the claim.

Folate Terminology

In this document the following terms are used:

- *folate* is used generically to refer to all forms of the vitamin, both naturally-occurring and synthetic, and its active derivatives;
- *folic acid* is used to refer to synthetic folate which is used in food fortification and supplements;

- *natural folate* is used to refer to folate found naturally in food and does not include folic acid added to food; and,
- *dietary folate* is used to refer to folate that is consumed via the diet, both naturally occurring and folic acid added through fortification. This term does not encompass folate consumed through supplements.

Areas Not Covered in this Review

It is intended that this review will focus on the extent to which scientific evidence supports the relationship between dietary folate and neural tube defects.

It is not intended that the review should focus on other issues including:

- Detailed appraisal of past US Food and Drug Administration reviews in relation to folic acid and neural tube defects.
- Assessment of the burden of disease or economic impact of neural tube defects.
- Assessment of the potential social and economic benefit from increased folate intakes.
- Assessment of dietary intake patterns for folate or of folate status of overseas populations.
- Issues determining whether folic acid supplementation is an effective mechanism in lowering the risk of neural tube defects.

I have also added the following two issues for exclusion, as these do not relate to whether scientific evidence supports the relationship between dietary folate and neural tube defects.

- Assessment of the potential risks associated with high folic acid intakes.
- Assessment of the role of vitamin B_{12} in the prevention of neural tube defects.

Part 1. Critical appraisal of previous review of this diet-health relationship

In this section the reviewer was asked for a critical appraisal of the review conducted in 2000 by Health Canada in relation to a health claim for folic acid and neural tube defects. Given that Health Canada is currently reviewing their work, this section of the review is not presently available.

Part 2. Review of evidence released since the time of the Canadian review

A MEDLINE search was conducted for the period of January 2000 to March 2005 using the terms folate or folic acid and neural tube defects, yielding 525 citations. This search was limited to randomized controlled trials, systematic reviews, prospective studies, case-control studies, English language, and human studies, yielding 45 citations. Of these, only 12 were directly relevant to relationship between dietary folate intake of women of childbearing age and risk of NTDs in the foetus. The remaining 33 studies were typically reviews, commentaries, or methodology papers. A Cochrane Database of Systematic Reviews search was also conducted for the period of January 2000 to March 2005 using the terms folate or folic acid and neural tube defects, yielding nine citations. Only one was relevant. In addition I was aware of three other papers that were relevant that that did not show up in the MEDLINE search.

A well-conducted systematic review of folate and NTDs by Lumley et al was published in 2001. Four randomized controlled trials were included with a total of 6,425 women. The authors found that periconceptional folic acid supplementation at 360 – 4000 µg/day reduced the incidence of NTDs (RR 0.28; 95% CI: 0.13, 0.58). This study supports the health claim for dietary folate as folic acid.

Two additional case-control studies have been published since 2000 (Moore et al, 2003; Thompson et al, 2003). Both studies reported that women in the highest quartile of total folate intake (supplements + diet) periconceptionally were at lower risk of an NTD affected pregnancy than women in the lowest quartile. In one study there was a dose response relationship between increasing total dietary folate intake and decreasing NTD risk. Neither study could indicate a benefit of supplements alone. One study indicated a benefit of dietary folate alone. These studies are supportive of the health claim for folic acid. Both studies used a food frequency questionnaire (FFQ) that did not distinguish between natural folate and folic acid.

Three studies have been published that examined NTD rates before and after mandatory folic acid fortification in North America. Mandatory fortification was estimated to provide an additional 100-200 µg/d folic acid to the US and Canadian

populations. In the US there was a modest 27% decline in NTD prevalence between a 24 month period pre-fortification (1995-1996) and a 24 month post-fortification period (1999-2000) (Centers for Disease Control and Prevention, 2004). In Ontario, Canada there was a 50% decline in NTD prevalence after folic acid fortification (Ray et al, 2002). In Newfoundland, Canada there was a 78% decline (Liu et al, 2004). One cannot conclude from these studies that folic acid fortification reduced the population rate of NTDs, only that the decline in NTDs was temporally associated with folic acid fortification. However, it would be hard to attribute the massive decline in NTD rate in Newfoundland to anything but fortification. Newfoundland had a pre-fortification NTD rate of 43.5 per 10,000 whereas the US had a rate of only 10.6 per 10,000. The very different reductions in NTD prevalence post-fortification suggest that the magnitude of reduction in NTD rate will be diminished in areas with a lower background prevalence of NTDs. Nevertheless, a decline in NTDs as a result of mandatory folic acid fortification supports the health claim for dietary folate (as added folic acid). Also it supports the notion that folic acid in amounts less than 400 µg/day is effective against NTDs.

In four case-control studies, maternal genotype for common polymorphisms of enzymes involved in folate metabolism or combinations of these polymorphisms were examined for their association with NTD risk (De Marco et al, 2001; De Marco et al, 2002; Relton, Wilding, Laffling et al, 2004; Relton, Wilding, Pearce et al, 2004). In almost all studies maternal homozygosity (*cf* wild-type) for these common polymorphisms was associated with increased risk. Although these studies are supportive of a relationship between folate and NTDs they do not really add anything further to the substantiation of the relationship. I have not included a detailed assessment of these studies.

A number of studies have examined potential undesirable effects of folic acid, including the issue of increased risk of multiple births in women who have taken folic acid supplements. . Since publication of the Canadian report, data from a Swedish study published in 2001 suggested that women (n=2,569) who took folic acid (typically 200 or 400 µg) were at increased risk of having dizygotic twins (RR 2.13; 95% CI: 1.64, 2.74) (Ericson et al, 2001). Further, in the systematic review by Lumley et al (2001) there was an overall non-significant increase in multiple gestation (RR 1.40; 95% CI: 0.93, 2.11) in women receiving folic acid either alone or

in combination with other vitamins in the three studies included. However, no increase in multiple births was reported in a non-randomized trial in China (n=242,015) in women taking 400 µg/d folic acid during pregnancy (0.59%) compared to women who did not (0.65%) (Li et al, 2003). Recently, the results of a follow-up study of women in a large 1960s randomized controlled trial of antenatal folic acid consumption were published (Charles et al, 2004). By September 2002, 210 women had died; 40 attributable to cardiovascular disease, 112 deaths to cancer, including 31 to breast cancer. All cause mortality and breast cancer mortality were greater in the group receiving 5 mg folic acid per day compared with placebo; although neither reached conventional statistical significance ($p>0.05$). Although these findings are interesting they could have occurred by chance. There was no pre-specified hypothesis and the number of deaths and breast cancer deaths were very low. The findings of increased twinning or breast cancer do not impact on the health claim.

In view of the recent evidence:

- The evidence that folic acid, either as supplements or folic acid fortified foods, is protective against NTDs remains convincing. The findings of a reduced prevalence of NTDs post-mandatory fortification supports the notion that folic acid foods are effective against NTDs and that folic acid at doses less than 400 µg/d is also effective. The finding of a smaller reduction in NTDs post-fortification in the US versus Newfoundland, Canada confirms the findings of the study in China indicating that the effect of folic acid on NTD reduction will be diminished in areas with a low background incidence.
- There is no new evidence to support a protective role for natural folate against NTDs and this evidence suggesting a relationship remains ‘possible’ at best.

Thompson, S.J., Torres, M.E., Stevenson, R.E., Dean, J.H. & Best, R.G. (2003). Periconceptional multivitamin folic acid use, dietary folate, total folate and risk of NTDs in South Carolina. *Ann Epidemiol 13*: 412-418.

Location, design and purpose	Subjects	Exposure	Duration
North Carolina, USA Case control study examine whether dietary folate or folic acid taken as part of a multivitamin supplement taken three months before conception and during the first three months of pregnancy reduces the occurrent risk of NTD affected pregnancies.	Cases were 179 women who had a NTD affected pregnancy between 1992-1997. Restricted to first occurrence, singleton, and isolated NTD. Controls were 288 women with live born births without NTD selected randomly and concurrently each year with cases. Comments: Sample size adequate but not all eligible women participated. 256 eligible women with NTDs, 185 agreed to participate (72.3%). Also excluded – 3 women who had taken anti-convulsant medication, 2 who had twins, and 1 who had an NTD herself. 398 control women eligible 289 agreed (72.6%) one excluded for anti-convulsant medication.	Each subject was interviewed to collect information on vitamin supplement use, socio-demographic factors, family medical history, pregnancy/fertility history, maternal illnesses, medication use, tobacco and alcohol use and environmental exposures. Subjects completed a FFQ.	An interviewer contacted women within two weeks of hospital discharge and within 4-6 weeks of an elective termination for a NTD. Information was collected on supplement use three months prior to conception until termination or delivery.

Thompson et al, 2003 (continued)

Diet	Results	Comments
Women completed the Harvard semi-quantitative FFQ for the period three months prior to conception through three months pregnancy. Folate intake divided into quartiles according to the distribution of control mothers. Each woman's total average daily supplemental folic acid intake for each of the six periconceptional months was derived from supplement composition and frequency of use information. Use was defined as: Regular use – taking multivitamins with 0.4 mg, 0.8 mg or more folic acid for at least three times per week. Some use – taking multivitamin folic acid for less than three times per week or in partial months. No vitamin use – no use at any time. Comments: FFQ would not have distinguished between natural folate and folic acid.	Cases who choose to participate did not differ from non-participants for age, type of NTD, or elective terminations. Case and control were similar for most demographic and behavioral characteristics except that a higher proportion of cases than controls were white and reported having been exposed to cigarette smoking of others. All odds ratios (OR) adjusted for covariates. Regular use of supplements during the periconceptional period showed no statistically significant reduction in NTD risk [OR=0.55; 95% CI: 0.25, 1.22]. A protective effect of dietary folate was only shown for those in the highest quartile of folate intake (0.457-3.125 mg), estimated from the FFQ, compared with those in the lowest quartile (0.015-0.235 mg) [OR=0.40; 95% CI: 0.19, 0.84]. For total folate intake (dietary + supplemental) again only the highest quartile had a reduced risk compared to the lowest quartile [OR=0.35; 95% CI: 0.17, 0.72].	18% of controls and 10% of cases took folic acid supplements the three months prior to conception. The low use of supplements in both cases and control may explain the failure to find an effect of supplements. The finding that the highest quartile of folic acid intake is associated with lowest NTD risk supports health claim. The lack of a dose response for folate and NTDs does not support the notion that lower amounts of folic acid (<400 µg), as might be provided under the health claim, are protective against NTDs. However, FFQ crude measure of folate intake. Study weak design compared to randomized controlled trials.

Moore, L.L., Bradlee, M.L., Singer, M.R., Rothman, K.J. & Milunsky, A. (2003). Folate intake and the risk of NTDs: An estimation of dose-response. *Epidemiology 14*: 200-205.

Location, design and purpose	Subjects	Exposure	Duration
Northeastern United States This is a re-analysis of an earlier published prospective cohort study. The purpose of the re-analysis was to try and determine whether periconceptional folate intake (supplemental or total folate) reduces NTD risk in a dose-responsive manner.	28,559 women in the early second trimester of pregnancy were asked to participate in a prospective study. All had alpha-fetoporotein screening or an amniocentesis. The reanalysis included 23,228 women who agreed to participate, had an informed pregnancy, and complete questionnaires. NTDs occurred in 49 pregnancies and the remaining women were the controls. The original study was assessed in the US document.	Interviews were carried out by phone by a nurse interviewer. Each woman was asked to provide detailed information about her use of multivitamins and other vitamin supplements, including folic acid and yeast (which contains folic acid). The women were asked for the brand of any supplement they took and the number of times it was taken per week. They were also asked when they had started taking the supplement and when and if there had been any change in its use. Medical and maternal history, and demographic and lifestyle information were collected.	Interviews were carried between the 15th and 20th gestational week. Information was collected on supplement use three months prior to conception through the first trimester.

Moore et al, 2003 (continued)

<table>
<tr><th>Diet</th><th>Results</th><th>Comments</th></tr>
<tr>
<td>50-item semi-quantitative FFQ adapted from Harvard questionnaire to emphasize food sources of folate. Women were asked to recall diet during the first eight weeks of pregnancy.

Comment: the two months prior to becoming pregnant are probably more important and are not assessed in this study.</td>
<td>Relative risks adjusted for covariates. There was no evidence of a dose-response relation according to intake of supplements alone or dietary folate alone; although for dietary folate the trends were supportive of a dose response. Total folate from supplements and food was associated with NTD risk in a dose responsive manner.

Prevalence and Relative Risk of NTD according to daily total folate intake
<table>
<tr><th>DFEs/Day</th><th>N</th><th>No of defects</th><th>RR (95% CI)</th></tr>
<tr><td>0-149</td><td>2041</td><td>7</td><td>1.0</td></tr>
<tr><td>150-399</td><td>10416</td><td>24</td><td>0.66 (0.28-1.6)</td></tr>
<tr><td>400-799</td><td>4147</td><td>10</td><td>0.70 (0.26-1.9)</td></tr>
<tr><td>800-1199</td><td>4017</td><td>6</td><td>0.44 (0.14-1.4)</td></tr>
<tr><td>≥1200</td><td>2607</td><td>2</td><td>0.23 (0.05-1.1)</td></tr>
</table>
</td>
<td>Not enough data to determine optimal periconceptional folate intake.

Study supports health claim by suggesting that lower levels of dietary folate (presumably folic acid) are protective.</td>
</tr>
</table>

Li, Z., Gindler,J., Wang, H., Berry, R.J., Li, S., Correa, A., Zheng, J., Erickson, J.D. & Wang, Y. (2003). Folic acid supplements during early pregnancy and likelihood of multiple births: a population cohort study. *Lancet 361*: 380-84.

Location, design and purpose	Subjects	Exposure	Duration
China Non-randomized intervention to determine if folic acid supplements increase the risk for multiple births. Carried out in two regions of China; a Northern province where the rate of NTDs was high (5-6 per 1,000) and two southern provinces where the rates were lower (1 per 1,000).	285,536 pregnant women and women preparing for marriage registered with a pregnancy monitoring system.	All women were asked to take pills containing 400 µg/d folic acid. Women were divided into groups based on their use (folic acid use versus no folic acid use). The median pill taking compliance among those with any folic acid use was >80%, with >90% of women taking pills to the end of the first trimester.	Subjects were asked to take one pill daily from the time of their premarital examination until the end of the first trimester. The median pill taking compliance among those with any folic acid use was >80%, with >90% of women taking pills to the end of the first trimester.

Li et al continued (2003)

Diet	Results	Comments
Not assessed.	There were 242,015 informed pregnancies. Women who took pills were on average two years younger and were more likely to be pregnant for the first time than non-users. Multiple births occurred in 1,496 pregnancies (0.62%). No difference between supplement versus non supplement users. The rate of multiple pregnancies was 0.59% and 0.65% in the supplement users and non-users, respectively (Rate ratio 0.91; 95% CI: 0.82, 1.00).	Suggests earlier concerns about increased twinning with folic acid from one earlier small randomized control trial are not founded. Concerns about increased twinning related to increased folic acid use as a result of health claim not substantiated.

Charles, D., Ness, A.R., Campbell, D., Davey Smith, G. & Hall, M.H. (2004).Taking folate in pregnancy and risk of maternal breast cancer. *BMJ 329*: 1375-1376.

Location, design and purpose	Subjects	Exposure	Duration
United Kingdom Follow-up of the participants in a large randomized controlled trial of antenatal folic acid consumption and pregnancy outcome in the 1960s. The effects of folate supplementation on death and cause of death are assessed in the participants.	Between June 1966 and June 1967, 3,187 women were identified as potentially eligible for a trial of folate supplementation and pregnancy outcome. The study aimed to recruit all pregnant women booking for antenatal care under 30 weeks gestation who were residents of Aberdeen, Scotland. 3,037 women recruited 2,928 were randomized.	Women were randomized to placebo (n=1,977), 200 µg folic acid (n=466), or 5 mg folic acid (n=485). The husband or partner's occupation at the time of delivery was used to determine social class. Linking the trial data to the Aberdeen maternity and neonatal databank added information on maternal smoking and maternal height. Compliance was assessed by self-report and by measurement of folate status.	Subjects were asked to take one pill daily. The length of time of folic acid supplementation is not given. No women withdrew from the trial. Death records were examined up until September 2002.

Charles et al continued (2004)

Diet	Results	Comments
Not assessed.	In the placebo group, 1.9% reported that they had not taken their tablets regularly compared with 1.7% in the group taking 0.2 mg folate and 3.2% in the group taking 5 mg. 210 women had died, 40 cardiovascular disease deaths, 112 deaths to cancer, 31 to breast cancer. All cause mortality and breast cancer mortality were greater in the group receiving 5 mg compared with placebo. Neither reached conventional level of significance. *Adjusted for maternal age, smoking, height, weight, social class, and systolic blood pressure; parity; and gestational age.	Preliminary findings only. Possibility of chance finding. Study had no pre-specified hypothesis. The number of deaths was small with only 31 breast cancer deaths. For both breast cancer and all cause mortality the confidence intervals are wide and include one. Raises concerns about the health claim increasing folic acid intakes and having an adverse affect on the target population. Findings too tentative for action at this time.

	Hazard Ratio (95% CI)	P for trend
All Cause mortality		
Placebo	1.00	0.13
0.2 mg folate	1.21 (0.83, 1.77)	
5 mg folate	1.42 (1.00, 2.04)	
Cardiovascular mortality		
Placebo	1.00	1.00
0.2 mg folate	1.02 (0.42, 2.48)	
5 mg folate	1.02 (0.42, 2.48)	
All cancer deaths		
Placebo	1.00	0.09
0.2 mg folate	1.20 (0.71, 2.02)	
5 mg folate	1.70 (1.06, 2.72)	
Breast cancer mortality		
Placebo	1.00	0.23
0.2 mg folate	1.56 (0.38, 3.41)	
5 mg folate	2.02 (0.88, 4.72)	

Lumley, J., Watson, L., Watson, M. & Bower, C. (2005). Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. The Cochrane Library.

Design, aim, and date	Criteria for Consideration	Search Strategy	Inclusion/Exclusion and Quality Assessment
Systematic Review The primary objective was to identify whether the prevalence of NTDs can be reduced by increased consumption of multivitamins or folate before pregnancy and in the first two months of pregnancy. Also • To identify whether the prevalence at birth of other birth defects can be reduced by the same intervention. • To identify whether the same intervention changes fertility or foetal survival. There were other objectives not directly related to the relationship between dietary folate and folic acid. Date of most recent update: 31 May 2001	Participants • Women planning on becoming pregnant who already had an NTD affected pregnancy. • Women planning on becoming pregnant who had not had an NTD affected pregnancy, or had not been pregnant before. Types of study • All studies with randomized or quasi-randomized allocation were considered. Types of intervention • Studies which compared periconceptional supplementation with multivitamins and placebo • Folate with placebo • Multivitamins without folate with folate • Different dosages of multivitamins or folate Outcomes • NTDs • Facial clefts, limb reduction defects, conotruncal heart defects, urogenital defects; all other birth defects • Spontaneous abortion • Multiple pregnancy • Preterm birth (<37 weeks gestation) • Perinatal and infant mortality • Time to conception	Searched the Cochrane Pregnancy and Childbirth Group trials register. Briefly, group searches MEDLINE, the Cochrane Controlled Trials Register and reviews the contents tables. Search strategy is not fully described in the paper but is available in another document.	The methods of data extraction and quality assessment were provided and were appropriate. Reasons given for inclusion and exclusion. Four relevant studies included: Czeizel, 1994; Kirke 1992; Laurence, 1981; MRC 1991. One study excluded; Ulrich 1999 (miscarriages, including late miscarriages were excluded, only 16.6% treated with folic acid prior to last menstrual period). China study not included presumably because it was not a randomized controlled trial. According to authors the methodological quality of the four included supplementation studies was variable. In two of the four prevention trials, the method of randomisation was unclear. Two had no clear sample size justification. Another did not reach its planned sample size because of large and unanticipated changes in the prevalence of the primary outcome.

Lumley et al continued, 2005

Results	Comment	
Total of 6,425 women in the four trials. Periconceptional folate supplementation reduced the incidence of neural tube defects (RR 0.28, 95% CI: 0.13, 0.58). Studies were underpowered to examine other birth defects. Folate supplementation did not significantly increase miscarriage, ectopic pregnancy or stillbirth. There was a large increase in multiple gestation but this was not significant 1.40 (95% CI: 0.93, 2.11).	Well-conducted systematic review of randomized controlled trials. Support the health claim that folic acid fortified foods will decrease NTD risk. Gives no information on minimum dose required.	

Three studies published since the Canadian review are relevant but are not classifiable under the guidelines in the FSANZ Substantiation Framework. Summaries and an interpretation of each follow.

Centers for Disease Control and Prevention. (2004). Spina bifida and anencephaly before and after folic acid mandate – United States, 1995-1996 and 1999-2000. *MMWR 53*: 362-365.

Mandatory folic acid fortification of cereal grain products went into effect in January 1998. The purpose of this study was to compare NTD rates before and after fortification. The number of annual NTDs was calculated for a 24-month period pre-fortification (1995-1996) and a 24-month post-fortification period (1999-2000). To calculate the number of NTD affected pregnancies (live births, stillbirths, fetal deaths, and elective terminations) data was obtained from eight population-based surveillance systems that systematically collect data from sources that perform diagnostic prenatal ultrasounds. There were another 15 population based systems that only reported the number of live births, stillbirths, and fetal deaths, but not prenatally ascertained cases. Based on the systems with pre-natal ascertainment there was a 27% decline in NTDs before and after fortification.

	Systems with prenatal ascertainment	Systems without prenatal ascertainment	Fetal deaths and terminations
Prefortification (1995-1996)			
Spina bifida			
Prevalence*	6.4	5.1	
Number	2490	1980	
Anencephaly			
Prevalence	4.2	2.5	
Number	1640	970	
Total	4130	2950	1180
Postfortification (1999-2000)			
Spina bifida			
Prevalence	4.1	3.4	
Number	1640	1340	
Anencephaly			
Prevalence	3.5	2.1	
Number	1380	840	
Total	3020	2180	840

*per 10000 live births
difference between sytems with and without prenatal ascertainment

Assessment and Implication

One cannot conclude from this study that folic acid fortification reduced the population rate of NTDs, only that the decline in NTDs was temporally associated with folic acid fortification. A concern about this study is that NTD rates had been falling prior to fortification and it is not clear what part of the decline after fortification was a continuation of this decline or was a direct result of fortification. A 27% reduction in NTDs is much less than the 50% reduction thought possible if all women received sufficient folic acid prenatally. One interpretation is that the level of folic acid fortification is not high enough. Another interpretation is that the impact of folic

acid on NTD rates will be less in area of lower incidence such as the US. The findings of an 80% reduction in NTDs post-fortification in Newfoundland, Canada (43.5 per 10,000 pre-supplementation 1991-1993) would seem to support the latter interpretation.

Ray, J.G., Meier, C., Vermeulen, M.J., Boss, S., Wyatt, P.R. & Cole, D.E. (2002). Association of neural tube defects and folic acid fortification in Canada. *Lancet 360*: 2047-2048.

Many Canadian women do not take folic acid before conception. In response the Canadian government mandated the addition of folic acid to grain products. By January 1998 most of Canada's cereal grain products were fortified with folic acid. This was estimated to provide an additional 0.1-0.2 mg folic acid to the Canadian population. The purpose of this study was to assess the prevalence of antenatally and postnatally diagnosed open NTDs before and after fortification in the province of Ontario.

Defects were detected through antenatal maternal serum screening at 15-20 weeks gestation, which is available to all Ontario women. Those with a positive test are referred to counselling at one of 17 genetics centres. These centres contribute data to a central database. Data from one of these centres was excluded because it was not complete. A postnatally diagnosed event comprised all liveborn and stillborn affected infants after 20 weeks gestation, identified by data linkage to the mother's provincial health insurance number. All defects were defined by the date of conception. Between January 1994 and March 2000, 336,963 women underwent screening. The authors compared prevalence of NTDs before (January 1994-December 1997) and after (January 1998-March 2000) folic acid fortification. The prevalence rate of NTDs declined from 11.3 to 5.8 per 10,000 pregnancies before and after fortification. The authors conclude that at a population level folic acid food fortification is associated with a reduction in NTDs.

	Period of Observation		Age-adjusted prevalence ratio
	Before Fortification	After Fortification	
Anencephaly or spina bifida			
Number of women with affected fetuses	248	69	
Prevalence (per 1000 pregnancies)	1.13	0.58	0.62 (0.40, 0.67)
Anencephaly			
Number of women with affected fetuses	84	19	
Prevalence (per 1000 pregnancies)	0.38	0.16	0.49 (0.29, 0.97)
Spina Bifida			
Number of women with affected fetuses	164	50	
Prevalence (per 1000 pregnancies)	0.75	0.42	0.69 (0.49, 0.97)

Assessment and Implication

One cannot conclude from this study that folic acid fortification reduced the population rate of NTDs, only that the decline in NTDs was temporally associated with folic acid fortification. My main concern about this study is that NTD rates had been falling prior to fortification and it is not clear what part of the decline after fortification was a continuation of this decline or was a direct result of fortification. If

one accepts that the decline is due to folic acid fortification it provides supporting evidence that folic acid added to food (versus folic acid supplements) is effective against NTDs. Also, it suggests that food fortified with folic acid will lower NTDs even in areas with a low background incidence (11.3 per 10,000 pregnancies before January 1998).

Liu, S., West, R., Randell, E., Longerich, L., Steel O'Connor, K., Scott, H., Crowley, M., Lam, A., Prabhakaran, V. & McCourt. (2004). A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. *BMC Pregnancy and Childbirth 4*: 20.

Canadian women are advised to take folic acid supplements pre-pregnancy. Many women do not take folic acid before conception. In response the Canadian government mandated the addition of folic acid grain products. By January 1998 most of Canada's cereal grain products were fortified with folic acid. This was estimated to provide an additional 0.1-0.2 mg folic acid to the Canadian population. A purpose of this study was to determine the rates of NTD before advice to take supplements, before fortification, and after fortification in the province of Newfoundland, a province with a historically high rate of NTDs.

Data on NTDs were compiled from the Newfoundland and Labrador Medical Genetics Program. Cases were identified in the following ways: provincial live birth and stillbirth notification forms, maternal fetal-medicine referrals, and letters sent to all provincial hospitals requesting data on NTD cases or terminations for NTDs. NTD cases included anencephaly, spina bifida and encephalocele diagnosed in live births, stillbirths, and fetuses from pregnancies terminated because of a prenatal diagnosis of an NTD. NTD rates were compared pre-supplementation (1991-1993), pre-fortification (1994-1997), and post-fortification (1998-2001). There was no change in NTD rates after advice was given to women to take supplements. Seventeen percent of women took folic acid supplements pre-fortification and 28% took supplements post-fortification. Fortification of the food supply was temporally associated with a 78 % (95% CI: 65, 86%) reduction in NTD rates.

Period	NTD s	Total no. of births	Rate per 1000 births
Pre-supplementation 1991-1993	90	20,711	4.35
Pre fortification 1994-1997	103	23,592	4.37
Post-fortification 1998-2001	19	19,816	0.96

Assessment and Implication

One cannot conclude from this study that folic acid fortification reduced the population rate of NTDs, only that the decline in NTDs was temporally associated with folic acid fortification. Nevertheless, it would be hard to attribute this substantial decline to anything but fortification. It provides evidence that folic acid added to food

(versus folic acid supplements) is effective against NTDs. Also, it demonstrates that food fortified with folic acid will have a dramatic impact on NTD rates in areas with a high background incidence (43.5 per 10,000 pre-supplementation 1991-1993).

Part 3. Relevance of the relationship to Australia and New Zealand

Folic acid taken during the periconceptional period has been shown to be effective against NTDs in a wide variety of study designs and settings. The intervention trials have been carried out in Hungary, China, and the United Kingdom. The large United Kingdom MRC trial was conducted in seven countries including a small arm in Australia. Accordingly, there is no reason to expect that increasing folic acid intake peri-conceptionally would not be effective against NTDs in New Zealand and Australia. The relationship between folate and NTDs has been examined in one Australian study. In a case-control (n=77 cases) study carried out in Western Australia increasing intake of folate (diet + supplements) during the first six weeks of pregnancy was protective against NTDs.

An important question to answer is; what magnitude of NTD reduction is possible with folic acid in Australia and New Zealand? I suspect it will be less than that achieved in the experimental trials. The magnitude of the reduction in NTDs, achievable with folic acid, will depend in part on the initial folate status of women and the background incidence of NTDs in the population. Red cell folate is inversely associated with NTD risk. The results from a representative survey of Dunedin women of childbearing age (18-45 y; n=216), of who 18% regularly took a folic acid containing supplements, indicate that over one third have red cell folate concentrations exceeding the level that confers a very low risk of NTDs (Ferguson et al, 2000). If the red cell folate concentrations of Dunedin women are similar to women in Australia and the rest of New Zealand it is possible that a substantial portion of women will derive no further benefit from additional folic acid.

The underlying recurrence rate in women not taking folic acid in the MRC trial and Hungarian trials was 350/10,000 and 25/10,000 births, respectively. In the northern region of China, with a NTD birth rate of 48/10,000, there was a 79% reduction in risk with the use of folic acid supplements. However, in the southern region, with a defect rate of 10/10,000, use of folic acid supplements did not significantly reduce the risk of NTDs (16%). Mandatory folic acid fortification has had a variable effect on NTD rates in North America**.** In the US there was a modest 27% decline in NTDs pre and post-fortification period. In Newfoundland, Canada there was a 78%

decline. Newfoundland had a pre-fortification NTD rate of 43.5 per 10,000 whereas the US had a rate of only 10.6 per 10,000. The evidence suggests that effectiveness of folic acid in preventing NTDs may be diminished in populations with a low background incidence of NTDs such as Australia and New Zealand. The birth rate for NTDs in Australia for the period 1991-1997 was 12.4/10,000. However, not all states record terminations of pregnancy due to NTD, thus the true rate may be higher (Lancaster and Hurst, 2001). Monitoring data from the Western Australia Birth Defects Registry indicates that NTD rates within this state during the period 1996-2000 were 12.9/10,000 (livebirths, stillbirths and terminations of pregnancies with NTD per 10,000 live and stillbirths) for non-Indigenous women, and 25.6/10,000 for Indigenous women (Bower et al, 2004). The birth rate for NTDs in New Zealand for the period 1995-1999 was 4/10,000. Estimates that include terminations in 1998 and 1999 suggest the overall rate for NTDs is 10/10,000 (New Zealand Ministry of Health, 2002).

There are no major differences in genetics, lifestyle, including dietary patterns and health practices between Australia/New Zealand and Canada that would have an impact on the Health Claim. The prevalence of the TT variant for the MTHFR polymorphism, which has been associated with increased NTD risk, is similar in Australia/New Zealand and Canada (Wilcken et al, 1996; Venn et al, 2003). A lower rate of NTDs has been reported in Maori and Pacific People (NZ Ministry of Health, 2002) and a higher rate in certain Aboriginal groups (Bower et al, 2004). The relevance of this to the health claim is not clear. Folate intakes in Canada (pre-fortification), New Zealand (Russell et al, 1999), and Australia (McLennan and Podger, 1998) are remarkably similar at around 200 µg/day for women of childbearing age. The 1997 New Zealand National Nutrition Survey found that for adults 15 years and older mean folate intake was 251 mg/day. A slightly higher intake figure was calculated for Australian adults (2 years and older) at 289mg/day (data derived using the FSANZ dietary modelling computer program, DIAMOND, and data from the 1995 Australian and 1997 New Zealand National Nutrition Surveys). Wright and coworkers (1998) have reported red cell folate data from US NHANES III pre-fortification is available. Although methodological issues confound the interpretation it would appear that the women in the Dunedin study have higher red cell folate (median 780 nmol/L) concentrations than US women (median 350 nmol/L).

The conclusions reached in Part 2 are relevant to Australians and New Zealanders.

Part 4. Relationship of folate intake with relevant biomarkers of disease outcome

Red cell folate is a potential biomarker. Red cell folate is a physiological marker that responds in a dose responsive manner to increased folic acid from supplements or fortified foods. Supplementing women with 100, 200, and 400 µg folic acid per day for six months increased red cell folate by 150, 365, and 495 nmol/L, respectively (Venn et al, 2002). Red cell folate concentration has been associated with NTD risk. Red cell folate was lower in women with an NTD affected pregnancy than control women in two case control studies (Smithells et al, 1976; Yates et al, 1987). In a case-control study of 56,049 women in Ireland, Daly et al (1995) reported that the risk of neural tube defects declined continuously as red blood cell folate concentrations increased; the lowest category of risk occurring in women with folate concentrations greater than 905 nmol/L.

Red Cell Folate (nmol/L)	Cases n (%)	Controls n (%)	Risk of NTD per 1000 births	95% CI
0-339	11 (13.1)	10 (3.8)	6.6	3.3-11.7
340-452	13 (15.5)	24 (9.0)	3.2	1.7-5.5
453-679	29 (34.5)	75 (28.2)	2.3	1.6-3.3
680-905	20 (23.8)	77 (29.0)	1.6	1.0-2.4
³906	11 (13.1)	80 (30.0)	0.8	0.4-1.5
Total	84 (100)	266 (100.0)	1.9	1.5-2.3

However, the validity of red cell folate as a marker of NTDs has not been rigorously evaluated. Red cell folate is not highly predictive of NTDs. Most women with a low red cell folate will not have an NTD affected pregnancy. Likewise some women with high red cell folates will have an NTD affected pregnancy. Indeed, in the Irish study there were as many NTD cases in the highest quintile as the lowest quintile of red cell folate. There is no biological basis for believing that the biomarker is on the causal pathway between exposure and the disease or health outcome (Note: at least not in the same sense as cholesterol is a biomarker of saturated fat and cardiovascular risk). There is no substantiated relationship between dietary folate and red cell folate as a biomarker of NTD risk.

Part 5. Overall conclusions

The purpose of this final part of the review is to draw together the findings of the preceding parts into a summary document:

Summary

Folate is a water-soluble B vitamin that occurs naturally in food. Folic acid is a synthetic form of folate. Because of its high stability and bioavailability, folic acid is the form of folate that is used in supplements and added to fortified foods. Folate helps produce and maintain new cells and is, therefore, important during periods of rapid growth such as pregnancy. Neural tube defects (NTD), serious birth defects that occur around 28 days post-conception, result in malformations of the brain (anencephaly) and spine (spina bifida). Infants with anencephaly die at birth or soon after and those with spina bifida are often afflicted with varying degrees of paralysis and disability. Over 30 years ago it was first suggested that folic acid taken periconceptionally could reduce a woman's chance of having an NTD-affected pregnancy.

There is now convincing evidence that folic acid taken prior to and during the first month of pregnancy can reduce a woman's risk of having an NTD-affected pregnancy. No fewer than 15 cross-sectional and case-control studies have examined this relationship and nearly all have been supportive. The most convincing evidence comes from three randomized controlled trials and a public health campaign conducted in China. In one study, periconceptional folic acid taken alone (4 mg) or as part of a multi-vitamin reduced the risk of NTD by 72% in women with a prior NTD-affected pregnancy. In another study of primary NTD prevention there were no cases of NTD in women receiving folic acid (800 µg) as part of a multivitamin supplement and six cases in women receiving a placebo, representing a complete protective effect (Czeizel & Dudas, 1992). In a population based prevention campaign in China, risk of an affected pregnancy was reduced by 80% in women taking 400 µg folic acid in a northern province of China. Although there is now convincing evidence that folate lowers NTD risk we are no closer at saying how folate prevents NTDs. Most women who have an NTD-affected pregnancy are not folate deficient. It has been suggested that additional folic acid during the peri-

conceptional period helps overcome some metabolic block in genetically sensitive women.

Improved folate status during the peri-conceptional period will not prevent all NTDs. Nevertheless, folic acid taken during the periconceptional period has been shown to be effective against NTD in a wide range of populations and study designs. There is no reason to expect that increasing folic acid intake peri-conceptionally would not be effective against NTD in Australia and New Zealand. NTD rates are low in Australia and New Zealand. Accordingly, the magnitude of reduction in NTD with folic acid may be diminished.

Public health advice is that women who are planning a pregnancy should take a folic acid supplement providing 400 µg/d folic acid at least one month before conception through the third month of pregnancy. Women planning a pregnancy should not rely on natural sources of folate or fortified foods for NTD prevention. Up to 50% of pregnancies are unplanned and as a result strategies are needed to provide additional folate to women who could become pregnant.

The strength of evidence indicating that natural folate is protective against NTDs is possible at best. Further, issues of bioavailability, stability, and measurement of natural folate make it is impossible to indicate the dose, if any, of natural folate required to prevent NTD. Consideration should be given to amount of folic acid a food should contain to be allowed to carry the claim. The minimum amount of folate required to prevent NTD is not known with any certainty. The lowest dose of folic acid demonstrated to be effective in an intervention trial is 400 µg/d. However, based on changes in red cell folate, doses of folic acid as low as 100-200 µg folic acid per day consumed chronically may confer some protection. A woman would have to eat approximately six and eleven servings of a fortified food containing, for example, 35 µg folic acid to achieve folic acid intakes of 200 and 400 µg, respectively.

References

Berry, R. J., Li, Z., Erickson, J. D., Li, S., Moore, C. A., Wang, H., et al (1999). Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med, 341*, 1485-1490.

Blakley, R. The biochemistry of folic acid and related pteridines. (1969). In North Holland Research Monographs Frontiers of Biology. Newbergen, H., & Taton E.L. Amsterdam: North Holland Publishing Company.

Bower, C., & Stanley, F. (1989). Dietary folate as a risk factor for neural-tube defects: Evidence from a case-control study in Western Australia. *Med J Aus, 150*, 613-619.

Bower, C., Eades, S., Payne, J., D'Antoine, H., Stanley F. (2004). Trends in neural tube defects in Western Australia in Indiengous and non-Indigenous populations, *Paediatric and Perinatal Epidemiology, 18,* 277-280

Brouwer, I. A., van Dusseldorp, M., West, C. E., Meyboom, S., Thomas, C. M., Duran, M., et al (1999). Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J Nutr, 129*, 1135-1139.

Centers for Disease Control and Prevention. (2004). Spina bifida and anencephaly before and after folic acid mandate – United States, 1995-1996 and 1999-2000. *MMWR, 53*, 362-365.

Charles, D., Ness, A. R., Campbell, D., Davey Smith, G., & Hall, M. H. (2004). Taking folate in pregnancy and risk of maternal breast cancer. *BMJ, 329*, 1375-1376.

Cuskelly, G. J., McNulty, H., & Scott, J. M. (1996). Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. *Lancet, 347*, 657-659.

Czeizel, A. E., & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med, 327*, 1832-1835.

Daly, L. E., Kirke, P. N., Molloy, A., Weir, D. G., & Scott, J. M. (1995). Folate levels and neural tube defects. Implications for prevention. *JAMA, 274*, 1698-1702.

Daly, S., Mills, J. L., Molloy, A. M., Conley, M., Lee, Y. J., Kirke, P. N., et al (1997). Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet, 350*, 1666-1669.

De Marco, P., Calevo, M. G., Moroni, A., Arata, L., Merello, E., Cama, A., et al (2001). Polymorphisms in genes involved in folate metabolism as risk factors for NTDs. *Eur J Pediatr Surg, 11,* S14-17.

De Marco, P., Calevo, M. G., Moroni, A., Arata, L., Merello, E., Finnell, R. H., et al (2002). Study of MTHFR and MS polymorphisms as risk factors for NTD in the Italian population. *J Hum Genet, 47*, 319-324.

Ericson, A., Kallen, B., & Aberg, A. (2001). Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res, 4*, 63-66.

Ferguson EL, Skeaff CM, Bourn DM, Dixon M. (2000). Folate Status of Representative Populations in Dunedin: Issues for folate fortification. Dunedin: Department of Human Nutrition and Department of Food Science, University of Otago.

Kirke, P. N., Daly, L. E., & Elwood, J. H. (1992). A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child, 67*, 1442-1446.

Lancaster, P., & Hurst, T. (2001) Trends in neural tube defects in Australia. National Food and Nutrition Monitoring and Surveillance Project.

Laurence, K. M., James, N., Miller, M. H., Tennant, G. B., & Campbell, H. (1981). Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. *Br Med J (Clin Res Ed), 282*, 1509-1511.

Li, Z., Gindler, J., Wang, H., Berry, R. J., Li, S., Correa, A., et al (2003). Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet, 361*, 380-384.

Liu, S., West, R., Randell, E., Longerich, L., Steel O'Connor, K., Scott, H., et al (2004). A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. *BMC Pregnancy and Childbirth, 4*, 20.

Lumley, J., Watson, L., Watson, M., & Bower, C. (2001). Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane Database Syst Rev*, CD001056.

McLennan, W., Podger, A. (1998). National Nutrition Survey. Nutrient Intakes and Physical Measurements. Australia. 1995. Canberra: Australian Bureau of Statistics.

McKillop, D. J., Pentieva, K., Daly, D., McPartlin, J. M., Hughes, J., Strain, J. J., et al (2002). The effect of different cooking methods on folate retention in various foods that are amongst the major contributors to folate intake in the UK diet. *Br J Nutr, 88*, 681-688.

Mills, J. L., Rhoads, G. G., Simpson, J. L., Cunningham, G. C., Conley, M. R., Lassman, M. R., et al (1989). The absence of a relation between the periconceptional use of vitamins and neural-tube defects. National Institute of Child Health and Human Development Neural Tube Defects Study Group. *N Engl J Med, 321*, 430-435.

Moore, L. L., Bradlee, M. L., Singer, M. R., Rothman, K. J., & Milunsky, A. (2003). Folate intake and the risk of neural tube defects: an estimation of dose-response. *Epidemiology, 14*, 200-205.

MRC Vitamin Study Research Group. (1991). Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet, 338*, 131-137.

New Zealand Ministry of Health. (2002) Improving Folate Intake in New Zealand: Policy implications. Wellington, New Zealand.

National Health and Medical Research Council. (1994) Folate Fortification. Reprot of the Expert Committee on Folate Fortification. Australian Government Publishing Service.

Pfeiffer, C. M., Rogers, L. M., Bailey, L. B., & Gregory, J. F., 3rd. (1997). Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. *Am J Clin Nutr, 66*, 1388-1397.

Puwastien, P., Pinprapai, N., Judprasong, K., & Tamura, T. (2005). International inter-laboratory analyses of food folate. *J Food Comp Analysis, 18*, 387-397.

Ray, J., Meier, C., Vermeulen, M., Boss, S., Wyatt, P., & Cole, D. (2002). Association of neural tube defects and folic acid fortification in Canada. *Lancet, 360*, 2047-2048.

Relton, C. L., Wilding, C. S., Laffling, A. J., Jonas, P. A., Burgess, T., Binks, K., et al (2004). Low erythrocyte folate status and polymorphic variation in folate-related genes are associated with risk of neural tube defect pregnancy. *Mol Genet Metab, 81*, 273-281.

Relton, C. L., Wilding, C. S., Pearce, M. S., Laffling, A. J., Jonas, P. A., Lynch, S. A., et al (2004). Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. *J Med Genet, 41*, 256-260.

Russell, D., Parnell, W., Wilson, N. NZ Food: NZ People Key results of the 1997 National Nutrition Survey. (1999) Wellington, New Zealand.

Shaw, G. M., Schaffer, D., Velie, E. M., Morland, K., & Harris, J. A. (1995). Periconceptional vitamin use, dietary folate, and the occurrence of neural tube defects. *Epidemiology, 6*, 219-226.

Smithells, R. W., Sheppard, S., & Schorah, C. J. (1976). Vitamin dificiencies and neural tube defects. *Arch Dis Child, 51*, 944-950.

Thompson, S. J., Torres, M. E., Stevenson, R. E., Dean, J. H., & Best, R. G. (2003). Periconceptional multivitamin folic acid use, dietary folate, total folate and risk of neural tube defects in South Carolina. *Ann Epidemiol, 13*, 412-418.

Venn, B. J., Green, T. J., Moser, R., McKenzie, J. E., Skeaff, C. M., & Mann, J. (2002). Increases in blood folate indices are similar in women of childbearing age supplemented with [6S]-5-methyltetrahydrofolate and folic acid. *J Nutr, 132*, 3353-3355.

Venn, B. J., Green, T. J., Moser, R., & Mann, J. Comparison of the effect of low-dose supplementation of L-5-methyltetrahydrofolate or folic acid on plasma homocysteine: a ranomized placebo controlled study. *Am J Clin Nutr,* 75, 658-662.

Werler, M. M., Shapiro, S., & Mitchell, A. A. (1993). Periconceptional folic acid exposure and risk of occurrent neural tube defects. *JAMA, 269*, 1257-1261.

Wilcken, D.E., Wang, X.L., Sim, A.S., & McCredie, R.M. (1996). Distribution in healthy and coronary populations of the methylenetetrahydrolate reductase (MTHFR) C677T mutation. Arteriosler Thromb Vasc Biol, 16, 878-882.

Wright, J.D., Bialostosky K., Gunter, E.W., Carroll, M.D. Najjar, M.F., Bowman, B.A., & Johnson, C.L. (1998). Blood and folate vitamin B-12: United States, 1988-94. Vital Health Stat 11. 1998.

Yates, J. R., Ferguson-Smith, M. A., Shenkin, A., Guzman-Rodriguez, R., White, M., & Clark, B. J. (1987). Is disordered folate metabolism the basis for the genetic predisposition to neural tube defects? *Clin Genet, 31*, 279-287.