**FORTIFICATION OVERAGES**
**OF THE FOOD SUPPLY**

**FOLATE**

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

Dr Barbara Thomson
Shirley Jones

March 2009

Client Report
FW08030

# FORTIFICATION OVERAGES OF THE FOOD SUPPLY

# FOLATE

Dr Stephen On
Food Safety Programme Leader

Dr Barbara Thomson
Project Leader

Peter Cressey
Peer Reviewer

**DISCLAIMER**

This report or document ("the Report") is given by the Institute of Environmental Science and Research Limited ("ESR") solely for the benefit of the New Zealand Food Safety Authority ("NZFSA"), Public Health Services Providers and other Third Party Beneficiaries as defined in the Contract between ESR and the NZFSA, and is strictly subject to the conditions laid out in that Contract.

Neither ESR nor any of its employees makes any warranty, express or implied, or assumes any legal liability or responsibility for use of the Report or its contents by any other person or organisation.

**ACKNOWLEDGEMENTS**

The assistance of staff at the Christchurch Science Centre, ESR, and in particular Shirley Jones and Lita Lee, with the purchase, moisture analysis and dispatch of samples is gratefully acknowledged.

The vitamin analyses were undertaken by PathWest Laboratory Medicine, Perth, Australia.

The authors acknowledge the New Zealand Ministry of Health as copyright owners and funders of the 1997 National Nutrition Survey and the 2002 Children's Nutrition Survey that were used as a basis for modelling dietary exposures.

**CONTENTS**

## LIST OF TABLES



## LIST OF FIGURES

## ABBREVIATIONS AND DEFINITIONS

| | |
|---|---|
| AI | Adequate intake, used when a Recommended Dietary Intake cannot be determined. Is based on observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate (NHMRC, 2006). |
| CI | Confidence interval. A statistical range with a specified probability (usually 95% or 99%) that the true value (usually the mean) lies within the interval. The boundaries of the confidence interval are the confidence limits (CL)s. |
| CNS | National Children's Nutrition Survey: a 24 hour diet recall survey of 3275 New Zealand school children 5-14 years of age, conducted in 2002. |
| CV | Coefficient of variation, equal to the standard deviation of results divided by the mean of results, expressed as a percentage. |
| EAR | Estimated average requirement. A daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group (NHMRC, 2006). |
| Dietary folate equivalent (DFE) | 1 µg DFE = 1µg food folate = 0.5µg folic acid on an empty stomach = 0.6µg folic acid consumed with meals or as fortified foods |
| Folate | A generic term referring to various forms of this water soluble B-group vitamin, both naturally occurring and synthetic, and it's active derivatives. |
| Folic acid | A synthetic form of folate that is used in food fortification. |
| Free folate | A measure of folate not bound to the food matrix and therefore an approximation of added folic acid. |
| FSC | Australia New Zealand Food Standards Code |
| Label claim | Nutrient value stated in the nutrition information panel on the product label. For the purposes of this report label claim refers to folate. |
| MFD | Manufactured Foods Database |
| NIP | Nutrition information panel |
| NNS | National Nutrition Survey: a 24 hour diet recall survey of 4636 adult New Zealanders 15+ years of age, conducted in 1997. |
| NZFSA | New Zealand Food Safety Authority |
| Overage | The measured amount that exceeds the amount (of a vitamin or nutrient) claimed on a label, in this case, the nutrition information panel of a food item. |

| | |
|---|---|
| RDI | Recommended dietary intake, the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals in a particular life stage and gender group (NHMRC, 2006). |
| UL | Upper level of intake, the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population (NHMRC, 2006). |
| Underage | The measured amount that is below the amount (of a vitamin or nutrient) claimed on a label, in this case, the nutrition information panel of a food item. |
| µg/100g | Micrograms per 100 grams |

## SUMMARY

The aim of the current project was to measure the levels of folate in a range of fortified food types and to compare these levels to those of levels claimed in the nutrition information panel (NIP) on the product labels, to underpin the development of food standards relating to nutrient fortification.

One hundred and fifty three samples from 33 different food products were purchased between November 2007 and February 2008 from Christchurch retail outlets. Products that made a claim for folate were targeted for analysis except for three bread products that were selected to provide information on levels of naturally occurring folate. For most of the products (27/33) five batches were purchased. All samples were analysed for folate and 27 bread samples were also analysed for free folate to provide an approximation of the level of folic acid. For one product only two batches were able to be purchased and these results were not included.

Folate was determined by tri-enzyme extraction and microbiological detection using *Lactobacillus casei* as the test organism. The variability between three and five batches of 32 food products, as measured in terms of coefficient of variation (CV), ranged from 7-61% with 36% of food products giving a CV of greater than 25%. The majority of this variability was attributed to the variability in folate concentrations between product batches.

For each of the foods the mean concentration of folate, with corresponding 95% confidence intervals (CIs), were calculated from the raw data. The label claim fell within this CI in 45% of cases (13/29). In 48% (14/29) the lower 95% confidence limit (CL) was above the claim and in 7% (2/29) the upper CL was below the label claim. In this document the former is referred to as an "overage" and the latter as an "underage". Overages[a] ranged from 18% to 328% of the label claim and underages[a] from -48% to -64%.

The level of naturally occurring folate ranged from 12 to 66µg/100g across the seven breads.

Whilst single, average servings of these products would not be expected to exceed upper levels (ULs) for folic acid, high consumers of the three products with the highest overages, may exceed the UL for folic acid, on some days from the consumption of this food alone. Based on interrogation of the most recent adult and children's national nutrition surveys this represents up to 1% of New Zealand adults (15 years and above) and 2% of children (5-14 years). In addition, people who consume average servings of all three of the products with the highest overages on the same day may exceed the UL for folic acid.

All analytical measurements have associated uncertainty arising from sampling, the analytical method and the manufacturing technique. For standard setting, consideration may be given to defining a range around the label claim that takes these uncertainties into account.

a=The difference between the mean and label claim divided by the label claim, expressed as a percent.

# 1 INTRODUCTION

Work is currently being conducted on the development of food standards relating to nutrient fortification. The establishment of safe upper levels for nutrients added to foods and meaningful label claims (in respect of the nutrient level) relies on robust data of current intake, based on consumption data and concentration information of measured levels, for the foods of interest.

The Australia New Zealand Food Standards Code (FSC) requires that most packaged foods must display a nutrition information panel (NIP) (FSANZ, 2008, Standard 1.2.1). Where a food product makes a vitamin or mineral claim, either a NIP is required, or the information must be available on request. The FSC requires an average quantity of that vitamin or mineral (FSANZ, 2008, Standard 1.3.2) to be stated in the NIP where "average" may be determined in one of three ways, namely, (a) the manufacturer's analysis of the food; or (b) calculation from the actual or average quantity of nutrients in the ingredients used; or (c) calculation from generally accepted data to best represent the quantity of the substance that the food contains, allowing for seasonal variability and other known factors that could cause actual values to vary (FSANZ, 2008, Standard 1.1.1).

The Manufactured Foods Database (MFD) is a compilation of food ingredient and composition data voluntarily provided by New Zealand food manufacturers and compiled by Nutrition Services, Auckland Hospital, under contract to the New Zealand Food Safety Authority (NZFSA). The MFD includes data on fortificants which is supplied by food manufacturers but does not identify how the level was calculated for any particular product

While there are sufficient data on the composition of unfortified foods, there are limited independent data on measured levels of fortificants in fortified foods in New Zealand. International evidence suggests that measured levels of fortificants can vary significantly, by up to 320% of the claimed label value (Whittaker et al, 2001). For New Zealand foods fortified with calcium, folic acid, iron, vitamins A, C and D or zinc and for selenium in infant formulae, overages of up to 530% have been found (Thomson, 2005, 2006, 2007).

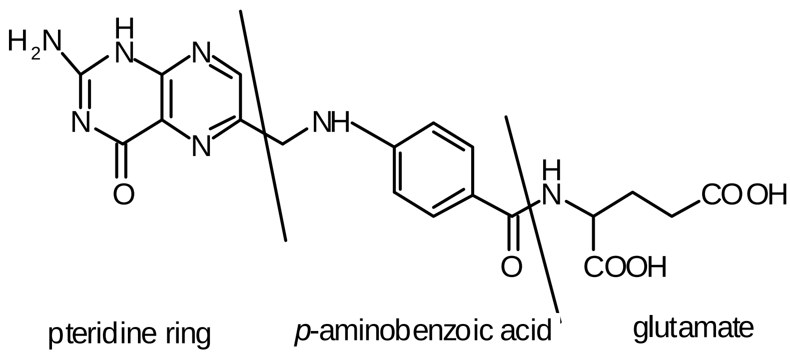


**Figure 1: Chemical structure of folic acid (pteroylglutamic acid, or PGA) showing the component pteridine ring, *p*-aminobenzoic acid and glutamate moieties.**

Folate is the commonly used generic term for both the naturally occurring and synthetic forms of this B-group vitamin comprising an aromatic pteridine ring linked to *p*-aminobenzoic acid and one or more glutamate residues. In foods and in the body, folates are usually in a reduced form, tetrahydrofolate, and conjugated with up to seven glutamate residues. Folic acid (pteroylglutamic acid, or PGA) (Figure 1) is a synthetic form used in supplements and food fortification as it is more stable than natural folates and is better absorbed (SACN, 2006).

Folate acts as a co-enzyme in the metabolism of nucleotides and amino acids and has an important role in methylation and gene expression. Hence folate is essential for cell division and cell maintenance. The need for folate is higher when cell turnover is increased, as in fetal development. Inadequate folate intake is reflected in low erythrocyte, serum or urinary folate levels, plasma homocysteine and blood status measures as well as clinical endpoints such as neural tube defects or chronic degenerative disease (NHMRC, 2006).

The estimated average requirement (EAR) of folate for adults under 50 years is based on metabolic balance studies (erythrocyte and plasma folate and homocysteine levels). For adults 51 years and over, the requirements are based on metabolic, observational and epidemiological studies (NHMRC, 2006). The adequate intake (AI) for 0-6 month infants is derived from the intake from breast milk based on the average concentration of folate in breast milk. The AI for 7-12 month infants is extrapolated up from young infants or down from adults.

There is a potential public health and safety issue associated with insufficient or over-consumption of some nutrients, and interactions between nutrients if levels are too high. For this reason, Recommended Dietary Intakes (RDI)s and Upper Levels of Intake (UL)s have been estimated for New Zealand and Australia, for a range of nutrients including folate (NHMRC, 2006). Details of these nutrient reference values for folate, are provided in Appendix 1.

Consumption of the amounts of folate normally found in foods or fortified foods is not currently associated with adverse effects. However, studies in the 1940s and 1950s of high intake of folic acid from dietary supplements showed adverse neurological effects in people with $B_{12}$ deficiency, a condition most commonly found in the elderly and rarely in the rest of the population. The UL for folic acid intake is based on neurological effects seen with $B_{12}$ deficiency, and because these data have at least some dose-response characteristics (NHMRC, 2006).

The aim of the current project was to measure the levels of folate in foods fortified with folic acid and to compare measured levels with levels claimed in the NIP on the product label. An analysis of levels of nutrient fortification is essential for undertaking a robust risk assessment of the consequences of nutrient additions to foods, both mandatory and voluntary, and will feed directly into the food standard setting process.

An attempt was also made to see if it was possible to distinguish the levels of naturally occurring folate and added folic acid. When a food product makes a nutrition claim for folate the NIP is required to list the level of folate i.e. both naturally occurring folate and added folic acid. It is not possible to distinguish the contributions of naturally occurring folate and added folic acid in any food product from the currently required labelling information.

# 2 MATERIALS AND METHODS

## 2.1 Selection of Foods for Inclusion in the Study

Foods that are fortified with folic acid were identified from the MFD and from supermarket browsing. These were grouped into food types and foods from each group were selected for analysis with consideration being given to both the relative popularity of the food and the inclusion of as wide a range of fortified foods as possible. The following sample plan was agreed in consultation between the NZFSA and ESR (Table 1). The description of most foods is self explanatory with the exception of food drinks, a term used in the MFD for products including manufactured beverages (eg. drinking chocolate, and sports drinks) and liquid meal replacements (eg. liquid breakfasts). Three samples of unfortified bread were selected to ascertain naturally occuring folate levels.

**Table 1: Foods listed as fortified with folic acid in 2003 and 2006 (Nutrition Services, 2003, 2006) and selection of products for folate analysis and comparison with label claims.**

| Food | Number of products 2003[a] | Number of products 2006[a] | Number of products for analysis[b] |
|---|---|---|---|
| Baby Foods | 2 (1) | 4 (2) | 1 (x5) |
| Biscuits | 1 (1) | 4 (1) | 1 (x5) |
| Breads | 7 (3) | 7 (3) | 5 (4x5, 1x2) |
| Unfortified | | | 3 (x5) |
| Breakfast cereals | 53 (10) | 69 (10) | 13 (x5) |
| Extracts of meat/yeast/vegetables | 2 (2) | 3 (3) | 2 (x5) |
| Food Drinks | 19 (4) | 21 (5) | 3 (2x5, 1x3) |
| Fruit Drink & Fruit Nectar Cordial | 1 (1) | 11 (3) | 1 (x3) |
| Fruit Juice | 1 (1) | 3 (2) | 1 (x5) |
| Miscellaneous | 5 (3) | 13 (4) | 2 (x3) |
| Pasta | 4 (1) | 2 (1) | 0 |
| Protein Products | 1 (1) | 2 (1) | 1 (x3) |
| Total | 96 (28) | 139 (35) | 33 ( 155) |

a= number of brands in parenthesis
b= number of batches per food product in parenthesis

## 2.2 Sampling and Sample Preparation

Samples were only analysed if folate was declared in the NIP, with the exception of 15 samples of unfortified bread that were analysed to ascertain background levels of naturally occurring folate.

Foods were purchased between November 2007 and February 2008. Single packets of each selected food item were purchased from Christchurch retail outlets. For the majority of food items (27/33), five batches were purchased, for five foods, three batches were purchased and for "Bread 4", only two batches were purchased before this product was discontinued. No similar product was found as a suitable replacement.

The level of folate claimed in the NIP on the product label was recorded, and used as the basis for comparison with the measured level.

For foods sold in a ready-to-eat form, such as breads, breakfast cereals and snack bars, the entire packet was ground in a domestic blender. The moisture content was determined for a sub-sample of one batch of each product. Approximately 50ml of the remaining powdered material was frozen at -15°C until dispatch to the analytical laboratory.

Sub-samples of powdered food drinks and meal replacements, extracts, drinks and liquid meal replacements were analysed, as received, for moisture. Sub-samples were frozen at -15°C until dispatch to the analytical laboratory by overnight courier.

All samples for folate analysis were dispatched frozen (except the extracts), to Auckland where they were repacked in dry ice for overnight courier to Melbourne and onward dispatch to Perth. If necessary, samples were held in frozen storage in Melbourne. Samples were sent in three consignments, on 11 December 2007, 14 January 2008 and 12 February 2008.

## 2.3 Laboratory Analytical Methods

### 2.3.1 Folate analysis

All samples were analysed for folate. Bread samples only were additionally analysed for free folate where free folate is a measure of folate not bound to the food matrix and therefore an approximation of added folic acid.

Folate (naturally occurring and added folic acid) was determined using the tri-enzyme extraction and microbiological detection using *Lactobacillus casei* as the test organism to achieve the detection limits necessary to quantify folate in the range of foods selected. This methodology is recommended for folate analysis in foods (Koontz et al, 2005) and, for example, is the basis for the AOAC Official Method 2004.05 for the analysis of folates in cereals and cereal foods. Free folate, such as added folic acid, was determined without the enzyme extractions that break down the food matrix.

Folate and free folate analysis was undertaken by Path West Laboratory Medicine, Perth, Australia (Method ref RPH. BI. VITM.0040, Davis et al, 1970, Tamura, 1990), in accordance with their NATA accreditation ISO/IEC 17025, for the microbiological assay of vitamins in food. The Royal Perth Hospital is the only known laboratory in Australasia that is accredited for folate analysis to the detection limits required for this study and was therefore the best available. Duplicates of all samples were tested, with 3 controls: a known food, a yeast and an enzyme. If the CV of the duplicate result exceeded 10%, the sample was retested.

### 2.3.2 Moisture

The moisture content of samples was determined by oven drying at 103 ± 3°C at ambient pressure to a constant weight (Kirk and Sawyer, 1991).

### 2.3.3 Quality control procedures

The following quality assurance procedures were undertaken to ensure robust results:

- The analytical repeatability was determined for folate by undertaking five analyses of each of three samples representing different food matrices (bread, cereal and meal replacements).
- Blind duplicates of nine different fortified food types were submitted for analysis.
- One bread and one breakfast cereal were run as consignment controls with each of the three sample consignments.

## 2.4 Data analysis

Results from the analysis of, particularly, complex matrices such as food samples will always have an associated degree of variability. Variability for the current samples is due to:

1. Intra-sample uncertainty, or repeatability- a measure of the measurement uncertainty in results for multiple analyses of the same sample. This is a measure of variability resulting from the analytical method and sub-sampling procedures.

2. Inter-sample variability – a measure of the variability between different batches of the same product. This includes the uncertainty of the analytical method and the variability of the manufacturing technique. The homogeneity of a product depends on when and how the fortificant is added and may differ for different products. Lack of homogeneity is one source of both intra- and inter-sample variability.

The mean and standard deviation of the reported sample results were calculated. From these the coefficient of variation (CV) and the 95% confidence interval and limits of the mean were also calculated according to standard statistical methods (TELARC, 1987).

Analytical repeatability was assessed on the basis of the CV.

The relationship between the label claim and the result was assessed on the basis of the former lying within the 95% confidence limits of the sample mean.

For those samples where the label claim was outside of the confidence limits, the percent overage or underage was calculated using the following formula.

$$\% \text{ overage/underage} = \frac{\text{mean concentration-label claim}}{\text{label claim}} \times 100$$

## 3 RESULTS

### 3.1 Changes in the Number and Types of Folic acid Fortified Foods

Review of the data provided by manufacturers and collated in the reports on "Fortified foods available in New Zealand", December, 2003 and 2006 (Nutrition Services, 2003, 2006) revealed the number of foods fortified with folic acid increased from 96 in 2003 to 139 in 2006, a 45% increase. These totals include multiple flavours of some foods namely, biscuits, food drinks, fruit drinks and meal replacements so that the diversity of products is exaggerated in the 45% increase. The number of food brands increased by 25% from 28 in 2003 to 35 in 2006 (Table 1).

Changes in the number of folic acid fortified foods were most apparent for breakfast cereals, fruit drinks/fruit nectar cordials and miscellaneous foods (Figure 2). The greatest change in both the number and diversity of products fortified with folic acid was observed for breakfast cereals (up by 30%), with the brands reporting folic acid fortification remaining constant at ten. The change in "miscellaneous" foods is due to the addition of five flavours of a protein dietary supplement powder.

The number of folic acid fortified bread brands and number of bread products has not changed from 2003 and 2006. However this will change due to bread being the food type targeted for mandatory fortification with folic acid from September 2009 (Food Safety Minister Annette King, 22 June 2007).

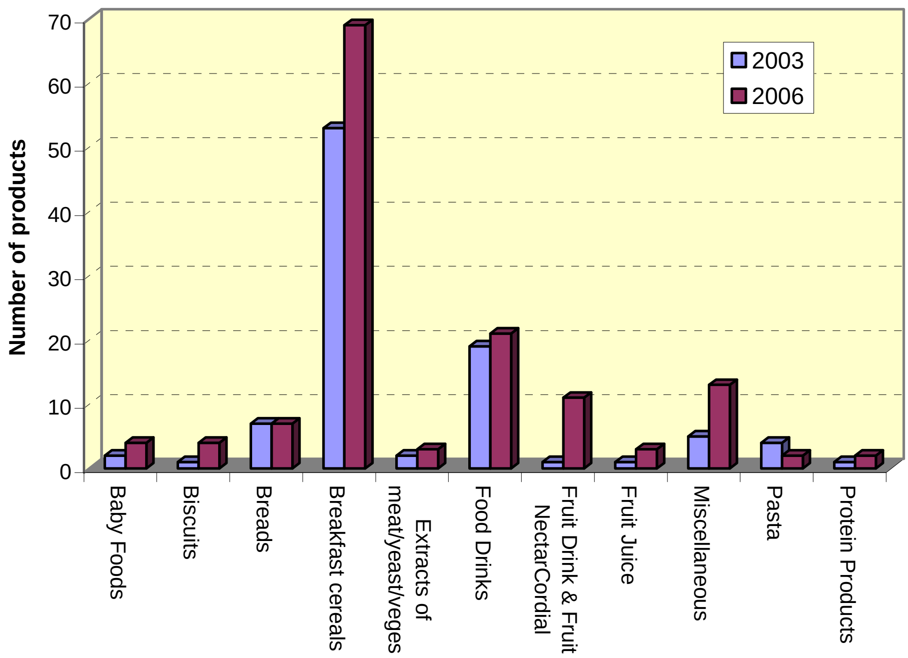


**Figure 2: Numbers of manufactured foods fortified with folic acid in 2003 and 2006 (Nutrition Services 2003, 2006)**

### 3.2 Assessment of Data Quality

The intra-sample variability or repeatability, expressed as %CV, was very good by international standards (Koontz et al., 2005, De Vries et al, 2005), ranging from 2-12% (Appendix 2.1). The repeatability of free folate measurements in bread, expressed as intra-sample %CV was slightly higher but also good at 18% (Appendix 2.1). The intra-sample %CVs for the blind duplicates ranged from 0-40% (Appendix 2.2) and were predictably higher than the repeatability results since there were two blind samples rather than five, resulting in less precise estimates of the mean and thence higher CVs. The variability of the blind duplicates was consistent with the variability observed for a number of unfortified foods analysed by commercial laboratories in the USA (Koontz et al., 2005). The CVs of the two control foods were 4 and 8% for folate in the bread and cereal respectively, showing excellent consistency between consignments. A CV of 25% for free folate in bread was achieved (Appendix 2.3). Together, these quality assurance data give confidence in the analytical results.

### 3.3 Concentration of Folate in Fortified foods

The mean concentration of folate in the selected foods as purchased ranged from a level of 28 µg/100g in an unfortified bread (an approximation of the naturally occurring folate level of bread), to 8560 µg/100g in one extract product (Table 2). A full set of results is included in Appendix 3. The label claims cited in Table 2 are those stated in the NIP on the food product label except breads 1-3 which made no label claims.

Two batches of food sample "bread 4" were found to contain 480 and 410µg/100g folate compared with a label claim of 200µg/100g. However, the CI for these two samples is too wide to be meaningful (0-754) and the assessment against label claim has not been included in Table 2.

**Table 2: Mean concentration of folate (µg/100g or µg/100ml) in fortified foods compared with label claim**

| Food | Label claim | Measured mean | Std Dev. | 95% CI[a] | % overage or underage | > or < CI |
|---|---|---|---|---|---|---|
| Baby food 1 | 145 | 210 | 24 | 182-238 | 45 | * |
| Biscuit | 280 | 256 | 68 | 175-337 | -9 | |
| Bread 1 | nil | 28 | 11 | 15-41 | NA | |
| Bread 2 | nil | 38 | 8.4 | 28-48 | NA | |
| Bread 3 | nil | 40 | 7.1 | 32-48 | NA | |
| Bread 4[b] | 200 | 445 | 49 | NR | NR | |
| Bread 5 | 200 | 236 | 29 | 201-271 | 18 | * |
| Bread 6 | 200 | 288 | 55 | 223-353 | 44 | * |
| Bread 7 | 200 | 104 | 49 | 45-163 | -48 | * |
| Bread 8 | 200 | 72 | 44 | 19-125 | -64 | * |
| Cereal 1 | 200 | 196 | 11 | 182-210 | -2 | |
| Cereal 2 | 167 | 497 | 51 | 435-559 | 198 | * |
| Cereal 3 | 167 | 234 | 26 | 203-265 | 40 | * |
| Cereal 4 | 222 | 440 | 83 | 341-539 | 98 | * |
| Cereal 5 | 333 | 414 | 34 | 374-454 | 24 | * |
| Cereal 6 | 222 | 288 | 77 | 195-381 | 30 | |

| Food | Label claim | Measured mean | Std Dev. | 95% CI[a] | % overage or underage | > or < CI |
|---|---|---|---|---|---|---|
| Cereal 7 | 250 | 360 | 45 | 306-414 | 44 | * |
| Cereal 8 | 333 | 442 | 103 | 318-566 | 33 | |
| Cereal 9 | 333 | 314 | 33 | 275-353 | -6 | |
| Cereal 10 | 333 | 830 | 75 | 740-920 | 149 | * |
| Cereal 11 | 333 | 240 | 78 | 146-334 | -28 | |
| Cereal 12 | 100 | 194 | 23 | 166-222 | 94 | * |
| Cereal 13 | 114 | 170 | 12 | 155-185 | 49 | * |
| Food drink 1 | 91 | 120 | 12 | 105-135 | 32 | * |
| Food drink 2 | 40 | 53 | 10 | 41-65 | 32 | * |
| Food drink 3c | 167 | 320 | 165 | 0-733 | 92 | |
| Fruit drink 1c | 20 | 40 | 17 | 0-83 | 100 | |
| Fruit juice | 40 | 48 | 13 | 32-64 | 20 | |
| Miscellaneous 1c | 38 | 60 | 17 | 17-103 | 58 | |
| Miscellaneous 2c | 125 | 153 | 15 | 115-192 | 23 | |
| Protein productc | 35 | 47 | 5.8 | 32-61 | 33 | |
| Extract 1 | 2000 | 8560 | 2410 | 5670-11460 | 328 | * |
| Extract 2 | 2000 | 2120 | 483 | 1540-2700 | 6 | |

NA= not applicable, NR=no result, CI=confidence interval
* = label claim outside 95% CI
a= mean ± 1.2 standard deviations of the measured concentration
b= 2 batches only, no CI included
c= 3 batches of these products were analysed, compared with 5 batches of other products, 95% confidence interval = mean ± 2.5 standard deviations of the measured concentration

A summary of the concentration of both folate and free folate for seven bread samples, excluding Bread 4, is shown in Table 3, where samples 1 to 3 were unfortified but samples 5 to 8 contained added folic acid. Individual results are provided in Appendix 3.2.

Free folate is a measure of the unbound folate that approximates to the level of added folic acid. Folic acid in the fortified breads (5 to 8) was mostly accounted for in the free folate fraction as expected. But a background level of 12- 26 µg/100g free folate was detected in the unfortified bread that did not claim to contain added folic acid. A correction for this background is necessary to assess added folic acid in fortified foods if based on the free folate measurement.

The difference between folate and free folate could potentially be used as a measure of naturally occurring folate in the food product. The level of naturally occurring folate ranged from 12 to 66µg/100g across the seven breads.

**Table 3: Comparison of folate and free folate in bread samples (µg/100g)**

| | Folate | | | Free folate | | | Folate - Free folate[a] |
|---|---|---|---|---|---|---|---|
| | Mean | Std dev. | %CV | Mean | Std dev. | %CV | |
| Bread 1 | 28 | 11 | 39 | 12 | 4.5 | 37 | 16 |
| Bread 2 | 38 | 8.4 | 22 | 22 | 8.4 | 38 | 16 |
| Bread 3 | 40 | 7.1 | 18 | 26 | 5.5 | 21 | 14 |
| Bread 5 | 236 | 29 | 12 | 175 | 50 | 28 | 61 |
| Bread 6 | 288 | 55 | 19 | 222 | 26 | 12 | 66 |
| Bread 7 | 104 | 49 | 47 | 68 | 47 | 69 | 36 |
| Bread 8 | 72 | 44 | 62 | 60 | 45 | 75 | 12 |

Bread 4 was not included as only 2 batches were available
a = Folate - free folate = approximation of naturally occurring folate
%CV = inter-sample variability (n=5), standard deviation/mean x 100

### 3.4 Batch Variability

A comparison of the results for different batches of the same product (Appendix 3) showed variability, measured as %CV, ranged from 6-62% for folate and 12-75% for free folate. This variability included both measurement (analytical) uncertainty and batch variability. The repeatability results where one sample was measured a number of times, and the food control samples that were analysed in each of the three consignments, showed measurement uncertainty contributed in the order of 11, 8 and 12 %CV for bread, cereal and a liquid meal replacement respectively (Appendix 2). These results suggest that the majority of the variability observed is due to variability in folate concentrations between different batches of product.

Variability in folate concentrations was greatest in two of the bread samples and for two of the food drink samples (Figure 3). The highest variability was observed for one of the food drink products. Differences in matrix effects for different foods is recognised in the international literature with optimal analytical conditions varying for particular food matrices (Koontz et al, 2005).

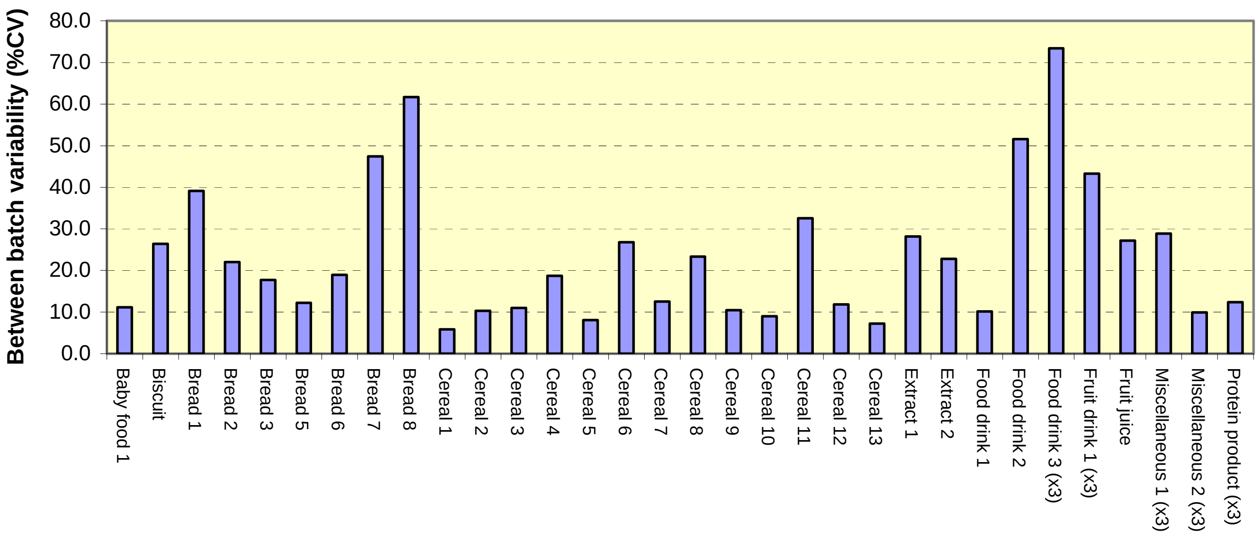


**Figure 3: Variability in folate between batches (5 unless otherwise noted), for different foods expressed as %CV.** Bread 4 not included as only 2 batches of this product were available.

## 3.5 Comparison with Label Claim

The mean concentrations of measured folate compared with label claims, are shown graphically in Figure 4. Error bars for ±1.2 standard deviations, where 5 batches per product were analysed (n=27) or ±2.5 standard deviations, where 3 batches per product were analysed (n=3) represent the measurement uncertainty and variability across multiple batches of the same product.

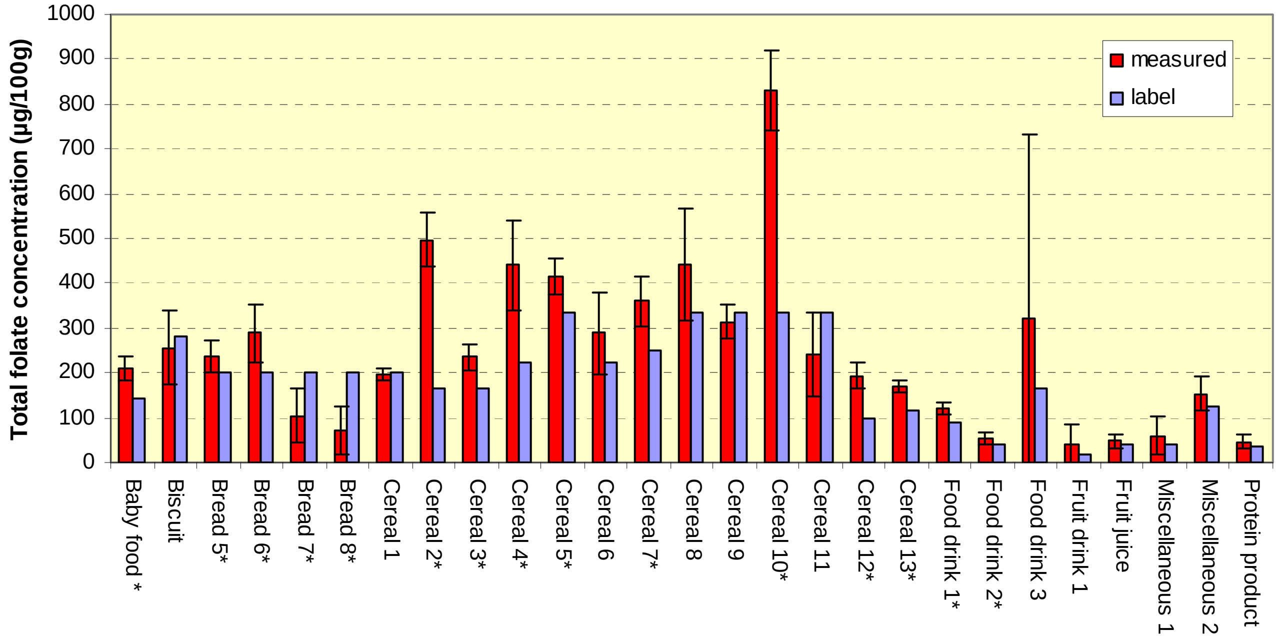


**Figure 4 Measured concentrations of folate for 27 food types, compared with label claim.** Errors bars are ±1.2 x standard deviation, except for Food drink 3, Fruit drink 1, Miscellaneous 1, Miscellaneous 2 and Protein product where errors bars are ±2.5 standard deviations. * denotes under or over label claim. Bread 4 not included as only 2 batches of this product were available. Breads 1-3 were not included as these breads made no label claim for folate.

The two yeast extracts are shown separately (Figure 5) since the concentrations of folate in these foods are almost an order of magnitude higher than the other foods and for this reason they dominate and mask all other foods when graphed together.

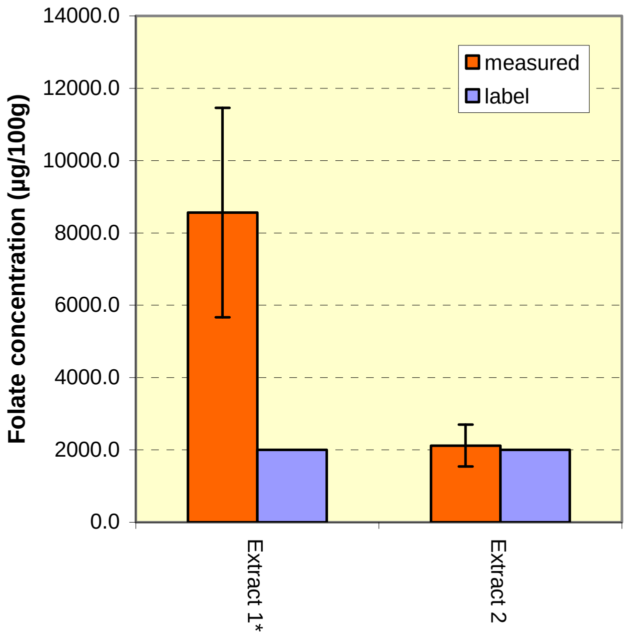


**Figure 5 Measured concentrations of folate for extract foods compared with label claim.** Errors bars are ±1.2 x standard deviation. * denotes over label claim.

The label claim fell within the 95% confidence interval in 45% (13/29) of products.

For 48% (14/29) of products the lower 95% confidence limit was above the claim with overages ranging from 18-328%. Three products, two cereals and one extract, contained in excess of 100% more folate than claimed on the label. The highest overage of 328% was for one extract product.

For 7% (2/29) of products the upper 95% confidence limit was below the label claim with underages of -48 and -64%. These were two bread products with approximately half the level of folate measured compared with that claimed.

### 3.6 Dietary modelling of exposure to folate

The RDI is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all healthy individuals in a particular life stage and gender group (NHMRC 2006). The UL is the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. For folic acid the UL varies for different population groups, ranging from 300µg/day for children 1-3 years to 1000µg/day for adults. The UL is expressed in terms of folic acid from food fortification and supplements (Appendix 1) and is based on adverse neurological observations seen with B12 deficiency following high intakes of folic acid supplements (NHMRC, 2006).

Three of the products analysed had folate concentrations in excess of 100% more than the label claim (Table 2). Dietary intakes of these three products were modelled with consumption information from the 1997 National Nutrition Survey that included adults 15 years and over (NNS; Russell et al., 1999) and the 2002 National Children's Nutrition Survey that included children 5-14 years (CNS; MoH, 2003). Respondents were grouped into age/gender groups that most closely matched those for which ULs have been estimated (Appendix 1).

The product with the highest percent folate overage, and the highest measured concentration of folate, was a yeast extract (extract 1) with a mean folate concentration of 8560µg/100g, more than four times the label claim (2000µg/100g). Analysis of the NNS and CNS showed that about 10% of adults and 6% of children eat a yeast extract at least once a day with average serving sizes of 3-6g for this type of product. Using these average serving sizes, the intakes of folic acid, from a single serving of extract 1, are estimated at 55% of the UL for men 19-70+ years, 41% for women 19-70+ years, 43% for children 14 years (from the CNS) and 9-13 years, and 86% of the UL for children 5-8 years.

Reported maximum serving sizes of a yeast extract ranged from 8-36g per day for different age/gender groups. According to the various data available, these high end consumers would have much higher folic acid intakes than those based on average serving sizes and could be at risk of exceeding the UL on days they consume extract 1. The data does not indicate whether this high level of intake reflects habitual consumption practices. However, assuming these high end consumers reflect the consumption practices of the NZ population, approximately 1% of people in each of the population groups above might be expected to exceed the UL for folic acid from the consumption of extract 1 alone, without allowing for other sources of folate.

Two cereal products showed folate overages greater than 100%, with mean folate concentrations of 497µg/100g for Cereal 2 and 830µg/100g for Cereal 10. The NNS and CNS provide limited consumption data for Cereal 2 or an equivalent product. Average serving sizes for those who did consume this, or an equivalent product, ranged from 38-47g, contributing from 20% to 58% of the UL for folic acid. Results from dietary modelling indicate no high end consumers (those who consume in excess of an average serving size) of this product would have exceeded the UL for folic acid from Cereal 2 alone.

The average serving size for Cereal 10, or an equivalent product, ranged from 28-40g, contributing from 24% to 77% of the UL for folic acid. However of the group of high end consumers none of the respondents 9 years and over would have exceeded the UL for folic acid, but approximately 2% of children 5-8 years old would have, from the consumption of Cereal 10 only.

Of the limited number of children who consumed an extract 1 product and a Cereal 10 type of product none would have exceeded the UL for either food, but when consumed in combination, 75% of the respondents would potentially have exceeded the UL for folic acid, illustrating the impact of consuming more than one fortified food on a daily basis.

# 4 DISCUSSION AND CONCLUSIONS

The number of food products fortified with folate increased from 96 in 2003 to 139 in 2006, a 45% increase. This apparent increase was due to multiple flavours of some products rather than an increase in the diversity of products available. The number of product brands increased by 25% from 28 in 2003 to 35 in 2006. These data reflect information that is voluntarily provided by food manufacturers to the MFD and may well not capture all folic acid fortified foods currently available for purchase (Nutrition Services, 2006).

The tri-enzyme extraction used in the current study is the recommended method for the analysis of folate in food as it gives the most complete extraction of naturally occuring folates from food matrices (DeVries et al, 2005). Both microbial (Koontz et al, 2005, Whittaker et al, 2001) and chemical analysis of the extracted material is used to quantify the folate, with chemical analysis being more prevalent (Gujska and Majewska 2005, Jastrebova et al, 2003, Őhrvik and Witthőft 2008, Osseyi et al, 2001, Pfeiffer et al, 1997, Phillips et al, 2005). Chemical analysis using high pressure liquid chromatography has the advantage of distinguishing individual naturally occuring folates and folic acid whereas quantitation from the growth of *Lactobacillus casei* gives a measure of folate only. The microbiological quantitation was the only accredited methodology available for the current study.

The use of a free folate concentration value is of limited usefulness as a way of determining naturally occuring levels of folate in bread for two reasons. Firstly, measureable levels of free folate were found in the unfortified product. If free folate is equivalent to folic acid, this value would be expected to be closer to zero. Secondly, where bread was fortified, the analytical variability, expressed as standard deviation, was greater than the naturally occurring level of folate, hence the background could not be determined with any degree of confidence (Table 3). From this study, it would be just as useful to measure folate in a wider range of unfortified breads than to measure folate and free folate in fortified breads and calculate the naturally occurring level from these measurements.

No analytical measurement is absolute. All analytical measurements have associated uncertainty arising from sampling, the analytical method and the manufacturing process. From this study, it is seen that variability, measured as %CV, ranged from 6-62% for total folate and 12-75% for free folate with the majority of the variability being a reflection of the variability of folate concentration between different batches of the same food.

Two thirds of foods containing folate had CV values of less than 25%. The low %CVs for the measurement uncertainties in the current study give confidence that the variability is mostly a reflection of batch variability. However there were still a third of samples that were more variable, particularly some breads and drinks. Variability of up to 54 %CV has been reported elsewhere and found to be higher for unfortified foods suggesting an issue with the effectiveness of extraction of the naturally occurring folates (Koontz et al, 2005, DeVries et al, 2005). This variability has been attributed to a number of factors including pH and incubation time of the extraction system, source and activity of the enzymes used, buffer concentrations, and matrix effects such that different foods may require different optimizations. No other studies of batch variability for folate fortified products have been found for comparison.

There are two important factors to consider if using a CI as the basis for assessing label compliance of fortificants. First is the number of samples analysed, since the width of the CI varies with the number of samples. For five samples the 95% CI is the mean ±1.2 standard deviations, whereas if three samples are measured, the 95% CI is more than twice as wide at mean ±2.5 standard deviations. This means that samples are more likely to comply if fewer samples are tested, as evidenced in Table 2 where each of the five food products for which three batches were analysed, had label claims within the 95% CI. Secondly, if product compliance is based on the CI, that is proportional to the standard deviation, a highly variable product (high standard deviation) will have a wider CI and therefore will more easily comply than a consistent product with a small standard deviation and tighter CI. Thus more variability favours the manufacturer, and this may not be desirable nor equitable.

The label claim was less than the lower 95% confidence limit in a total of 48% (14/29) of the foods sampled with overages ranging from 18-328%. A degree of overage is expected to allow for potential loss during the manufacturing process and on storage. This is to ensure the product contains at least as much as claimed on the label by the end of its shelf life. This raises a question - is it appropriate to define an acceptable tolerance between a label claim and a measured level during the shelf life of a fortified product? The FSC does not prescribe any tolerances around the average quantity used in making a label claim.

Internationally, the Danish regulators allow a tolerance of 80-150% for added vitamins and minerals (EC, 2006). The asymmetrical tolerance, with more overage than underage permitted, is to allow for any loss of nutrient over time. If these criteria are applied to the results of the current study, 2/29 foods would be below and 5/29 foods above the label claim. The UK takes a different approach, allowing a tolerance of +100% or -50% for water soluble vitamins and minerals (i.e. the B vitamin group and minerals) and ±30% for fat soluble vitamins (i.e. Vitamins A,D, and E) (EC, 2006). Applying these UK tolerances, one food would be under and three foods above at a 95% confidence interval. The Canadian authorities permit a tolerance of 50% over a label claim for added vitamins or minerals where a minimum limit applies (CFIA, 2003) or 50% under a claim where a maximum limit applies. The food must contain not less than the label claim. Applying these criteria to the current study based on label claim because there are no maximum or minimum limits in New Zealand would mean two foods were under and five foods over the label claims.

Manufacturers routinely add vitamins and minerals in excess of the label claim to allow for any losses during the manufacturing process, seasonal variation or storage to ensure that the label claim is met at the end of shelf life. Cooking, canning, drying/dehydrating, exposure to oxygen, light, heat and pressure can reduce the levels of folic acid in foods (Gujska and Majewska 2005, Hawkes and Villota, 1989, Indrawati et al, 2004). For example, losses of 12-21% of folic acid from flour to bread have been reported (Gutska and Majewska 2005, Osseyi et al 2001). Most of the literature reports on the effect of a process on the stability of folate and relatively little data was found on the degradation of either naturally occuring folate or added folic acid during storage. One study of frozen and fresh fruits and vegetables found no change in a naturally occurring folate, (5-methyltetrahydofolate), in any samples after 12 months storage at -60°C in the dark (Phillips et al, 2005). Negligible loss (0-4%) of folic acid was measured in orange juice fortified at 40 and 60µg/100g and stored, sheltered from light at below 8°C until the end of its shelf life (35 days) (Őhrvik and Witthőft, 2008). No consistent degradation of folate was observed in 15 fortified foods including baby food, cereals, drinks, a meat extract and a protein product, over a six month period using the same methodology as the current study, (Thomson, 2005). Therefore, at the present time, there is no evidence of significant losses of folate on storage.

Whilst single, average servings of these products would not be expected to exceed ULs for folic acid, analysis of the data available suggests that high consumers of the three products with the highest overages, may exceed the UL for folic acid, on some days from the consumption of this food alone. Based on interrogation of the most recent adult and children's national nutrition surveys (Russell et al, 1999, MoH, 2003) this represents up to 1 percent of adults and 2% of children. In addition, people who consume average servings of the high folate containing products in combination may exceed the UL for folic acid.

It must be recognized that the accuracy of the dietary modelling is only as good as the underpinning data. Just as analytical results have associated variability, so too the consumption information has limitations.

None of these assessments include any contribution to folate exposure from the consumption of other fortified products, naturally occurring folate in other foods or folic acid supplements. These consumers, will of course, have higher intakes of folate.

# 5 REFERENCES

CFIA (Canadian Food Inspection Agency). (2003) Nutrition labelling compliance test. Available at http://www.inspection.gc.ca/english/fssa/labeti/nutricon/nutricone.shtml

Davis RE, Nicol DJ and Kelly A. (1970) An automated method for the measurement of folate activity. Journal of Clinical Pathology, 23(1):47-53.

De Vries JW, Rader JI, Keagy PM, Hudson CA. (2005) Microbiological assay-trienzyme procedure for total folates in cereals and cereal foods;collaborative study. Journal of AOAC International, 88(1): 5-15.

EC (European Commission) (2006) Directive 90/496/EEC on Nutrition labelling for foodstuffs: discussion paper on revision of technical issues. Available at http://ec.europa.eu/food/food/labellingnutrition/nutritionlabel/discussion_paper_rev_tech_issues.pdf Accessed 28/07/08.

FSANZ (Food Standards Australia and New Zealand). (2008) Australia and New Zealand Food Standards Code 2002. Available at www.foodstandards.gov.au

Gujska E and Majewska K. (2005) Effect of baking process on added folic acid and endogenous folates stability. Plant Foods for Human Nutrition, 60:37-42.

Hawkes JG and Villota R. (1989) Folates in foods; reactivity, stability during processing, and nutritional implications. Critical Reviews in Food Science and Nutrition, 28(6):439-538.

Indrawati, Arroqui C, Messagie I, Nguyen MT, Van Loey A, Hendrickx M. (2004) Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. Journal of Agricultural and Food Chemistry, 52: 485-492.

Jastrebova J, Witthöft C, Grahn A, Svensson U, Jägerstad M. (2003) HPLC determination of folates in raw and processed beetroots. Food Chemistry, 80:579-588.

Kirk RS, Sawyer R. (1991) Pearson's composition and analysis of foods. 9th Edition. UK: Longman Scientific & Technical, pp498.

Koontz JL, Phillips KM, Wunderlich KM, Exler J, Holden JM, Gebhardt SE, Haytowitz DB. (2005) Comparison of total folate concentrations in foods determined by microbiological assay at several experienced US commercial laboratories. Journal of AOAC International, 88(3): 805-813.

MoH (Ministry of Health). (2003) NZ Food NZ Children: Key results of the 2002 National Children's Nutrition Survey. Wellington: Ministry of Health.

NHMRC (National Health and Medical Research Council). (2006). Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes. Canberra and Wellington: National Health and Medical Research Council and Ministry of Health. Available at www.moh.govt.nz

Nutrition Services. (2003) Fortified foods available in New Zealand. December 2003. Wellington: Ministry of Health.

Nutrition Services. (2006) Fortified foods available in New Zealand. December 2006. Wellington: Ministry of Health.

Őhrvik V and Witthöft C. (2008) Orange juice is a good folate source in respect to folate content and stability during storage and simulated digestion. European Journal of Nutrition, 47:92-98.

Osseyi ES, Wehling RL, Albrecht JA. (2001) HPLC determination of stability and distribution of added folic acid and some endogenous folates during breadmaking. Cereal Chemistry, 78(4):375-378.

Pfeiffer CM, Rogers LM, Gregory III JF. (1997) Determination of folate in cereal-grain food products using trienzyme extraction and combined affinity and reversed-phase liquid chromatography. Journal of Agricultural and Food Chemistry, 45:407-413.

Phillips KM, Wunderlich KM, Holden JM et al., (2005) Stability of 5-methyltetrahydrofolate in frozen fresh fruits and vegetables. Food Chemistry, 92:587-595.

Russell DG, Parnell WR, Wilson NC. (1999) NZ Food:NZ people. Key results of the 1997 National Nutrition Survey. Wellington: Ministry of Health.

SACN (Scientific Advisory Committee on Nutrition) (2006) Folate and disease prevention. United Kingdom: The Stationery Office.

Tamura T. (1990) Microbiological assay of folates. Folic Acid metabolism in Health and Disease. Contemporary issues in clinical nutrition. Eds. Picciano MF,Stokstad ELR and Gregory III JF. New York: Wiley- Liss, Inc.

TELARC (Testing Laboratory Registration Council of New Zealand) (1987). Precision and limits of detection for analytical methods. Auckland, New Zealand: Testing Laboratory Registration Council of New Zealand.

Thomson BM. (2005) Fortification overages of the food supply. Folate and iron. ESR Client Report FW 0536. Wellington: NZFSA

Thomson BM. (2006) Fortification overages of the food supply. Vitamin A, Vitamin D and calcium. ESR Client Report FW 0637. Wellington: NZFSA.

Thomson BM. (2007) Fortification overages of the food supply. Vitamin C, zinc and selenium. ESR Client Report FW 0745. Wellington: NZFSA.

Whittaker P, Tufaro PR, Rader JI. (2001) Iron and folate on fortified cereals. Journal of the American College of Nutrition; 20 (3):247-254.

## APPENDIX 1: NEW ZEALAND AND AUSTRALIAN EARS, RDIS AND ULS FOR DIETARY FOLATE EQUIVALENTS (NHMRC, 2006)

| **Age/gender group** | | **Dietary Folate equivalents (µg/day)** | | **Folic acid (µg/day)** |
|---|---|---|---|---|
| | | EAR | RDI | UL* |
| Infants | 0-6 mo. | | AI =65 | BM |
| | 7-12 mo. | | AI = 80 | B/F |
| Children | 1-3 yrs | 120 | 150 | 300 |
| | 4-8 yrs | 160 | 200 | 400 |
| Boys | 9-13 yrs | 250 | 300 | 600 |
| | 14-18 yrs | 330 | 400 | 800 |
| Girls | 9-13 yrs | 250 | 300 | 600 |
| | 14-18 yrs | 330 | 400 | 800 |
| Men | 19-30 yrs | 320 | 400 | 1000 |
| | 31-50 yrs | 320 | 400 | 1000 |
| | 51-70 yrs | 320 | 400 | 1000 |
| | >70 yrs | 320 | 400 | 1000 |
| Women | 19-30 yrs | 320 | 400 | 1000 |
| | 31-50 yrs | 320 | 400 | 1000 |
| | 51-70 yrs | 320 | 400 | 1000 |
| | >70 yrs | 320 | 400 | 1000 |
| Pregnant | 14-18 yrs | 520 | 600 | 800 |
| | 19-30 yrs | 520 | 600 | 1000 |
| | 31-50 yrs | 520 | 600 | 1000 |
| Lactating | 14-18 yrs | 450 | 500 | 800 |
| | 19-30 yrs | 450 | 500 | 1000 |
| | 31-50 yrs | 450 | 500 | 1000 |

AI = Adequate intake,
BM = amount normally received from breast milk of healthy women;
B/F = amount in breast milk and food,
EAR = Estimated average requirement,
Dietary folate equivalent (DFE) 1 µg DFE = 1µg food folate = 0.5µg folic acid on an empty stomach = 0.6µg folic acid consumed with meals or as fortified foods
RDI = Recommended Daily Intake,
UL = Upper level of intake

## APPENDIX 2: QUALITY ASSURANCE DATA

### 2.1 Repeatability for folate (μg/100g)

| Food type | Label claim | Analysis 1 | 2 | 3 | 4 | 5 | Mean | Std dev. | %CV |
|---|---|---|---|---|---|---|---|---|---|
| Bread - folate | 200 | 310 | 280 | 240 | 240 | 290 | 272 | 31.1 | 11 |
| Bread –free folate | NA | 250 | 270 | 260 | 260 | 370 | 282 | 49.7 | 18 |
| Breakfast cereal | 167 | 230 | 220 | 220 | 220 | 220 | 222 | 4.5 | 2 |
| Liquid meal replacement | 40 | 40 | 50 | 40 | 50 | 40 | 44 | 5.5 | 12 |

NA = not applicable, only folate claimed (200 μg/100g)
%CV = standard deviation/mean x 100

### 2.2 Blind duplicates for folate (μg/100ml or 100g).

| Food sample | Result 1 | Result 2 | Result 3 | Mean | Std dev. | %CV |
|---|---|---|---|---|---|---|
| Baby food | 190 | 310 | 240 | 247 | 60 | 24 |
| Biscuit | 270 | 270 | NR | 270 | 0 | 0 |
| Bread 2 | 30 | 70 | NR | 50 | 28 | 57 |
| Bread 5 | 210 | 310 | NR | 260 | 71 | 27 |
| Cereal 2 | 520 | 450 | NR | 485 | 49 | 10 |
| Cereal 5 | 410 | 420 | NR | 415 | 7 | 2 |
| Cereal 9 | 270 | 200 | NR | 235 | 49 | 21 |
| Extract 2 | 2390 | 2830 | NR | 2610 | 311 | 12 |
| Fruit juice | 50 | 90 | NR | 70 | 28 | 40 |
| Protein product | 50 | 50 | NR | 50 | 0 | 0 |

NR = no result
An additional analysis of the baby food was undertaken

### 2.3 Consignment control samples (μg/100ml or 100g).

| Food sample | Consignment 1 | Consignment 2 | Consignment 3 | Mean | Std dev. | %CV |
|---|---|---|---|---|---|---|
| Bread 5 - folate | 272 | 260 | 250 | 261 | 11.0 | 4 |
| Bread 5 – free folate | 282 | 170 | 250 | 234 | 57.7 | 25 |
| Cereal 3 | 222 | 190 | 200 | 204 | 16.4 | 8 |

## APPENDIX 3: FOLATE CONCENTRATION (µG/100G OR 100ML) IN INDIVIDUAL SAMPLES

### 3.1 Folate

| Food | % moisture | Label Claim | Measured Mean | Std dev. | %CV | 95% confidence interval[a] | % overage or underage |
|---|---|---|---|---|---|---|---|
| Baby food | | | 190 | | | | |
| | | | 200 | | | | |
| | | | 240 | | | | |
| | | | 190 | | | | |
| | | | 230 | | | | |
| | **3.7** | **145** | **210** | **23.5** | **11.2** | **182-238** | **45** |
| Biscuit | | | 250 | | | | |
| | | | 270 | | | | |
| | | | 350 | | | | |
| | | | 250 | | | | |
| | | | 160 | | | | |
| | **1.7** | **280** | **256** | **67.7** | **26.4** | **175-337** | **-9** |
| Bread 1 | | | 30 | | | | |
| | | | 30 | | | | |
| | | | 30 | | | | |
| | | | 10 | | | | |
| | | | 40 | | | | |
| | **40.4** | **nil** | **28** | **10.9** | **39.1** | **15-41** | **NA** |
| Bread 2 | | | 40 | | | | |
| | | | 30 | | | | |
| | | | 50 | | | | |
| | | | 40 | | | | |
| | | | 30 | | | | |
| | **38.9** | **nil** | **38** | **8.4** | **22.0** | **28-48** | **NA** |
| Bread 3 | | | 40 | | | | |
| | | | 40 | | | | |
| | | | 30 | | | | |
| | | | 50 | | | | |
| | | | 40 | | | | |
| | **38.7** | **nil** | **40** | **7.1** | **17.7** | **32-48** | **NA** |
| Bread 4 | | | 480 | | | | |
| | | | 410 | | | | |
| | | | no sample | | | | |
| | | | no sample | | | | |
| | | | no sample | | | | |
| | **37.7** | **200** | **445** | **49.5** | **11.1** | **NR** | **NR** |
| Bread 5 | | | 260 | | | | |
| | | | 200 | | | | |
| | | | 210 | | | | |
| | | | 260 | | | | |
| | | | 250 | | | | |
| | **39.6** | **200** | **236** | **28.8** | **12.2** | **201-271** | **18** |
| Bread 6 | | | 230 | | | | |
| | | | 290 | | | | |
| | | | 240 | | | | |
| | | | 320 | | | | |
| | | | 360 | | | | |
| | **38.1** | **200** | **288** | **54.5** | **18.9** | **223-353** | **44** |
| Bread 7 | | | 140 | | | | |

| Food | % moisture | Label Claim | Measured Mean | Std dev. | %CV | 95% confidence interval[a] | % overage or underage |
|---|---|---|---|---|---|---|---|
| | | | 50 | | | | |
| | | | 140 | | | | |
| | | | 140 | | | | |
| | | | 50 | | | | |
| | **38.2** | **200** | **104** | **49.3** | **47.4** | **45-163** | **-48** |
| Bread 8 | | | 40 | | | | |
| | | | 150 | | | | |
| | | | 60 | | | | |
| | | | 50 | | | | |
| | | | 60 | | | | |
| | **37.0** | **200** | **72** | **44.4** | **61.6** | **19-125** | **-64** |
| Cereal 1 | | | 200 | | | | |
| | | | 200 | | | | |
| | | | 180 | | | | |
| | | | 210 | | | | |
| | | | 190 | | | | |
| | **6.9** | **200** | **196** | **11.4** | **5.8** | **182-210** | **-2** |
| Cereal 2 | | | 480 | | | | |
| | | | 580 | | | | |
| | | | 500 | | | | |
| | | | 440 | | | | |
| | | | 485 | | | | |
| | **3.2** | **167** | **497** | **51.4** | **10.3** | **435-559** | **198** |
| Cereal 3 | | | 222 | | | | |
| | | | 230 | | | | |
| | | | 260 | | | | |
| | | | 200 | | | | |
| | | | 260 | | | | |
| | **2.4** | **167** | **234.4** | **25.8** | **11.0** | **203-265** | **40** |
| Cereal 4 | | | 540 | | | | |
| | | | 340 | | | | |
| | | | 440 | | | | |
| | | | 500 | | | | |
| | | | 380 | | | | |
| | **6.9** | **222** | **440** | **82.5** | **18.7** | **341-539** | **98** |
| Cereal 5 | | | 410 | | | | |
| | | | 400 | | | | |
| | | | 380 | | | | |
| | | | 470 | | | | |
| | | | 410 | | | | |
| | **2.6** | **333** | **414** | **33.6** | **8.1** | **374-454** | **24** |
| Cereal 6 | | | 210 | | | | |
| | | | 200 | | | | |
| | | | 350 | | | | |
| | | | 320 | | | | |
| | | | 360 | | | | |
| | **5.6** | **222** | **288.0** | **77.3** | **26.8** | **195-381** | **30** |
| Cereal 7 | | | 350 | | | | |
| | | | 410 | | | | |
| | | | 400 | | | | |
| | | | 300 | | | | |
| | | | 340 | | | | |
| | **9.2** | **250** | **360** | **45.3** | **12.6** | **306-414** | **44** |
| Cereal 8 | | | 370 | | | | |
| | | | 400 | | | | |

| Food | % moisture | Label Claim | Measured Mean | Std dev. | %CV | 95% confidence interval[a] | % overage or underage |
|---|---|---|---|---|---|---|---|
| | | | 380 | | | | |
| | | | 440 | | | | |
| | | | 620 | | | | |
| | **4.7** | **333** | **442** | **103** | **23.3** | **318-566** | **33** |
| Cereal 9 | | | 270 | | | | |
| | | | 320 | | | | |
| | | | 320 | | | | |
| | | | 300 | | | | |
| | | | 360 | | | | |
| | **4.4** | **333** | **314.0** | **32.9** | **10.5** | **275-353** | **-6** |
| Cereal 10 | | | 920 | | | | |
| | | | 880 | | | | |
| | | | 740 | | | | |
| | | | 770 | | | | |
| | | | 840 | | | | |
| | **5.9** | **333** | **830.0** | **74.8** | **9.0** | **740-920** | **149** |
| Cereal 11 | | | 210 | | | | |
| | | | 370 | | | | |
| | | | 170 | | | | |
| | | | 250 | | | | |
| | | | 200 | | | | |
| | **5.5** | **333** | **240.0** | **78.1** | **32.5** | **146-334** | **-28** |
| Cereal 12 | | | 160 | | | | |
| | | | 210 | | | | |
| | | | 210 | | | | |
| | | | 210 | | | | |
| | | | 180 | | | | |
| | **6.7** | **100** | **194** | **23.0** | **11.9** | **166-222** | **94** |
| Cereal 13 | | | 170 | | | | |
| | | | 190 | | | | |
| | | | 160 | | | | |
| | | | 170 | | | | |
| | | | 160 | | | | |
| | **3.8** | **114** | **170** | **12.2** | **7.2** | **155-185** | **49** |
| Extract 1 | | | 6570 | | | | |
| | | | 6880 | | | | |
| | | | 10200 | | | | |
| | | | 7170 | | | | |
| | | | 12000 | | | | |
| | **39.7** | **2000** | **8564** | **2410** | **28.1** | **5672-11456** | **328** |
| Extract 2 | | | 2180 | | | | |
| | | | 2390 | | | | |
| | | | 1270 | | | | |
| | | | 2380 | | | | |
| | | | 2380 | | | | |
| | **34.5** | **2000** | **2120** | **483** | **22.8** | **1540-2700** | **6** |
| Food drink 1 | | | 120 | | | | |
| | | | 140 | | | | |
| | | | 120 | | | | |
| | | | 110 | | | | |
| | | | 110 | | | | |
| | **2.8** | **90.9** | **120** | **12.2** | **10.2** | **105-135** | **32** |
| Food drink 2 | | | 44 | | | | |
| | | | 50 | | | | |
| | | | 70 | | | | |

| Food | % moisture | Label Claim | Measured Mean | Std dev. | %CV | 95% confidence interval[a] | % overage or underage |
|---|---|---|---|---|---|---|---|
| | | | 50 | | | | |
| | | | 50 | | | | |
| | **82.1** | **40** | **52.8** | **10.0** | **18.9** | **41-65** | **32** |
| Food drink 3 | | | 400 | | | | |
| | | | 430 | | | | |
| | | | 130 | | | | |
| | **3.0** | **167** | **320** | **165.2** | **51.6** | **0-733** | **92** |
| Fruit drink 1 | | | 20 | | | | |
| | | | 50 | | | | |
| | | | 50 | | | | |
| | **97.6** | **20** | **40** | **17.3** | **43.3** | **0-83** | **100** |
| Fruit juice | | | 40 | | | | |
| | | | 30 | | | | |
| | | | 50 | | | | |
| | | | 60 | | | | |
| | | | 60 | | | | |
| | **89.7** | **40** | **48** | **13.0** | **27.2** | **32-64** | **20** |
| Miscellaneous 1 | | | 50 | | | | |
| | | | 50 | | | | |
| | | | 80 | | | | |
| | **13.1** | **38** | **60** | **17.3** | **28.9** | **17-103** | **58** |
| Miscellaneous 2 | | | 150 | | | | |
| | | | 170 | | | | |
| | | | 140 | | | | |
| | **6.2** | **125** | **153** | **15.3** | **10.0** | **115-192** | **23** |
| Protein product | | | 40 | | | | |
| | | | 50 | | | | |
| | | | 50 | | | | |
| | **88.1** | **35** | **47** | **5.8** | **12.4** | **32-61** | **33** |

%CV= standard deviation/mean x 100
NA = not applicable, NR = no result
a= mean ± 1.2 standard deviations of the measured concentration

## 3.2 Folate and free folate in bread samples (µg/100g)

| Food | Measured Folate | Measured Free folate | Std dev. Free folate | %CV Free folate |
|---|---|---|---|---|
| Bread 1 | 30 | 20 | | |
| | 30 | 10 | | |
| | 30 | 10 | | |
| | 10 | 10 | | |
| | 40 | 10 | | |
| | **28** | **12** | **4.5** | **37** |
| Bread 2 | 40 | 20 | | |
| | 30 | 30 | | |
| | 50 | 20 | | |
| | 40 | 10 | | |
| | 30 | 30 | | |
| | **38** | **22** | **8.4** | **38** |
| Bread 3 | 40 | 30 | | |
| | 40 | 20 | | |
| | 30 | 20 | | |
| | 50 | 30 | | |
| | 40 | 30 | | |
| | **40** | **26** | **5.5** | **21** |
| Bread 4 | 480 | 590 | | |
| | 410 | 420 | | |
| | no sample | | | |
| | no sample | | | |
| | no sample | | | |
| | **445** | **505** | **120.2** | **24** |
| Bread 5 | 260 | 170 | | |
| | 200 | 160 | | |
| | 210 | 130 | | |
| | 260 | 155 | | |
| | 250 | 260 | | |
| | **236** | **175** | **49.7** | **28** |
| Bread 6 | 230 | 230 | | |
| | 290 | 250 | | |
| | 240 | 180 | | |
| | 320 | 230 | | |
| | 360 | 220 | | |
| | **288** | **222** | **25.9** | **12** |
| Bread 7 | 140 | 80 | | |
| | 50 | 10 | | |
| | 140 | 100 | | |
| | 140 | 120 | | |
| | 50 | 30 | | |
| | **104** | **68** | **46.6** | **69** |
| Bread 8 | 40 | 30 | | |
| | 150 | 140 | | |
| | 60 | 40 | | |
| | 50 | 50 | | |
| | 60 | 40 | | |
| | **72** | **60** | **45.3** | **75** |